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Molecular systematics of Salmonidae: combined nuclear data yields a robust phylogeny

Bernard J. Crespi* and Michael J. Fulton

Behavioural Ecology Research Group, Department of Biosciences, Simon Fraser University, 8888 University Drive, Burnaby, BC, Canada V5A 1S6

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Abstract

The phylogeny of salmonid fishes has been the focus of intensive study for many years, but some of the most important relationships within this group remain unclear. We used 269 Genbank sequences of mitochondrial DNA (from 16 genes) and nuclear DNA (from nine genes) to infer phylogenies for 30 species of salmonids. We used maximum parsimony and maximum likelihood to analyze each gene separately, the mtDNA data combined, the nuclear data combined, and all of the data together. The phylogeny with the best overall resolution and support from bootstrapping and Bayesian analyses was inferred from the combined nuclear DNA data set, for which the different genes reinforced and complemented one another to a considerable degree. Addition of the mitochondrial DNA degraded the phylogenetic signal, apparently as a result of saturation, hybridization, selection, or some combination of these processes. By the nuclear-DNA phylogeny: (1) (*Hucho hucho*, *Brachymystax lenok*) form the sister group to (*Salmo*, *Salvelinus*, *Oncorhynchus*, *H. perryi*); (2) *Salmo* is the sister-group to (*Oncorhynchus*, *Salvelinus*); (3) *Salvelinus* is the sister-group to *Oncorhynchus*; and (4) *Oncorhynchus masou* forms a monophyletic group with *O. mykiss* and *O. clarki*, with these three taxa constituting the sister-group to the five other *Oncorhynchus* species. Species-level relationships within *Oncorhynchus* and *Salvelinus* were well supported by bootstrap levels and Bayesian analyses. These findings have important implications for understanding the evolution of behavior, ecology and life-history in Salmonidae.
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Keywords: Salmonidae; Phylogeny; Total evidence; Anadromy

1. Introduction

The family Salmonidae comprises three subfamilies, Coregoninae (whitefish and ciscoes), Thymallinae (grayling), and Salmoninae (char, trout, and salmon). The most speciose of these, Salmoninae, includes five genera distributed throughout the Northern Hemisphere, *Brachymystax* (lenok), *Hucho* (huchen and taimen), *Oncorhynchus* (Pacific trout and salmon), *Salmo* (Atlantic salmon and brown trout), and *Salvelinus* (char) (Hart, 1973; Hendry and Stearns, 2003; Scott and Crossman, 1973). Salmonid fishes have long been of great interest due to the commercial and recreational value of some species, and they are becoming increasingly important as model systems for addressing a wide range of evolutionary and ecological questions (Elliot, 1994; Groot and Margolis, 1991; Hendry and Stearns, 2003). Inference of a robust phylogeny for this group is important for comparative analyses of salmonid adaptations (e.g., Crespi and Teo, 2002; Fleming, 1998), comparative genomics (e.g., Woram et al., 2003), studies involving inference of ancestral states (e.g., McDowall, 1997; McLennan, 1994; Stearley, 1992), and evaluation of conservation priorities (Crandall et al., 2000).

Despite the importance of salmonids to humans, and to terrestrial and marine ecosystems, their evolutionary history has remained a matter of considerable dispute for many years (e.g., Domanico et al., 1997; McKay et al., 1996; McPhail, 1997; Norden, 1961; Oakley and Phillips, 1999; Phillips and Oakley, 1997; Phillips and Pleyte, 1991; Regan, 1914; Utter et al., 1973; Utter and Allendorf, 1994). Previous species-level and genus-level phylogenetic research on salmonid fishes have provided insights into some relationships, but numerous questions remain, most notably the among-genus diver-

* Corresponding author. Fax: +604-291-3496.
E-mail address: crespi@sfu.ca (B.J. Crespi).

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gences, and species-level relationships within *Oncorhynchus* and *Salvelinus*.

The lack of a comprehensive, well-resolved and well-supported phylogeny for Salmonidae can be largely attributed to previous studies using relatively small subsets of extant salmonid diversity, and only one or at most several genes or other character sets (e.g., morphology or karyology). To overcome these limitations, we have assembled and analyzed all available DNA-sequence data for the species in this family. The main goals of our study are twofold: (1) to use these data to infer the best tree for the family as a whole, and for particular lineages; and (2) to assess what additional data (i.e., sequence from which genes) are needed to achieve a species-level tree for the entire group.

2. Methods

2.1. Data set

We compiled all of the available sequence data for salmonid fishes and one outgroup (*Plecoglossus altivelis*) (Salmoniformes: Osmeridae), which comprised 269 sequences of mitochondrial DNA (from 16 genes) and nuclear DNA (from 9 genes) for 31 species (Table 1). The bulk of these data were from Genbank, with several additional sequences graciously provided to us by T. Oakley and R. Phillips. Some species for which very little data were available (e.g., only one or several genes) were not included. However, some species with substantial amounts of missing data were included, as inclusion of such taxa has been shown to increase phylogenetic accuracy and is not expected to produce misleading results (Wiens and Reeder, 1995; Wiens, 1998a). Complete mitochondrial DNAs were available for *C. lavaretus*, *O. mykiss*, *O. tshawytscha*, *P. altivelis*, *S. salar*, *Sv. alpinus*, and *Sv. fontinalis*. The MHC genes used were chosen randomly, one for each species, from the larger sample of alleles in Genbank.

The sequences were aligned gene by gene using Clustal X (Thompson et al., 1997) and by eye, and regions with ambiguous alignments (e.g., parts of the DLOOP) were excluded. The full data set had 27,593 base pairs, and it is available as a NEXUS file from BC.

2.2. Phylogenetic analyses

We used maximum parsimony and maximum likelihood in PAUP (Swofford, 2002) and Bayesian analysis in MrBayes (http://brahms.biology.rochester.edu/software.html) (Alfaro et al., 2003; Hall, 2001; Huelsenbeck et al., 2001; Rannala and Yang, 1996; Yang and Rannala, 1997) for our analyses. We analyzed each gene separately (for genes with at least 13 taxa represented, and for vitellogenin and MHC), the full mitochondrial data set, the full nuclear-gene data set, and all of the data combined. For most of the analyses of mitochondrial data, *P. altivelis* was used as the outgroup. However, because data from this species were not available for nuclear genes, *C. lavaretus*, *B. lenok*, or *H. perryi* were also used as outgroups, depending on which was available and closest to the ingroup based on previous studies. MrMODELTEST (http://www.ebc.uu.se/systzoo/staff/nylander.html) was used to choose the most appropriate models of molecular evolution for the likelihood analyses of each separate gene, and for the combined data sets (Posada and Crandall, 1998, 2001; Sullivan and Swofford, 2001). To assess the robustness of the inferred trees, we used bootstrapping with 500 replicates under maximum parsimony and bootstrapping with 200 replicates for maximum likelihood. Maximum likelihood bootstrapping was not computationally feasible for the combined data sets. For the Bayesian analyses, we used at least 5000 trees after stabilization of the likelihoods to compute the a posteriori probabilities, which can be interpreted as the probabilities that particular clades are correct. These probabilities tend to be less conservative than maximum-likelihood bootstrap values (Alfaro et al., 2003; Douady et al., 2003). Although they tend to identify more correct monophyletic groups than do parsimony or likelihood bootstrapping in simulations, Bayesian support values may also overestimate the degree of clade support, especially for lineages descending from short internodes (Alfaro et al., 2003; Douady et al., 2003).

We have taken a 'conditional combination' approach (Bull et al., 1993; De Queiroz et al., 1995; Huelsenbeck et al., 1996) to analyze data derived from multiple genes and loci. This approach involves an assessment of congruence, using various means, prior to a decision to combine data sets or analyze them separately. We follow this approach for two reasons. First, some genes or loci may produce inconguent and incorrect results, due to such processes as sampling error, hybridization, natural selection, rate variation among lineages, variation in base composition, or a high degree of saturation (Sanderson and Schaffer, 2002; Slowinksi and Page, 1999). Second, given only minor effects from such processes in a combined data set, a total evidence analysis should yield the best results, because different genes should provide resolution and support in different regions of the tree (Bull et al., 1993; Huelsenbeck et al., 1996).

Our analysis of congruence was constrained by the variable sets of taxa for which data were available for each gene, which precluded direct comparisons of genes on a pairwise basis with ML tests (e.g., Huelsenbeck and Bull, 1996) or other methods (see Barker and Lutzoni, 2002). As a result, we evaluated the degree of congru-

Table 1
Genbank sequences used in the analyses

	DLOOP	12S	16S	ND1	ND2	CO1
B. lenok	AF125519	AF125513	AF125513			
C. artedi	AF246932			AF246933		
C. autumnalis	AJ250996					
C. clupeaformis	AF239253					
C. kiyi	U95191					
C. lavaretus	AB034824	AB034824	AB034824	AB034824	AB034824	AB034824
H. hucho						
H. perryi						
O. clarki	AF254863		AF296347	AF254865		
O. gorbuscha			AF296345			
O. keta	AB039901	AF113119	AF296344			
O. kisutch	AF318037		AF296342			
O. masou	AF429780	AF113117	AF125510			
O. mykiss	L29771	L29771	L29771	L29771	L29771	L29771
O. nerka	U59926		AF296343			
O. rhodurus						
O. tshawytscha	AF392054	AF392054	AF392054	AF392054	AF392054	AF392054
P. altivelis	AB047553	AB047553	AB047553	AB047553	AB047553	AB047553
P. coulteri	AY008713					
P. williamsoni	AY008696					
S. orhidana						
S. salar	AF133701	AF133701	AF133701	AF133701	AF133701	AF133701
S. trutta	U62286			AF117718		M64917
Sv. alpinus	AF154851	AF154851	AF154851	AF154851	AF154851	AF154851
Sv. confluentus		AF126004				
Sv. fontinalis	AF154850	AF154850	AF154850	AF154850	AF154850	AF154850
Sv. leucomaenis	AF297988		AF060445			
Sv. malma	AF298043					
Sv. namaycush	AF297989					
T. arcticus	AF329990		AF076906	AF076908		
T. thymallus	AF329989		AF036381	AF036381		

	CO2	Atp8	Atp6	CO3	ND3	ND4L
B. lenok						
C. artedi			AF246934			
C. autumnalis						
C. clupeaformis					AJ133367	
C. kiyi						
C. lavaretus	AB034824	AB034824	AB034824	AB034824	AB034824	AB034824
H. hucho						
H. perryi						
O. clarki				AF294830	AF312575	
O. gorbuscha				AF294831	AF055090	
O. keta			D84147	D84147	AF055089	D84147
O. kisutch				AF294829	AF055092	
O. masou			D63336	D63336	U28364	D63336
O. mykiss	L29771	L29771	L29771	L29771	L29771	L29771
O. nerka				AF294832	AF055091	
O. rhodurus					U28363	
O. tshawytscha	AF392054	AF392054	AF392054	AF392054	AF392054	AF392054
P. altivelis	AB047553	AB047553	AB047553	AB047553	AB047553	AB047553
P. coulteri						
P. williamsoni					AJ133369	
S. orhidana						
S. salar	AF133701	AF133701	AF133701	AF133701	AF133701	AF133701
S. trutta			X76247		U61181	
Sv. alpinus	AF154851	AF154851	AF154851	AF154851	AF154851	AF154851
Sv. confluentus						
Sv. fontinalis	AF154850	AF154850	AF154850	AF154850	AF154850	AF154850
Sv. leucomaenis						
Sv. malma						
Sv. namaycush					U61182	

Table 1 (*continued*)

	CO2	Atp8	Atp6	CO3	ND3	ND4L	
T. arcticus							
T. thymallus							
	ND4	ND5	ND6	Cyt b	GH1c	GH2c	
B. lenok			AF125052	AF125213	AF005919	AF005917	
C. artedi							
C. autumnalis				AJ251592			
C. clupeaformis							
C. kiyi							
C. lavaretus	AB034824	AB034824	AB034824	AB034824		AB001865	
H. hucho				AF172397		AF005907	
H. perryi				D58396	AF005920	AF005908	
O. clarki		AY032633	AY032633	AY032633	AF005924	AF005913	
O. gorbuscha		U66039	U66039	AF165077	AF005926	AF075572	
O. keta			AF125051	AF165078	AF005927	L04688	
O. kisutch				AF165079	AF005925	U04931	
O. masou			AF125050	D58403			
O. mykiss	L29771	L29771	L29771	L29771	AF005923	J03797	
O. nerka				AJ314568	U14551	U14535	
O. rhodurus							
O. tshawytscha	AF392054	AF392054	AF392054	AF392054	Oakley	AF005914	
P. altivelis	AB047553	AB047553	AB047553	AB047553			
P. coulteri				AY008700			
P. williamsoni				AY008701			
S. orhidana				AF202033		AF005915	
S. salar	AF133701	AF133701	AF133701	AF133701	Oakley	M21573	
S. trutta				X77526		AF005912	
Sv. alpinus	AF154851	AF154851	AF154851	AF154851	AF005921	AF005909	
Sv. confluentus						AF005911	
Sv. fontinalis	AF154850	AF154850	AF154850	AF154850			
Sv. leucomaenis				D58397			
Sv. malma							
Sv. namaycush					AF005922	AF005910	
T. arcticus				AF319544	Oakley		
T. thymallus		AF270858		AF270689			
	GH2d	ITS1	ITS2	18s	GH1d	VIT	MHC
B. lenok				AF243426			
C. artedi							
C. autumnalis							
C. clupeaformis							
C. kiyi							
C. lavaretus	AB001865					AF454745	
H. hucho							
H. perryi		M94900	AF174612				
O. clarki							
O. gorbuscha		AF170535	AF170539				U34717
O. keta	L04688	Oakley	AF170538				U34703
O. kisutch		Oakley	AF170540	AF030250		AF454747	U34692
O. masou		AF170536	AF170542	AF243427			U34697
O. mykiss	J03797	AF170533	AF170543	AF243428		AJ011689	U34715
O. nerka	U14535	Oakley	AF170537		U14551		U34711
O. rhodurus							
O. tshawytscha		AF170534	AF170541			AF454748	U34719
P. altivelis							
P. coulteri	AY008709						
P. williamsoni	AY008695						
S. orhidana		AF201313					
S. salar	M21573	AF201312		AJ427629	Phillips		X70166
S. trutta		AF072862		X98839	Phillips	AF454750	
Sv. alpinus		AF059899	AF174609	AF469620	Phillips	AF454751	
Sv. confluentus		M94902	AF174613		Phillips		
Sv. fontinalis		M94903	AF174611		Phillips	AF454752	

Table 1 (*continued*)

	CO2	Atp8	Atp6	CO3	ND3	ND4L
Sv. leucomaenis		M94904	AF174607		Phillips	
Sv. malma		M94905	AF174608		Phillips	
Sv. namaycush		M94906	AF174610		Phillips	
T. arcticus						
T. thymallus						AF454753

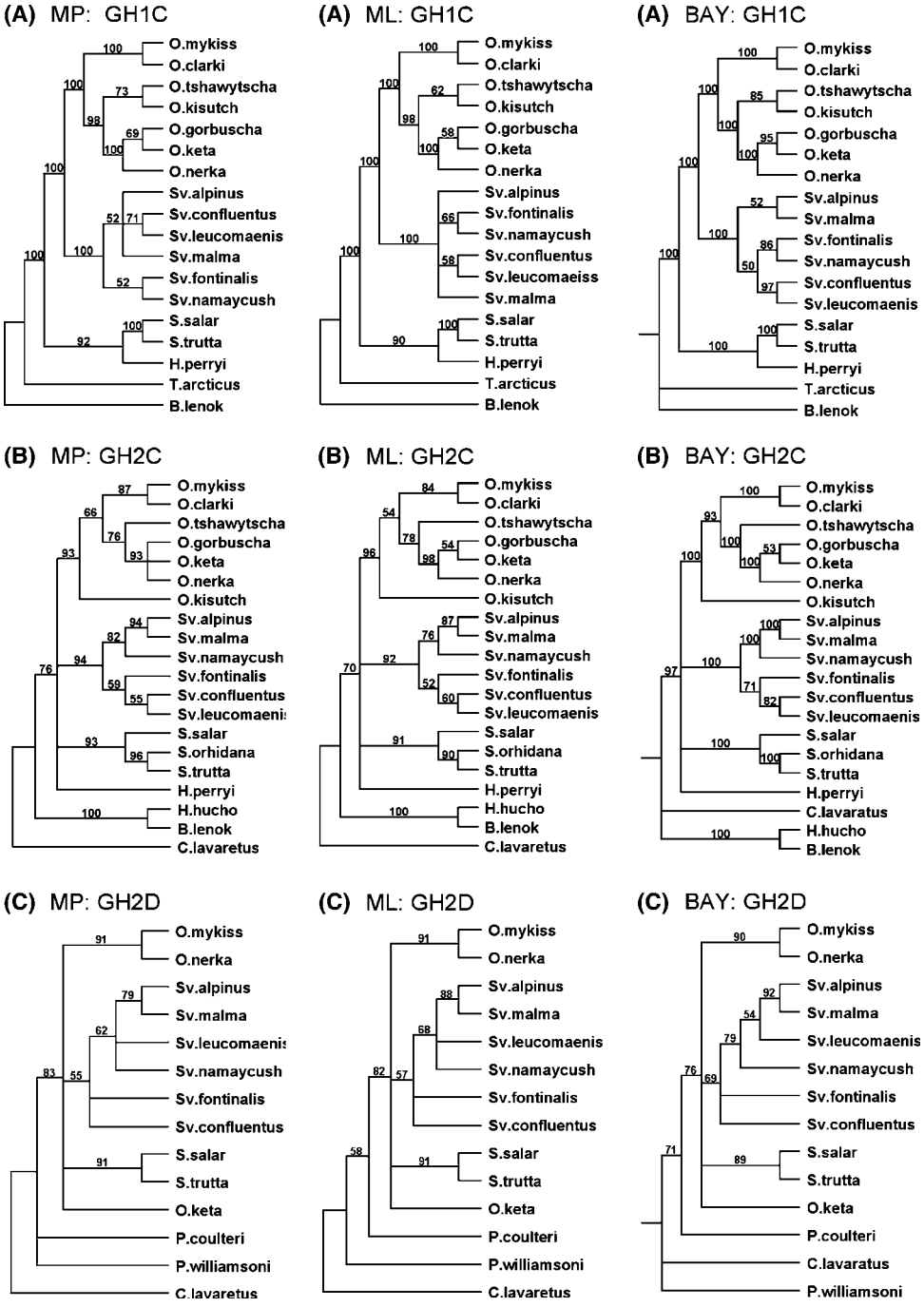

Fig. 1. Bootstrap majority-rule phylogenies inferred from individual-gene DNA data sets. MP = maximum parsimony, ML = maximum likelihood, BAY = Bayesian analysis. GH = growth hormone, ITS = internal transcribed spacer, VIT = vitellogenin, MHC = major histocompatibility complex. ML bootstraps were not computationally feasible for the DLOOP data set.

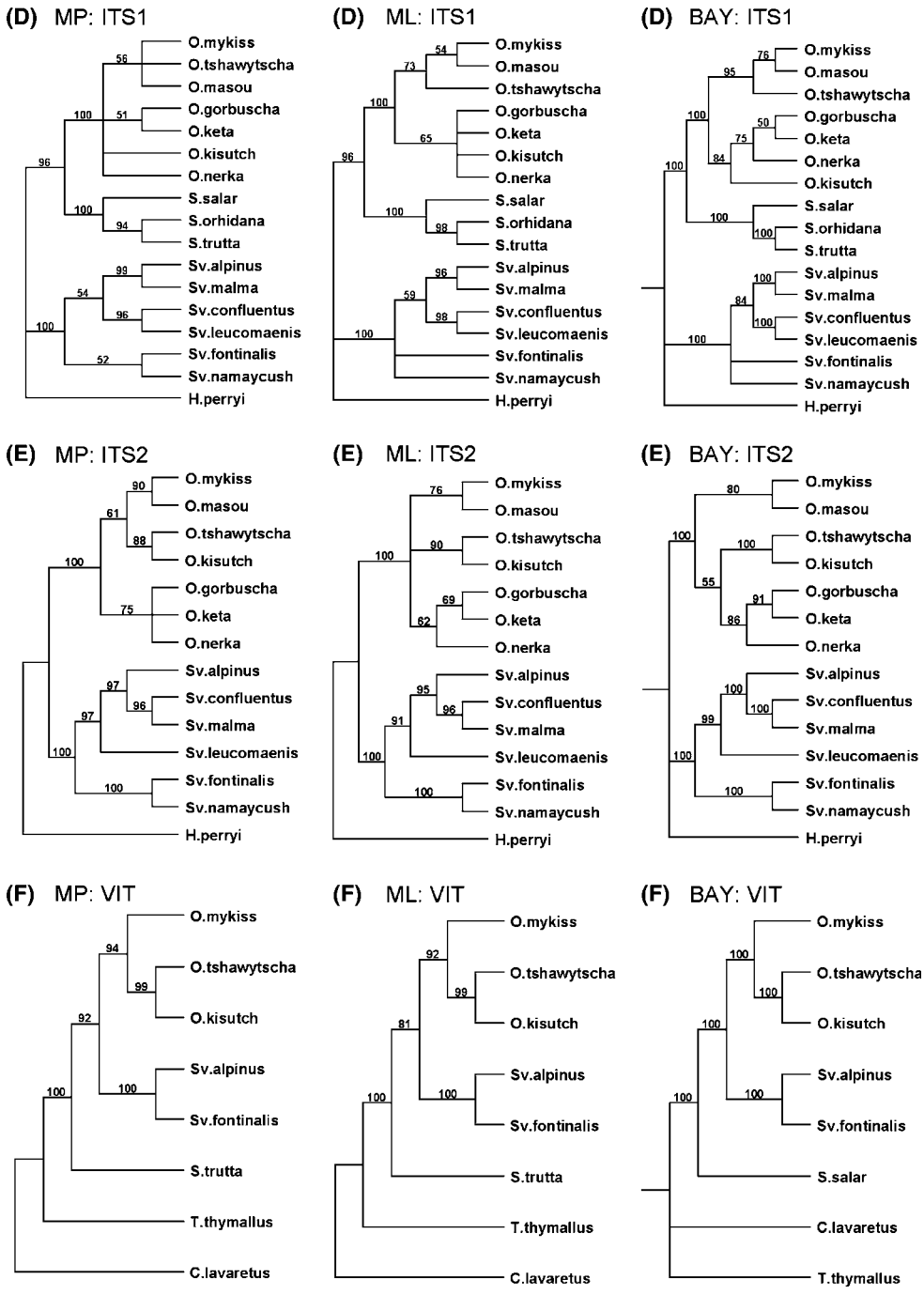

Fig. 1. (*continued*)

ence between the results from analyses of separate genes via inspection of bootstrap or a posteriori probability values, to determine how many different genes and loci supported a given monophyletic group and to what extent, and to identify any strongly supported nodes that differed among genes (De Queiroz et al., 1995).

Given potential incongruence in one or more parts of a phylogeny, we agree with Wiens (1998b) that combining data may still represent the best strategy for inferring the most-accurate tree, subject to the caveat that the gene or genes involved in possible incongruence should be considered questionable and may require removal from a combined analysis. In addition to analyzing each gene separately, we also compared the degrees of resolution and support obtained from analyses of the combined mitochondrial data set, the combined nuclear data set, the combined nuclear data set without the MHC data (since salmonid MHC is known to be under strong balancing selection, Miller and Withler, 1996), all of the data except MHC, and all of

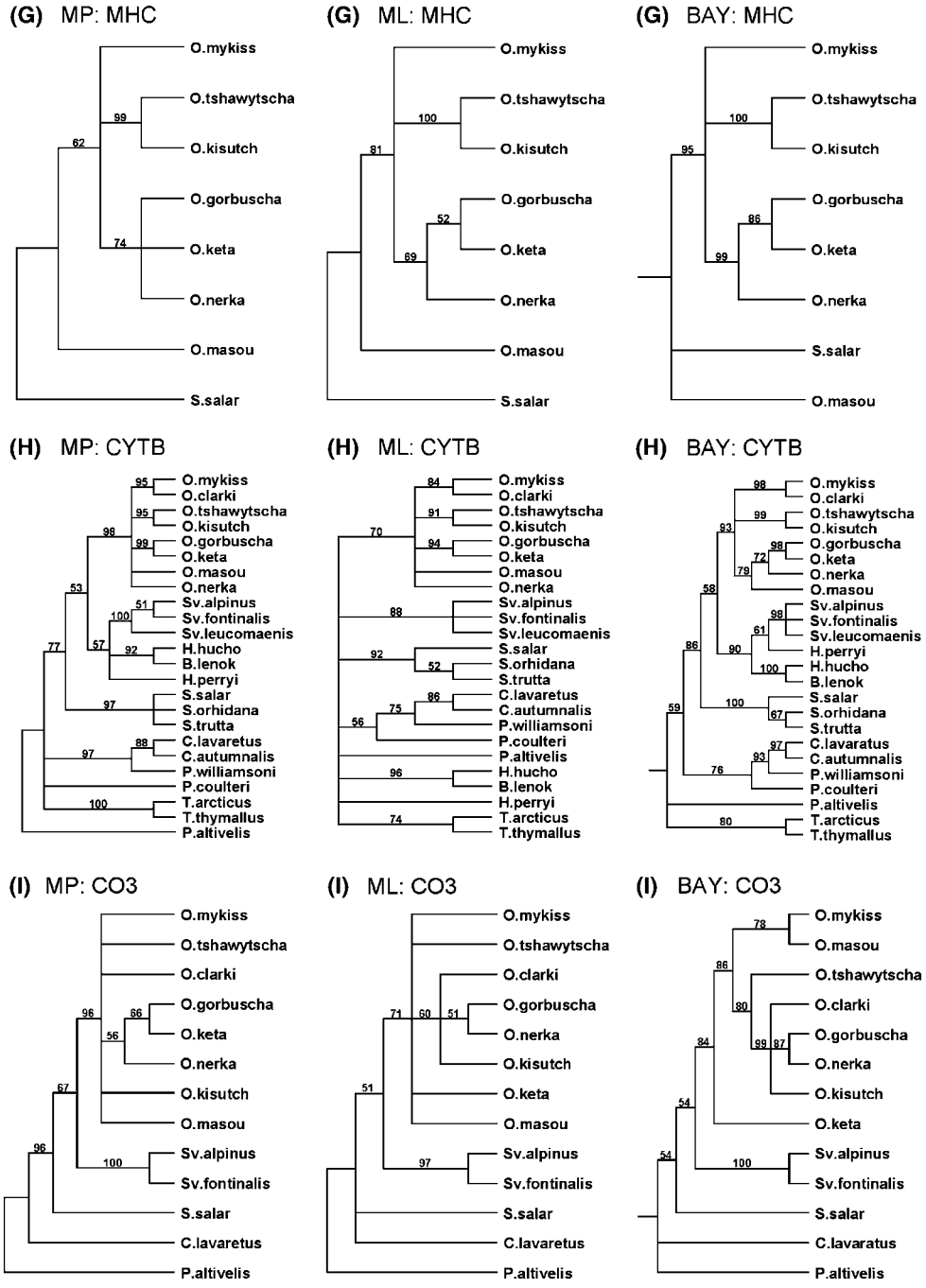

Fig. 1. continued)

the data combined. Cunningham (1997) has shown that congruence and phylogenetic accuracy tend to be positively correlated, such that strongly supported combined-data trees are likely to reflect congruence among trees from the individual data sets. Finally, alternative a priori hypotheses for the placement of particular species and sets of species were also evaluated using the SH test (Shimodaira and Hasegawa, 1999) and the Templeton test (Templeton, 1983), implemented in PAUP* as described below.

3. Results

3.1. Phylogenetic analyses of individual and combined data

Bootstrap majority-rule consensus trees from analyses using maximum parsimony and maximum likelihood, and a posteriori clade support values from Bayesian analysis, are shown in Fig. 1 for each of the individual genes, and Fig. 2 shows maximum-parsimony

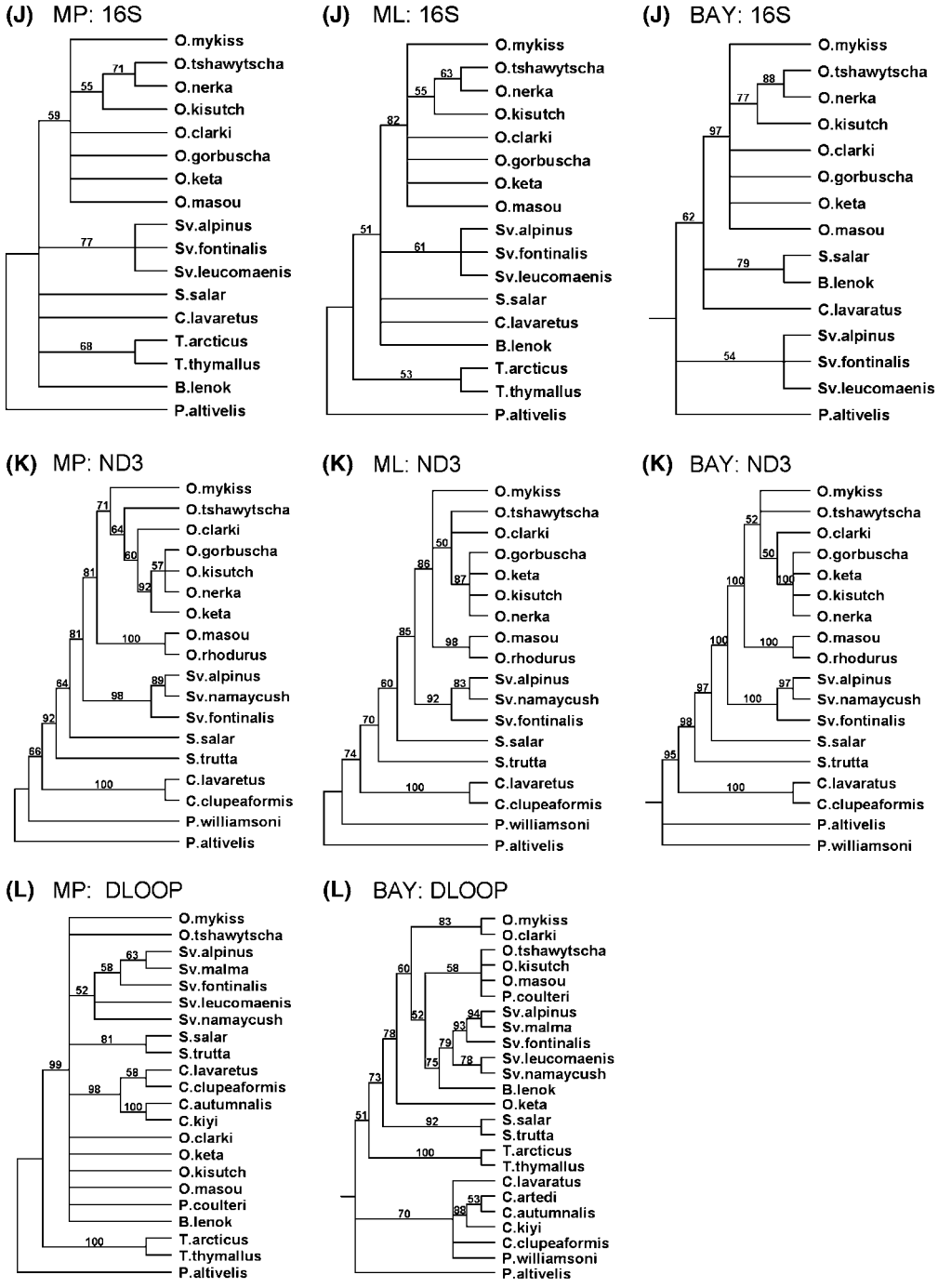

Fig. 1. (*continued*)

bootstraps and Bayesian support for the combined data sets. The individual-gene trees differ considerably in the species included and the degree of support for various relationships. We assessed the degree of support for the main phylogenetic hypothesis among Salmonidae by collating the bootstrap and Bayesian a posteriori values relevant to each putative monophyletic group of interest (Table 2). This analysis allows us to assess the degree to which the results from the different data sets are congruent, reinforce one another, or provide conflicting signal.

3.2. *Oncorhynchus*

The monophyly of *Oncorhynchus*, which is strongly supported by virtually all previous studies (Murata et al., 1993, 1996, 1998; Oakley and Phillips, 1999; Oleinik, 1997; Phillips and Oakley, 1997 and references therein),

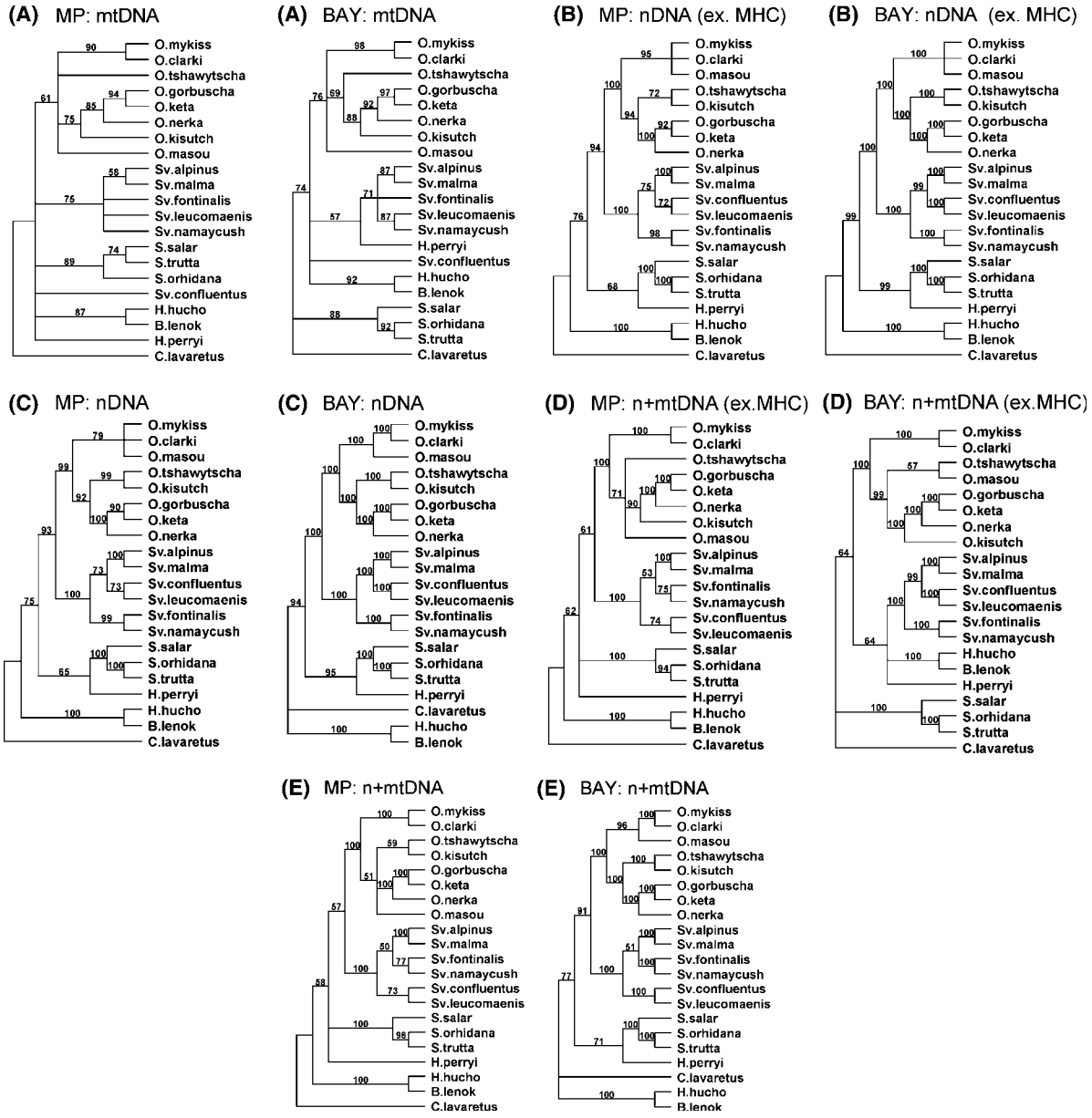

Fig. 2. Bootstrap majority-rule and Bayesian phylogenies for the combined data sets: (A) all mtDNA, (B) all nuclear DNA excluding MHC, (C) all nuclear DNA, (D) all nuclear DNA and mtDNA excluding MHC, (E) all data.

was also supported in our analyses by all of the individual genes and by the combined data sets (Table 2). However, the strength of support varied considerably across data sets, with the nuclear genes tending to provide higher bootstrap and Bayesian a posteriori values than the mitochondrial genes, and the combined mtDNA data set (Fig. 2A) returning the weakest support.

The monophyly of *Oncorhynchus* excluding *O. clarki*, *O. masou*, and *O. mykiss* was strongly supported here by GH1C and by the combined nuclear DNA. By contrast, this group was not supported by analyses of ITS1, CO3, ND3, and GH2C, the other genes that provided any degree of bootstrap majority-rule resolution for this clade. However, the monophyly of this group was not strongly contradicted by these analyses, as the relevant bootstrap values were low. The reduced support for this clade, compared to the genus as a whole, is due primarily to the presence of *O. mykiss*, *O. clarki* or *O. masou* among the other *Oncorhynchus* in some of the trees.

The clades (*O. nerka*, *O. gorbuscha*, *O. keta*) and (*O. gorbuscha*, *O. keta*) have been inferred in most previous studies of *Oncorhynchus* (Domanico and Phillips, 1995; Kitano et al., 1997; McKay et al., 1996; Murata et al., 1993, 1996, 1998; Oakley and Phillips,

Table 2
Bootstrap values from maximum likelihood (200 replicates, in plain text) or Bayesian analyses (majority-rule of at least 5000 trees, in italics), and maximum parsimony (500 replicates, in parentheses) analyses, for relevant clades in Figs. 1 and 2

All *Oncorhynchus* (Pacific salmon and Pacific trout)	
(*O. tshawytscha*, *O. kisutch*, *O. nerka*, *O. gorbuscha*, *O. keta*, *O. mykiss*, *O. clarki*, *O. masou*)	
GH1C	100 *100* (100)
ITS1	100 *100* (100)
ITS2	100 *100* (100)
GH2C	96 *100* (93)
CYTB	70 *93* (98)
CO3	71 *84* (96)
ND3	86 *52* (81)
16S	82 *97* (59)
MHC	(62)
All mt DNA	*76* (61)
All nuclear DNA (excl. MHC)	*100* (100)
All nuclear DNA	*100* (99)
All nuclear DNA (excl. MHC) + mtDNA	*100* (100)
All nuclear + mt DNA	*100* (100)
Pacific salmon excluding *O. masou*	
(*O. tshawytscha*, *O. kisutch*, *O. nerka*, *O. gorbuscha*, *O. keta*)	
GH1C	98 *100* (98)
ITS2	*55*
GH2C	Not monophyletic 54 *93* (66)
CO3	Not monophyletic 60 *99*
ITS1	Not monophyletic 73 *95* (56)
CYTB	Not monophyletic *79*
ND3	Not monophyletic *100*
All nuclear DNA (excl. MHC)	*100* (94)
All nuclear DNA	*100* (92)
All nuclear DNA (excl. MHC) + mtDNA	Not monophyletic (includes *O. masou*, 99)
(*O. nerka*, *O. gorbuscha*, *O. keta*)	
GH1C	100 *100* (100)
GH2C	98 *100* (93)
MHC	69 *99* (74)
ITS2	62 *86* (75)
CYTB	*72*
ITS1	65 *84* includes *O. kisutch*
ND3	87 *100* (92) includes *O. kisutch*
CO3	Not monophyletic 60 *99* (monophyletic, 56)
16S	Not monophyletic *O. nerka* with *O. tshawytscha, O. kisutch* 55,63 *77,88* (55,71)
All mt DNA	*92* (85)
All nuclear DNA (excl. MHC)	*100* (100)
All nuclear DNA	*100* (100)
All nuclear DNA (excl. MHC) + mtDNA	*100* (100)
All nuclear + mt DNA	*100* (100)
(*O. gorbuscha*, *O. keta*)	
CYTB	94 *98* (99)
ITS2	69 *91*
GH1C	58 *95* (69)
GH2C	54 *53*
MHC	52 *86*
ITS1	*50* (51)
ND3	(Not monophyletic *O. gorbuscha* with *O. nerka*, *O. kisutch*: 57)
CO3	Not monophyletic 60 *99* (monophyletic, 66)
All mt DNA	*97* (94)
All nuclear DNA (excl. MHC)	*100* (92)
All nuclear DNA	*100* (90)
All nuclear DNA (excl. MHC) + mtDNA	*100* (100)
All nuclear + mt DNA	*100* (100)
(*O. tshawytscha*, *O. kisutch*)	
MHC	100 *100* (99)
VIT	99 *100* (99)

Table 2 (*continued*)

ITS2	90 *100* (88)
CYTB	91 *99* (95)
GH1C	62 *85* (73)
CO3	Not monophyletic 60 *99*
16S	Not monophyletic, includes *O. nerka*: 55,63 *77,88* (55,71)
ITS1	Not monophyletic 73 *95* (56)
GH2C	Not monophyletic 78 *100* (76)
ND3	Not monophyletic 87 *100* (92)
All mt DNA	Not monophyletic *88* (75)
All nuclear DNA (excl. MHC)	*100* (72)
All nuclear DNA	*100* (99)
All nuclear DNA (excl. MHC) + mtDNA	Not monophyletic *100* (90)
All nuclear + mt DNA	*100* (59)
Pacific trout and *O. masou*	
(*O. mykiss*, *O. masou*)	
ITS2	76 *80* (90)
CO3	*78*
ITS1	54 *76* (56, includes *O. tshawytscha*)
ND3	Not monophyletic *52*
MHC	Not monophyletic 81 *95* (62)
DLOOP	Not monophyletic *83*
CYTB	Not monophyletic *98*
All mt DNA	Not monophyletic *98* (90) (*O. mykiss* with *O. clarki*)
All nuclear DNA	Not monophyletic *100* (*O. mykiss* with *O. clarki*)
All nuclear DNA (excl. MHC) + mtDNA	Not monophyletic *100* (100) (*O. mykiss* with *O. clarki*)
All nuclear + mtDNA	Not monophyletic *100* (100) (*O. mykiss* with *O. clarki*)
(*O. mykiss*, *O. clarki*)	
GH1C	100 *100* (100)
CYTB	84 *98* (95)
GH2C	84 *100* (87)
DLOOP	*83*
ND3	Not monophyletic 50 *50*
CO3	Not monophyletic 60 *99*
All mt DNA	*98* (90)
All nuclear DNA (excl. MHC) + mtDNA	*100* (100)
All nuclear DNA	*100*
All nuclear + mt DNA	*100* (100)
(*O. mykiss*, *O. clarki*, *O. masou*)	
ND3	*O. masou* as sister-taxon to all other *Oncorhynchus*; Not monophyletic *52*
CO3	Not monophyletic 60 *99* ML, MP: *O. masou* basal but unresolved in *Oncorhynchus*
CYTB	ML, MP: *O. masou* basal but unresolved in *Oncorhynchus* Not monophyletic *79*
ND3	Not monophyletic 50; *O. masou* basal in *Oncorhynchus*; (MP: *O. masou* sister to other *Oncorhynchus*, 71)
All nuclear DNA (excl. MHC)	*100* (95)
All nuclear DNA	*100* (79)
All nuclear DNA (excl. MHC) + mtDNA	Not monophyletic *99* (71), *O. masou* with O. *tshawytscha*
All nuclear + mt DNA	*96*, Not monophyletic (51), *O. masou* basal in Pacific salmon
Salvelinus	
(*Sv. alpinus*, *Sv. malma*, *Sv. confluentus*, *Sv. leucomaenis*, *Sv. fontinalis*, *Sv. namaycush*)	
GH1C	100 *100* (100)
ITS1	100 *100* (100)
ITS2	100 *100* (100)
GH2C	92 *100* (94)
GH2D	57 *69* (55)
All nuclear DNA (excl. MHC)	*100* (100)
All nuclear DNA	*100* (100)
All nuclear DNA (excl. MHC) + mtDNA	*100* (100)
All nuclear + mt DNA	*100* (100)

Table 2 (*continued*)

(*Sv. alpinus*, *Sv. malma*, *Sv. confluentus*, *Sv. leucomaenis*)	
ITS2	91 *99* (97)
ITS1	59 *84* (54)
GH1C	Not monophyletic *50* (monophyletic: 52)
GH2D	Not monophyletic 68 *79* (62)
GH2C	Not monophyletic 76 *100* (82)
All mtDNA	Not monophyletic *87* (75)
All nuclear DNA (excl. MHC)	*99* (75)
All nuclear DNA	*100* (73)
All nuclear DNA (excl. MHC) + mtDNA	*99*
All nuclear + mt DNA	Not monophyletic *51* (50)
(*Sv. alpinus*, *Sv. malma*)	
ITS1	96 *100* (99)
GH2C	87 *100* (94)
GH2D	88 *92* (79)
DLOOP	(63)
GH1C	*52*
ITS2	Not monophyletic 96 *100* (96)
All mt DNA	*87* (52)
All nuclear DNA (excl. MHC)	*100* (100)
All nuclear DNA	*100* (100)
All nuclear DNA (excl. MHC) + mtDNA	*100* (100)
All nuclear + mt DNA	*100* (100)
(*Sv. confluentus*, *Sv. leucomaenis*)	
ITS1	98 *100* (96)
GH1C	58 *97* (71)
GH2C	60 *82* (55)
ITS2	Not monophyletic 96 *100* (97)
GH2D	Not monophyletic 68 *79* (62)
All mt DNA	Not monophyletic *87* (75)
All nuclear DNA (excl. MHC)	*100* (72)
All nuclear DNA	*100* (73)
All nuclear DNA (excl. MHC) + mtDNA	*100* (74)
All nuclear + mt DNA	*100* (73)
(*Sv. fontinalis*, *Sv. namaycush*)	
ITS2	100 *100* (100)
GH1C	66 *86* (52)
ITS1	(52)
GH2D	Not monophyletic 68 *79* (62)
GH2C	Not monophyletic 76 *100* (82)
ND3	Not monophyletic 83 (89)
All mtDNA	Not monophyletic *87*
All nuclear DNA (excl. MHC)	*100* (98)
All nuclear DNA	*100* (99)
All nuclear DNA (excl. MHC) + mtDNA	*100* (75)
All nuclear + mt DNA	*100* (77)
Sister-group to *Oncorhynchus* (*Oncorhynchus, Salvelinus*)	
GH1C	100 *100* (100)
VIT	81 *100* (92)
ND3	85 *100* (81)
DLOOP	*78* (includes *P. coulteri*, *B. lenok*, *Oncorhynchus* paraphyletic)
CO3	51 *54* (67)
CYTB	*58* (53) (*Salvelinus* lineage includes *Hucho* and *B. lenok)*
All nuclear DNA (excl. MHC)	*100* (94)
All nuclear DNA	*100* (93)
All nuclear DNA (excl. MHC) + mtDNA	*64, Salvelinus* includes *Hucho* and *B. lenok* (61)
All nuclear + mt DNA	*91* (57)
(*Oncorhynchus*, *Salmo*)	
ITS1	96 *100* (96)

Results from genes for which relationships remain unresolved in the 50% majority-rule consensus trees are not shown. For clades that are not monophyletic, the highest bootstrap or a posteriori Bayesian value supporting the lack of monophyly is listed. Genes are listed from top to bottom in order of the degree to which they tend to support (top) or contradict (bottom) the presence of the monophyletic group shown.

1999; Oohara et al., 1997, 1999; Osinov, 1999; Phillips and Oakley, 1997; Shedlock et al., 1992 and references therein) and have yet to be strongly contradicted. In our analyses, we found support for (*O. nerka*, *O. gorbuscha*, *O. keta*) from the nuclear genes GH1C, GH2C, MHC, and ITS2, as well as from each of the combined data sets. The results that were incongruent with (*O. nerka*, *O. gorbuscha*, *O. keta*) tended to involve relatively low bootstrap and Bayesian values, excepting those from ND3 and GH2D. The group (*O. gorbuscha*, *O. keta*) was accorded bootstrap and Bayesian support from CYTB, ITS2, GH1C, GH2C, ITS1, and MHC though most of these support values were rather low. However, the contradictory results from ND3 and CO3 were weak, and the combined data sets provided very strong support for this clade.

The sister-taxon status of *O. tshawytscha* with *O. kisutch* is supported by considerable previous phylogenetic work, and has yet to be contradicted strongly (Du et al., 1993; Kitano et al., 1997; McKay et al., 1996; Murata et al., 1993, 1996, 1998; Oakley and Phillips, 1999; Oohara et al., 1997, 1999; Osinov, 1999; Phillips and Oakley, 1997 and references therein). This clade was supported in our analyses by MHC, VIT, ITS2, CYTB, GH1C, the combined nuclear data sets, and the complete data set. By contrast, analyses of CO3, 16S, ITS1, GH2C, ND3, the mtDNA data, and the complete data set without MHC, yielded results contradictory to the presence of this clade. However, the bootstrap and Bayesian values for these genes tended to be relatively low compared to the clade support values from the genes that supported it. Moreover, the phylogenetic positions of these two species were inconsistent across CO3, 16S, ITS1, GH2C, and ND3 (Fig. 1), such that there was no evidence for one or more specific alternative placements. Considered together, these results are consistent with the sister-taxon status of *O. tshawytscha* and *O. kisutch*, although support for this grouping is not entirely unambiguous.

The phylogenetic placement of *O. masou*, in relation to the other *Oncorhynchus*, has not received strong support from previous analyses (e.g., Kitano et al., 1997; McKay et al., 1996; Murata et al., 1996; Oleinik, 2000; Oohara et al., 1997, 1999). To elucidate the relationships of *O. mykiss*, *O. clarki* and *O. masou* among themselves and to the other *Oncorhynchus*, we jointly evaluated the degrees of support for the clades (*O. mykiss*, *O. clarki*), (*O. mykiss*, *O. masou*), and (*O. mykiss*, *O. clarki*, *O. masou*) (Table 2). The sister-taxon relationship of *O. mykiss* with *O. clarki* was clearly upheld by the analyses of GH1C, CYTB, GH2C, the mtDNA data, and the full combined data set, and it was relatively weakly incongruent with results from ND3 and CO3. In addition, a relationship of *O. mykiss* with *O. masou* was inferred from ITS2 and (marginally) from ITS1. Considering all three species together, we note that by most single-gene analyses, *O. masou* appears relatively basal in *Oncorhynchus* but its position is unresolved, as are the positions of *O. mykiss*, *O. clarki*, or both in the analyses of CO3, 16S, and ND3. A basal but weakly resolved position for *O. masou* is also apparent in analyses of the combined mtDNA data (Fig. 2A), the full data set with MHC excluded (Fig. 2D), and the the full data set (Fig. 2E) using maximum parsimony.

The clearest results for the position of *O. masou* come from analyses of the nuclear DNA data set, with or without MHC (Fig. 2B and C). These analyses provided strong bootstrap support, Bayesian support, or both for the monophyly of (*O. mykiss*, *O. clarki*, *O. masou*). The Bayesian analyses also strongly supported sister-taxon status of *O. mykiss* and *O. clarki*. These results indicate that the various individual genes provide no support or weak to moderate support for the clade (*O. masou*, (*O. mykiss*, *O. clarki*)), and that no evidence strongly

Table 3
Results from Templeton and SH tests that in each case compare the best (unconstrained) tree to an alternative, constraint tree: (a) best unconstrained tree vs. best tree showing *O. masou* not with *O. mykiss* and *O. clarki*, (b) best unconstrained tree vs. best tree with *Salmo* as sister-group to *Oncorhynchus*, (c) best unconstrained tree vs. best tree where *O. kisutch* and *O. tshawytscha* are not sister-taxa

		Constraint		
		(a) Not (*O. masou*, *O. mykiss*, *O. clarki*)	(b) (*Salmo*, *Oncorhynchus*)	(c) Not (*O. kisutch*, *O. tshawytscha*)
Data set				
Nuclear data without MHC	MP	1703 vs. 1709 $P = 0.058$	1703 vs. 1718 $P = 0.022$	1700 vs. 1702 $P > 0.40$
	ML	22712.21 vs. 22729.14 $P = 0.030$	22712.21 vs. 22733.51 $P = 0.046$	22712.21 vs. 22718.89 $P > 0.10$
All nuclear data	MP	1911 vs. 1914 $P > 0.40$	1911 vs. 1926 $P = 0.022$	1908 vs. 1919 $P = 0.012$
	ML	25687.05 vs. 25696.96 $P = 0.087$	25687.05 vs. 25708.15 $P = 0.050$	25687.05 vs. 25717.39 $P = 0.018$

For Templeton tests (MP), tree lengths are shown, and for SH tests (ML), likelihoods are shown. For the MP analysis of (c), *H. hucho* was excluded due to apparent long-branch attraction into *Oncorhynchus* in the constraint tree. Qualitatively similar results were obtained using the constraint tree "Not (*Oncorhynchus, Salvelinus*)".

contradicts these relationships. However, taken together, the nuclear ones reinforce and complement one another sufficiently to provide strong evidence that *O. masou* belongs with the Pacific trout.

3.3. Salvelinus

The monophyly of *Salvelinus* was supported by all of the genes for which data on this genus was available, though support from GH2D, and from the combined mitochondrial DNA data set, was relatively weak (Table 2). Some of the relationships within *Salvelinus* were reasonably clear, but for others different genes yielded incongruent results. Thus, the monophyly of (*Sv. alpinus*, *Sv. malma*, *Sv. confluentus*, *Sv. leucomaenis*) was supported strongly by ITS2, moderately supported by the complete nuclear DNA data set, and weakly supported by ITS1 and GH1C. However, the differing results from analyses of GH2D and GH2C, the combined mtDNA data set, and the full data set, preclude unambiguous interpretation of this result. Similarly, the groups (*Sv. alpinus*, *Sv. malma*), (*Sv. confluentus*, *Sv. leucomaenis*), and (*Sv. fontinalis*, *Sv. namaycush*) were each upheld, often with high bootstrap and Bayesian support values, for one or more genes, but analyses of one or more other genes yielded notably incongruent results. For each of these cases, the combined data sets, especially the nuclear data, lent strong or moderate support to the group, but this support tended to stem from only one or two genes that may or may not be indicating the correct phylogeny.

The simplest interpretation of these heterogeneous results is that one or more of the genes does not reflect the species tree for *Salvelinus*. In particular, the data from ITS2 disrupts the monophyly of both (*Sv. alpinus*, *Sv. malma*) and (*Sv. confluentus*, *Sv. leucomaenis*), because it strongly groups *Sv. confluentus* with *Sv. alpinus* and *Sv. malma*, and the data from GH2C, GH2D, and ND3 prevents (*Sv. alpinus*, *Sv. malma*, *Sv. confluentus*, *Sv. leucomaenis*) and (*Sv. fontinalis*, *Sv. namaycush*) from each being monophyletic, because they position *Sv. namaycush* strongly with *Sv. alpinus* and *Sv. malma*. As discussed below, these findings are consistent with the hypothesis that extensive hybridization has obfuscated relationships among species of *Salvelinus* as inferred from DNA-sequence data.

3.4. Relationship of Oncorhynchus to Salvelinus and Salmo

A sister-taxon relationship between *Oncorhynchus* and *Salvelinus* is strongly supported by GH1C, VIT, ND3, and the combined nuclear data sets, and weakly supported by CO3 and CYTB (Table 2). By contrast, the ITS1 data groups *Oncorhynchus* and *Salmo* with such a high degree of confidence that is it clearly incongruent with the results from these other data sets. This analysis of ITS1 appears problematic, however, because our analyses of other genes have shown that *H. perryi* may not be an appropriate outgroup for an analysis of intergeneric relationships between *Oncorhynchus*, *Salmo*, and *Salvelinus*. CYTB groups *H. perryi* with *Salvelinus* with 57% bootstrap confidence in the parsimony analysis, and GH1C and the complete nuclear DNA data set group *H. perryi* with *Salmo*, with 90–92% and 66% confidence respectively. Thus, given that *H. perryi* may belong in the ingroup, the analysis of ITS1 cannot be used to address relationships between *Oncorhynchus*, *Salmo*, and *Salvelinus*. The fish species that are closest to this set of taxa, but definitely not in the ingroup, are Cichlidae, which are highly divergent in ITS1 (i.e., on the order of 50% or more divergent in nucleotide sequence). Use of *Neochromis nigricans* (Genbank U67338) as an outgroup, aligned to the taxa in the ITS1 data set using Clustal X, yielded a phylogeny with *Oncorhynchus* and *Salmo* as sister-taxa but with only 58% bootstrap support from maximum parsimony analysis (500 replicates).

3.5. Statistical tests of alternative hypotheses

We used SH tests and Templeton tests to evaluate alternative hypotheses for three important questions in salmonid phylogenetics: (1) the phylogenetic position of *O. masou*, (2) the sister-group to *Oncorhynchus*, and (3) the monophyly of (*O. tshawytscha*, *O. kisutch*) (Table 3). These tests used the two combined nuclear-gene data sets, which, as described below, provide what we believe is the best estimate of salmonid phylogeny.

The monophyly of (*O. masou*, *O. mykiss*, *O. clarki*) was statistically supported by the SH test of the nuclear data without MHC, and support was marginally non-significant ($0.05 < P < 0.10$) for the Templeton test of this data set and the SH test of the full nuclear data set (Table 3). These results are consistent with erosion of bootstrap and Bayesian support for (*O. masou*, *O. mykiss*, *O. clarki*) with the addition of the MHC data (Fig. 2).

A sister-taxon relationship between *Oncorhynchus* and *Salmo* was statistically rejected at the 0.05 level by all four of the analyses (Table 3). These results concur with the strong bootstrap and Bayesian values for (*Oncorhynchus*, *Salvelinus*) shown in Fig. 2, and they show that the apparently incongruent results from ITS1 do not substantially disrupt the monophyly of these two genera.

The relationship (*O. tshawytscha*, *O. kisutch*) was statistically supported by both the SH test and the Templeton test using the full nuclear data set (Table 3). By contrast, the SH test on the data set excluding MHC gave a result that was non-significant ($P = 0.14$), and the Templeton test result provided no support for the

monophyly of this pair of species. Overall, these findings are consistent with the moderate (72%) maximum-parsimony bootstrap support for (*O. tshawytscha*, *O. kisutch*) in the nuclear data set excluding MHC (Fig. 2D), the strong Bayesian support for this group in this data set, and the 100% bootstrap and Bayesian support for this group from the combined nuclear data set (Fig. 2C).

4. Discussion

This is the first study of salmonid phylogenetics that uses virtually all of the DNA sequence data currently available. Our analyses of the data from each gene separately, followed by combined analyses of the mitochondrial data, the nuclear data, and the full combined data set, showed that the mitochondrial data yielded levels of resolution and support that were substantially lower than the nuclear data, and that the nuclear data showed higher levels of resolution and support than did the nuclear and mitochondrial data combined (Fig. 2). These findings indicate that although some of the individual mitochondrial genes provide good evidence for some salmonid relationships (Table 2), the mitochondrial data taken together reduced the strength of the phylogenetic signal. The high noise to signal ratio of the mitochondrial data is probably due to saturation, effects of hybridization, selection (Bernatchez et al., 1995; Wilson and Bernatchez, 1998) or some combination of these processes, and it was not alleviated by removal of third-codon positions for protein-coding genes (results not shown). Such a lack of clear, strong signal in mitochondrial data has probably been responsible for much of the ongoing uncertainty regarding the molecular phylogenetics of Salmonidae.

In the combined nuclear DNA data sets, the different genes reinforced and complemented one another to a considerable degree, yielding generally well-resolved and well-supported trees (Fig. 2B and C). These trees agree with the results of most previous studies, but also help to resolve some long-standing uncertainties regarding the placement of *O. masou*, the phylogeny of the Pacific salmon, relationships within *Salvelinus*, and the sister-taxon to *Oncorhynchus*.

4.1. Oncorhynchus masou

By our combined nuclear phylogenies, *Oncorhynchus masou* forms a monophyletic group with *O. mykiss* and *O. clarki*, and these three taxa comprise the sister-group to the five other *Oncorhynchus* species. This result appears to provide a striking case of data from different genes complementing and reinforcing one another. Thus, none of the genes analyzed separately provides information on the monophyly of this group of three species, but GH1C, GH2C, ITS1, ITS2, and CYTB each supported the monophyly of (*O. mykiss*, *O. clarki*) or (*O. mykiss*, *O. masou*). Taken together, the nuclear DNA indicated good support for this clade from maximum-parsimony bootstraps (95% without the MHC data, and 79% with MHC) and Bayesian support values (100% for other data sets). Moreover, the Bayesian analysis of the full nuclear data set also provided 100% support for (*O. mykiss*, *O. clarki*), which is consistent with numerous previous studies (e.g., Kitano et al., 1997; McKay et al., 1996; Oakley and Phillips, 1999; Oleinik, 1997; Oohara et al., 1997, 1999; Phillips and Ráb, 2001). Results of the SH and Templeton tests (Table 3) are also consistent with the group (*O. masou*, *O. mykiss*, *O. clarki*), although only the SH test on the data set excluding the questionable MHC data achieved statistical significance.

Previous studies have generally considered *O. masou* to be basal within the Pacific salmon or within *Oncorhynchus* as a whole (Kitano et al., 1997; McKay et al., 1996; Murata et al., 1996; Oohara et al., 1997, 1999; see also Oleinik, 2000). Our findings provide the first firm evidence for its phylogenetic position within the clade of Pacific trout. This inference is consistent with diverse additional forms of evidence from allozymes, morphology, behavior, biogeography, and life history (Table 4), and it should motivate more-detailed evaluation of the evolution of phenotypic traits within this lineage.

4.2. Pacific salmon

Our analyses provide a fully resolved and well-supported multi-gene phylogeny for *Oncorhynchus* excluding *O. masou*, *O. mykiss*, and *O. clarki*. The relationships among *O. tshawytscha*, *O. kisutch*, *O. nerka*, *O. gorbuscha*, and *O. keta* shown in Fig. 2B and C have been believed for some time from a variety of morphological, genetic, and other data (Domanico and Phillips, 1995; Domanico et al., 1997; Kitano et al., 1997; McKay et al., 1996; Murata et al., 1993, 1996, 1998; Oakley and Phillips, 1999; Oleinik, 1997; Oohara et al., 1997, 1999; Osinov, 1999; Phillips and Oakley, 1997; Phillips and Pleyte, 1991; Shed'ko et al., 1996; Shedlock et al., 1992; Smith and Stearley, 1989; Takasaki et al., 1994; Thomas and Beckenbach, 1989; Thomas et al., 1986; Utter et al., 1973; Utter and Allendorf, 1994). However, previous analyses have lacked unambiguous or strong support for at least one of the nodes, usually many more.

We suspect that the prior lack of conclusive results for the phylogeny of Pacific salmon has been due to a combination of saturation of mitochondrial DNA (e.g., McKay et al., 1996), such that it provides little evidence for more-basal nodes, possible selection on mtDNA (e.g., Bernatchez et al., 1995; Wilson and Bernatchez, 1998), and potential hybridization of *O.*

Table 4
Evidence from previous studies that (*O. masou, O. mykiss, O. clarki*) represents a monophyletic group

Evidence	References
(1) Chromosome number of *O. masou* ($2n = 66$) is most similar to that of *O. mykiss* (58–64) and *O. clarki* (64–68)	Phillips and Ráb (2001)
(2) *O. masou* similar to *O. mykiss* in muscle proteins and to *O. mykiss* and *O. clarki* in allozymes	Tsuyuki and Roberts (1966), Utter et al. (1973)
(3) *O. masou* is 'most troutlike' of *Oncorhynchus* in morphology and behavior	Neave (1958), Yoshiyasu (1973), Stearley (1992)
(4) Some *O. masou* males and females are iteroparous	Tanaka (1965), Tsuyuki and Roberts (1966), Kato (1991), Healey et al. (2001)
(5) Male *O. masou* interbreed best with female *O. mykiss*, compared to crosses with other salmonids (i.e., low levels of post-zygotic isolation in laboratory studies)	Chevassus (1979)
(6) *O. mykiss*, *O. clarki*, and *O. masou* have similar life histories, with freshwater residence times 1–2+ years, freshwater populations common	Rounesfell (1958), Willson (1997)
(7) *O. masou* feed and mature during freshwater spawning migration, like *O. mykiss* and *O. clarki* but unlike other Pacific salmon	Miller and Brannon (1981), Groot and Margolis (1991)
(8) Distribution of *O. masou* is precisely parapatric to that of *O. mykiss*, with line of demarkation near Amur River, Sea of Othotsk	Lee et al. (1980), Kato (1991)

tshawytscha or *O. kisutch* with one or more of the other three species. Indeed, *O. tshawytscha* and *O. kisutch* show a curious tendency to group with *O. keta*, *O. gorbuscha*, and *O. nerka* in analyses of the GH2C, 16S, and ND3 data sets. Given that the fertility of some of the crosses between (*O. tshawytscha* or *O. kisutch*) and (*O. keta*, *O. gorbuscha*, or *O. nerka*) is currently high (Chevassus, 1979), it should have been even higher in the past, and hybridization events could have led to the moderate degree of discordance between gene trees observed here (see also Rosenfield et al., 2000). Regardless of such apparent incongruities, the clade (*O. tshawytscha*, *O. kisutch*) is strongly supported (99–100% bootstrap or Bayesian support values) by all of the analyses of the full nuclear data set, by the Bayesian analysis of the nuclear data set with MHC excluded, and by the SH and Templeton tests for the full nuclear data set.

4.3. Salvelinus

Our combined nuclear DNA data sets provide a well-resolved and generally well-supported phylogeny for within the genus *Salvelinus*. This phylogeny is generally concordant with the results of most previous molecular-genetic studies (reviewed in Phillips and Oakley, 1997; Westrich et al., 2002), and also helps in diagnosing some incongruent findings from single-gene studies. A close relationship between *Sv. alpinus* and *Sv. malma* is well supported by morphology (Behnke, 1984; Cavender, 1980), karyotypes (Cavender, 1984; Phillips et al., 1989), allozymes (Crane et al., 1994), and all studies using DNA sequence. *Sv. confluentus* and *Sv. leucomaenis* are also usually grouped together by morphology and allozymes (Table 6). However, *Sv. confluentus* groups strongly with *Sv. alpinus* and *Sv. malma* by analyses of mtDNA restriction sites, ND3, ITS2, and satellite DNA

Table 5
Summary of evidence from previous studies for a sister-taxon relationship between *Oncorhynchus* and *Salvelinus* (rather than *Salmo* and *Oncorhynchus*)

Evidence	References
(1) Vitellogenin gene organization groups *Oncorhynchus* and *Salvelinus* together	Buisine et al. (2002)
(2) Microsatellite gene structure groups *Oncorhynchus* and *Salvelinus* together	Angers and Bernatchez (1997)
(3) The number of chromosome arms in the karyotype is the same (100) in basal *Salvelinus, O. masou*, and the Pacific salmon; inferred to have changed to 104 in branch leading to (*O. mykiss*, *O. clarki*).	Phillips and Ráb (2001)
(4) Some *Salvelinus*, *O. mykiss*, *O. clarki*, and *O. masou* have similar life histories, with freshwater residence times 1–2+ years, freshwater populations common	Kato (1991), Groot and Margolis (1991), Stearley (1992), Willson (1997)
(5) Some morphological traits that link *Salmo* and *Oncorhynchus* are related to large size and breeding competition, and thus may be convergent	Stearley and Smith (1993) (as reinterpreted here)
(6) *Oncorynchus* and *Salvelinus* have diversified mainly in the Pacific and Nearctic respectively, whereas *Salmo* is in the Palearctic and Atlantic	Angers and Bernatchez (1997)
(7) *Oncorhynchus* and *Salvelinus* both diversified over the same general time period (roughly 6–15 mya), from fossil and molecular-clock evidence	Cavender and Miller (1972), Cavender (1980), Smith et al. (1982), Shedlock et al. (1992), McKay et al. (1996), Oohara et al. (1997)
(8) Independent evolution of highly developed anadromy in *Oncorhynchus* and *Salmo* in different ocean basins is not unexpected on ecological grounds	Northcote (1978), Stearley (1992), Hansen and Quinn (1998)

Table 6
Evidence from sources other than DNA sequence for relationships among species of *Salvelinus*, compared to total evidence nuclear-DNA tree inferred here

Evidence and reference	Phylogeny
Morphology (Behnke, 1984)	*fontinalis*, *namaycush*, ((*confluentus*, *leucomaenis*), (*alpinus*, *malma*))
Morphology (Stearley, 1992)	((*leucomaenis*, *fontinalis*, (*confluentus*, *namaycush*)), (*alpinus*, *malma*)
Morphology and karyology (Cavender and Kimura, 1989)	(*fontinalis*, *namaycush*), ((*confluentus*, *leucomaenis*), (*alpinus*, *malma*))
Satellite DNA (Hartley and Davidson, 1994)	*leucomaenis*, (*fontinalis*, (*namaycush*, (*alpinus*, *malma*, *confluentus*)))
Allozymes (Crane et al., 1994)	*fontinalis*, (*namaycush*, ((*confluentus*, *leucomaenis*), (*alpinus*, *malma*)))
Karyology (Phillips et al., 1989, 2002)	*fontinalis*, *namaycush*, ((*confluentus*, *leucomaenis*), (*alpinus*, *malma*))
This study (Fig. 2B and C)	(*fontinalis*, *namaycush*), ((*confluentus*, *leucomaenis*), (*alpinus*, *malma*))

Our nuclear DNA trees were compatible with the results of Phillips et al. (1989, 2002), and identical to the results of Cavender and Kimura (1989).

(Grewe et al., 1990; Hartley and Davidson, 1994; Phillips et al., 1994, 1995), but it groups with *Sv. leucomaenis* by analysis of ITS1 and allozymes (Crane et al., 1994; Phillips et al., 1994). *Sv. fontinalis*, *Sv. namaycush*, and *Sv. leucomaenis* have the same chromosome number, which appears to be primitive within the genus (Phillips et al., 1994); in *Sv. fontinalis* and *Sv. namaycush* this karyotype comprises 104 chromosome arms, while *Sv. leucomaenis* and *Sv. confluentus* have 100 arms and *Sv. malma* and *Sv. alpina* have 98. *Sv. fontinalis* and *Sv. namaycush* are basal to the other four species in most previous DNA studies (and allozymes: Crane et al., 1994), though they form strongly supported sister taxa only by the ITS1 analysis of Pleyte et al. (1992). Taken together, DNA-sequence studies of relationships within *Salvelinus* have yielded strikingly incongruent results, especially with regard to the positions of *Sv. confluentus*, *Sv. fontinalis*, and *Sv. namaycush*.

The discordance among phylogenetic studies of *Salvelinus* appears to be the result of hybridization (Phillips et al., 1994, 1995; Westrich et al., 2002). The main evidence for this hypothesis comes from the many examples of ancient and current hybridization between *Salvelinus* species. Ancient introgression of mtDNA has been demonstrated for *Sv. alpinus* and *Sv. fontinalis* (Bernatchez et al., 1995; Glémet et al., 1998), and for *Sv. alpinus* and *Sv. namaycush* (Wilson and Bernatchez, 1998), and ongoing hybridization and introgression have been reported for *Sv. alpinus* and *Sv. namaycush* (Wilson and Hebert, 1993), *Sv. malma* and *Sv. confluentus* (Baxter et al., 1997), and *Sv. confluentus* and *Sv. fontinalis* (Kanda et al., 2002; Redenbach and Taylor, 2002; Spruell et al., 2001). Moreover, most laboratory crosses between *Sv. alpinus*, *Sv. malma*, *Sv. fontinalis*, and *Sv. namaycush* and other *Salvelinus* result in fertile offspring (Chevassus, 1979). The extent of hybridization in *Salvelinus* appears to be higher than within *Oncorhynchus* (e.g., Allendorf and Leary, 1988; Campton and Utter, 1985; Chevassus, 1979; Dangel et al., 1973; Smith, 1992; Taylor, 2003), though it may be comparable to levels in the coregonids (Ferguson et al., 1978). Introgression of mtDNA in *Salvelinus* may also in some cases be driven by selection (Bernatchez et al., 1995; Wilson and Bernatchez, 1998), which would tend to amplify its effects.

Substantial levels of hybridization throughout the evolutionary history of a group make phylogenetic inference problematic (Arnold, 1992). Indeed, given extensive ongoing hybridization, even the geographic location of *Salvelinus* samples used for DNA sequencing could substantially affect the inferences. Our combined nuclear data phylogenies, especially those inferred from Bayesian analyses, provide very good resolution and support overall. However, support for the grouping of (*Sv. alpinus*, *Sv. malma*, *Sv. confluentus*, *Sv. leucomaenis*) appears to derive predominantly from a single gene (ITS2). Similar considerations apply to the support for (*Sv. confluentus*, *Sv. leucomaenis*), mainly from ITS1, and (*Sv. fontinalis*, *Sv. namaycush*), with support mainly from ITS2. Indeed, when the ITS1 and ITS2 data are excluded, the only group within *Salvelinus* that is accorded maximum-parsimony bootstrap support over 70% is (*Sv. alpinus*, *Sv. malma*) (93%). We believe that the best strategy in such cases is the gathering of DNA-sequence data from as many independently evolving nuclear DNA loci as possible, as well as liberal use of other types of character, such as genome structure, karyotypes and allozymes. Taken together, previous phylogenetic inferences from the use of morphology, allozymes and karyological characters (Table 6) are consistent with our phylogeny, although levels of support for the topologies from such sources of data are difficult to ascertain. Given this concordance among diverse data types, we believe that our DNA phylogeny of *Salvelinus* (Fig. 2B and C) is very likely to be correct. However, additional data from nuclear genes are needed to rigorously test this hypothesis.

4.4. Sister-group to Oncorhynchus

The sister-group to *Oncorhynchus* has long been believed to be *Salmo* (e.g., Murata et al., 1996; Phillips and Oakley, 1997; Phillips and Pleyte, 1991; Stearley and Smith, 1993), although analysis of data from the GH1C gene by Oakley and Phillips (1999) provided evidence that *Salmo* and *Oncorhynchus* are not sister taxa. Our analyses concur with this result of Oakley and

Phillips, and show that a sister-taxon relationship between *Oncorhynchus* and *Salvelinus* is well supported by data from three genes (GH1C, VIT, and ND3), by the combined data sets that exclude mitochondrial DNA, and by SH and Templeton tests using these combined data sets. Evidence for a sister-taxon relationship between *Oncorhynchus* and *Salvelinus* also comes from data on gene organization, karyology, life history, morphology, biogeography, and ecology (Table 5). The primary molecular-genetic evidence against (*Oncorhynchus*, *Salvelinus*) is the results from analysis of ITS1, which show strong bootstrap support for a sister-taxon relationship between *Oncorhynchus* and *Salmo* (Fig. 1D). However, we note that this analysis is compromised by use of an outgroup (*H. perryi*) that may belong among the ingroup taxa; when the closest available fish species outside of these genera is used (a cichlid), support for (*Oncorhynchus*, *Salmo*) is substantially reduced. Considered together, our analyses, and data from previous studies, constitute strong evidence for a sister-taxon relationship between *Oncorhynchus* and *Salvelinus*. These results should compel further evaluation of the data from morphology (e.g., position of the vomerine teeth) that has been used to support a sister-taxon relationship between *Oncorhynchus* and *Salmo*.

4.5. Implications for the evolution of salmonid fishes

Our results present a number of interesting implications for understanding the evolution of salmonid life history, behavior, and diversification. First, the finding that *Salvelinus* forms the sister-group to *Oncorhynchus* indicates that anadromy, in the form of long ocean migrations followed by a return to the natal stream, migration tightly linked to reproduction, and semelparity or a very low degree of iteroparity, has evolved at least twice, once in *Salmo* and once in *Oncorhynchus* (Oakley and Phillips, 1999; Stearley, 1992). This parallel evolution of life history also involves large body size for age in both sexes, due to extensive feeding at sea, and strong male–male competition, probably a result of high breeding densities (Crespi and Teo, 2002; Stearley, 1992). Such parallel changes in behavior and life history are ultimately a consequence of the parallel ecological opportunities favoring anadromy in the north Pacific and north Atlantic oceans (Dodson, 1997; Gross et al., 1988; Hansen and Quinn, 1998; McDowall, 1988; Northcote, 1978).

Second, the sister-taxa *Oncorhynchus* and *Salvelinus* have apparently diversified in parallel on a large scale: each genus has given rise to exclusively freshwater species (reproductively isolated kokanee forms of *O. nerka*, *Sv. namaycush*), forms with interior (freshwater) and sea-run populations (e.g., in *O. mykiss* and *Sv. malma*), and exclusively Asian species (*O. masou*, *Sv. leucomaenis*) (Rounesfell, 1958; Stearley, 1992; Willson, 1997). These similarities are consistent with the parallel radiation of the two genera from a common ancestor, subject to relatively similar selective pressures, opportunities for dispersal, and vicariant events.

Third, given that *O.masou* groups with *O. mykiss* and *O. clarki* rather than with the other so-called Pacific salmon, semelparity has apparently evolved twice in *Oncorhynchus*, once in the lineage leading to *O. masou*, and once in the lineage leading to (*O. tshawytscha*, *O. kisutch*, *O. nerka*, *O. gorbuscha*, *O. keta*). Alternatively, *O. masou* may be less strictly semelparous than is currently believed, as females of some populations of landlocked *O. masou* exhibit a small degree of iteroparity (Healey et al., 2001), and one of the main forms of evidence in the literature for semelparity in *O. masou* appears to have been its presumed phylogenetic position among the Pacific salmon (Kato, 1991).

4.6. Congruence, total evidence, and clade support

Our analyses raise a number of issues regarding the use of multiple data sets and criteria for evaluating congruence. First, our results provide good examples of both the strengths and limitations of a conditional combining approach to phylogenetic congruence. Overall, the data sets from the nuclear genes complemented and reinforced one another to yield a robust tree, which is what one hopes that combining of data will achieve (Bull et al., 1993; Cunningham, 1997). By contrast, the results from mtDNA genes tended to contradict the results from nuclear genes, and in the combined data sets the inclusion of mtDNA reduced the degree of resolution and bootstrap or Bayesian support. But because the individual data sets overlapped only partially in the taxa that they included, it was not possible to apply statistically based congruence tests (e.g., Huelsenbeck and Bull, 1996) to our data sets, which could more-objectively justify the exclusion of mtDNA or other data sets such as MHC.

Second, the use of bootstrap or Bayesian a posteriori support values to evaluate congruence is subject to important caveats. Majority-rule bootstrap trees may differ from best or strict consensus trees, or the bootstrap profiles (sets of bootstrapped trees) from analyses of different genes may exhibit little or no overlap (Page, 1996; Sanderson, 1989). Moreover, low bootstraps across a clade can be due to only one or few 'rogue' species whose position is especially uncertain due to long-branch attraction, a paucity of data, or other processes (Page, 1996; Sanderson and Schaffer, 2002). For our separate and combined data sets, the bootstrap majority-rule consensus trees were almost always the same as the best or strict consensus trees, subject to the lack of resolution shown in many of the bootstrap trees; these findings suggest that rogue species are not unduly influencing our results. Such limitations do not apply to Bayesian a posteriori probability values, which appear

to provide a more accurate metric of support for the true tree due to their lack of bias and higher sensitivity to phylogenetic signal (Alfaro et al., 2003). However, such Bayesian probability values are less conservative than bootstraps, and given the vagaries of such factors as sampling error and imprecise model specification, Bayesian probability values may in some cases imply strong support for relationships that are accorded only weak support by bootstraps (Alfaro et al., 2003; Douady et al., 2003) or by other metrics such as tree length. In our view, these considerations imply that high Bayesian clade support values should be interpreted cautiously, and that they should be accorded high confidence only in conjunction with high likelihood or parsimony bootstraps, or results from SH or Templeton tests.

Third, our analyses of the combined nuclear data sets show that in some cases, adding data from an additional gene can substantially increase maximum-parsimony bootstrap support for some nodes while notably decreasing support for others. Thus, the exclusion of the MHC data from our combined nuclear data set yields a tree with very strong maximum-parsimony bootstrap support for (*O. mykiss*, *O. masou*, *O. clarki*, 95% maximum-parsimony bootstrap) but only moderate support for (*O. tshawytscha*, *O. kisutch*, 72%), while its inclusion gives the reverse: weaker support for the former clade (79%), but very high support for the latter (99%). Because MHC is known to be under strong selection in salmonids (Miller and Withler, 1996), we hesitate to include it without reservations, even if when analyzed separately these data provide a tree that does not appear to be unequivocally incongruent with others.

In contrast to these parsimony results, the Bayesian a posteriori probabilities for (*O. mykiss*, *O. masou*, *O. clarki*) and for (*O. tshawytscha*, *O. kisutch*) remained high (100%) whether or not the MHC data was included (Fig. 2). These results appear to reflect the higher sensitivity of Bayesian analysis (vs. maximum parsimony bootstraps) to phylogenetic signal, its increased accuracy in recovering monophyletic groups, and the high susceptibility of maximum parsimony analysis to long-branch attraction, which can erode bootstrap support for the affected clades (Alfaro et al., 2003). Overall, we find it difficult to argue against the monophyly of both (*O. mykiss*, *O. masou*, *O. clarki*) and (*O. tshawytscha*, *O. kisutch*), as there is considerable support for each clade and no notably conflicting results.

4.7. Optimizing future studies

One of the most important results of this study is its role in mapping the best route for future molecular-phylogenetic studies of salmonids, with the ultimate goal of a robust tree for all species and subspecies in the family. In our study, the most-informative genes were GH1C, GH2C, VIT, CYTB, ITS1, ITS2, and MHC. Of these genes, evidence for incongruence was observed with the results from ITS1, ITS2, and MHC. Since the apparent cause of the incongruence can be surmised in each case (i.e., hybridization and selection respectively), we believe that the data from these genes should be treated with reservations in combined analyses. Despite such cautions, in each of these cases the apparent incongruence involved the placement of only a few species, the data from the other genes appeared to supercede the problematic effects, and the inclusion of the data from these genes thus increased the robustness of the combined nuclear data tree overall. These results imply that the sequencing of some or all of the genes GH1C, GH2C, VIT, ITS1, ITS2, and possibly CYTB and MHC, for an enlarged set of salmonid species, is likely to provide the best estimate for the phylogeny of Salmonidae, until additional nuclear markers are developed. Indeed, a combined data set that includes only these genes provides almost as well-resolved and supported a phylogeny as the full nuclear data set.

When data from the same large suite of taxa are available for a collection of genes, statistically based methods for the analysis of congruence can also be applied, to better assess the extent to which different data partitions agree on one true species tree. Such a data set should also allow robust inference of the nature and timing of major events in salmonid diversification, using data from fossils (Behnke, 1992; Cavender, 1980; Cavender and Miller, 1972; McPhail, 1997; Smith and Stearley, 1989; Stearley and Smith, 1993), paleoclimatology (Pearcy, 1992), geology (Montgomery, 2000), paleobiogeography (McPhail, 1997; Minckley et al., 1986; Neave, 1958), and the biogeography and ecology of the Esociformes, a freshwater group that has recently been shown to be the sister taxon to salmonids (Ishiguro et al., 2003). In conjunction with recent methods for use of DNA sequence to infer divergence times (Arbogast et al., 2002; Sanderson, 2002), such data will provide a comprehensive, interdisciplinary picture of the adaptive radiation of Salmonidae.

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References

Alfaro, M.E., Zoller, S., Lutzoni, F., 2003. Bayes or bootstrap? A simulation study comparing the performance of Bayesian Markov

chain Monte Carlo sampling and bootstrapping in assessing phylogenetic confidence. Mol. Biol. Evol. 20, 255–266.

Allendorf, F.W., Leary, R.F., 1988. Conservation and distribution of genetic variation in a polytypic species, the cutthroat trout. Cons. Biol. 2, 170–184.

Angers, B., Bernatchez, L., 1997. Complex evolution of a salmonid microsatellite locus and its consequences in inferring allelic divergence from size information. Mol. Biol. Evol. 14, 230–238.

Arbogast, B.S., Edwards, S.V., Wakeley, J., Beerli, P., Slowinski, J.B., 2002. Estimating divergence times from molecular data on phylogenetic and population genetic timescales. Ann. Rev. Ecol. Syst. 33, 707–740.

Arnold, M.L., 1992. Natural hybridization as an evolutionary process. Ann. Rev. Ecol. Syst. 23, 237–261.

Barker, F.K., Lutzoni, F.M., 2002. The utility of the incongruence length difference test. Syst. Biol. 51, 625–637.

Baxter, J.S., Taylor, E.B., Devlin, R.H., Hagen, J., McPhail, J.D., 1997. Evidence for natural hybridization between dolly varden (*Salvelinus malma*) and bull trout (*Salvelinus confluentus*) in a northcentral British Columbia watershed. Can. J. Fish. Aquat. Sci. 54, 421–429.

Behnke, R.J., 1984. Organizing the diversity of the Arctic char complex. In: Johnson, L., Burns, B.L. (Eds.), Biology of the Arctic Charr. Proceedings of the International Symposium on Arctic Charr. University of Manitoba Press, Winnipeg, pp. 3–21.

Behnke, R.J., 1992. Native trout of Western North America. Am. Fish. Soc. Monograph 6, 1–275.

Bernatchez, L., Glemet, H., Wilson, C.C., Danzmann, R.G., 1995. Introgression and fixation of Arctic char (*Salvelinus alpinus*) mitochondrial genome in an allopatric population of brook trout (*Salvelinus fontinalis*). Can. J. Fish. Aquat. Sci. 52, 179–185.

Bull, J.J., Huelsenbeck, J.P., Cunningham, C.C., Swofford, D.P., Waddell, P.J., 1993. Partitioning and combining data in phylogenetic analysis. Syst. Biol. 42, 384–397.

Buisine, N., Trichet, V., Wolff, J., 2002. Complex evolution of vitellogenin genes in salmonid fishes. Mol. Genet. Genomics 268, 535–542.

Campton, D.E., Utter, F.M., 1985. Natural hybridization between steelhead trout (*Salmo gairdneri*) and coastal cutthroat trout (*Salmo clarki clarki*) in two Puget Sound streams. Can. J. Fish. Aquat. Sci. 42, 110–119.

Cavender, T.M., 1980. Systematics of *Salvelinus* from the North Pacific basin. In: Balon, E.K. (Ed.), Charrs: Salmonid Fishes of the Genus *Salvelinus*. The Hague, Junk, pp. S295–S322.

Cavender, T.M., 1984. Cytotaxonomy of North American *Salvelinus*. In: Johnson, L., Burns, B. (Eds.), Biology of the Arctic Char. Proceedings of the International Symposium on Arctic Char, Winnipeg, Manitoba. Manitoba Press, Winnipeg, pp. 431–445.

Cavender, T.M., Kimura, S., 1989. Cytotaxonomy and interrelationships of Pacific basin *Salvelinus*. Physiol. Ecol. Jpn. Special 1, 49–68.

Cavender, T.M., Miller, R.R., 1972. *Smilidonichthys rastrosus*, a new Pliocene salmonid fish. Museum of Natural History, University of Oregon, Bulletin 18.

Chevassus, B., 1979. Hybridization in salmonids: results and perspectives. Aquaculture 17, 113–128.

Crandall, K.A., Bininda-Emonds, O.R.P., Mace, G.M., Wayne, R.K., 2000. Considering evolutionary processes in conservation biology. Trends Ecol. Evol. 15, 290–295.

Crane, P.A., Seeb, L.W., Seeb, J.E., 1994. Genetic relationships among *Salvelinus* species inferred from allozyme data. Can. J. Fish. Aquat. Sci. 51, 182–197.

Crespi, B.J., Teo, R., 2002. Comparative phylogenetic analysis of the evolution of semelparity and life history in salmonid fishes. Evolution 56, 1008–1020.

Cunningham, C.W., 1997. Is congruence between data partitions a reliable predictor of phylogenetic accuracy? Empirically testing an iterative procedure for choosing among phylogenetic methods. Syst. Biol. 46, 464–478.

Dangel, J.R., Macy, P.T., Withler, F.C. 1973. Annotated bibliography of interspecific hybridization of fishes of the subfamily Salmoninae. NOAA, Nat. Mar. Fish. Ser. Tech. memorandum NMFS NWFC-1, 48 pp.

De Queiroz, A., Donoghue, M.J., Junhyong, K., 1995. Separate versus combined analysis of phylogenetic evidence. Ann. Rev. Ecol. Syst. 26, 657–682.

Dodson, J.J., 1997. Fish migration: an evolutionary perspective. In: Godin, J.-G.J. (Ed.), Behavioural Ecology of Teleost Fishes. Oxford University Press, Oxford, UK, pp. 10–36.

Domanico, M.J., Phillips, R.B., 1995. Phylogenetic analysis of Pacific salmon (genus *Oncorhynchus*) based on mitochondrial DNA sequence data. Mol. Phylogenet. Evol. 4, 366–371.

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

Douady, C.J., Delsuc, F., Boucher, Y., Doolittle, W.F., Douzery, E.J.P., 2003. Comparison of Bayesian and maximum likelihood bootstrap measures of phylogenetic reliability. Mol. Biol. Evol. 20, 248–254.

Du, S.J., Devlin, R.H., Hew, C.L., 1993. Genomic structure of growth hormone genes in chinook salmon (*Oncorhynchus tshawytscha*): presence of two functional genes, GH-I and GH-II, and a male-specific pseudogene, GH-¥. DNA Cell Biol. 12, 739–751.

Elliott, J.M., 1994. Quantitative Ecology and the Brown Trout. Oxford University Press, Oxford, UK.

Ferguson, A., Himberg, K.-J.M., Svardson, G., 1978. Systematics of the Irish pollan (*Coregonus pollan* Thompson): an electrophoretic comparison with other holarctic Coregoninae. J. Fish Biol. 12, 221–233.

Fleming, I.A., 1998. Pattern and variability in the breeding system of Atlantic salmon (*Salmo salar*), with comparisons to other salmonids. Can. J. Fish. Aquat. Sci. 55 (Suppl. 1), 59–76.

Glémet, H., Blier, P., Bernatchez, L., 1998. Geographical extent of Arctic char (*Salvelinus alpinus*) introgression in brook char populations (*S. fontinalis*) from eastern Quebec, Canada. Mol. Ecol. 7, 1655–1662.

Grewe, P.M., Billington, N., Hebert, D.N., 1990. Phylogenetic relationships among members of *Salvelinus* inferred from mitochondrial DNA divergence. Can. J. Fish. Aquat. Sci. 47, 984–991.

Groot, C., Margolis, L., 1991. Pacific Salmon Life Histories. University of British Columbia Press, Vancouver, BC.

Gross, M.R., Coleman, R.M., McDowall, R.M., 1988. Aquatic productivity and the evolution of diadromous fish migration. Science 239, 1291–1293.

Hall, B.G., 2001. Phylogenetic Trees Made Easy. Sinaeur, Sunderland, MA.

Hansen, L.P., Quinn, T.P., 1998. The marine phase of the Atlantic salmon (*Salmo salar*) life cycle, with comparisons to Pacific salmon. Can. J. Fish. Aquat. Sci. 55 (Suppl. 1), 104–118.

Hart, J.L., 1973. Pacific Fishes of Canada. Fisheries Research Board of Canada, Ottawa.

Hartley, S.E., Davidson, W.S., 1994. Distribution of satellite DNA sequences isolated from Arctic char, *Salvelinus alpinus*, in the genus *Salvelinus*. Can. J. Fish. Aquat. Sci. 51 (Suppl. 1), 277–283.

Healey, M., Kline, P., Tsai, C.F., 2001. Saving the endangered Formosa landlocked salmon. Fisheries 26, 6–14.

Hendry, A.P., Stearns, S.C., 2003. Evolution Illuminated: Salmon and their Relatives. Oxford University Press, Oxford, UK.

Huelsenbeck, J.P., Bull, J.J., 1996. A likelihood ratio test to detect conflicting phylogenetic signal. Syst. Biol. 45, 92–98.

Huelsenbeck, J.P., Bull, J.J., Cunningham, C.W., 1996. Combining data in phylogenetic analysis. Trends Ecol. Evol. 11, 152–158.

Huelsenbeck, J.P., Ronquist, F., Nielsen, R., Bollback, J.P., 2001. Bayesian inference of phylogeny and its impact on evolutionary biology. Science 294, 2310–2314.

Ishiguro, N.B., Miya, M., Nishida, M., 2003. Basal euteleostean relationships: a mitogenomic perspective on the phylogenetic reality of the "Proacanthopterygii". Molec. Phylogenet. Evol. 27, 476–488.

Kanda, N., Leary, R.F., Allendorf, F.W., 2002. Evidence of introgressive hybridization between bull trout and brook trout. Trans. Am. Fish. Soc. 131, 772–782.

Kato, F., 1991. Life histories of Masu and Amago Salmon (*Oncorhynchus masou* and *Oncorhynchus rhodurus*). In: Groot, C., Margolis, L. (Eds.), Pacific Salmon Life Histories. UBC Press, Vancouver, BC, pp. 447–520.

Kitano, T., Matsuoka, N., Saitou, N., 1997. Phylogenetic relationship of the genus *Oncorhynchus* species inferred from nuclear and mitochondrial markers. Genes Genet. Syst. 72, 25–34.

Lee, D.S., Gilbert, C.R., Hocutt, C.H., Jenkins, R.E., McAllister, D.E., Stauffer Jr., J.R., 1980. Atlas of North American Freshwater Fishes. North Carolina State Museum of Natural History.

McDowall, R.M., 1988. Diadromy in Fishes: Migrations Between Freshwater and Marine Environments. Timber Press, Portland, OR.

McDowall, R.M., 1997. The evolution of diadromy in fishes (revisited) and its place in phylogenetic analysis. Rev. Fish. Biol. Fish. 7, 443–462.

McKay, S.J., Devlin, R.H., Smith, M.J., 1996. Phylogeny of Pacific salmon and trout based on growth hormone type-2 and mitochondrial NADH dehydrogenase subunit 3 DNA sequences. Can. J. Fish. Aquat. Sci. 53, 1165–1176.

McLennan, D.A., 1994. A phylogenetic approach to the study of fish behavior. Rev. Fish. Biol. Fish. 4, 430–460.

McPhail, J.D., 1997. The origin and speciation of *Oncorhynchus* revisited. In: Stouder, D.J., Bisson, P.A., Naiman, R.J. (Eds.), Pacific Salmon and their Ecosystems: Status and Future Options. Chapman and Hall, New York, pp. 29–38.

Miller, K.M., Withler, R.E., 1996. Sequence analysis of a polymorphic Mhc class II gene in Pacific salmon. Immunogenetics 43, 337–351.

Miller, R.J., Brannon, E.L., 1981. The origin and development of life history patterns in Pacific salmonids. In: Brannon, E.L., Salo, E.O. (Eds.), Salmon and Trout Migratory Behavior Symposium. University of Washington Press, Seattle, WA, pp. 296–309.

Minckley, W.L., Hendrickson, D.A., Bond, C.E., 1986. Geography of western North American freshwater fishes: description and relationships to intracontinental tectonism. In: Hocutt, C.H., Wiley, E.O. (Eds.), Zoogeography of North American Freshwater Fishes. Wiley, New York, pp. 519–614.

Montgomery, D.R., 2000. Coevolution of the Pacific salmon and Pacific Rim topography. Geology 28, 1107–1110.

Murata, S., Takasaki, N., Okazaki, T., Kobayashi, T., Numachi, K., Chang, K., Okada, N., 1998. Molecular evidence from short interspersed elements (SINEs) that *Oncorhynchus masou* (cherry salmon) is monophyletic. Can. J. Fish. Aquat. Sci. 55, 1864–1870.

Murata, S., Takasaki, N., Saitoh, M., Okada, N., 1993. Determination of the phylogenetic relationships among Pacific Salmonids by using short interspersed elements (SINEs) as temporal landmarks of evolution. Proc. Natl. Acad. Sci. USA 90, 6995–6999.

Murata, S., Takasaki, N., Saitoh, M., Tachida, H., Okada, N., 1996. Details of retropositional genome dynamics that provide a rationale for a genetic division: the distinct branching of all the Pacific salmon and trout (*Oncorhynchus*) from the Atlantic salmon and trout (*Salmo*). Genetics 142, 915–926.

Neave, F., 1958. The origin and speciation of *Oncorhynchus*. Trans. R. Soc. Canada 52, 25–39.

Norden, C.R., 1961. Comparative osteology of representative salmonid fishes, with particular reference to the grayling (*Thymallus arcticus*) and its phylogeny. J. Fish. Res. Bd. Canada 18, 679–791.

Northcote, T.G., 1978. Migratory strategies and production in freshwater fishes. In: Gerking, S.D. (Ed.), Ecology of Freshwater Fish Production. Blackwell Scientific Publishers, Oxford, UK, pp. 326–359.

Oakley, T.H., Phillips, R.B., 1999. Phylogeny of Salmonine fishes based on growth hormone introns: Atlantic (*Salmo*) and Pacific (*Oncorhynchus*) Salmon are not sister taxa. Mol. Phylogenet. Evol. 11, 381–393.

Oleinik, A.G., 1997. Molecular phylogeny of salmonids: results of an analysis of nuclear and mitochondrial DNA. Genetika 33, 173–177.

Oleinik, A.G., 2000. On the rates of evolution of the mitochondrial and nuclear genomes in Salmonid fishes. Biologiya Morya (Vladivostok) 26, 410–416.

Oohara, I., Sawano, K., Okazaki, T., 1997. Mitochondrial DNA sequence analysis of the masu salmon—phylogeny in the genus *Oncorhynchus*. Mol. Phylogenet. Evol. 7, 71–78.

Oohara, I., Sawano, K., Okazaki, T., Kobayashi, T., 1999. Reexamination of the molecular phylogeny of the masu salmon of the genus *Oncorhynchus*. In: Séret, B., Sire, J.-Y. (Eds.), Proc. 5th Indo-Pacific Fish Conference, Nouméa, New Caledonia, pp. 417–426.

Osinov, A.G., 1999. Salmonid fish of the genera *Salmo*, *Parasalmo*, and *Oncorhynchus*: genetic divergence, phylogeny and classification. J. Ichthyol. 39, 571–587.

Page, R.D.M., 1996. On consensus, confidence, and 'total evidence'. Cladistics 12, 83–92.

Pearcy, W.G., 1992. Ocean ecology of north Pacific salmonids. University of Washington Press, Seattle, WA.

Phillips, R.B., Manley, S.A., Daniels, T.J., 1994. Systematics of the salmonid genus *Salvelinus* inferred from ribosomal DNA sequences. Can. J. Fish. Aquat. Sci. 51, 198–204.

Phillips, R.B., Makoto, M.P., Reed, K.M., 2002. Characterization of charr chromosomes using fluorescence in situ hybridization. Environ. Biol. Fishes 64, 223–228.

Phillips, R.B., Oakley, T.H., 1997. Phylogenetic relationships among the Salmoninae based on their nuclear and mitochondrial sequences. In: Kocher, T.D., Sepien, C.A. (Eds.), Molecular Systematics of Fishes. Academic Press, San Diego, CA, pp. 145–162.

Phillips, R.B., Pleyte, K.A., 1991. Nuclear DNA and salmonid phylogenetics. J. Fish Biol. 39, 259–275.

Phillips, R.B., Pleyte, K.A., Ihssen, P.E., 1989. Patterns of chromosomal nucleolar organizer region (NOR) variation in fishes of the genus *Salvelinus*. Copeia 1989, 47–53.

Phillips, R.B., Ráb, P., 2001. Chromosome evolution in the Salmonidae (Pisces): an update. Biol. Rev. 76, 1–25.

Phillips, R.B., Sajdak, S.L., Domanico, M.J., 1995. Relationships among charrs based on DNA sequences. Nordic J. Freshwater Res. 71, 378–391.

Pleyte, K.A, Duncan, S.D., Phillips, R.B., 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.

Posada, D., Crandall, K.A., 2001. Selecting the best-fit model of nucleotide substitution. Syst. Biol. 50, 580–601.

Rannala, B., Yang, Z., 1996. Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. J. Mol. Evol. 43, 304–311.

Redenbach, Z., Taylor, E.B., 2002. Evidence for historical introgression along a contact zone between two species of char (Pisces: Salmonidae) in northwestern North America. Evolution 56, 1021–1035.

Regan, C.T., 1914. The systematic arrangement of the fishes of the family Salmonidae. Ann. Mag. Nat. History 13, 405–408.

Rosenfield, J.A., Todd, T., Greil, R., 2000. Asymmetric hybridization and introgression between pink salmon and chinook salmon in the Laurentian Great Lakes. Trans. Am. Fish. Soc. 129, 670–679.

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Rounesfell, G.A., 1958. Anadromy in North American Salmonidae. US Fish Wildlife Ser. Bull. 58, 171–185.
Sanderson, M.J., 1989. Confidence limits on phylogenies: the bootstrap revisited. Cladistics 5, 113–129.
Sanderson, M.J., 2002. Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. Mol. Biol. Evol. 19, 101–109.
Sanderson, M.J., Schaffer, H.B., 2002. Troubleshooting molecular phylogenetic analyses. Ann. Rev. Ecol. Syst. 33, 49–72.
Scott, W.B., Crossman, E.J., 1973. Freshwater Fishes of Canada. Bull. 184, Fisheries Research Board of Canada, Ottawa.
Shed'ko, S.V., Ginatulina, L.K., Parpura, I.Z., Ermolenko, A.V., 1996. Evolutionary and taxonomic relationships among Far-Eastern salmonid fishes inferred from mitochondrial DNA divergence. J. Fish Biol. 49, 815–829.
Shedlock, A.M., Parker, J.D., Crispin, D.A., Pietsch, T.W., Burmer, G.C., 1992. Evolution of the salmonid mitochondrial control region. Mol. Phylogenet. Evol. 1, 179–192.
Shimodaira, H., Hasegawa, M., 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Mol. Biol. Evol. 16, 1114–1116.
Slowinski, J., Page, R.D.M., 1999. How should species phylogenies be inferred from sequence data? Syst. Biol. 48, 814–825.
Smith, G.R., 1992. Introgression in fishes: significance for paleontology, cladistics, and evolutionary rates. Syst. Biol. 41, 41–57.
Smith, G.R., Swirydczuk, K., Kimmul, P.G., Wilkinson, B.H., 1982. Fish biostratigraphy of late Miocene to Pleistocene sediments of the western Snake River plain, Idaho. Cenozoic Geol. Idaho, Idaho Bureau Mine Geol. Bull. 26, 519–541.
Smith, G.R., Stearley, R.F., 1989. The classification and scientific names of rainbow and cutthroat trouts. Fisheries 14, 4–10.
Spruell, P., Bartron, M.L., Kanda, N., Allendorf, F.W., 2001. Detection of hybrids between bull trout (*Salvelinus confluentus*) and brook trout (*Salvelinus fontinalis*) using PCR primers complementary to interspersed nuclear elements. Copeia 2001, 1093–1099.
Stearley, R.F., 1992. Historical ecology of Salmoninae, with special reference to *Oncorhynchus*. In: Mayden, R.L. (Ed.), Systematics, Historical Ecology, and North American Freshwater Fishes. Stanford University Press, Stanford, CA, pp. 622–658.
Stearley, R.F., Smith, G.R., 1993. Phylogeny of the Pacific trouts and salmons (*Oncorhynchus*) and genera of the family Salmonidae. Trans. Am. Fish. Soc. 122, 1–33.
Sullivan, J., Swofford, D.L., 2001. Should we use model-based methods for phylogenetic inference when we know that assumptions about among-site rate variation and nucleotide substitution pattern are violated? Syst. Biol. 50, 723–729.
Swofford, D.L., 2002. PAUP: Phylogenetic Analysis Using Parsimony, version 4.0b10. Illinois Natural History Survey. Champaign, IL.
Tanaka, S., 1965. A review of the biological information on masu salmon (*Oncorhynchus masou*). In: Salmon of the North Pacific Ocean. Part IX. Coho, chinook and masu salmon in offshore waters. Int. North Pacific Fish. Comm. Bull. 16, 75–135.
Takasaki, N., Murata, S., Saitoh, M., Kobayashi, T., Park, L., Okada, N., 1994. Species-specific amplification of tRNA-derived short interspersed repetitive elements (SINEs) by retroposition: a process of parasitization of entire genomes during the evolution of salmonids. Proc. Natl. Acad. Sci. USA 91, 10153–10157.
Taylor, E.B., 2003. Evolution in mixed company: evolutionary inferences from studies of natural hybridization in Salmonidae. In: Hendry, A., Stearns, S. (Eds.), Evolution Illuminated: Salmon and their Relatives. Oxford University Press, Oxford, UK.
Templeton, A.R., 1983. Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and apes. Evolution 37, 221–244.
Thomas, W.K., Beckenbach, A.T., 1989. Variation in salmonid mitochondrial DNA: evolutionary constraints and mechanisms of substitution. J. Mol. Evol. 29, 233–245.
Thomas, W.K., Withler, R.E., Beckenbach, A.T., 1986. Mitochondrial DNA analysis of Pacific salmonid evolution. Can. J. Zool. 64, 1058–1064.
Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucl. Acids Res. 25, 4876–4882.
Tsuyuki, H., Roberts, E., 1966. Inter-species relationships within the genus *Oncorhynchus* based on biochemical systematics. J. Fish. Res. Bd. Canada 23, 101–107.
Utter, F.M., Allendorf, F.W., Hodgins, H.O., 1973. Genetic variability and relationships in Pacific Salmon and related trout based on protein variations. Syst. Zool. 22, 257–270.
Utter, F.M., Allendorf, F.W., 1994. Phylogenetic relationships among species of *Oncorhynchus*: a consensus view. Conserv. Biol. 8, 864–867.
Westrich, K.M., Konkol, N.R., Matsuoka, M.P., Phillips, R.B., 2002. Interspecific relationships among charrs based on phylogenetic analysis of nuclear growth hormone intron sequences. Environ. Biol. Fishes 64, 217–222.
Wiens, J.J., 1998a. Does adding characters with missing data increase or decrease phylogenetic accuracy? Syst. Biol. 47, 625–640.
Wiens, J.J., 1998b. Combining data sets with different phylogenetic histories. Syst. Biol. 47, 568–581.
Wiens, J.J., Reeder, T.W., 1995. Combining data sets with different numbers of taxa for phylogenetic analysis. Syst. Biol. 44, 548–558.
Willson, M.F., 1997. Variation in salmonid life histories: patterns and perspectives. Res. Pap. PNW-RP-498, US Department of Agriculture, Forest Service, Pacific Northwest Research Station, Portland, OR.
Wilson, C.C., Bernatchez, L., 1998. The ghost of hybrids past: fixation of Arctic charr (*Salvelinus alpinus*) mitochondrial DNA in an introgressed population of lake trout (*S. namaycush*). Mol. Ecol. 7, 127–132.
Wilson, C.C., Hebert, P.D.N., 1993. Natural hybridization between Arctic charr (*Salvelinus alpinus*) and lake trout (*S. namaycush*) in the Canadian Arctic. Can. J. Fish. Aquat. Sci. 50, 2652–2658.
Woram, R.A., Gharbi, K., Sakamoto, T., Hoyheim, B., Holm, L.E., Naish, K., McGowan, C., Ferguson, M.M., Phillips, R.B., Stein, J., Guyomard, R., Cairney, M., Taggart, J.B., Powell, R., Davidson, W., Danzmann, R.G., 2003. Comparative genome analysis of the primary sex-determining locus in salmonid fishes. Genome. Res. 13, 272–280.
Yang, Z., Rannala, B., 1997. Bayesian phylogenetic inference using DNA sequences: a Markov Chain Monte Carlo method. Mol. Biol. Evol. 14, 717–724.
Yoshiyasu, K., 1973. Starch-gel electrophoresis of hemoglobins of freshwater salmonid fishes in southwest Japan—II. Genus *Oncorhynchus* (salmon) Bull. Jpn. Soc. Sci. Fish. 39, 97–114.