Phylogeny Inference Among Alaskan/Circumpolar Salmonidae Via Gene Sequences

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Introduction

Many species belonging to the salmonidae family are ecologically and economically important, but the genetic relationships linking them together, especially for those native to Alaskan waters, are relatively unknown. Based on the methods of Li et al (2008), three gene fragments (*svep1*, *a2bi17*, *ptc*) were selected for comparison based on their known reliability as indicators of genetic variance. These fragments were amplified via polymerase chain reaction (PCR) for 25 genomic DNA samples of nine salmonid, four umbrid, two esocid, and one petromyzontid species. The PCR products were sequenced via terminator dye method. Only two of the samples were forward-sequenced successfully, meaning a Salmonidae phylogeny could not be constructed from the data provided.

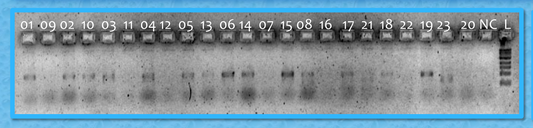
Table 1. List of species included in this study and their taxonomy.

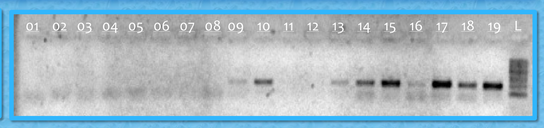
Family	Genus	Species	Common Name
Salmonidae	Thymallus	grutii	Amur grayling
Salmonidae	Thymallus	arcticus	Arctic grayling
Salmonidae	Brachymystax	lenok	Lenok trout
Salmonidae	Salvelinus	namaycush	Lake trout
Salmonidae	Salvelinus	alpinus	Arctic charr
Salmonidae	Coregonus	pidschian	Humpback whitefish
Salmonidae	Coregonus	laurettae	Bering cisco
Salmonidae	Hucho	taimen	Siberian salmon
Salmonidae	Parahucho	perryi	Sakhalin taimen
Umbridae	Dallia	pectoralis	Alaska blackfish
Umbridae	Umbra	limi	Central mudminnow
Umbridae	Umbra	pygmaea	Eastern mudminnow
Umbridae	Novumbra	hubbsi	Olypmic mudminnow
Esocidae	Esox	lucius	Northern pike
Esocidae	Esox	niger	Chain pickerel
Petromyzontidae	Lampetra	alaskensis	Brook lamprey

PCR Amplification Results

- PCR for *svep1* and *ptc* genes were not suitable for sequencing (insufficient, varying product base-pair length, not enough samples amplified).
- Overall, PCR amplification of *a2bi17* gene produced products for 14 of 25 DNA samples.
- F340/R1294 primer combination amplified *a2bi17* gene for all nine salmonid species and one outgroup species (*D. pectoralis*).

Images 1 & 2. Electrophoresed gels for *a2bi17* PCR, F340/R1294 primers. NC = negative control; L = 1kb DNA ladder





Methods

Genomic DNA Preparation
Tissue samples were obtained from various locations and sources (ANSP, UAM, Host lab). DNA was extracted from the tissue samples using QiaGen® QIAamp Mini Kits according to protocol.
DNA samples targeted for amplification via PCR using oligonucleotide primers specific to *svep1*, *a2bi17*, and *ptc* genes (Table 4).

PCR Amplification
- *svep1* gene amplified twice via PCR for 20 of the 25 DNA samples.
- *a2bi17* gene amplified seven times via PCR for all 25 DNA samples.
- *ptc* gene amplified once via PCR for all 25 DNA samples.

All PCR reactions checked for products via 0.8% agarose gel electrophoresis and ethidium bromide staining.

DNA Sequencing
PCR products prepared for sequencing with a one-quarter BigDye terminator dye sequencing reaction as per the "Cycle Sequencing Reactions for Single/Double Stranded DNA" protocol provided by the UAF Institute of Arctic Biology Core Lab. One reaction mix created for each primer (1 forward, 1 reverse).

a2bi17 DNA sequence fragments purified/precipitated via "Ethanol/EDTA/Sodium Acetate Precipitation" from *BigDye Terminator v3.1 Cycle Sequencing Kit Protocol*. Sequenced by UAF IAB Core Lab using an ABI3100 Genetic Analyzer.

Since most of the *a2bi17* sequencing results were unreadable, no phylogeny was constructed.

Table 2. List of PCR components and concentrations.

Reaction Component	Initial (Stock) Conc.	Final (Mix) Conc.
Deionized H_2O (ddH_2O)	N/A	N/A
GoTaq Flexi Buffer	5X	1X
Deoxyribonucleotides (dNTPs)	40mM	0.8mM
Mg^{+2} ion solution	25mM	3mM
Forward Primer	10µM	0.4µM
Reverse Primer	10µM	0.4µM
Go Taq Polymerase	5U/µl	0.025U/µl

Table 3. General PCR thermal cycler conditions used to amplify *svep1*, *a2bi17*, and *ptc* genes.

Thermal Cycler Phase	Temperature (°C)	Duration (mm:ss)	Number of Iterations
Pre-Incubation	94/95	01:30/02:00	N/A
Denaturation	94	00:30	35x/40x
Annealing	50/52	00:30	35x/40x
Extension	72	01:00	35x/40x
Final Extension	72	04:00/07:00	N/A
Post-Incubation	4	Indefinite	N/A

Table 4. List of primers used to target each gene.

Gene	Forward Primer	Reverse Primer
svep1	7960F	8889R
a2bi17	F340, F373	R1265, R1294
ptc	F120	R1248

Table 5. Thermal cycler conditions for BigDye ® terminator dye cycle sequencing reaction mix.

Thermal Cycler Phase	Temperature (°C)	Duration (mm:ss)	Number of Iterations
Pre-incubation	96	N/A	N/A
Step 1	96	00:30	N/A
Step 2	96	00:10	25x
Step 3	50	00:30	25x
Step 4	60	04:00	25x

Table 6. Component list for each BigDye® terminator dye cycle sequencing reaction mix.

Reaction Component	Amount	Concentration (Stock)	Concentration (Mix)
BigDye® Terminator Dye	2µl	5X	1X
Sequencing Buffer	1µl	5X	0.5X
Primers	1µl	10µM	3.2pmol
Template PCR Products	1µl	~800ng/µl	~10ng/µl
Deionized water	5µl	N/A	N/A

A2bi17 Sequencing Results

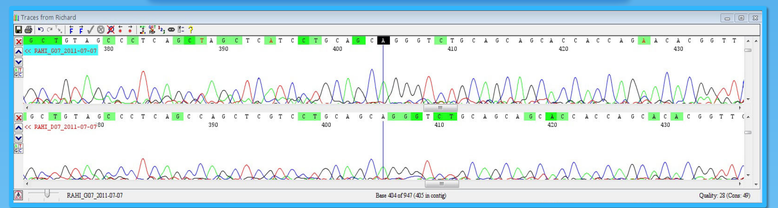


Image 4. Readable *a2bi17* forward sequence from *Salvelinus Alpinus* (Arctic charr) and *Brachymystax lenok* (Lenok trout) samples

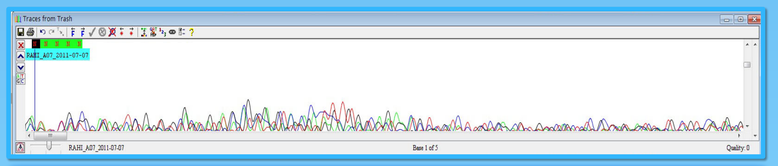


Image 5. Unreadable "trash" *a2bi17* sequence from *Thymallus grutii* (Amur grayling)

Phylogeny Results

No phylogeny inferred due to insufficient *a2bi17* gene sequencing data.

Discussion/Conclusion

- *Svep1* double-band products may be due to varying intron length
- Inconsistent base-pair length of *ptc* products may be due mispriming of homologous loci, varying intron length
- Unreadable *a2bi17* sequencing results may be due to improper primer annealing, homologous loci being sequenced, etc.
- Sequencing data is insufficient to infer Salmonidae phylogeny, although two samples were successfully forward-sequenced
- Effective Salmonidae phylogeny may have been constructed if a2bi17 products had sequenced properly

References

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