Determining Phylogeny of Salmonidae Using DNA sequence analysis of VCPIP and ENC genes

Melissa Streitmatter, Robert Marcotte, and Andres Lopez

University of Alaska Fairbanks, Institute of Arctic Biology and Department of Biology & Wildlife

Introduction

In this experiment, some species of the family Salmonidae, Thymalliane, Petromyzontidae, Umbridae, and Esocidae were compared to study the phylogeny of Salmoniae.. Salmonidae are a family of ray-finned fish that includes salmon, trout, chars, freshwater whitefishes, and graylings.Tissue samples from these fish were obtained from University of Alaska Museum (UAF) and Academy of Natural Sciences Philadelphia. DNA was extracted from these tisses and the VCPIP and ENC genes of the DNA were studied. The DNA was amplified using PCR and analyzed by gel photos produced from gel electrophoresis. After maximum product from DNA was obtained, DNA was sequenced so that a phylogenic tree for the family Salmonidae could be created. Salmonidae have also been studied in the past using techniques such as morphology and microarrays.

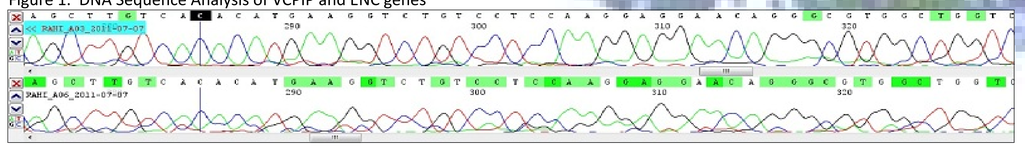
Materials and Methods

- Tissue samples and species type shown in Table 1.
- Tissue extracted by Robert Marcotte (UAF, Dr. A. Lopez lab) or Richard Bekeris and myself using a Qiagen QIAamp® DNA Mini kit.
- PCR amplifying VCPIP and ENC genes was performed on all DNA samples.
- Gel electrophoresis was run using a solution of 0.8% (w/v) agarose gel to visualize sequences.
- A nanodrop spectrometer was used only on samples 21 to 25 to quantify the concentration in ng/µL to determine if DNA was present, as product had not been showing up in gel photos.
- Once maximum product was produced, samples that showed product were selected to be sequenced.
- Samples were put into a sequencing reaction mix and ran in a thermocycler.
- DNA was purified using sodium acetate (3M)-ethanol precipitation.
- DNA was sequenced and phylogenetic tree for Salmonidae was created

Table 1. Summary of tissue samples and species type

Tube #	Sample (Scientific Name)	Common Name
01	*Thymallus Grubbi I*	Amur Grayling
02	*Thymallus Grubii II*	Amur Grayling
03	*Hucha Taimen I*	Taimen
04	*Hucha Taimen II*	Taimen
05	*Brachymystax Lenok I*	Lenok
06	*Brachymystax Lenok II*	Lenok
07	*Salvelinus Namaycush I*	Lake Trout
08	*Salvelinus Namaycush II*	Lake Trout
09	*Salvelinus Namaycush III*	Lake Trout
10	*Salvelinus Alpinus*	Arctic Char
11	*Lampetra Alaskense I*	Alaskan Brook Lamprey
12	*Lampetra Alaskense I*	Alaskan Brook Lamprey
13	*Coregonus Pidschian I*	Humpback Whitefish
14	*Coregonus Pidschian II*	Humpback Whitefish
15	*Coregonus Laurettae I*	Bering Cisco
16	*Coregonus Laurettae II*	Bering Cisco
17	*Thymallus Articus*	Arctic Grayling
18	*Hucho Perryi*	Japanese Huchen
19	*Dallia Pectoralis*	Alaska Blackfish
20	*Esox Lucius*	Northern Pike
21	*Umbra Pygmae*	Eastern Mudminnow
22	*Exos Niger*	Chain Pickerel
23	*Umbra Limi I*	Umbra Mudminnow
24	*Umbra Limi II*	Umbra Mudminnow
25	*Novumbra Hubbsi*	Olympic Mudminnow

Figure 1. DNA Sequence Analysis of VCPIP and ENC genes



Results

PCR of VCPIP and ENC genes inconsistently produced product. Results of all PCR trials shown in Table 2. Results of DNA Sequence Analysis of VCPIP and ENC genes shown in Figure 1.

Of 48 sequencing reactions, most were successful, but only a few sequences from a few pairs of forward and reverse primer pairs were aligned; 5 pairs from VCPIP and 7 pairs from ENC were aligned. 5 more sequences were added to the VCPIP collection, using sequence results from only a single direction. The final alignment length for VCPIP was about 639 base pairs and 707 base pairs for ENC. For VCPIP there were 302 polymorphisms among those samples and for ENC there were 218 polymorphisms.

Using a neighbor-joining method, two unrooted trees were created; rooted trees were made using D. *Pectoralis* as an outgroup (Figures 2,3)

Table 2. Summary of base pair lengths from PCR trials using VCPIP and ENC genes

Sample Number	VCPIP 84F, 946R				ENC 86F, 982R			
Run #	1	2	3	4	1	2	3	4*
01	None	700	N/A	None	None	400,600	None	N/A
02	700	None	None	None	600	400,600	None	N/A
03	None	700	None	None	None	400,600	600	None
04	None	700	N/A	None	600,700	400,600	600	580
05	None	700	N/A	None	600,700	N/A	N/A	580
06	None	700	N/A	None	None	400,600	600	580
07	None	700	None	None	None	400,600	600	580
08	None	700	N/A	None	600,700	N/A	None	580
09	None	None	None	N/A	600,700	400,600	None	580
10	None	700	N/A	N/A	None	400,600	None	N/A
11	None	None	None	N/A	600	None	None	N/A
12	None	None	None	N/A	600	None	None	N/A
13	None	None	None	N/A	600	None	None	N/A
14	None	700	N/A	None	600,700	400,600	None	N/A
15	700	700	N/A	None	600,700	400,600	None	N/A
16	None	None	None	N/A	None	400,600	None	N/A
17	700	700	N/A	580	None	400,600	None	N/A
18	None	None	none	N/A	600,700	400,600	None	N/A
19	None	700	700	580	600,700	N/A	N/A	None
20	None	700	None	580	600,700	N/A	N/A	N/A
21	None	N/A	None	N/A	none	400,600	None	None
22	None	N/A	none	N/A	600,700	N/A	N/A	N/A
23	None	N/A	None	N/A	600,700	N/A	N/A	None
24	None	N/A	None	N/A	600	400,600	None	None
25	None	N/A	None	N/A	none	N/A	N/A	N/A

*Reverse Primer 975R was used instead of 982R

Figure 2. Rooted phylogenetic tree of certain species of Salmonidae using ENC gene

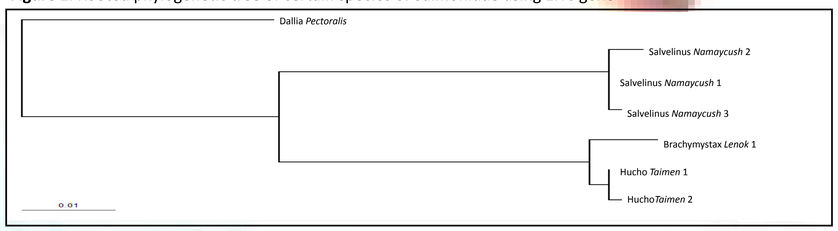
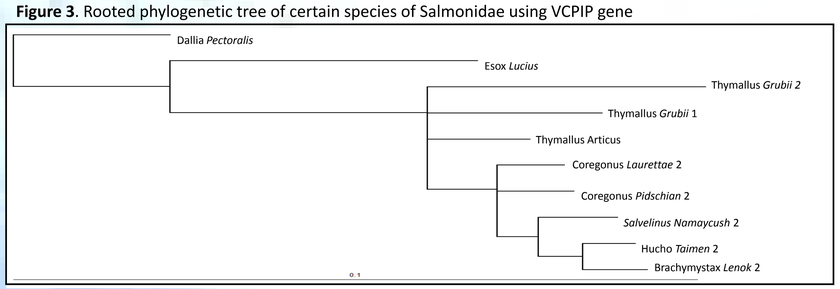


Figure 3. Rooted phylogenetic tree of certain species of Salmonidae using VCPIP gene



Discussion

- The reason for no product showing up in the gels was that the primers did not anneal correctly possibly because of polymorphisms in the primer region.
- As expected, all individuals of a single species were closely monophyletic, as shown in Figures 2 and 3.
- In comparing both phylogenetic trees, it is seen that *Brachymystax Lenok* and *Hucho Taimen* were more closely related to each other than either one is to *Salvelinus Namaycush*.
- Of the ENC phylogeny, the placement of 3 genera was consistent with 3 out of 4 trees shown in the Stearly and Smith (1993) article.
- The VCPIP tree is different from all 3 trees shown in the Stearly and Smith (1993) article, specifically with the placement of *Coregonus* and *Thymallus*.
- All trees based on mitochondrial DNA from Crespi and Fulton (2004) agreed with the VCPIP tree .
- None of the nuclear loci studied agreed with the VCPIP tree.
- GH1C gene was the only nuclear gene that disagreed with the ENC tree
- No comparisons can be made with VCPIP tree using Koop (2008) but it is noted that *Thymallus* is a more basil group than *Coregonus*.
- No comparisons can be made with ENC tree because it is dealing with 3 different genera that are not present in the trees created by Koop.

Conclusion

- All individuals of a single species were closely monophyletic.
- The ENC and VCPIP trees did not contradict each other.
- Based on the trees presented in this poster and the trees from the three articles, it is shown that the trees constructed from genetic data were more closely related to the VCPIP and ENC trees than the trees constructed using morphology.

Literature Cited

Crespi, Bernard J. and Fulton, Michael J. 2003. Molecular systematics of Salmonidae: combined nuclear data yields a robust phylogeny. *Molecular Phylogenetics and Evolution.* www.sciencedirect.com

Koop, Ben F. 2008. A salmonid EST gemonic study: genes, duplications, phylogeny, and microarrays. *BMC Genomics* 9:545

Stearley, R. F. and Smith, G. R.1993. Phylogeny of the Pacific Trouts and Salmons (Oncorhynchus) and Genera of the Family Salmonidae. *Transactions of the American Fisheries Society* 122:1: 1-33

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