



# DNA Barcoding of Eight North American Coregonine Species\*

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## Introduction:

Coregonine fishes occur throughout the Northern Hemisphere with over 30 species in three genera. This level of diversity presents a challenge for species identification due to the fact that phenotypic characteristics vary depending on the environment, life stage and migratory behavior. Due to increased subsistence and commercial demand in Alaska, research and management applications require correct identification of many individuals at a time. Here we present a genetic tool based on mitochondrial COI (the DNA barcode gene region) variation for rapid and cost-effective identification of co-occurring coregonine species.

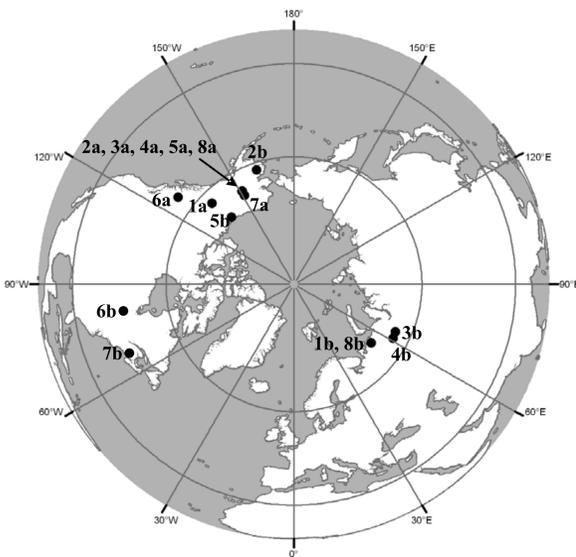
## Objectives:

- 1) Develop an RFLP assay based on the sequence variation of COI for identification of eight coregonine species common to Arctic and sub-Arctic North America that overlap in Alaska.
- 2) Evaluate the performance of the RFLP assay using a blind test

## Methods:

### Samples and DNA Sequencing:

- Sixteen individuals representing two specimens for each of the eight species were collected from North American and Russian locations.
- A 650 base pair segment of the COI gene was sequenced for each of the 16 individuals with universal primers.



Map Loc.	Species Name	Common Name
1 a,b	<i>Coregonus autumnalis</i>	Arctic cisco
2 a,b	<i>Coregonus laurettae</i>	Bering cisco
3 a,b	<i>Coregonus pidschian</i>	humpback whitefish
4 a,b	<i>Coregonus nasus</i>	broad whitefish
5 a,b	<i>Coregonus sardinella</i>	least cisco
6 a,b	<i>Prosopium coulterii</i>	pygmy whitefish
7 a,b	<i>Prosopium cylindraceum</i>	round whitefish
8 a,b	<i>Stenodus leucichthys</i>	inconnu/sheefish

## RFLP Assay Development and Testing:

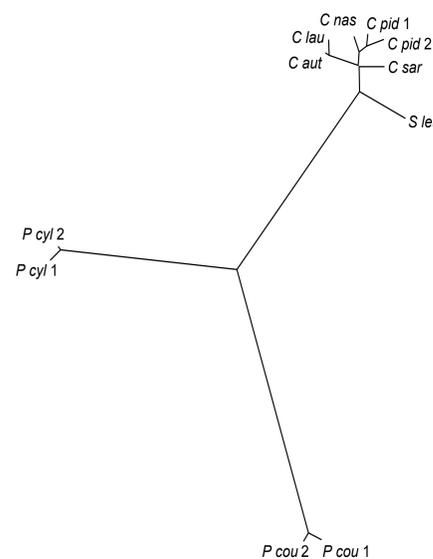
- Sequences were aligned to identify unique sequences, which were submitted to GenBank.
- Sequences were screened to identify enzyme recognition sites that produced species-specific restriction fragments and to determine the length (in base pairs) of the fragments.
- A suite of five restriction enzymes was selected and a blind test was conducted on 50 individuals from Alaska representing all eight species.



Enzyme *BsmI* diagnostic for *C. nasus* and *C. pidschian*

## Results:

- 1) Sequence variation and RFLP assay development
  - Eleven unique COI sequences were identified out of the 16 individuals.
  - Mean pairwise sequence divergence for all eight species was 7.04% and ranged from 0.46% to 14.23%.
  - Restriction site mapping revealed an average of 126 enzymes with recognition sites in the COI amplicon for all species
  - A final suite of four restriction enzymes in a step-wise assay identified each of the eight species.



1.0  
A neighbor-joining tree of COI sequence divergences using the Kimura 2-parameter model

## 2) RFLP assay performance

- Forty-nine of the 50 individuals were successfully amplified and 48 of those were correctly assigned to species.

- One putative *C. laurettae* was twice identified as a

*C. autumnalis* in separate assays following independent DNA extraction. This may be due to hybridization between the two species, which has been reported by previous studies.

## Conclusions:

- We have developed a tool that can distinguish seven of the eight target whitefish species with 100% accuracy. *C. laurettae* and *C. autumnalis* are regarded as a single group for species identification purposes with this tool.
- Further investigation is needed with regard to hybridization in whitefish, particularly in *C. laurettae* and *C. autumnalis*.
- Development of additional assays may be required to distinguish these eight species in geographical regions where other coregonine species occur. Future work will require assessment of COI diversity at the local population level and should include both larger sample sizes and additional blind tests for an RFLP assay

## Acknowledgements:

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Step: enzyme	Species (*diagnostic)	cut sites	COI fragments (base pairs)
1: <i>RsaI</i>	<i>P. coulterii</i> *	2	343-233-130
	<i>S. leucichthys</i> *	1	552-154
	<i>P. cylindraceum</i>	0	
	<i>C. sardinella</i>	0	
	<i>C. autumnalis</i>	0	
	<i>C. laurettae</i>	0	
2: <i>HaeIII</i>	<i>P. cylindraceum</i> *	5	295-162-110-81-42-16
	<i>C. sardinella</i> *	5	251-152-87-81-75-60
	<i>C. autumnalis</i>	5	295-152-87-81-75-16
	<i>C. laurettae</i>	5	295-152-87-81-75-16
	<i>C. nasus</i>	5	173-152-141-87-78-75
3: <i>BseRI</i>	<i>C. autumnalis</i> *	0	
	<i>C. laurettae</i> *	1	378-328
4: <i>BsmI</i>	<i>C. nasus</i> *	1	449-257
	<i>C. pidschian</i> *	0	