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 species 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.

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.

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 enzyme 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.

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