

**DNA barcoding of whitefish (Coregoninae)
collected from freshwaters in the Yukon Territory**

Eric B. Taylor
Department of Zoology,
Biodiversity Research Centre and Native Fishes Research Group
University of British Columbia
6270 University Blvd.,
Vancouver, BC V6T 1Z4
Tel: 604-822-9152
Fax: 604-822-2416
etaylor@zoology.ubc.ca

For

Susan Thompson
Yukon Department of Renewable Resources
Whitehorse, Yukon Territory
April 8, 2005

MRC-05-01

Introduction

Nine species of whitefishes (Coregoninae: *Coregonus*, *Prosopium*, and *Stenodus*) occur to varying degrees within freshwaters or nearshore coastal waters of Yukon Territory (Scott and Crossman 1973). There are five species of *Coregonus*: the Arctic (*C. autumnalis*), Bering (*C. laurettae*), and least ciscoes (*C. sardinella*), and the lake (*C. clupeaformis*), and broad whitefishes (*C. nasus*). There are three species of *Prosopium*: the pygmy (*P. coulteri*), round (*P. cylindraceum*), and mountain whitefishes (*P. williamsoni*). Finally, there is the monotypic member of *Stenodus*, the inconnu (*S. leucichthys*). While some species have fairly narrow distributions in Yukon (e.g. the mountain whitefish) or are morphologically distinctive (e.g., the inconnu) making identification fairly straightforward, others have broad, overlapping distributions and may be morphologically more similar (particularly as juveniles) making identification more challenging. For instance, the round and pygmy whitefish co-exist in Aishihik Lake and are commonly distinguished morphologically by lateral line scale counts that may be difficult to enumerate in small fish particularly under field conditions.

An alternative to morphological identification is the use of DNA-based diagnostic markers. So-called DNA barcoding, for instance, makes use of DNA sequence differences between species that can be rapidly assayed in tissues sampled non-lethally (Marshall 2005). This report summarized analyses conducted on 20 whitefish samples collected by the Yukon Department of Renewable Resources from various localities and submitted to the laboratory of E.B. Taylor at the University of BC for DNA-based identification.

Materials and methods

Fish samples

Fish samples consisted of adipose or pelvic fin clips or (for juveniles) whole bodies stored in 95% ethanol.

DNA analysis

DNA was extracted using a portion of these samples and the Qiagen DNA extractions kits and stored at -20°C . A small portion (500 base pairs) of the cytochrome *b* gene of the mitochondrial DNA genome was amplified using the polymerase chain reaction (PCR). Mitochondrial DNA is a genome inherited maternally and independently of the nuclear genome and is often employed in species-level studies in fishes. In addition, several partial cytochrome *b* sequences, that correspond to a portion of the region studied in this report, have been obtained from whitefishes (e.g., broad whitefish, least cisco, round whitefish) and deposited in the GENBANK international database (see <http://www.ncbi.nlm.nih.gov/>). In addition, others have been obtained previously in the Taylor lab (e.g., lake whitefish, pygmy whitefish, mountain whitefish).

Cytochrome *b* fragments were obtained using the primers cytb2 and GluDG as outlined in Johnson and Taylor (2004). These PCR products were purified using Qiagen kits and eluted into 40 μl of distilled sterilized water. The cytb2 primer was used in a standard cycle sequencing reaction using the Applied Biosystems Inc. "Big Dye" cycle sequencing kit and assayed on an ABI automated sequencer.

Sequences were aligned using Bioedit (version 6, Hall 1999) and were trimmed to accommodate the lengths of sequences in the GENBANK database (289 base pairs). Divergences among mtDNA sequences were calculated using the Kimura 2-parameter quantity and similarity among all sequences was summarized cluster analysis using the unweighted pair group method with arithmetic mean (UPGMA).

Results

Partial cytochrome *b* sequences were obtained from 20 Yukon Territory whitefish. Two samples (No. 5, and 7) were of poor quality such that complete sequence alignments could not be obtained for these two specimens. Sufficient sequences were, however, obtained to reliably diagnose these specimens (see below).

Kimura 2 parameter divergence estimates ranged from 0.0 (between two least cisco) and 16.6% (between a pygmy whitefish (“yukonwtfsh10”) and a lake whitefish (“yukonwhfish16”, Table 2).

The UPGMA tree of similarity among sequences resolved the species identity of all Yukon Territory whitefish (Fig. 1, Table 3). Among all sequences there were two major groups corresponding to the division between *Prosopium* and *Coregonus*. Within the *Prosopium* group, there were three clear groupings corresponding to: pygmy whitefish, mountain whitefish, and round whitefish (Fig. 1). Within the *Coregonus* group, there were four major subgroups corresponding to Arctic cisco, least cisco, broad whitefish, and lake whitefish (Fig. 1).

The two specimens for which complete sequences were not obtained were still able to be diagnosed as a mountain whitefish (“yukonwtfsh5”) and a broad whitefish (“yukonwtfsh7”), respectively, owing to their possession of nucleotides diagnostic of these species at several positions in the sequence (e.g., Fig. 2).

Discussion

The analyses presented straightforward interpretation of the species identifications. The general relationships amongst the whitefish haplotypes were consistent with previous phylogenetic results for mtDNA (Bernatchez et al. 1991) and some nuclear DNA sequences (Sajdak and Phillips 1997). For instance, all analyses point to the relatively close relationship between broad and lake whitefish and the more distinct relationship between these two species and the Arctic and least ciscos. The present analysis is unique, however, in being the first to include the pygmy whitefish and to indicate its rather divergent position within *Prosopium*.

Finally, one small caution that should be mentioned is the fact that mtDNA is maternally inherited and is subject to transfer between species if hybridization between them occurs (e.g., Redenbach and Taylor 2003). As mtDNA is haploid and inherited from a single parent only, it is not subject to elimination by recombination and the hybrid retains the mtDNA lineage of the female parent involved in the hybridization. Consequently, if hybridization is

though to occur in some systems (e.g. Reist et al. 1992), mtDNA only assays may compromise species identifications. Morphological analyses of key characters, where possible, is also advisable and nuclear assays, although somewhat more involved, can be employed if necessary in these situations.

Acknowledgements

I thank the Yukon Territorial Government and Susan Thompson, Fish and Wildlife Branch, Yukon Department of Renewable Resources for providing funding and samples for the development of this database.

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Table 1. List of specimens examined (DNA number), acronym, their geographic locality putative field identification, and DNA-based identification.

DNA Sample	Acronym	Locality	Field ID	DNA ID
1 ¹	yukonwtfsh1	6 Mile River	Least cisco	Least cisco
2	yukonwtfsh2	6 Mile River	Least cisco	Least cisco
3	yukonwtfsh3	Dawson City/Liard	Least or Bering cisco	Least cisco
4	yukonwtfsh4	Yukon R./Dawson City	Lake or broad whitefish	Lake whitefish
5	yukonwtfsh5	Tributary of Liard, Watson Lake, YT	Mountain or round whitefish	Mountain whitefish
6	yukonwtfsh6	Yukon R./Dawson City	Pygmy or round whitefish	Round whitefish
7	yukonwtfsh7	Yukon R./Dawson City	Lake or broad whitefish	Broad whitefish
8	yukonwtfsh8	Yukon R./Dawson City	Lake or broad whitefish	Lake whitefish
9	yukonwtfsh9	Aishihik Lake, YT?	Pygmy whitefish	Round whitefish
10	yukonwtfsh10	Aishihik Lake, YT?	Pygmy whitefish	Pygmy whitefish
11 ²	yukonwtfsh11	Aishihik Lake, YT?	Pygmy whitefish	Pygmy whitefish
12	yukonwtfsh12	Aishihik Lake, YT	Round whitefish	Round whitefish
13	yukonwtfsh13	Aishihik Lake, YT	Round whitefish	Round whitefish
14	yukonwtfsh14	Aishihik Lake, YT	Round whitefish	Round whitefish
15	yukonwtfsh15	Aishihik Lake, YT	Lake whitefish	Lake whitefish

¹1 and 2 two specimens examined from a total of six collected and all identified as least cisco.

²11, 12, and 13 are three specimens examined from a total of seven collected.

yukonwtf6 0.0105 0.0144 0.0035 0.1359 0.1363 0.1403 0.1319 0.1359 0.0000 0.0000 0.1192 0.0035 0.0035 0.1359 0.1319 0.1319 0.1153
0.0432 0.1400 0.1129 0.0141 0.0439 0.1418 0.1606 0.1509

yukonwtf9 0.0105 0.0144 0.0035 0.1359 0.1363 0.1403 0.1319 0.1359 0.0000 0.0000 0.1192 0.0035 0.0035 0.1359 0.1319 0.1319 0.1153
0.432 0.1400 0.1129 0.0141 0.0439 0.1418 0.1606 0.1509

yukonwtf10 0.1325 0.1318 0.1232 0.1594 0.1474 0.1474 0.1471 0.1594 0.1192 0.1192 0.0000 0.1232 0.1232 0.1594 0.1471 0.1471 0.0175
0.1371 0.1635 0.0177 0.1364 0.1351 0.1663 0.0448 0.1666

yukonwtf13 0.0070 0.0108 0.0000 0.1400 0.1403 0.1444 0.1359 0.1400 0.0035 0.0035 0.1232 0.0000 0.0000 0.1400 0.1359 0.1359 0.1192
0.0469 0.1441 0.1169 0.0105 0.0476 0.1461 0.1650 0.1509

yukonwtf14 0.0070 0.0108 0.0000 0.1400 0.1403 0.1444 0.1359 0.1400 0.0035 0.0035 0.1232 0.0000 0.0000 0.1400 0.1359 0.1359 0.1192
0.0469 0.1441 0.1169 0.0105 0.0476 0.1461 0.1650 0.1509

yukonwtf15 0.1497 0.1536 0.1400 0.0000 0.0140 0.0282 0.0175 0.0000 0.1359 0.1359 0.1594 0.1400 0.1400 0.0000 0.0175 0.0175 0.1390
0.1296 0.0035 0.1450 0.1536 0.1316 0.0000 0.2048 0.0424

yukonwtf2 0.1455 0.1451 0.1359 0.0175 0.0175 0.0210 0.0000 0.0175 0.1319 0.1319 0.1471 0.1359 0.1359 0.0175 0.0000 0.0000 0.1349
0.1215 0.0210 0.1327 0.1495 0.1233 0.0182 0.1957 0.0139

yukonwtf3 0.1455 0.1451 0.1359 0.0175 0.0175 0.0210 0.0000 0.0175 0.1319 0.1319 0.1471 0.1359 0.1359 0.0175 0.0000 0.0000 0.1349
0.1215 0.0210 0.1327 0.1495 0.1233 0.0182 0.1957 0.0139

kootpygm 0.1285 0.1277 0.1192 0.1390 0.1272 0.1312 0.1349 0.1390 0.1153 0.1153 0.0175 0.1192 0.1192 0.1390 0.1349 0.1349 0.0000
0.1249 0.1430 0.0106 0.1324 0.1268 0.1450 0.0525 0.1584

mtnwtfl 0.0549 0.0562 0.0469 0.1296 0.1299 0.1299 0.1215 0.1296 0.0432 0.0432 0.1371 0.0469 0.0469 0.1296 0.1215 0.1215 0.1249
0.0000 0.1337 0.1226 0.0547 0.0000 0.1353 0.1666 0.1468

yukonwtf8 0.1538 0.1579 0.1441 0.0035 0.0175 0.0318 0.0210 0.0035 0.1400 0.1400 0.1635 0.1441 0.1441 0.0035 0.0210 0.0210 0.1430
0.1337 0.0000 0.1491 0.1495 0.1316 0.0000 0.2002 0.0424

yukonwtf11 0.1262 0.1253 0.1169 0.1450 0.1330 0.1330 0.1327 0.1450 0.1129 0.1129 0.0177 0.1169 0.1169 0.1450 0.1327 0.1327 0.0106
0.1226 0.1491 0.0000 0.1257 0.1196 0.1467 0.0524 0.1454

yukonwtf12 0.0178 0.0219 0.0105 0.1536 0.1540 0.1582 0.1495 0.1536 0.0141 0.0141 0.1364 0.0105 0.0105 0.1536 0.1495 0.1495 0.1324

0.0547 0.1495 0.1257 0.0000 0.0515 0.1507 0.1521 0.1599
mtnwtf2 0.0557 0.0571 0.0476 0.1316 0.1319 0.1319 0.1233 0.1316 0.0439 0.0439 0.1351 0.0476 0.0476 0.1316 0.1233 0.1233 0.1268
0.0000 0.1316 0.1196 0.0515 0.0000 0.1353 0.1588 0.1468
yukonwtf16 0.1515 0.1606 0.1461 0.0000 0.0145 0.0293 0.0182 0.0000 0.1418 0.1418 0.1663 0.1461 0.1461 0.0000 0.0182 0.0182 0.1450
0.1353 0.0000 0.1467 0.1507 0.1353 0.0000 0.1990 0.0424
Ak50pygm 0.1712 0.1714 0.1650 0.2048 0.1962 0.1962 0.1957 0.2048 0.1606 0.1606 0.0448 0.1650 0.1650 0.2048 0.1957 0.1957 0.0525
0.1666 0.2002 0.0524 0.1521 0.1588 0.1990 0.0000 0.2433
csardinloc 0.1616 0.1685 0.1509 0.0424 0.0499 0.0426 0.0139 0.0424 0.1509 0.1509 0.1666 0.1509 0.1509 0.0424 0.0139 0.0139 0.1584
0.1468 0.0424 0.1454 0.1599 0.1468 0.0424 0.2433 0.0000

Figure 1. Clustering tree of pairwise Kimura 2-parameter genetic distances among Yukon whitefish mtDNA sequence haplotypes. Acronyms are defined in Table 1. Names within red outlined boxes are known sequence standards obtained from GENBANK (<http://www.ncbi.nlm.nih.gov/>) or from Taylor (unpublished data). Haplotypes are defined in Table 1 except for those from GENBANK or Taylor (unpublished): MCRWF2C = round whitefish. YRWFCOMP = round whitefish, RWFCOMP = round whitefish, LWFCOMP = lake whitefish. BWFCOMP = broad whitefish, cautumn = Arctic cisco, kootpygm = pygmy whitefish (Kootenay Lake, BC), mtnwtfh1 = mountain whitefish, mtnwtfh2 = mountain whitefish, Ak50pygm = pygmy whitefish (Chignik Lake, Alaska), csardinloc = least cisco (Lockwood et al. 1993). Scale bar is in units of Kimura 2 parameter distance.

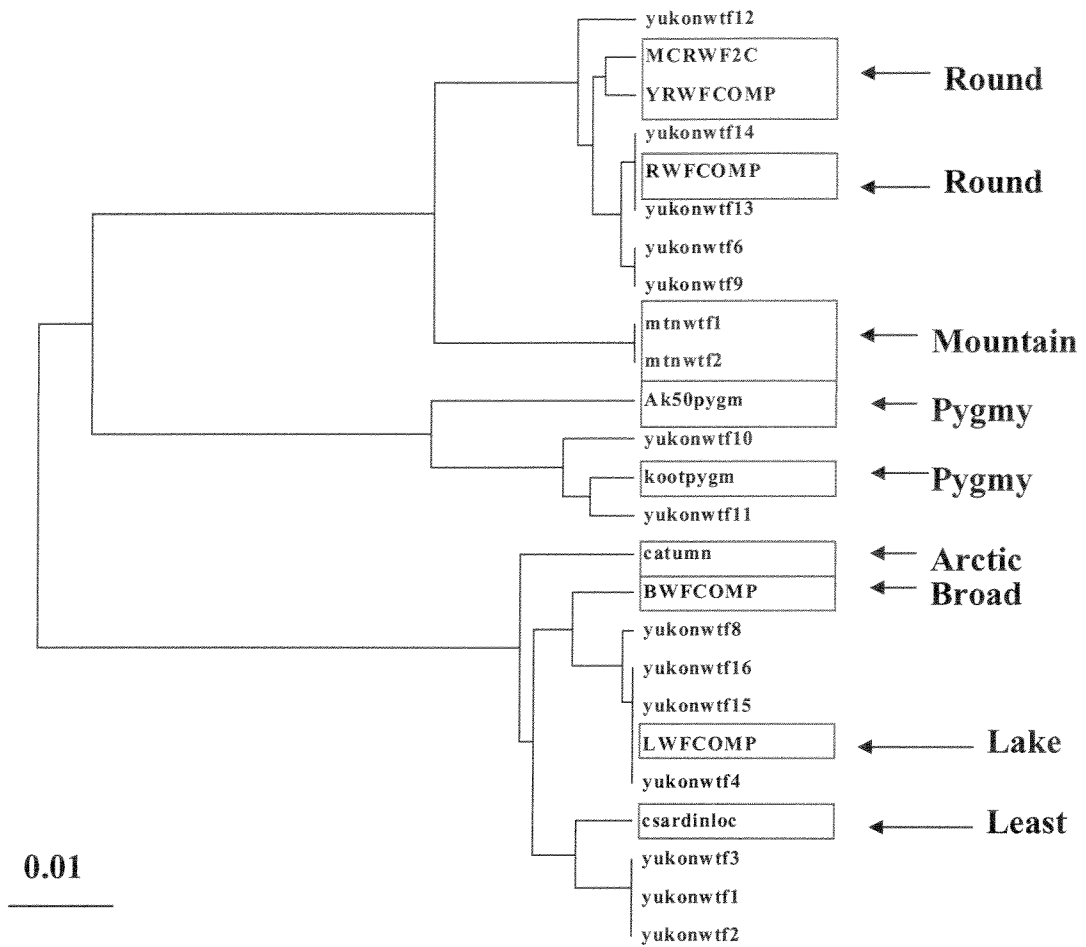


Figure 2. Partial cytochrome *b* alignments between (a) round whitefish and mountain whitefish and (b) broad and lake whitefishes to illustrate diagnostic (←→) positions in the sequences found in yukonwtfsh5 and yukonwtfsh7 indicating they are mountain and broad whitefish, respectively. The diagnostic differences occur between positions 150 and 300 of the total aligned sequences in (a) and between 250 and 275 in (b). Haplotypes are defined in Table 1.



(b)

LWFCOMP	TCACGCAGTAATCGTTAGAAATCTTCCCCCA
Yukonwtfsh4
Yukonwtfsh8
Yukonwtfsh15
Yukonwtfsh16
BWFCOMPC.....
<u>Yukonwtfsh7</u>C.....

