

INTRODUCTION

The correct identification of species is a crucial component to any research on fish biology or fisheries. The identification of fish species can be difficult because of the large number of species, and because the body forms of most fishes change dramatically during development. Molecular-based approaches, such as DNA barcodes, have the potential to transform the task of identification by providing rapid diagnosis of species identity across all life stages including eggs and larvae as well as from fish fragments. This ability will yield more precise data on recruitment, ecology and geographic ranges of fisheries resources, and improved knowledge of nursery areas and spawning grounds, thereby impacting fisheries management and conservation. In addition, it will enable identification of prey in stomach contents, and assist in the detection of cryptic species. Forensic applications may extend from monitoring of fish quotas and by-catch to inspection of fisheries markets and fisheries products. The progress and applications of the Consortium for the Barcode of Life Initiative for the global identification of all marine fish using DNA barcodes are presented in relation to the marine fishes of Atlantic Canada.

METHODS

SAMPLE COLLECTION

Specimens from the NW Atlantic (Figure 1) were targeted. Fish were collected through collaboration with a number of organizations but predominantly Fisheries and Oceans Canada (DFO - Maritimes, Gulf of Saint Lawrence, Quebec, Newfoundland and Labrador and Arctic) and NOAA (Gulf of Maine, Cape Hatteras). Fish were caught in groundfish surveys, coastal tagging surveys, inshore species diversity surveys, educational programs with schoolchildren and from various individuals working on specific species (sharks, skates, tunas etc.) as well as interested fishermen.

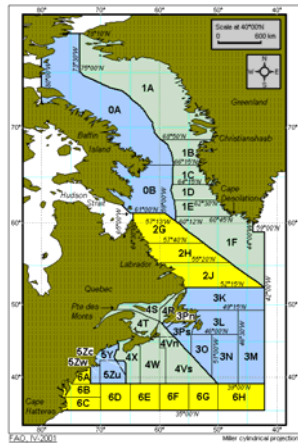


Figure 1. AREA OF THE NW ATLANTIC ILLUSTRATING NAFO (NORTH ATLANTIC FISHERIES ORGANIZATION) ZONES WHERE MARINE FISH ARE BEING TARGETED. MOST SAMPLES ARE CAUGHT WITH THE HELP OF DFO IN CANADA AND NOAA IN THE UNITED STATES.

ARCHIVING

The archiving of associated voucher specimens is carried out in collaboration with The Atlantic Reference Centre (ARC) of the Huntsman Marine Science Centre, in St. Andrews, New Brunswick. Once tissue is obtained for DNA analysis, the voucher thawed, photographed, fixed (in 10% formaldehyde and subsequently preserved in isopropanol), catalogued and an authoritative ID is obtained before final archiving in the museum collection. (Most of the specimens are tentatively IDed prior to arrival at the ARC and this original ID is what the DNA barcodes are initially compared to.)

DNA EXTRACTION AND AMPLIFICATION

DNA was extracted using a glass milk protocol (Elphinstone et al. 2003) on a Perkin Elmer MultiPROBE II PLUS Robot. PCR products were amplified using the published Fish Universal Cytochrome Oxidase I (CO1) primers (Ward et al., 2005) or modified M13 tailed primers (Ivanova et al. 2006). PCR products were cleaned and sequenced using standard protocols.

RESULTS

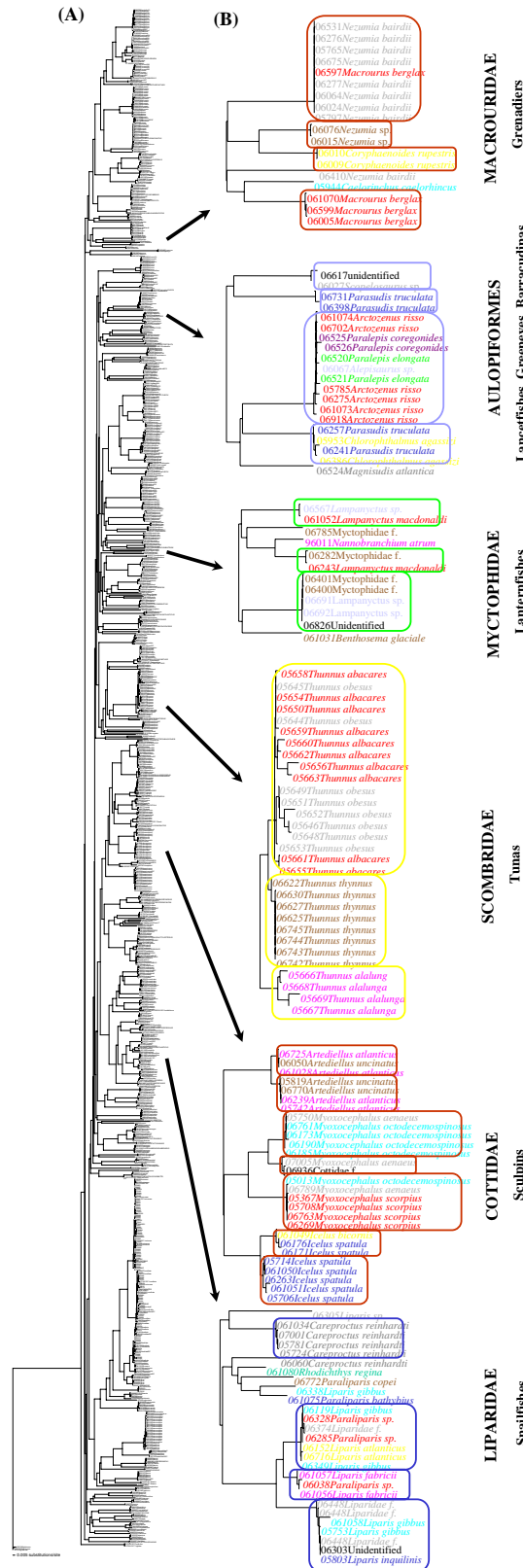


Figure 2. (A) NEIGHBOR-JOINING PHYLOGRAM FOR 1040 ATLANTIC CANADIAN MARINE FISH SPECIMENS. (B) SPECIES IDENTITY PROBLEMS WITHIN FIVE FAMILIES (COTTIDAE, LIPARIDAE, MACROURIDAE, MYCTOPHIDAE AND SCOMBRIDAE) AND ONE ORDER (AULOPIFORMES) ARE SHOWN IN MORE DETAIL WHERE TWO OR MORE INDIVIDUALS EXHIBIT $\geq 1\%$ SEQUENCE DIVERGENCE. THEY ARE GROUPED WITHIN COLOURED RECTANGLES.

RESULTS

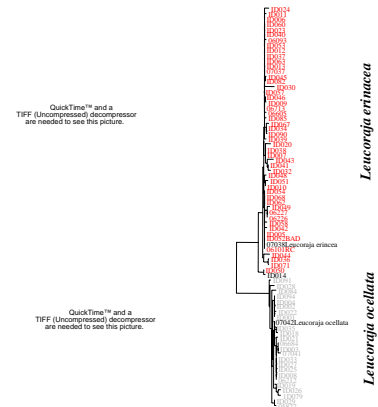
To date tissue from 2251 specimens have been collected with voucher specimens for 1578 of these, representing 284⁺ species groups. (*to be confirmed by examination of DNA barcodes and phenotype if voucher available). Of these 193 species are represented by more than one individual.

Where sufficient individuals from a particular species are available, the DNA barcodes indicated that further examination is required within a number of families including (1) Agonidae (Alligatorfishes and poachers), *Aspidophoroides monopterygius* and *Leptagonus decagonus* (2) Ammodytidae (Sandlances), *Ammodytes dubius*, *Ammodytes americanus* (3) Cottidae (Sculpins), *Icelus spatula*, *Icelus bicornis*, *Myoxocephalus octodecemspinosus*, *Myoxocephalus aeneus*, *Myoxocephalus scorpius* (5) Lotidae (Rocklings), *Gaidropsarus argenteus*, *Gaidropsarus ensis* (6) Macrouridae (Grenadiers), *Macrourus berglax*, *Nezumia bairdii*, *Nezumia* sp. (7) Chlorophthalmidae (Greeneyes), *Parasudis triculenta*, *Chlorophthalmus agassizi* (8) Paralepididae (Barracudinas), *Paralepis elongata*, *Paralepis coregonides*, *Arctocentrus risso* (9) Rajidae (Skates), *Amblyraja hyperborea*, *Amblyraja jenseni*, *Leucoraja ocellata*, *Leucoraja erinacea* (10) Scombridae (Tunas), *Thunnus obesus*, *Tunnus albacares*, (11) Liparidae (Snailfishes), *Careproctus reinhardi*, *Liparis gibbus*, *Liparis fabricii*, *Liparis inquilinus*. Six of these families are illustrated in Figure 2.

122 unidentified specimens from DFO groundfish surveys (1996-2006) were obtained and 78 yielded DNA barcodes. When compared to sequences in the BOLD database, 44 could be assigned to species level, 21 to genus, 3 to family and 10 did not correspond to DNA barcodes thus far generated.

DNA barcodes were successfully used to identify the species of juvenile skates caught in the western Atlantic (Figure 3.). The data used will help to develop a key for on-board identifications.

Figure 3. SKATE IDENTIFICATION. 108 JUVENILE SKATES WERE OBTAINED AND DNA BARCODES GENERATED. THE BARCODES WERE COMPARED TO THOSE FROM VOUCHER SPECIMENS AND USED TO IDENTIFY IMMATURE SPECIMENS. PHENOTYPIC CHARACTERS FROM THESE SPECIMENS WILL BE USED BY RESEARCHERS TO DEVELOP AN ON-BOARD KEY FOR SPECIES DISCRIMINATION.



Images of winter and little skate (country of Jordan & Evermann, drawing by H. L. Todd and Bigelow & Schroeder, drawing by E. N. Fischer, respectively).

CONCLUSIONS

DNA barcoding discriminates most Atlantic marine fish species. In those cases where individuals from 'nominal' species do not cluster, species misidentification is the most parsimonious explanation and these specimens are being re-examined to confirm this. The availability of specimens for re-examination underscores the need for expertly taxonomically identified voucher specimens in the DNA barcoding process. The information from misidentifications can be used to develop better methods for on-board identifications and correct errors in current survey data, which is a primary source of information for marine fish.

A concerted effort is needed to obtain specimens from the rarer and/or deep water species in the NW Atlantic as most of the common species have been collected.

ACKNOWLEDGEMENTS

We would like to thank everyone who was involved in the collection and archiving of fish. This research was supported through funding to the Canadian Barcode of Life Network from Genome Canada through the Ontario Genomics Institute.

NSERC, and other sponsors listed at www.BOL.NET.ca.