Application of DNA Barcoding Techniques For Speciation

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Objectives

– Background of catfish speciation
– Background of DNA barcoding
– FSIS Verification of catfish barcoding method
– Future plans and questions – extension to other regulatory species?
Background of Catfish Regulation

- In 2001, the FDA changed the definition of catfish to **exclude** all catfish that are not members of the family Ictaluridae, i.e. North American catfishes only.

- This prohibited all catfishes not of North American origin from labeling their product as such, **but** also limited the regulatory scope.
Farm Bill 2008

- Prior to 2008: processors subject to announced or unannounced inspections by FDA had the option of a voluntary, fee-for-service grading program from National Marine Fisheries Service (NMFS)

- The *proposed rule* amends the 1906’s Federal Meat Inspection Act (FMIA) such that *catfish* are subject to examination and inspection by USDA/FSIS when processed for use as human food in process
Definition Blues: Is It a catfish or isn’t it?

FSIS currently has two proposed definitions of catfish under review:

1. Maintain the narrow definition that includes only members of the North American catfish family (Ictaluridae)

OR

2. Broaden the definition to include all “true” taxonomic catfish or members of the order Siluriformes
Channel Catfish – *Ictalurus punctatus*

Image source: http://fish.dnr.cornell.edu/nyfish/ictaluridae/channelcatfish.html
Protein Methodology (IEF)

AOAC Method 980.16: has been in use since the 1980s, currently the gold standard for uncooked fillet (although not extended to cooked)

Protocol: Cold water extraction of sarcoplasmic proteins, separation of proteins by isoelectric point by PAGE, staining and visualization of gel with 0.1% acid violet 17

Comparison of banding pattern of authenticated standards with that of unknown samples

Process banding patterns with software
Example of Processed IEF Gel

Lane 2: pl marker (44488)
Lane 4: CATStd070909-02 (Blue Catfish)
Lane 5: CATStd070909-01 (Channel Catfish)
Lane 6: CATStd070909-03 (Hybrid [Blue x Channel])
Lane 8: pl marker (44488)
Lane 10: Kroger 031009-05 Confirmed Hybrid Catfish
Lane 11: OSEL Kroger 073009 Confirmed Channel Catfish
Lane 12: CATStd070809-04 (Tilapia)
Lane 13: CATStd020309-02 (Red Snapper)
Lane 14: CATStd020309-01 (Red Hind Grouper)
Lane 16: pl marker (44488)

Ran by: K. Thronsen 08 25 09
DNA-based Speciation Technology

- DNA-based methods: much more recent and quickly becoming the preferred approach
- Primary reasons for this shift are:
  1. Protein degrades more readily than DNA
  2. DNA sequences are more robust and less subjective than protein banding patterns
  3. DNA sequence-based methodologies are easy to standardize and amenable to the use of a common reference database
What is DNA Barcoding?

COI is one of 13 protein-coding genes in the mitochondrial genome. Mitochondria are cellular organelles that contain their own stands of DNA.

Source: http://www.dnabarcoding.ca/primer/COIProtein.html
What is DNA Barcoding?

- From Handy et al 2011:

  “a process by which species discriminations are achieved through the use of short, standardized gene fragments. (sic) For animals, a target gene region has been selected: a fragment consisting of 648-655 base pairs starting near the 5’ end of the COI mitochondrial gene”
Blue Catfish – *Ictalurus furcatus*

Image source: http://www.tpwd.state.tx.us/huntwild/wild/species/blc/
DNA Barcoding Workflow

Image source: http://www.springerimages.com/Images/RSS/1-10.1007_s_10530-010-9709-8-0
FSIS Barcoding Method Protocol

1. Extract genomic DNA
2. Amplify cytochrome oxidase I (COI) gene with M13-tailed primers (from Ivanova et al 2007 and Baldwin et al 2009)
3. Cleanup with Exo-SAP™
4. Sequencing reaction with M13 primers, cleanup with Performa DTR™
5. Sequencing by capillary electrophoresis
6. Data analysis using software program
1. DNA Extraction
   Image Source: Qiagen website

2. COI PCR
FSIS Method Work Flow

3. Exo-sap™ cleanup
Image Source: http://www.affymetrix.com/estore/browse/brand/usb/product.jsp?productId=131310#1_1

4. Seq rxn and Performa DTR™ cleanup
5. Sequencing by capillary electrophoresis
   Image source: taken by BG

6. Data analysis with BioNumerics™ software
   Image source: screenshot acquired by BG
Verification Results I

- Internal sequence library that now contains barcodes from a total of 31 different fish species including:
  (a) 2 main commercial domestic catfish species: channel catfish (*Ictalurus punctatus*) and blue catfish (*I. furcatus*)
  (b) 17 species of non-commercial Ictalurids from the genera *Noturus* (madtoms) and *Ameiurus* (bullheads)
  (c) 2 imported commercial species of Asian catfishes from the family Pangasiidae, 1 species of tilapia, and 9 species of commercially important saltwater fishes
Verification Results II

- Method approved by internal quality assurance division for inclusion in FSIS Chemistry Laboratory Guidebook as CLG-FPCR.00 in March 2012
- Currently training two analysts in FPCR
Iridescent shark - *Pangasianodon hypophthalamus*

Image source: [http://www.jjphoto.dk/fish_archive/warm_freshwater/pangasius_hypophthalmus.htm](http://www.jjphoto.dk/fish_archive/warm_freshwater/pangasius_hypophthalmus.htm)
Future Barcoding Plans

• Potential extension of the method to **single** species meat products of various matrices
  
  These include: bovine, caprine, equine, ovine, porcine, and poultry food products processed by a variety of approaches

• Preliminary results have been largely successful and include a total of nine species
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Thanks for your attention.
Any questions?