Using Genetics for Species Identification

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Important Point to Consider

Not all specimens need to be genetically identified
  - Body/Fin Identification guides can exclude most samples
    - Use of genetic ID to spot check visual ID’s?
Two types of DNA

—Nuclear
  • one copy per cell
    – nucleus
  • Half from each parent

—Mitochondrial
  • Multiple copies per mitochondria
    – Multiple mitochondria per cell
  • Inherited only from the mother
Many popular methods

— Frozen
  • Good preservation
  • Requires electricity
    – Power failures = sample loss

— Ethanol
  • 95% ethanol is a good long term preservative
    – Vodka (low concentration ethanol) is okay for short term preservation
  • Flammable – shipping restrictions

— Saturated salt solution or dry salt
  • Good short term preservation
  • Not good for long term preservation
    – Some sample degradation occurs with time

— FTA filter paper
  • Special filter paper dries and preserves liquid samples (blood, lysed tissue)
  • Easy and inexpensive storage of genetic material
  • Not good for long term preservation in humid environments

— Dried
  • Rapid drying (freeze drying) preserves samples okay
  • Not good for long term preservation in humid environments
Many methods of DNA extraction are available

— Tissue digestion
  • Sample + buffer + proteinase K
    – Overnight incubation at 55°C
  • Boiling (Chelex method)
    – 60°C for several minutes and 100°C for several minutes

— Removal of unwanted material and Purification of DNA
  • Organic separation
    – Phenol/chloroform
    – Ethanol precipitation
    – Several hours
  • Commercial Kits
    – Silica membrane spin columns (QIAGEN’s DNeasy kit)
    – Approximately 1 hour

— Quantification of DNA
  • UV absorbance (260nm) for DNA quantity
  • UV absorbance ratio (260nm/280nm) for DNA purity (protein contamination)
Polymerase Chain Reaction (PCR)

1. DNA strands separate at high temperature (>90°C) — “Denaturation”
2. Unique primers (short pieces of DNA) anneal to specific locations (50-65°C) — “Annealing”
3. DNA Polymerase adds nucleotides to the end of the primers copying the DNA (70-72°C) — “Elongation”

Steps 1-3 repeated many times
- Copy number doubles with each cycle
  - 35 cycles = 17,000,000,000 copies
  - 40 cycles = 550,000,000,000 copies
Genomics

Whole genomes, transcriptomes

• 100’s of millions of base pairs
• Identify genes under selection
• Better understand speciation drivers
• Clarify taxonomic relationships
Uses of DNA information

Phylogenetic analyses
  — Relationships between species
    • Evolutionary studies
    • Discovery of new species

Population Genetic Studies
  — Relationships between populations/stocks
    • Defining management units

Paternity Analyses
  — Mating systems
  — Population size estimates

Genetic tags
  — Replacement for standard tags
  — Unique code for every animal

Species Identification
  — Validate fishery observer species identifications
  — Identify animal parts
    • Shark fins, meat
  — Identify hard to identify life stages
    • Eggs, Larvae, Juveniles
Phylogenetic Studies

DNA sequencing of several genes
  —Mitochondrial
    • e.g. cytochrome b, cytochrome c oxidase, NADH
  —Nuclear
    • e.g. recombination activating gene (RAG1), internal transcribe spacer (ITS2), TMO4C4

Measures of genetic similarity, construction of trees and statistical testing
  —Maximum parsimony, Maximum Likelihood, Bayesian Inference
Fig. 3. Inferred phylogeny based on mixed model Bayesian analysis of the concatenated data and BEST analysis for species of the genus Sphyrna, Eusphyra blochii (Eusphyra) and the outgroup Carcharhinus acronotus. Numbers above nodes are the posterior probabilities (100 x) from the mixed model analysis that contained the group and numbers below nodes are BEST credibility values (100 x). Cephalofacial shapes and relative body sizes of the taxa are indicated. Scale bar applies to body sizes. Geographic distributions of the species are shown. Head shapes and distribution maps modified from Compagno (1984).
Identifying “cryptic” species

Quattro et al. 2006
Identifying “cryptic” species

Sphyra gilberti sp. nov., a new hammerhead shark (Carcharhiniformes, Sphyridae) from the western Atlantic Ocean

JOSEPH M. QUATTRO¹, WILLIAM B. DRIGGERS III², JAMES M. GRADY³, GLENN F. ULRICH⁴ & MARK A. ROBERTS¹

Quattro et al. 2006
Identifying Population Units

Duncan et al. 2006
Species Identification

Every species has a unique DNA sequence
  — Which gene do we sequence?
    • Cytochrome c oxidase subunit I (COI)

Reference specimens
  — We need to know what the DNA sequences for all potential species before we can identify unknown samples
    • Museum collections of reference material

Reference database
  — DNA sequences from all possible species needed for accurate identifications
    • GENBANK, Barcode of Life Database (BOLD)

Genetic methods for Identification
  — DNA sequencing
  — Restriction fragment length polymorphism (RFLP)
  — Species specific PCR
DNA Sequencing for Species Identification

Sequencing the COI gene with universal PCR primers and comparing to reference databases

Best method
- Provides more information
  - All species can be identified and population specific information can be obtained
- High accuracy
- Preferred method for forensic identification

Expensive
- Trained technicians
- Dedicated laboratory space
- Equipment:
  - Standard lab equipment & PCR machine $8-10k USD
  - DNA sequencer $150-250k USD
  - Chemicals for sequencing $10-15 USD per sample
  - Computers and software

Time
- 2 or 3 days
**Elasmobranchii (class) - Chordata:**

### Taxon Description (Wikipedia)

*Elasmobranchii* is a subclass of Chondrichthyes or cartilaginous fish, that includes the sharks (Selachii) and the rays and skates (Batoidea). [Full article at Wikipedia](https://en.wikipedia.org/wiki/Elasmobranchii)

### BOLD Stats

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<th>Specimen Records: 21,413</th>
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</table>

### Species List - Progress

- **Access Published & Released Data**

### Contributors (Specimens & Sequencing)

#### Specimen Depositories:

- Florida State University [9538]
- CSIRO, Australian National Fish Collection [1647]
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- Biodiversity Institute of Ontario [1012]
- Save the Seas Foundation, Shark Conservation Consortium [937]
- South African Institute for Aquatic Biodiversity [4690]
- United Arab Emirates University [458]
- University of Bologna [427]
- 116 Others [4103]

#### Sequencing Labs:

- Biodiversity Institute of Ontario [8388]
- Florida State University [4708]
- Mixed from GenBank [1028]
- University of Guelph [732]
- Mixed from GenBank, HCMC [178]
- Smithsonian Institution, Laboratories of Analytical Biology [174]
- Muséum National d’Histoire Naturelle, Station de Biologie... [111]
- University of Bologna [101]
- 39 Others [710]
DNA sequence entry form

DNA sequence from shark fin
Species Identification

100% match to silky shark
RFLP and Species Specific PCR

Use DNA differences to design species specific PCR primers or choose restriction enzymes to cut DNA at specific sites

Limited to small groups of species
Prone to false positive and false negative results
— Identifications for law enforcement should be verified by sequencing

Inexpensive:
— Minimal training required
— Laboratory is portable
— Standard lab equipment & PCR machine $8-10k USD
— Chemicals for assay $0.50-$1.50 USD per sample

Time:
— 2 to 12 hours
Species Specific PCR

Universal primers
— Generate 1 large PCR band “control”

Species specific internal primers
— Generate a single smaller band that is a specific size for different species

Figure 2. Schematic representation of the shark nuclear 5.8S and 28S ribosomal RNA genes and ITS2 locus showing relative annealing sites and orientation of primers used in the octaplex-PCR assays. Shark universal primers (FISH5.8SF and FISH28SR) are shown as solid arrows. The six species-specific primers are shown as open arrows. Abbreviations: SF Mako, shortfin mako; LF Mako, longfin mako.

Shivji et al. 2002
**Isurus paucus**

**Lamna nasus**

**Carcharhinus obscurus / C. galapagensis / C. longimanus**

**Isurus oxyrinchus**

**Prionace glauca**

**Carcharhinus falciformis**

Shivji et al. 2002
Shark Group Specific PCR

Useful for Identification of fins or meat

4 categories

— *Isurus* spp.
— *Carcharhinus* spp.
  - Also *Prionace glauca*
— *Alopias* spp.
— *Sphyrna* spp.

Caballero et al 2012
Unambiguous identification of *Alopias* species

— *Alopias vulpinus* 76bp
— *A. pelagicus* 198bp
— *A. superciliosus* 264bp

No amplification in non target species

Caballero et al 2012
Basic Lab Methods

- Species Specific PCR techniques are simple
- Infrastructure is minimal
- Assays are cheap and fast
  - < $1 USD/ sample
  - results in 2-3 hours
DNA Extraction
(making shark fin soup)

1. Cut off a small piece
2. Place tissue in tube
3. Close the tube
DNA Extraction (making shark fin soup)

Place tubes in PCR machine

Select and Run Program
PCR Preparation

Mix PCR ingredients

Aliquot into tubes

Add DNA
PCR Amplification

Place tubes in PCR machine

Select and Run Program
Gel Electrophoresis

DNA has a negative charge
— electricity forces DNA through the gel towards the cathode (positive terminal)

Pieces of DNA can be separated by using electrophoresis through a gel matrix
— Agarose or Polyacrylamide gels
  • Small pieces travel faster than large pieces
  • Higher percentage of agarose/polyacrylamide provides higher resolution of small pieces

After Electrophoresis the DNA is stained with a DNA specific dye
— Ethidium Bromide/Gel Red/SyBR Green

DNA/Dye complex will fluoresce with UV light and can be photographed
Compare unknowns to references for identification

*Sphyrna mokarran*
*Sphyrna lewini*
*Sphyrna zygaena*

Abercrombie et al. 2005
NOAA SWFSC as an International Partner

Advice
Collaborations
Training in basic genetic lab techniques
  • DNA Extraction
  • PCR
  • Gel Electrophoresis
  • DNA sequencing
  • Microsatellite genotyping
Thank You

Questions?

Southwest Fisheries Science Center, NOAA Fisheries Service