DNA Barcoding of hammerhead sharks (family Sphyrnidae) Character-based identification using single nucleotide diagnostics

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.: BACKGROUND

- The shark fin industry is now a major driving force of shark mortality
  - Sharks are susceptible to overexploitation due to slow growth and delayed maturation
  - Estimated shark biomass in the fin trade is 1.7 million tonnes year^{-1}
- Most molecular methods for shark identification are limited to a select number of known taxa and are not comprehensive
  - DNA barcoding provides a simple, rapid, and effective way to monitor shark species in the fin trade for forensic and conservation purposes
- Non-identical matches of unknown samples to reference sequences using a distance measure are problematic for confident identification
- Single nucleotide variations can be used in a character-based diagnostic system to complement the existing barcode identification engine
  - Akin to morphological methods – presence/absence of traits
  - **Single nucleotide diagnostics (SNDs)** – can be thought of as SNPs for the species level (Figure 1)
  - DNA characters can be combined to create compound diagnostics (DeSalle et al 2005)
  - Helps place non-identical matches to species

AAAGTACCCACGGACTATAGGCAC
AATGTACCCACGGACCATAGGCAC
AAGGTACCCACGGACCACAGGCAC

Figure 1. Each row represents a different species. Nucleotides highlighted in yellow represent SNDs- single nucleotide variations that are unique to the species. Nucleotides highlighted in green represent a compound diagnostic that is unique when considered in conjunction.

.: METHODS

- 164 hammerhead shark DNA samples (family Sphyrnidae) were analyzed
  - Across 7 species (of 9 total, 8 known and 1 cryptic species)
  - Average of 23.4 samples per species (range of 1 to 65)
  - Globally distributed samples available for 3 species (Figure 2)
- Approximately 25mg of tissue used for genomic DNA extraction
  - DNeasy Tissue Kit (QIAGEN Inc, Valencia, California)
- 652bp fragment near the 5' terminus of the cytochrome c oxidase I (COI) gene amplified and bi-directionally sequenced
  - Used a primer cocktail (Ivanova et al 2007)
- SNDs identified for samples while pooled with sequences originating from species of the same order (i.e. Carcharhiniformes)
  - 356 sequences in total
    - 155 hammerhead sequences
    - Remaining sequences from unpublished projects and Ward et al 2005

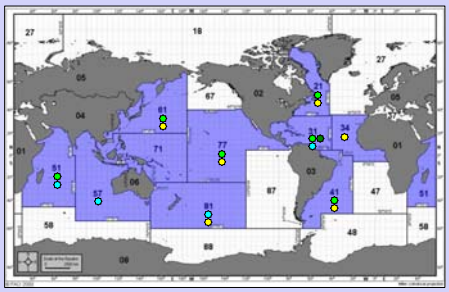
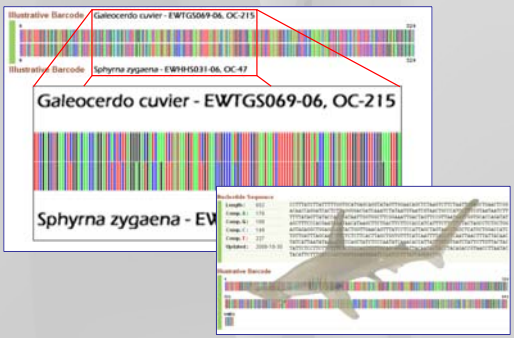


Figure 2. Food and Agriculture Organization (FAO) fisheries regions. FAO regions where samples originated from are highlighted in blue. Markers on the map indicate regions where the three species with globally distributed samples were collected. Green markers represent scalloped hammerheads (*Sphyrna lewini*). Turquoise markers represent great hammerheads (*Sphyrna mokarran*). Yellow markers represent smooth hammerheads (*Sphyrna zygaena*). The dark green marker represents the cryptic scalloped hammerhead species.



.: RESULTS

- COI sequences successfully recovered from 94.5% of the hammerhead specimens (155/164) (Figure 3)
  - Average of 22.1 samples per species, all 7 species represented
- Mean sequence divergence (Figure 4)
  - Intra-specific: 1.481% (0.030% standard error)
  - Inter-specific: 9.362% (0.009% standard error)

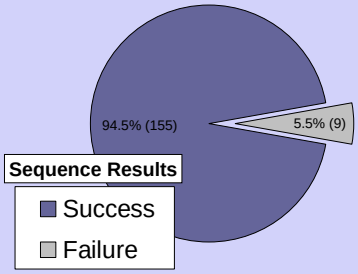


Figure 3. Proportion of successful PCR amplification and sequencing of the 164 samples of hammerhead sharks.

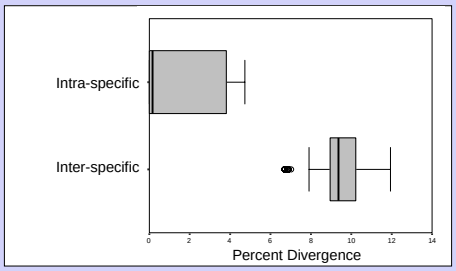


Figure 4. Boxplot of intra-specific and inter-specific sequence divergence. Boxes represent the central 50% of the data. Whiskers represent minimum and maximum values. The black bar within each box represents the median. The circles represent outliers.

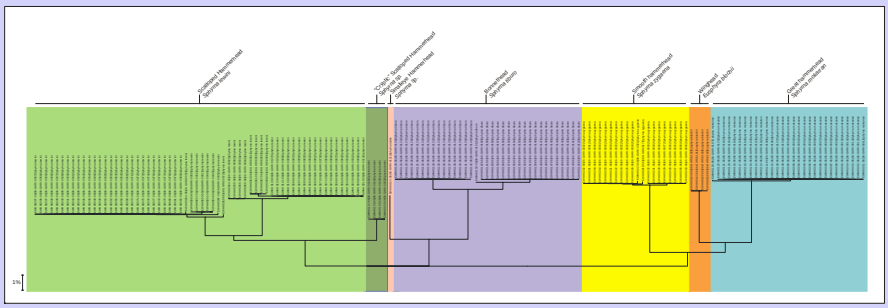


Figure 5. This neighbour-joining tree constructed under the Kimura 2-Parameter model illustrates the pattern of COI sequence divergence for the 155 hammerhead sequences examined in this study. Of note is the distinct clustering of *S. lewini* into two groups, which are correlated with the FAO region of sample collection (not shown on tree). The third group of *S. lewini* support the existence of a cryptic hammerhead species, as described by Quattro et al (2006). Other species also exhibit some phylogeographic variation.

- All 7 hammerhead species (including the cryptic species) examined in this study had at least one SND (Figure 6)
  - I.e. there was at least one single nucleotide variation that was unique to each species
  - A simple compound diagnostic, 2 nucleotides in length, was able to distinguish between geographic lineages of Scalloped hammerheads (labeled as a *Sphyrna lewini* 1 and 2)

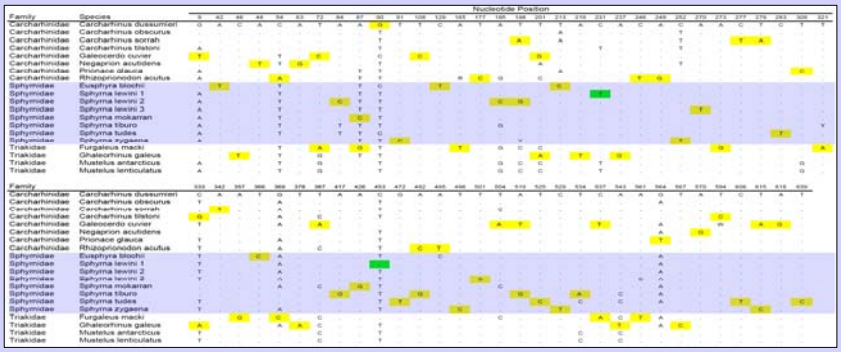


Figure 6. Parsimonious informative characters from the 652bp barcode sequences for 20 species of sharks (order Carcharhiniformes). Characters highlighted in yellow are single nucleotide diagnostics (SNDs). That is, these characters are unique to the corresponding species. Characters highlighted in green mark sites that form a compound diagnostic when taken in conjunction.

.: CONCLUSIONS

- DNA barcodes effectively identify hammerhead shark species
  - Distinguishes recently reported cryptic species (Quattro et al 2006) and contains phylogeographic signal in some species
  - Identification by distance measures or a character-based approach using SNDs holds under large sample sizes with global distribution
- SNDs are affected by the number of species considered simultaneously (fewer SNDs when there are more species)
  - More SNDs identified when species are pooled under a less encompassing taxonomic category (e.g. family instead of order)
  - More complex compound diagnostics, of longer length, can be used for increased resolution
  - Therefore, SNDs identified in this study are conservative
  - Traditional barcode distance measure can quickly place an unknown specimen to the genus level as a first step
- Identification of SNDs in the future is amenable to computer automation

.: REFERENCES

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