Genetic barcoding as a monitoring tool

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Outline

- What is genetic barcoding?
- How is it standardized?
- How are barcodes identified/developed?
- Is it successful?
- Use in ecological monitoring
- Implications for the San Francisco Estuary

What is genetic barcoding?

- Species identifiers made of short, genetic (DNA) sequences
  - Animals: 650 base pair portion of mitochondrial cytochrome oxidase 1 gene (CO1 or cox1)
  - Plants: not yet standard; several candidate regions
  - Original intent was to <u>inventory biodiversity</u>

What is genetic barcoding?

- DNA barcodes combine four factors:
  - Standardization on a particular gene region
  - Ability to use high-throughput techniques to screen “unknowns” for barcodes
    - 100s to 1000s per day
  - Barcodes tied to voucher specimens and expert verification
  - Reference barcodes are subject to enforcement of data standards

Standardization

- **CBOL**: The Consortium for the Barcode of Life is an international initiative devoted to developing DNA barcoding as a global standard for the identification of biological species. http://www.barcoding.si.edu/

- **BOLD**: The Barcode of Life Data Systems is an online workbench that aids collection, management, analysis, and use of DNA barcodes. [~714,000 records to date] http://www.barcodinglife.org

Standardization (cont.)

Many specialized databases:

- **CMarZ**: The Census of Marine Zooplankton Network is an association committed to completing a global biodiversity of holozooplankton. http://www.cmarz.org

- **FISH-BOL**: The Fish Barcode of Life Initiative is a global effort to coordinate an assembly of a standardised reference sequence library for all fish species. http://www.fishbol.org

How are barcodes identified/developed? I.

- Individual researchers sequence the CO1 region in their target animals
  - For all species to be identified
  - Include species' that could be confused with the target species
  - Closely related species to ensure there is a unique barcode
  - Several (to many) individuals of each species to capture within-species barcode variation

How are barcodes identified/developed? II.

- Barcodes deposited in databases (BOLD and/or others)
- Voucher specimens and/or photographs are deposited
- Taxonomy may be vetted by experts

How are barcodes identified/developed? III.

- Barcode sequences within a species are compared to related species to ensure unique identity

- "Mini-barcodes" (~150 bp) may be developed from full-length barcodes for use in degraded samples

  - Must be vetted in control experiments
  - Considered DNA barcodes *sensu lato*
  - Useful for stomach contents, fossil or forensic samples, etc.

Is it successful?

- Estimates of 95% to 97% accuracy in taxa with good species delineation by traditional methods
  - Have led to discovery of new species that were cryptic morphologically
  - CAUTION: DNA barcodes are NOT a substitute for traditional taxonomy by standard morphological and molecular methods

Is it successful?

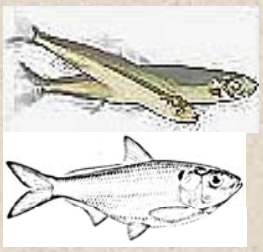
- Troublesome or unsuccessful in some cases:
  - Recently diverged species (in evolutionary time)
  - Hybridizing species
  - Species complexes (e.g., potatoes and many other plants, some butterflies, some fishes)
  - Taxa with poorly developed taxonomy (poorly described)

Use in ecological monitoring

- Original intent was to inventory biodiversity
  - Success limited to well-defined and well-described groups
  - For poorly-described groups, alternative is to turn a taxon's barcode into a
    - molecular operational taxonomic unit (MOTU)
    - If unique and associated with a taxon, even if poorly known

Use in ecological monitoring

- Ecological applications:



  - Species monitoring
    - presence/absence

  - Species identity within a sample (e.g.)
    - stomach contents
    - environmental sample
    - plankton tow

Use in ecological monitoring

- Ecological applications (cont.):
  - Identity of morphologically indistinguishable life stages or tissues (e.g.):
    - eggs
    - larvae
    - juveniles
    - egg cases
    - food or harvest products
    - partially decomposed or destroyed tissue

Use in ecological monitoring

- Examples
  - Gorokhova, 2006. Used DNA (mini-barcode) to identify *Cercopagis pengoi*, an invasive cladoceran, in food webs
    - Found stomachs of *Neomysids*
    - Represents novel prey for native fish and zooplanktivores
  - Tobe et al. 2009. Use DNA to identify and quantify diet of larval krill (*Euphausia superba*)
    - Screened for dominant copepod species
    - Stomach contents were confirmed by microscopy
  - Passmore et al. 2006. Used DNA to identify diatom prey of Antarctic krill (*Euphausia superba*)
    - Successful to identify prey
    - More reliable than microscopy
    - Simple barcoding unable to reliably assess relative abundance

Implications for San Francisco Estuary

- Food web studies involving imperiled fishes
- Plankton samples or environmental samples
  - Once developed, screening would be faster and cheaper than microscopy

- Identification of eggs, larvae, juveniles
- Screening for potential invasive species (all life stages)
- Identification of fish parts destroyed in screens or other devices

Implications for San Francisco Estuary

- Databases are keen to include San Francisco Estuary taxa, especially zooplankton
- With extra method development, DNA methods can be used to quantitate relative abundances of items in a sample
- Once developed and databased, DNA barcodes become public and benefit many applications

Questions?