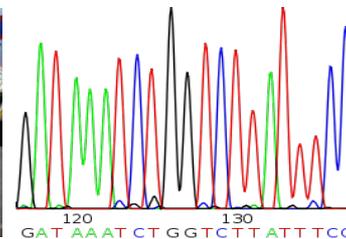
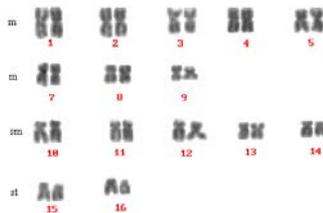


FRM-111 (1+2) "Taxonomy of Finfish"

Introduction to Modern Taxonomic Tools

Dr. Mamta Singh
Assistant Professor
Fisheries Resource Management
College of Fisheries, Kishanganj
Bihar Animal Sciences University, Patna

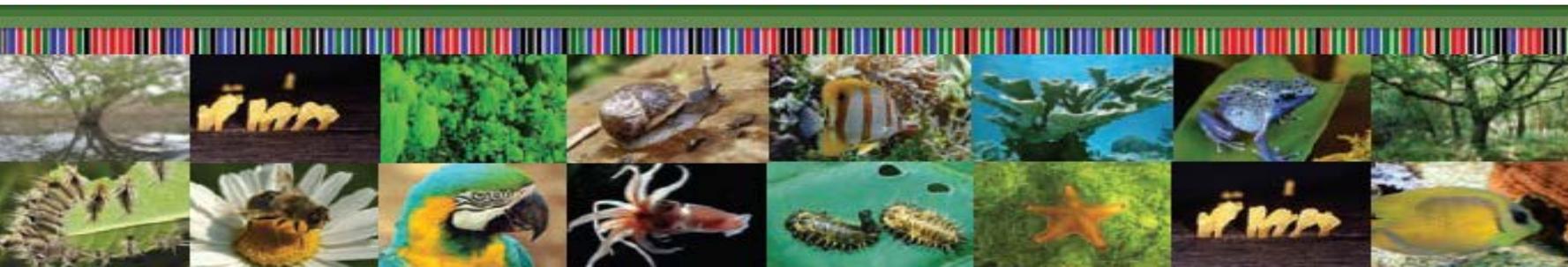
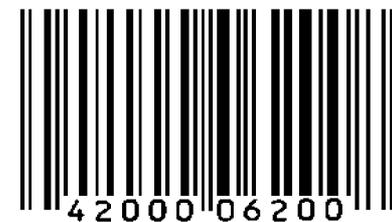


Barcoding: DNA-based analysis

During the last decade, **DNA-based analysis** have radically influenced nearly **all areas of biological research** – including taxonomy and systematics.

In 2003, researchers at the University of Guelph in Ontario, Canada, proposed “**DNA barcoding**” as a way to identify species.

Barcoding uses a very short genetic sequence from a standard part of the genome the way a supermarket scanner distinguishes products using the black stripes of the **Universal Product Code**.





■ DNA Barcoding: Documenting Biodiversity with a Gene Sequence



“DNA barcoding” is an exciting new tool for taxonomic research. The DNA barcode is a very short, standardized DNA sequence in a well-known gene. It provides a way to identify the species to which a plant, animal or fungus belongs. The Consortium for the Barcode of Life (CBOL) is promoting international partnerships that will enable people in all countries to better understand and protect their biodiversity.



iBOL WORKING GROUP | 1.1 VERTEBRATES

Fish barcode of life (FISH-BOL)

international
BARCODE
OF LIFE



progress

specimens barcoded: 96425

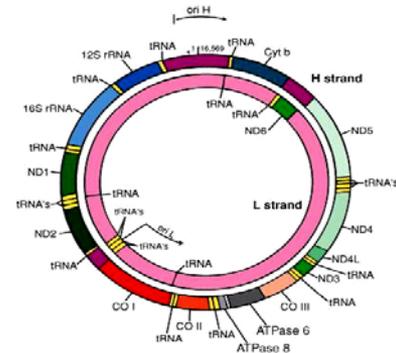
species barcoded: 10267

unnamed barcode
clusters found: 2029



What should be the Properties of gene used for Barcoding

- ✓ Should be present in all the taxa of interest
- ✓ Provide a large variation between species yet a relatively small amount of variation within a species.



Which gene?

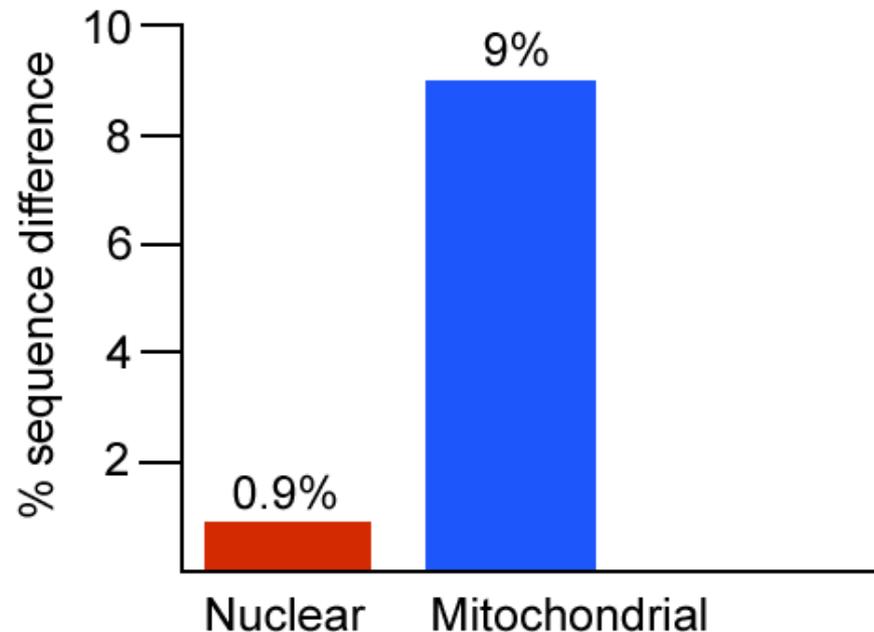
The mitochondrial gene of animals is a better target than the nuclear gene because:

1. Lack of introns: Making amplification straightforward. Nuclear genes are often interrupted by introns, making amplification difficult or unpredictable.



2. Faster mutation and Evolution rate: Greater differences among species i.e. 5 to 10-fold higher in mitochondrial than in nuclear genes.

Average sequence differences in nuclear and mitochondrial DNA
between human and chimp

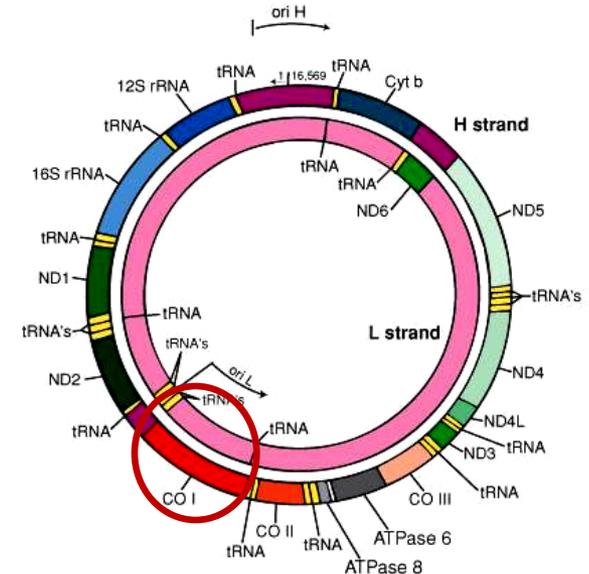
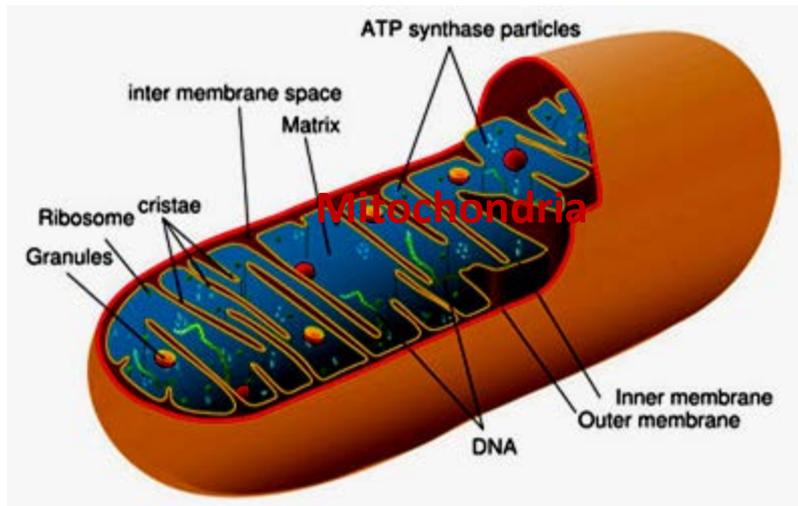


3. Limited exposure to recombination due to haploid mode of inheritance (maternal genome)

4. Copy number: There are 100-10,000 more copies of mitochondrial than nuclear DNA per cell, making recovery, especially from small or partially degraded samples, easier and cheaper.

DNA Barcode

The gene region that is being used as the standard barcode for almost all animal groups is a **655 base-pair** region in the mitochondrial **cytochrome c oxidase 1** gene (COI). COI is proving highly effective in identifying birds, butterflies, fish, flies and many other animal groups.



Mitochondrial Genome

Components of DNA Barcoding

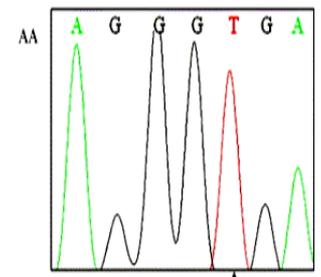
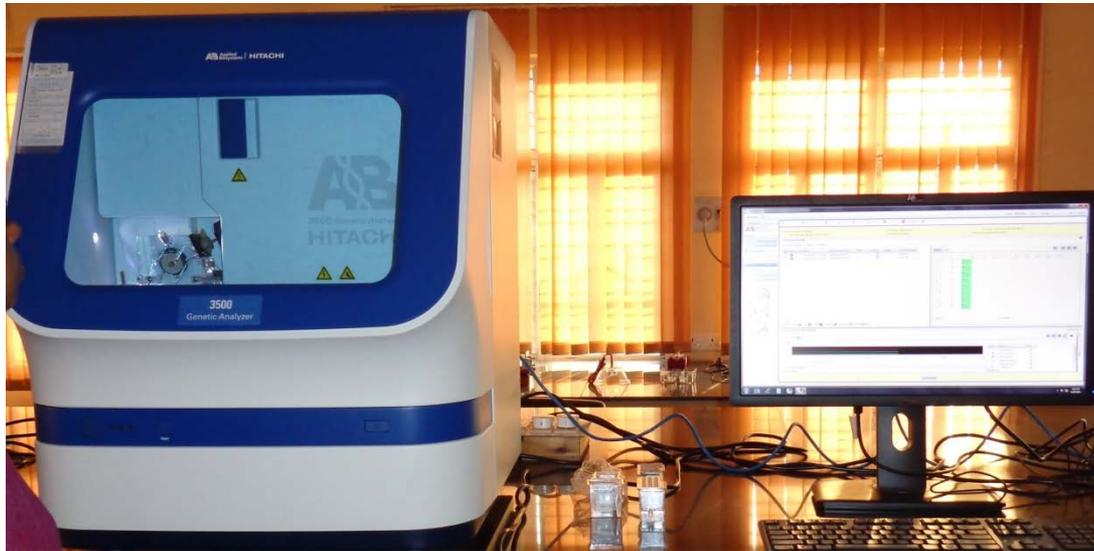
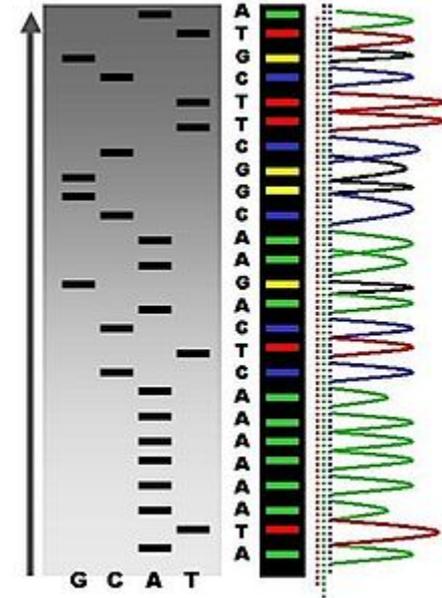
The Specimens collection: From Natural habitat, museums, herbaria, zoos, aquaria, frozen tissue collections, seed banks and other repositories of biological materials are treasure for specimens.

The Laboratory Analysis: to obtain DNA barcode sequences from specimens. The best equipped molecular biology labs can produce a DNA barcode sequence in a few hours.

The Database: One of the most important components of the Barcode. [Barcode of Life Database](#) (BOLD) was created and is maintained by University of Guelph in Ontario. It offers researchers a way to collect, manage, and analyze DNA barcode data.

The Data Analysis: Specimens are identified by finding the closest matching reference record in the database

Infrastructure for DNA Barcoding



Steps involve in DNA Barcoding

- ✓ **Specimen Collection**
- ✓ **Tissue collection (Blood, Muscle, Scales, Fins)**
- ✓ **DNA Isolation and Quantification**
- ✓ **Amplification of partial Cytochrome Oxidase I (COI) gene (PCR)**
- ✓ **Purification of Amplified (PCR) Products**
- ✓ **Labeling of amplified products with fluorescent dye**
- ✓ **Purification of labeled amplified Sequence**
- ✓ **DNA (Nucleotide) Sequencing**
- ✓ **Analysis of Generated data or sequence**

Nucleotide sequence of DNA barcode of *Channa marulius*: *COI* 655 base pair

CCTTTATTTAGTATTTGGTGCTTGAGCCGGATAGTAGGCACCG
CCCTAAGTCTCTTAATTCGGGCAGAACTAAGCCAGCCCGGCG
CCCTATTAGGCGATGATCAGATCTATAATGTAATTGTAACAGCG
CATGCCTTTGTAATAATTTTCTTCATGGTCATGCCAATAATAATT
GGAGGCTTTGGAAACTGACTAGTCCCATAATAATTGGCGCCC
CTGACATAGCATTCCCCCGCATGAATAACATAAGCTTCTGATTA
CTTCCTCCCTCTTTTTCTTCTCCTGCTAGCCTCCTCCGCAGTAG
AAGCCGGAGCCGGCACTGGGTGGACCGTTTACCCGCCCTGG
CCAGCAACCTAGCTCACGCAGGGGCTTCTGTAGATTTAACTAT
CTTCTCCCTGCATCTTGCAGGTGTTTCCTCAATCCTAGGTGCT
ATTA ACTTCATTACA ACTATTATTAACATAAAAACCCCTGCCAT
TTCTCAGTATCAGACACCCCTGTTTGTCTGAGCCATTCTGATT
ACTGCCGTACTTCTACTCCTCTCCCTCCCCGTACTGGCCGCCG
GCATTACAATGCTACTCACAGACCGAAACCTAAACACCACCTT
CTTCGACCCCGCCGGCGGAGGAGATCCAATTCTTTATCCAACA
CCTGTTC

Homology Search in database (GenBank) using online tool BLASTn

Sequences producing significant alignments:

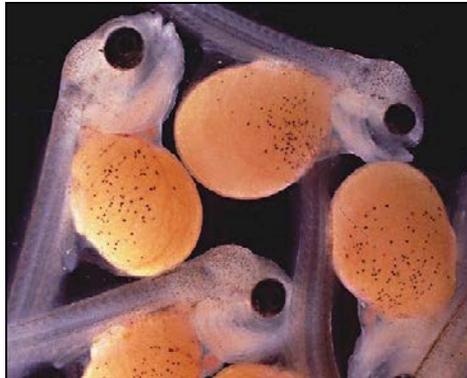
Select: [All](#) [None](#) Selected:0

Alignments Download GenBank Graphics Distance tree of results							
	Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/>	Channa marulius voucher MUND02 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1150	1150	98%	0.0	99%	JX260841.1
<input type="checkbox"/>	Channa marulius voucher MUND01 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1137	1137	97%	0.0	99%	JX260840.1
<input type="checkbox"/>	Channa marulius voucher NF420 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1133	1133	99%	0.0	99%	JX983244.1
<input type="checkbox"/>	Channa marulius voucher NF22 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1031	1031	99%	0.0	96%	JX983243.1
<input type="checkbox"/>	Channa marulius voucher NE-CM5 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	800	800	85%	0.0	92%	HM117196.1
<input type="checkbox"/>	Channa marulius voucher NE-CM4 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	800	800	85%	0.0	92%	HM117195.1
<input type="checkbox"/>	Channa marulius voucher NE-CM3 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	800	800	85%	0.0	92%	HM117194.1
<input type="checkbox"/>	Channa marulius voucher NE-CM2 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	800	800	85%	0.0	92%	HM117193.1
<input type="checkbox"/>	Channa marulius voucher CARE CM1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	800	800	85%	0.0	92%	EU342199.1
<input type="checkbox"/>	Channa marulius voucher CARE CM2 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	800	800	85%	0.0	92%	EU342200.1
<input type="checkbox"/>	Channa marulius voucher NE-CM1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	794	794	85%	0.0	92%	HM117192.1

Biological specimens come in many forms



Adults



Juvenile stages

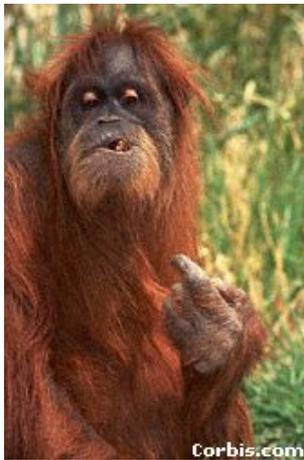
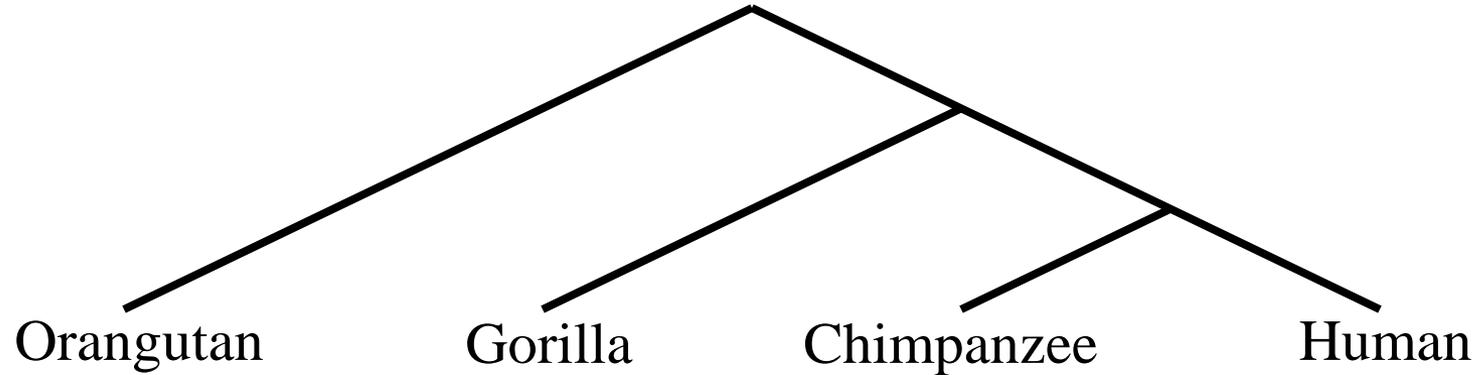


Processed products

Barcoding works on all forms



Phylogeny (Evolutionary Relationship)



Specimen Identification



Puntius filamentosus

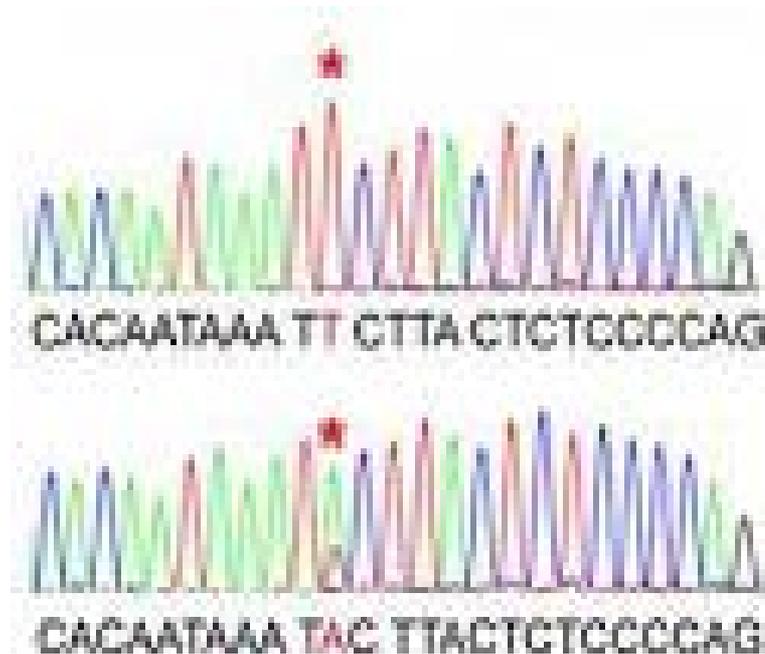
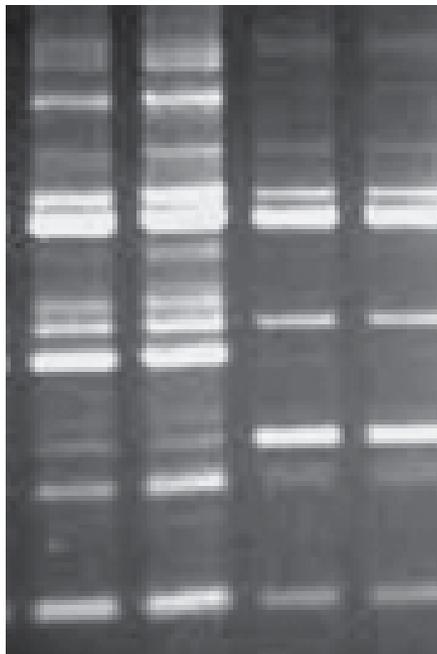


Puntius assimilis

Stock Identification

Fish Stocks (subpopulation) of a particular species are usually identified by their location. They are genetically discrete from other stocks.

This difference in gene or DNA can be determined by DNA barcoding to identify the stocks



An example of Forensic Application of DNA Barcoding

Identification of Endangered Whale Shark (*Rhincodon typus*).

- Flesh, suspected as that of the Wildlife protected **whale shark (*Rhincodon typus*)** seized from fishermen by the Forest Range Officer (Govt. of Kerala), Kannur, Kerala.
- The Judicial First Class Magistrate, Thalassery, Kannur, Kerala approached NBFGR for sample analysis and confirmation of species (Case No. R.P.330/08, dt 29. 09. 2008).
- Based on DNA sequencing of COI (655bp), 16S rRNA(525bp) and Cyt b(541bp) genes and comparing with the sequences earlier generated by NBFGR (FJ375724, FJ375725, FJ375726) from a stranded whale shark (from North Kerala, March, 2006), **the suspected sample was identified as that of endangered Whale Shark (*Rhincodon typus*).**

