Training on "Molecular Identification and DNA Barcoding of Insect Pests and Natural Enemies including Invasive species"
(Under ICAR-NBAIR HRD)
18. 11. 209 to 27. 11. 2019

# Mitochondrial Genome and its application in DNA Barcoding


Presented by :
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# Broad topics of the training

| | |
|---|---|
| DNA isolation Techniques | Dr. R. Gandhi Gracy & Mr. Venugopala |
| PCR Techniques | Dr. M. Mohan, Mr. Arya, Dr Nisha Nayyar, |
| Sequencing | Dr Gracy, Mr Arya, Dr Jyoti |
| Databases, NCBI Database: BLAST | Dr. Pratheepa, Dr Ashika, Dr Jyoti |
| NCBI-BankIt / DNA Bar coding | Dr. Gandhi Gracy, Dr. Ashika and Mr. Venugopala |
| BOLD, DNA Barcoding | Dr Venkatesan, Dr Nayyar |
| Introduction to Schools of Taxonomy | Dr. David. K. J. |
| Integrative Taxonomy | Dr Ankita Gupta |
| Molecular Phylogeny- ( MEGA 7) | Dr. Aditi |
| Introduction to Schools of Taxonomy | Dr Joshi |
| Evolutionary Biology and Molecular Phylogeny | Dr. Praveen Karanth, IISC |
| Mitochondrial DNA and DNA Bar Coding | M. NAGESH |
| Invertebrate Molecular Biology: an overview | M. Nagesh |

# Brief review

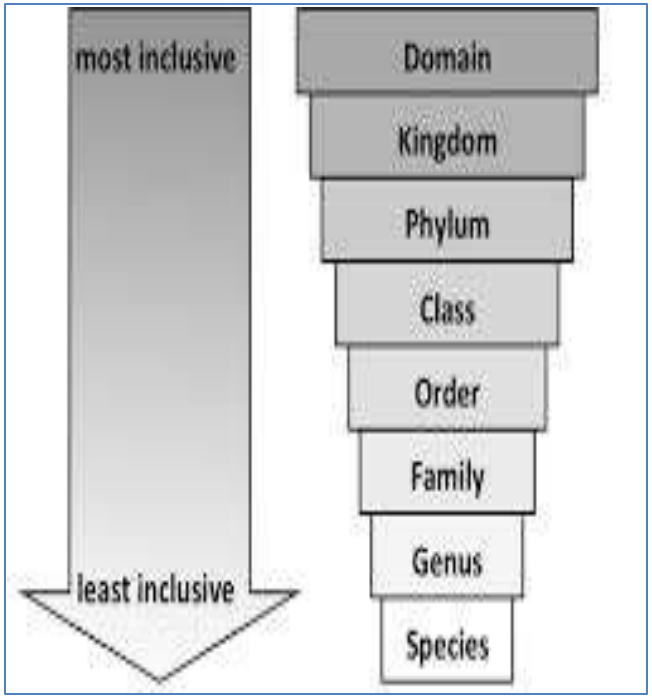
**BIODIVERSITY**

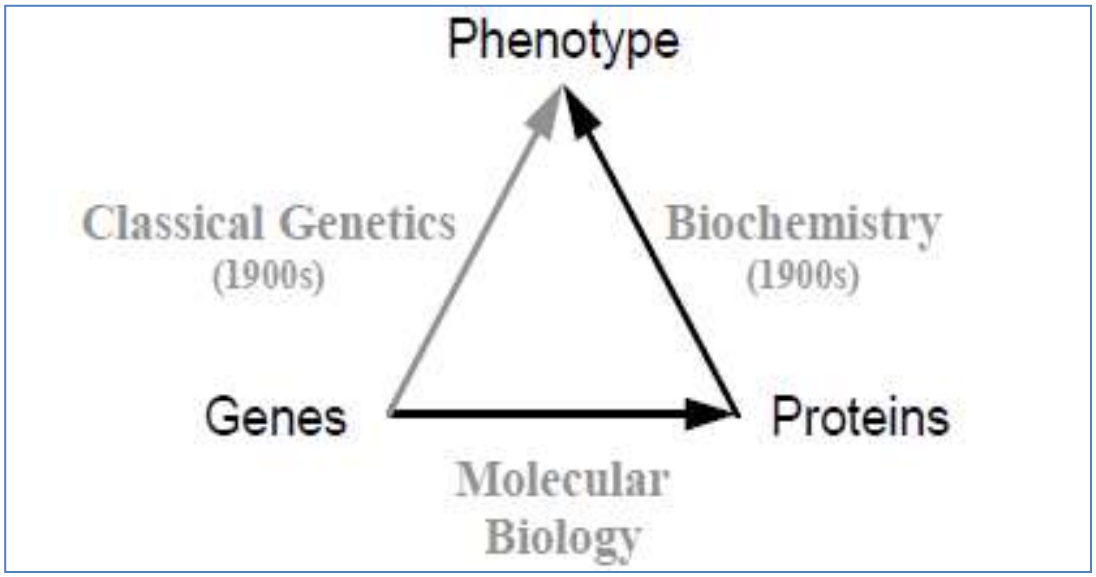
**CLASSIFICATION – TAXONOMY – SYSTEMATICS – PHYLOGENY**

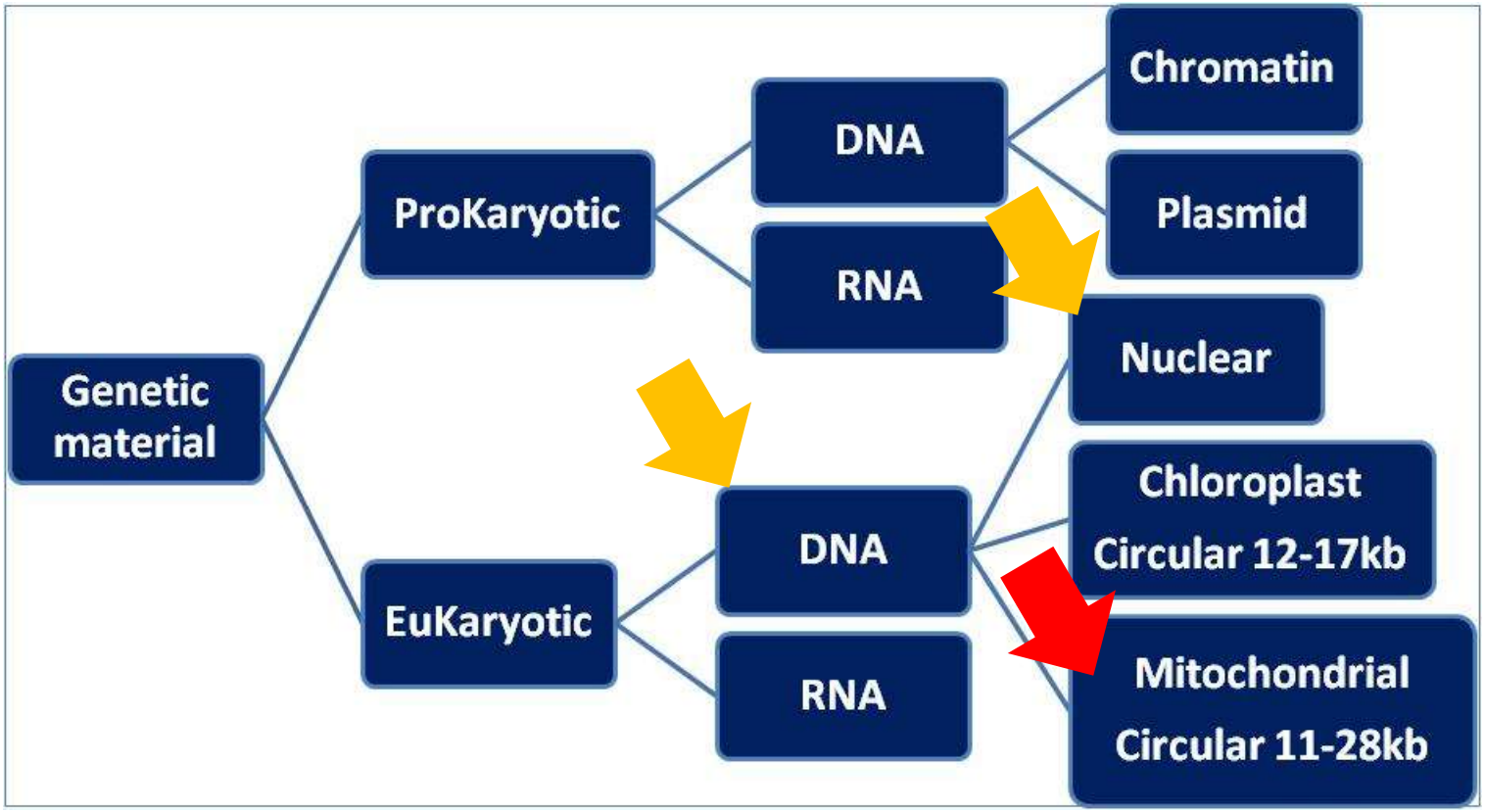
**VARIABILITY & SIMILARITY**

**Classification: Arrangement of organisms in to groups**
**Taxonomy _ identification of groups based on features/characters/traits**

| Taxonomy | Systematics |
|---|---|
| Classification in to taxa | Evolutionary relationships |
| Part of Systematics | Relationships |
| Classification, naming | Classification, naming, cladistics, phylogeny, evolution |

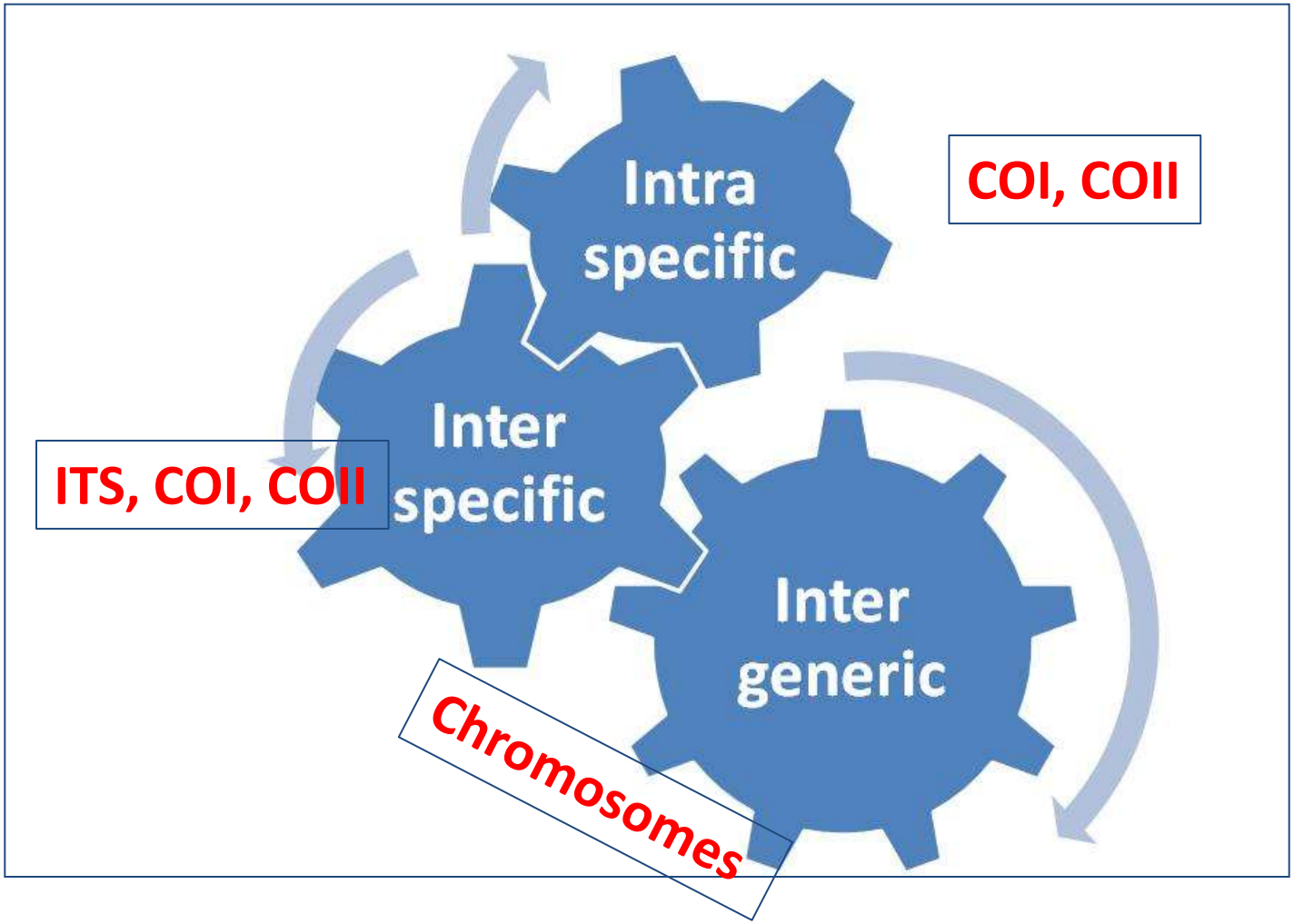



Phenotype
Classical Genetics
(1900s)
Biochemistry
(1900s)
Genes
Proteins
Molecular
Biology


Genetic material
ProKaryotic
EuKaryotic
DNA
RNA
DNA
RNA
Chromatin
Plasmid
Nuclear
Chloroplast
Circular 12-17kb
Mitochondrial
Circular 11-28kb

# Simplified understanding of capturing - Integrative Taxonomy

# Mitogenomics



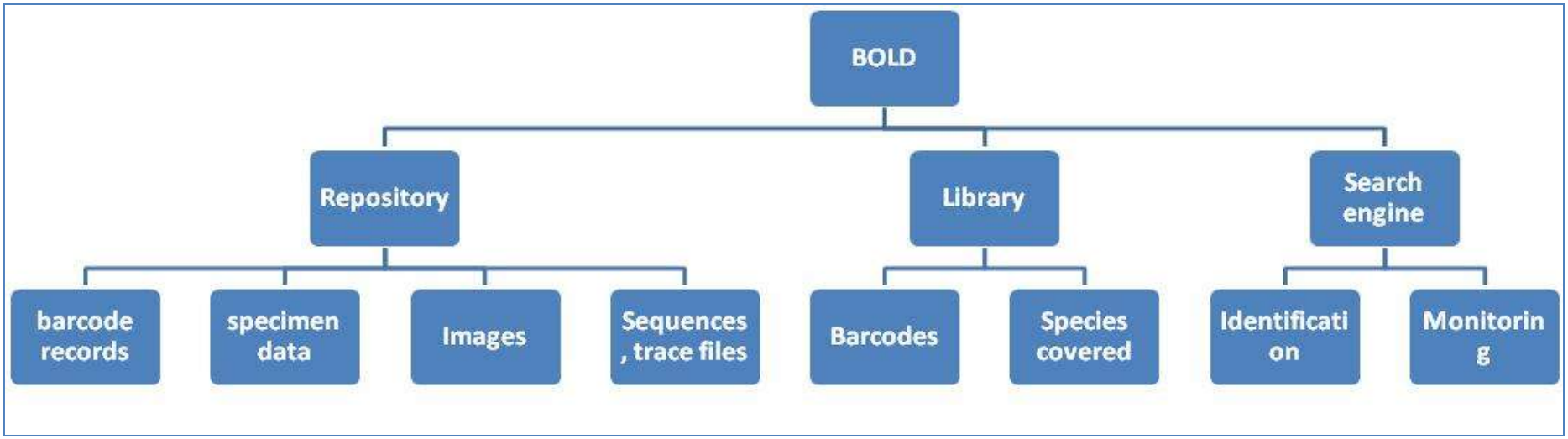
# Global initiative and purpose

# iBOL: International BAR CODE OF LIFE
# BOLD: Barcoding of Life Database

## iBOL: International BAR CODE OF LIFE

- Established in 2008
- developing globally accessible, dna-based systems for the discovery and identification of all multicellular life
- BARCODE 500K (2010-2015) -500,000Barcodes
- BIOSCAN (2019-2026) barcode coverage to 2.5 million species
- Planetary Biodiversity Mission by 2045

# BOLD: Barcode of Life Database System




## Significance of mtDNA

**Step 1:** Isolate DNA
**Step 2:** PCR Amplify target DNA barcode region
**Step 3:** Sequence PCR products
**Step 4:** Compare resulting sequences against ref databases to find matching species

# Essential functions of mitochondria

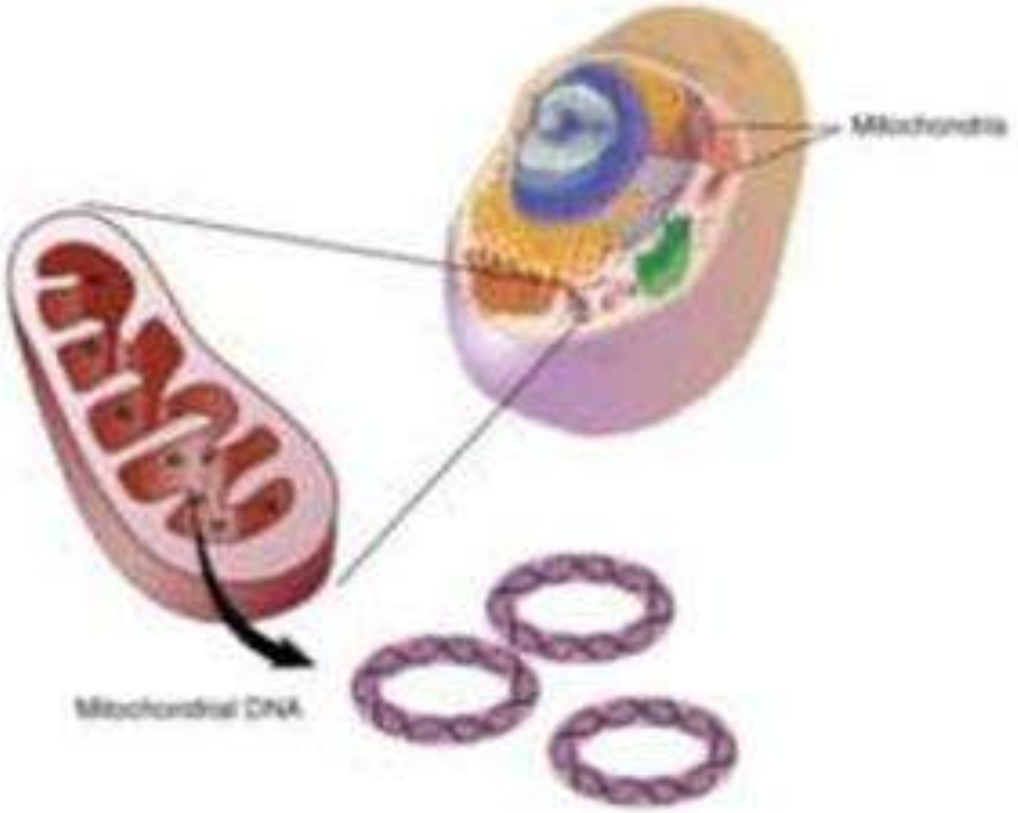
Biosynthesis of
- amino acids
- nucleotides
- steroid hormones
- heme

ATP synthesis

Oxidation of fatty acids

Apoptotic cell death

# Mitochondrial DNA



# Characteristics of animal mtDNAs

- Circular
- Small in size ~16 kb in man
- 5-10 copies of mtDNA / mitochondrion
- ~1,000 mitochondria / cell
- ~1% of cellular DNA
- Encode:
  - 13 proteins
  - large and small rRNA
  - tRNAs
- NO INTRONS- polycistronic mRNAs

# Mitochondrial genetic code

**vertebrates**

| Codon | Mitochondrial | Universal |
|---|---|---|
| UGA | Tryptophan | Stop |
| AUA | Methionine | Isoleucine |
| AGA | Stop | Arginine |
| AGG | Stop | Arginine |

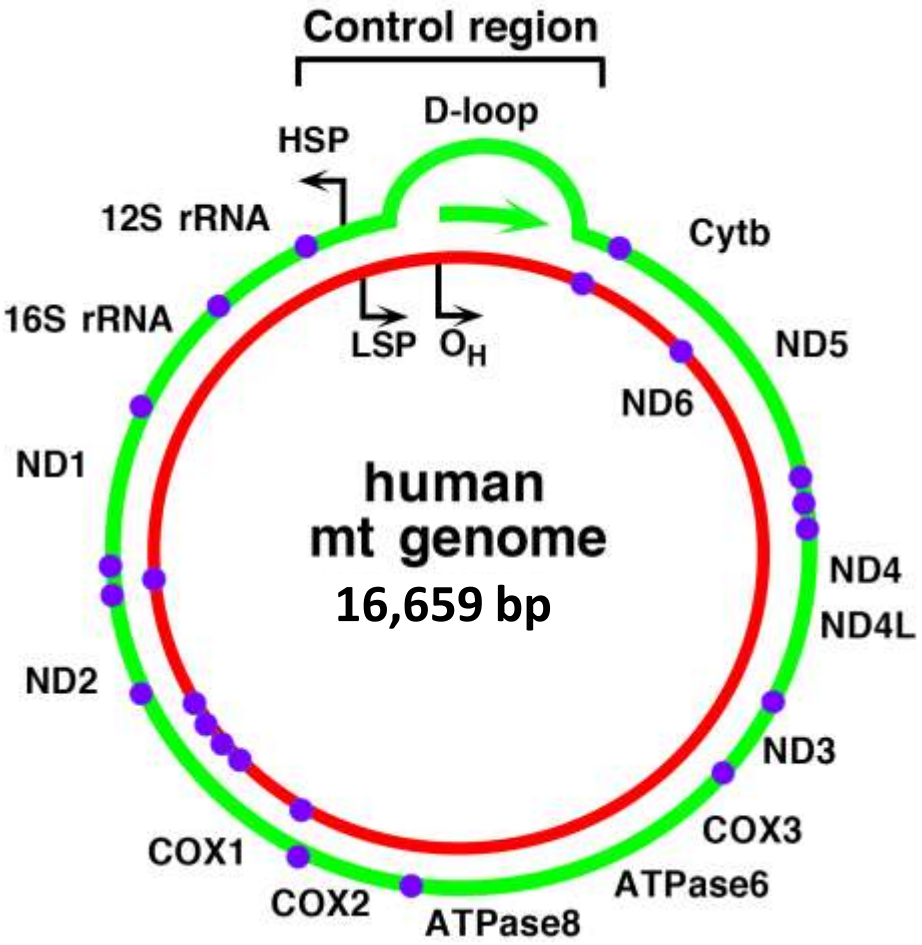
13 protein, two ribosomal RNA and 22 tRNA genes typically found in animal mitochondrial genomes

| Protein encoded | Designation for animal mtDNA | Synonym |
|---|---|---|
| Cytochrome oxidase subunit I, II, III | *COI, COII, COIII* | *cox1, cox2, cox3* |
| Cytochrome*b* apoenzyme | *Cytb* | *cob* |
| NADH dehydrogenase subunits 1-6, 4L | *ND1-6, 4L* | *nad1-6, 4L* |
| ATP synthase subunits 6, 8 | *A6, A8* or *ATP6, ATP8* | *atp6, atp8* |
| Large ribosomal subunit RNA | *lrRNA* | *rnl* |
| Small ribosomal subunit RNA | *srRNA* | *rns* |
| 18 Transfer RNAs each specifying a single amino acid | Corresponding one-letter amino acid code | *trnX* |
| Two transfer RNAs specifying leucine | Differentiated by codon recognized, *L(CUN)* and *L(UUR)* | Differentiated by subscript |
| Two transfer RNAs specifying serine | Differentiated by codon recognized, *S(AGN)* and *S(UCN)* | Differentiated by subscript |

*Nucleic Acids Research*, Volume 27, Issue 8, 1 April 1999, Pages 1767–1780, https://doi.org/10.1093/nar/27.8.1767

# Organization of the mitochondrial genome

**D-loop**
**non-coding region**




- tRNAs

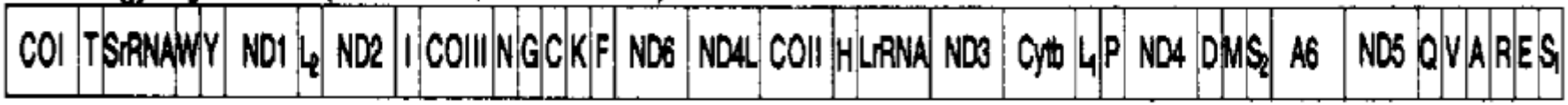
**D-loop: displacement loop**
**HSP and LSP: heavy- and light- strand promoters for transcription**
**$O_H$: origin of replication**

# Mitochondrial gene arrangements in nematodes

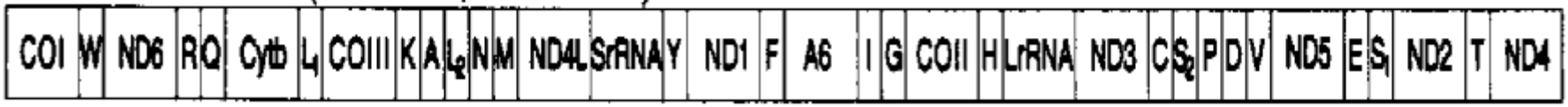
C

*Caenorhabditis elegans* and *Ascaris suum* (Nematoda, Secernentea)


*Meloidogyne javonica* (Nematoda, Secernentea)




*Onchocerca volvulus* (Nematoda, Secernentea)

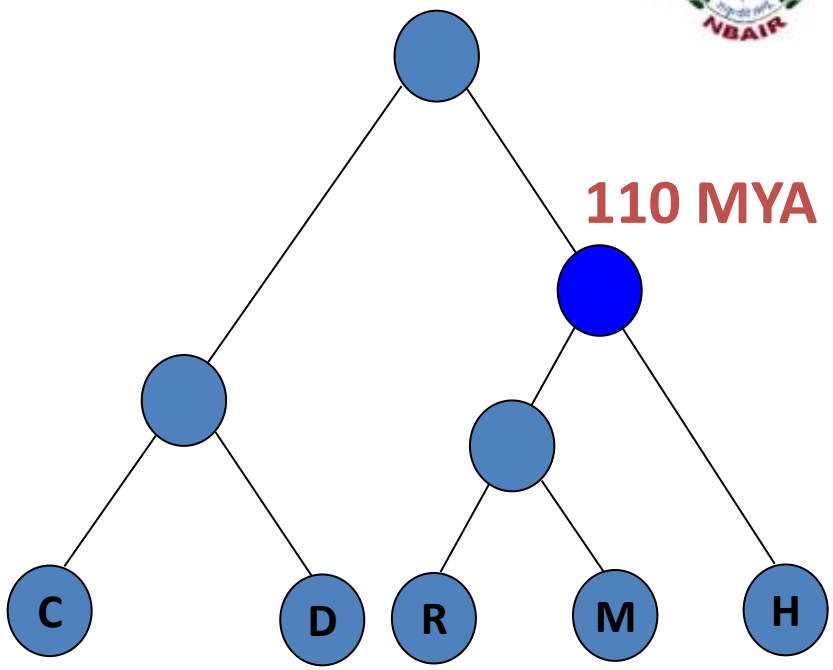



*Nucleic Acids Research*, Volume 27, Issue 8, 1 April 1999, Pages 1767–1780, https://doi.org/10.1093/nar/27.8.1767

# Why mtDNA important

- Owing to their bacterial origin, mitochondria actually have their own DNA
- inherited exclusively from one's mother
- mtDNA can be inherited, variants in this DNA can also be passed down from generation to generation
- it mutates at a rapid pace than nuclear genome "molecular clock"

- Given
  - a phylogenetic tree
  - branch lengths (rt)
  - a time estimate for one (or more) node




- Can we date other nodes in the tree?
- **Yes**... if the rate of molecular change is *constant* across all branches

# Why mtDNA for mol.clock & phylogeny

- DNA mutation rate slow and inheritance pattern, functional and structural genes
- Meiosis & natural assortion
- DNA repair

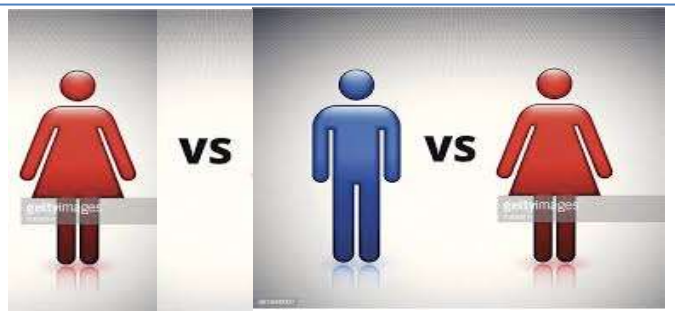
## Recognized as Mol. Operational Tax. Unit - MOTU

- **Mt DNA Inheritable**
- **Inheritance pattern – maternal,** homoplasmic & Heteropasmy
- **Non Mendelian inheritance**
- **Predictability of mutations/generation -10times**
- **Inter and intraspecific variance**
- **Small numbers of sequences**

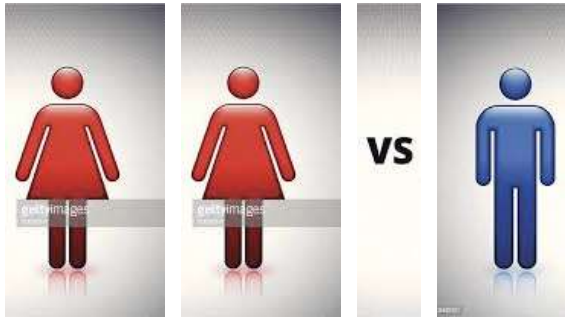
# How many parents are required for a single baby to be born?

Can you imagine a situation like this?


Egg F1 + sperm = embryo Mother

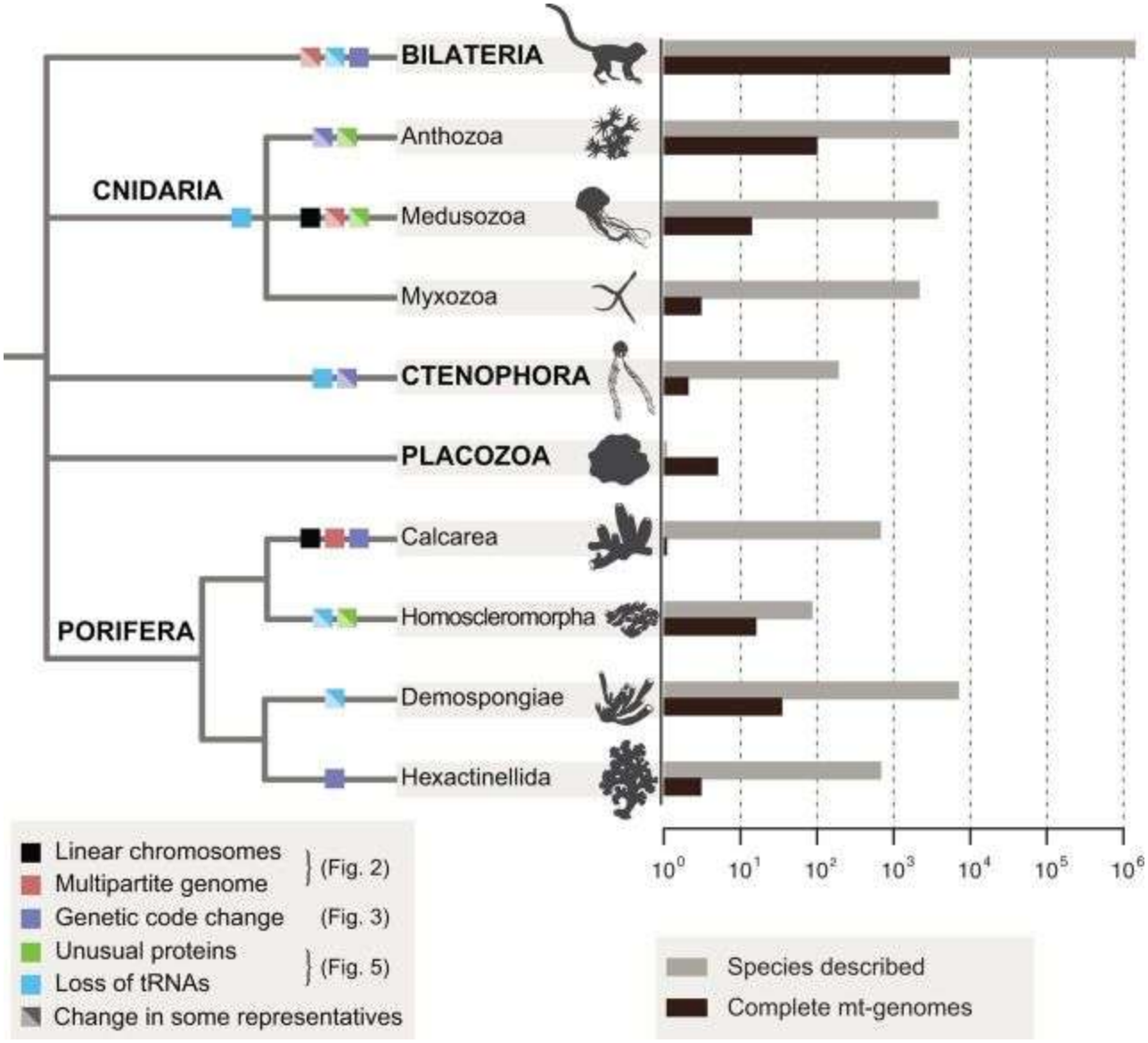



Egg F1 + sperm = embryo to Fem2




N from Egg M + Egg of F2 + sperm = embryo to Fem2

Leigh syndrome - mutations in mitochondrial DNA


BILATERIA
CNIDARIA
Anthozoa
Medusozoa
Myxozoa
CTENOPHORA
PLACOZOA
PORIFERA
Calcarea
Homoscleromorpha
Demospongiae
Hexactinellida
Linear chromosomes
Multipartite genome
(Fig. 2)
Genetic code change
(Fig. 3)
Unusual proteins
Loss of tRNAs
(Fig. 5)
Change in some representatives
$10^0$ $10^1$ $10^2$ $10^3$ $10^4$ $10^5$ $10^6$
Species described
Complete mt-genomes

# Phylogeny & Barcoding markers

- Usually single gene/sequence in mtDNA & nDNA as taxonomic unit or multigene sequence phylogeny – why
- Now mitgenome

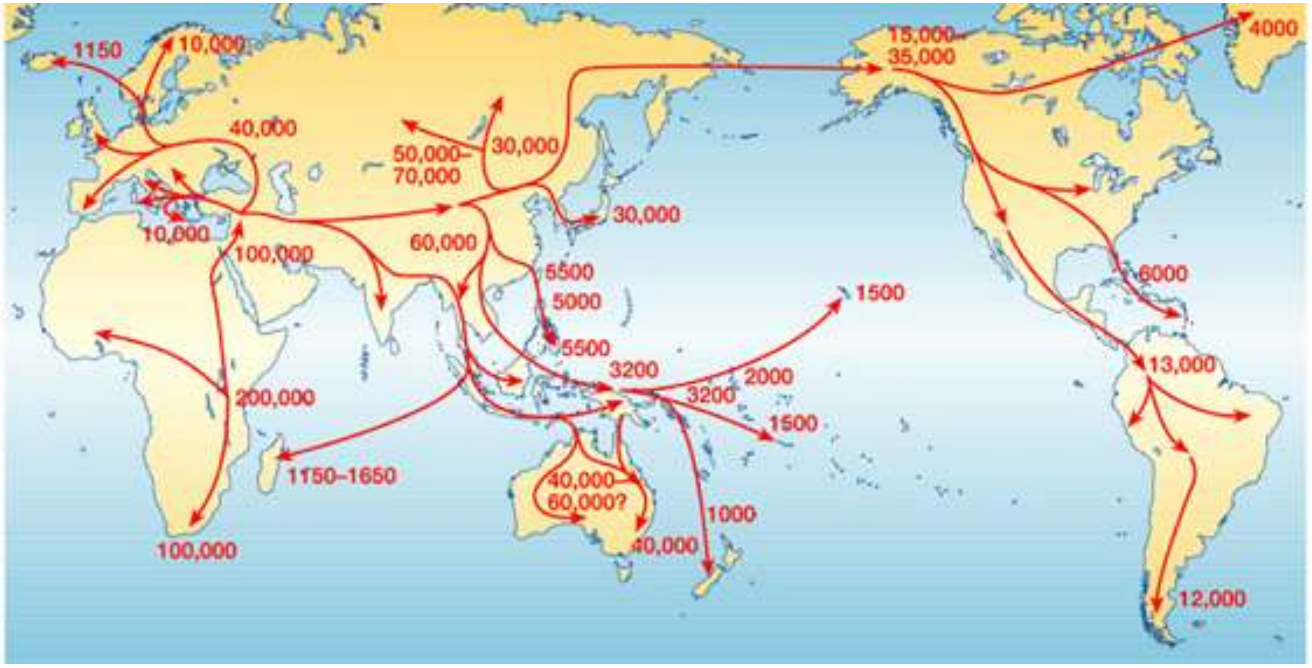
# Human mitochondrial DNA

- Multicopy (466-806 nucleoids /cell)
- 16,569 bp length and 0.68μM diameter
- Genes lack introns
- Maternally inherited
- Sequenced in 1981 (Nature,1981, 290:457-65)
- Mutation rate ~1/33 generations
- Heteroplasmy (original and mutated forms co-exist)
- More stable for forensic analysis

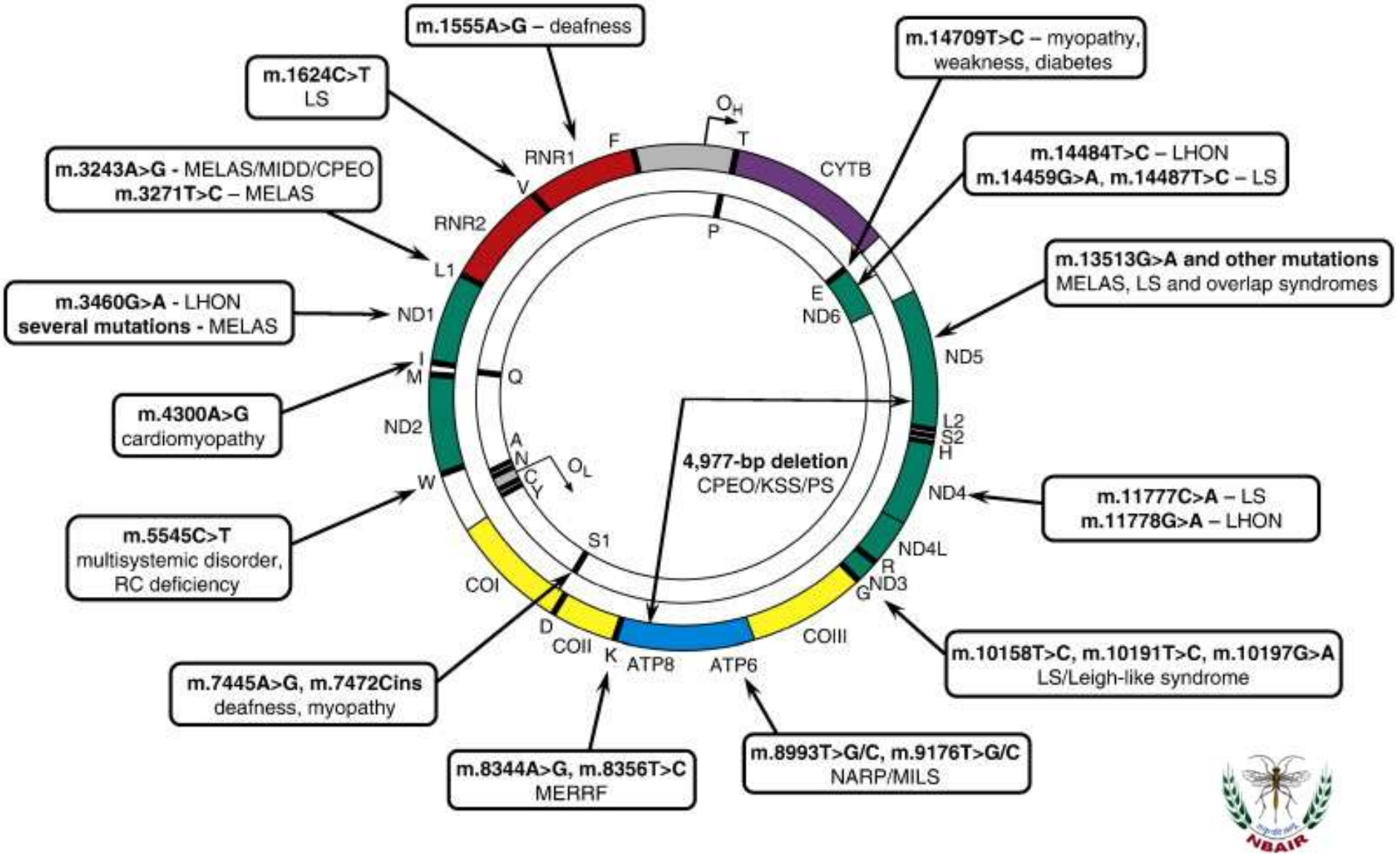
# Mitochondrial 'Eve'

common female ancestor who lived in Africa

- African Origin Model suggests that our species evolved from a small African population that subsequently colonised the whole world,
- Coalescence analysis indicates that all mtDNA in modern humans can be traced back to a single female (~100-150,000 years ago)



# Mitochondrial DNA mutations directly linked to human disease



## Mit Genomics is now central focal point

**Variability**
**Phylogeny**
**Molecular clock**
**Repository**
**Diseases**
**Global biodiversity and genomic resources**

# Thank you

**Acknowledgements:**
**Dr. NAGESH thankfully acknowledges**
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**Staff, Division of Genomic Resources**
**Staff, PME Cell**