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

Introduction to Modern Taxonomic Tools

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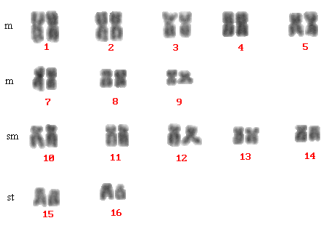
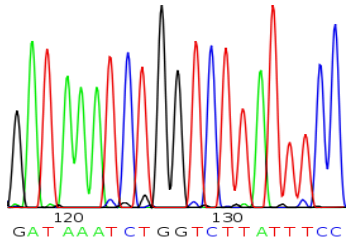
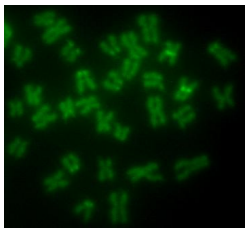






WHY Modern Tools ?

- ✓ **Earth - populated by millions of species.**
- ✓ **Their discrimination is no easy task.**
- ✓ **Taxonomists can critically identify only 0.01% of the estimated 10–15 million species.**
- ✓ **Fast & reliable**

Limitations of Classical Taxonomic Tools or Morphological Identification

1) **Presence of phenotypic plasticity and genetic variability** in the characters used for species recognition can lead to incorrect identifications

2) **Overlooks morphologically cryptic taxa (hidden species)** which are common in many groups including fishes (the lack of phenotypic differences among cryptic species)

3) Morphological keys are often effective only for a particular life stage or gender, many individuals cannot be identified or misidentified

Fig 2: Male of *Xenentodon cancila* with Hump

Fig 3: Female of *Xenentodon cancila* without Hump

4) The use of taxonomic keys often demands such a **high level of expertise**

Modern Taxonomic Tools

1. Karyotaxonomy

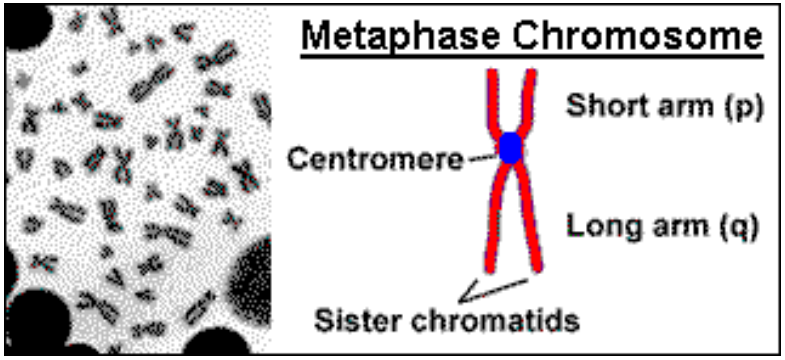
2. DNA Barcoding

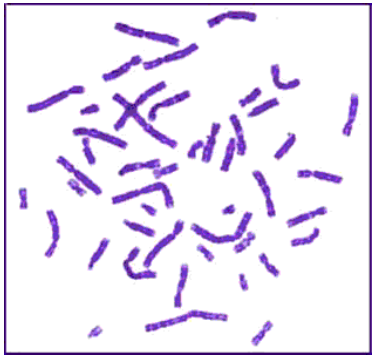
3. DNA Markers

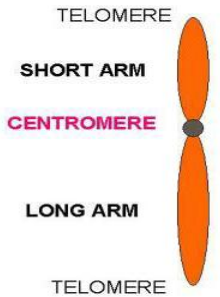
4. Protein Markers

1. Karyotaxonomy:

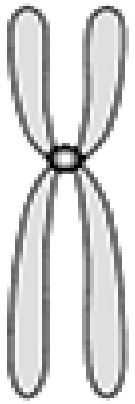
Species Identification by karyotyping







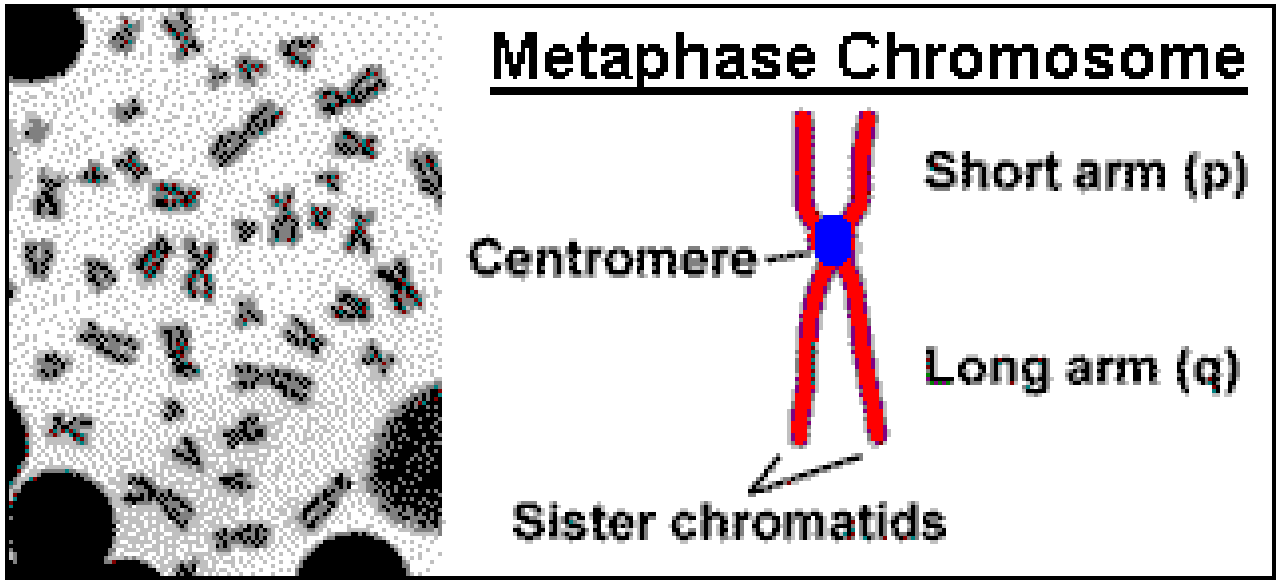
Chromosome

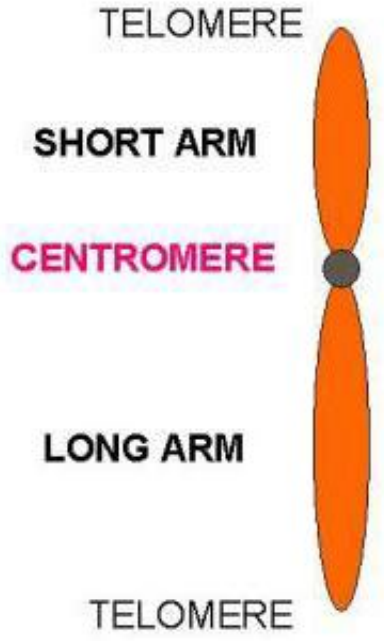


- **It was the Swiss botanist Karl Wilhelm von Nageli in who first described thread-like structures in the nuclei of plant cells in the 1842, and what he called "transitory cytoblasts" are now known as chromosomes.**

- **Later, in 1888, Waldeyer coined the term "chromosome" after staining techniques had been developed to make them more discernible (chromos = Greek for colour; soma = Greek for body)**

Chromosomes: Genetic material (DNA) packaged into thread like structure i.e. DNA tightly coiled many times around Proteins called histone.



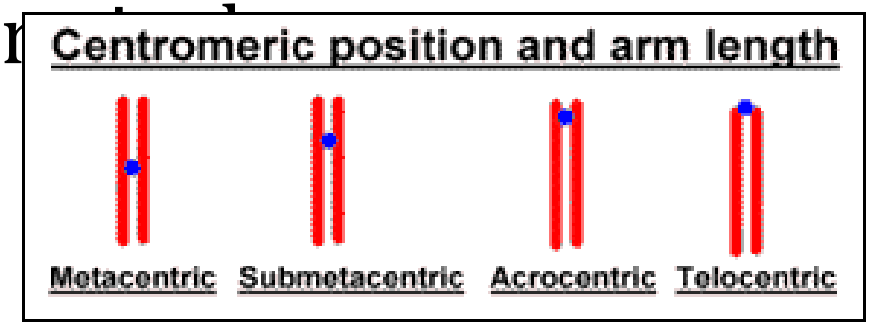


Chromosomes are visible during cell division only

Centromere

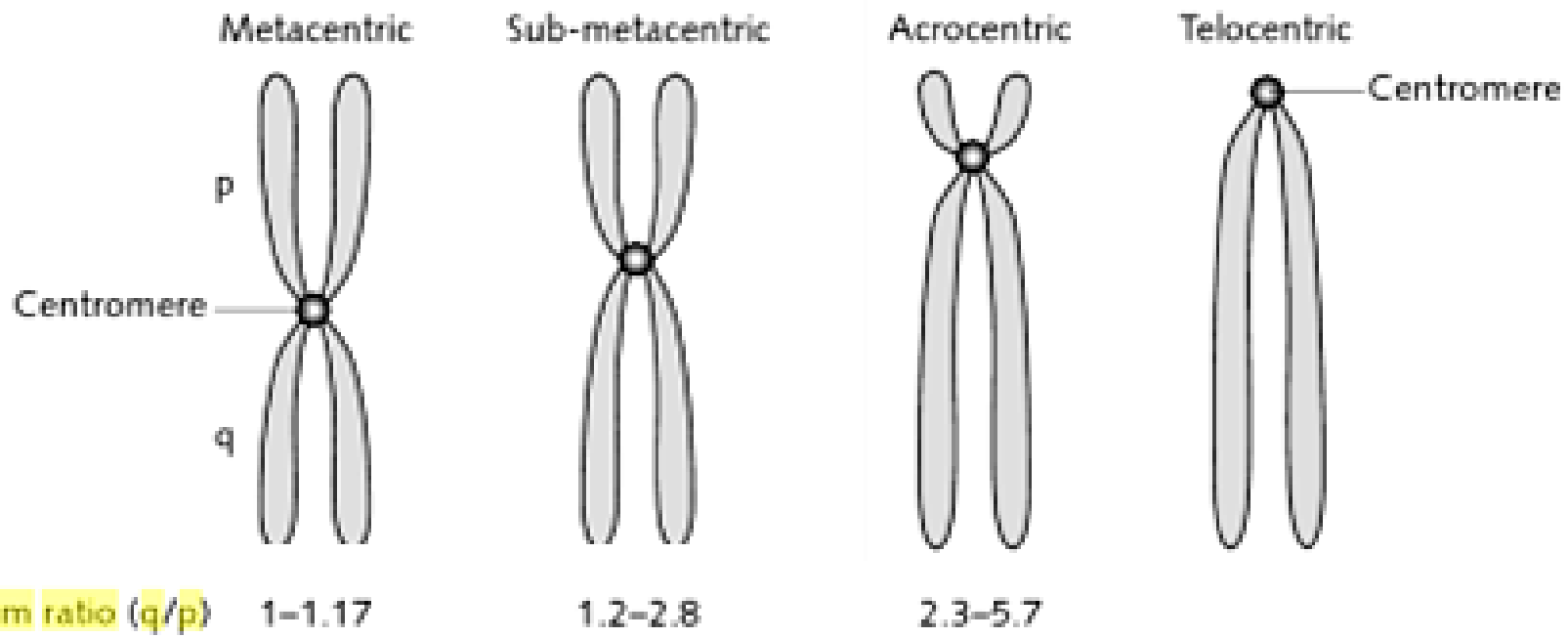
- **Centromere is region of the chromosome that attaches to the spindle fibres during cell division**

- The position of the centromere
- –**Metacentric**: in the middle
- -**Submetacentric:** off the middle
- –**Subtelocentric:** Sub ter
- –**Telocentric:** Terminal



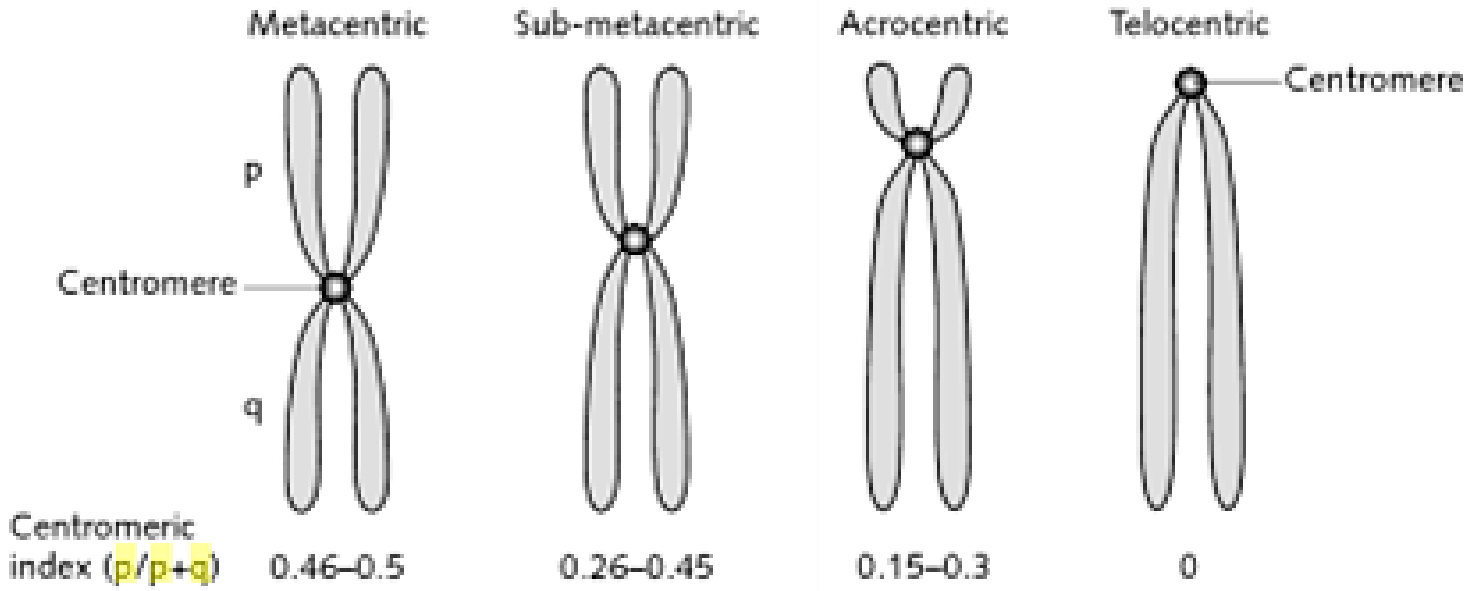
Arm Ratio

Ratio between q (long) arm and p (Short) arm (q/p)



Centromeric Index

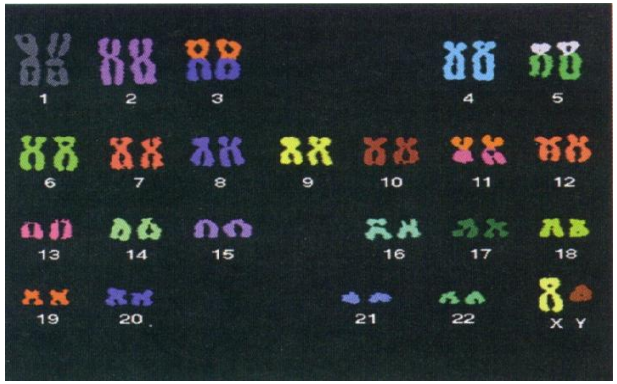
The ratio of the length of the short arm of the chromosome to that of the total chromosome; ordinarily expressed as a percentage. **CI = (short arm length ÷ [short arm length + long arm length]) × 100**



Karyotype

- **Refers to the chromosome complement of a cell or a whole organism**

- **In particular , it shows the number, size, and shape of the chromosomes as seen during metaphase of mitosis**



Karyotyping

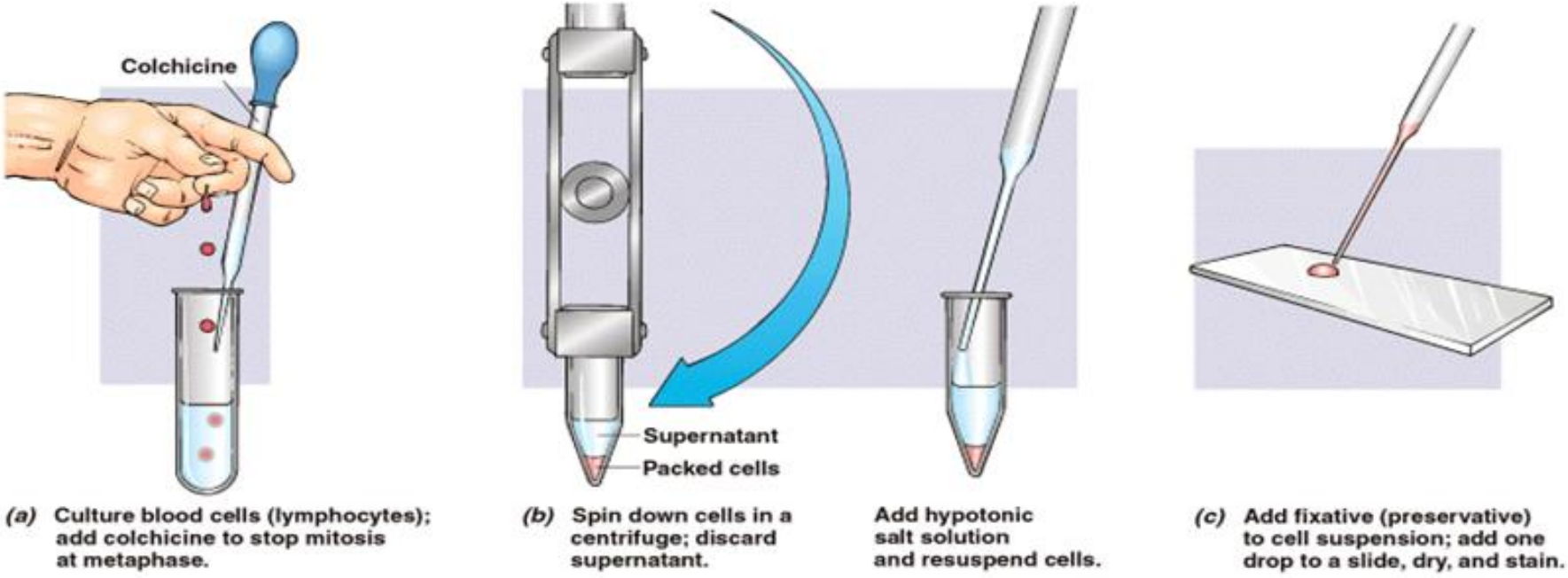
- **A Karyotype is an organized profile of an individiual chromosome.**
- **A pictorial display of metaphase chromosomes from a mitotic cell**
- **Karyotyping is the process of finding the chromosomal characteristics of a cell or organism or individual**
- **In karyotyping, chromosomes are stained to show structure that is used to arrange the chromosome in their pairs (Homologous chromosomes- pairs)**
- **In karyotype chromsomes are numbered and arranged according to the position of centromere from largest to smallest.**
- **If sex chromosome present, it will be placed last.**

Preparing a Karyotype

Metaphase cells are required to prepare a standard karyotype, and virtually any population of dividing cells could be used.

- **Blood cells**
- **Kidney cells (anterior kidney)**

How to prepare metaphase spread



(a) Culture blood cells (lymphocytes); add colchicine to stop mitosis at metaphase.

(b) Spin down cells in a centrifuge; discard supernatant.

Add hypotonic salt solution and resuspend cells.

(c) Add fixative (preservative) to cell suspension; add one drop to a slide, dry, and stain.

Mitotic inhibitor: Colchicine

Hypotonic solution: 0.56% KCl

Fixative: Cornay's fixative (methanol: acetic acid) 3:1

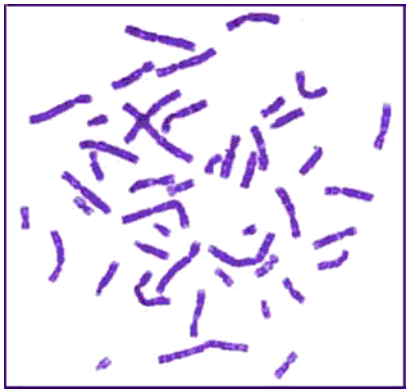
Stain: 6% Giemsa

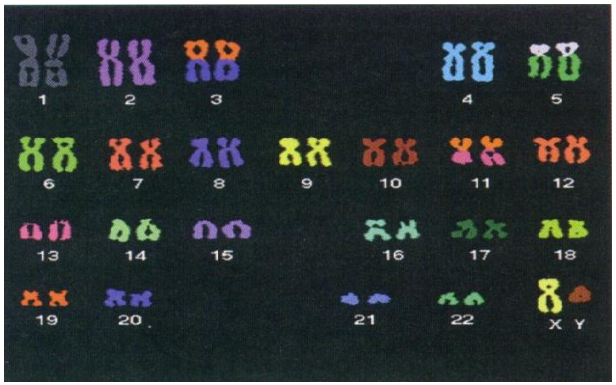
How to prepare karyotype

- **Karyotypes are prepared from the of cells that exhibit complete somatic chromosome number and characteristic chromosome morphology.**

- **Homologous chromosomes are placed together and arrange them on the basis of morphology and decreasing order of size.**

- **Arm ratio (q/p) is used to classify the chromosomes as metacentric (m), submetacentric (sm), acrocentric (st), and telocentric (t).**





Karyotype formula

metacentric Chromosome (m)+submetacentric chromosome (sm) + actrocentric or subtelocentric (st) + telocentric chromosome (t)

Karyotype formula=m+sm+st+t

Fundamental Arm number (FN)

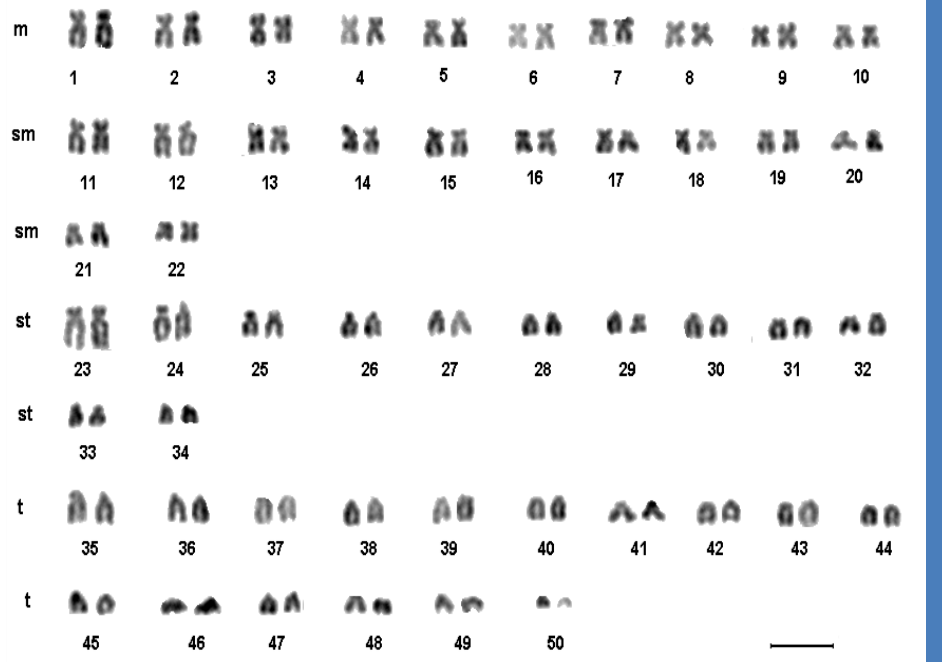
FN=2xM+2xSM+ST+T

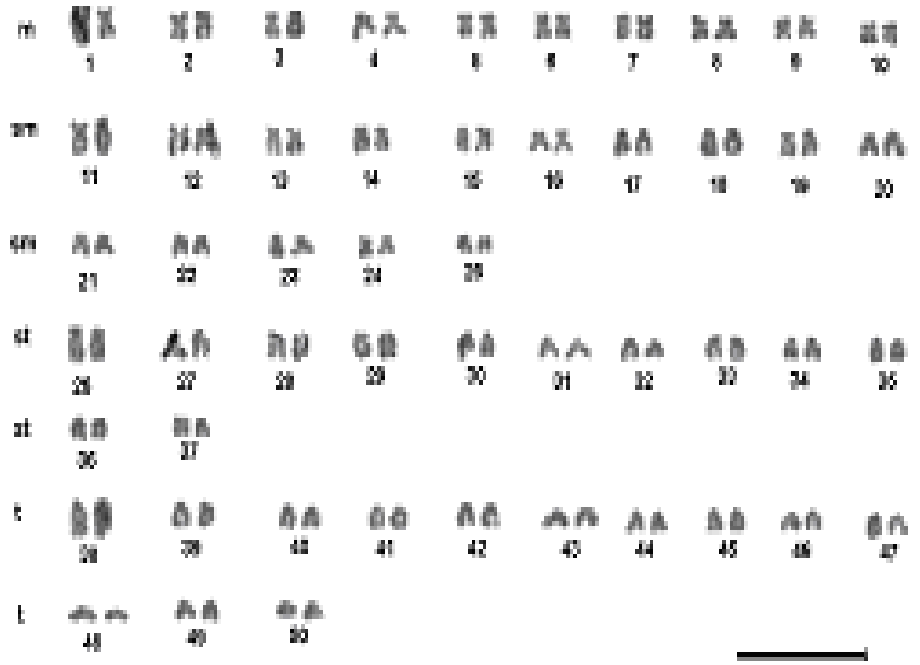
Karyotaxonomy: Species Identification

- ***Channa punctatus***: 2n=32
- ***Channa marulius***: 2n=44
- ***Channa striatus***: 2n=42

- ***Tor tor***: 2n=100
- ***Tor putitora***: 2n=100
- ***Tor chelynoides***: 2n=100

Karyotyping & Conventional banding in *Tor tor & Tor chelynoides (Mahseer)*





Tor tor

Karyotype Formula

20m+24sm+24st+32t (FN=144)

Tor chelynoides

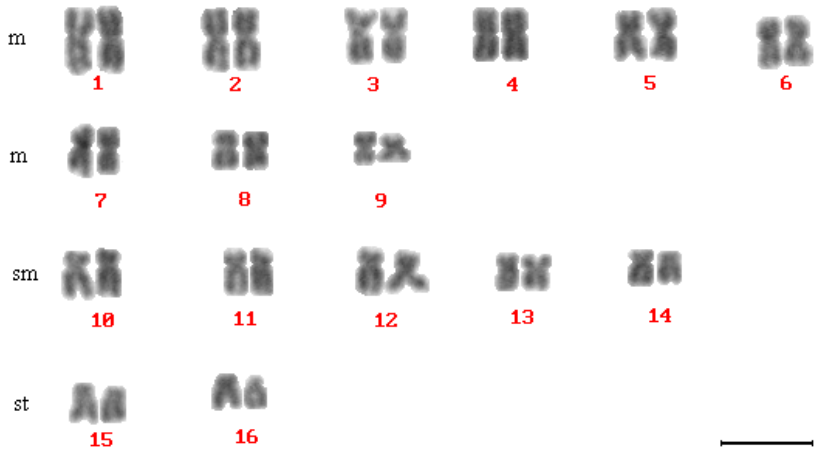
Karyotype Formula

20m+30sm+24st+26t (FN=150)

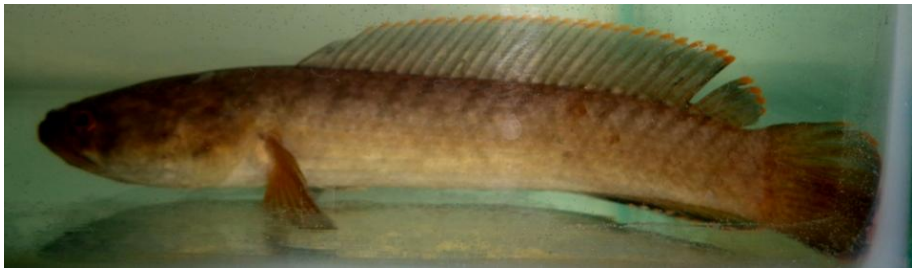
Next level of Karyotaxonomy

- **CMA3 (Chromomycin A3) staining: Constitutive heterochromatin**
- **AgNOR Staining: Silver staining of Nucleolar organizer region**
- **FISH: Fluorescence *in situ* hybridization (Nucleic acid hybridization)**

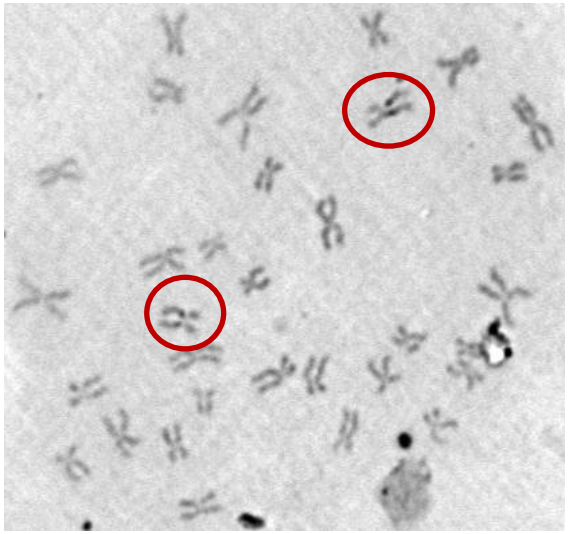
Cytogenetic characterization of snakehead *Channa punctata*



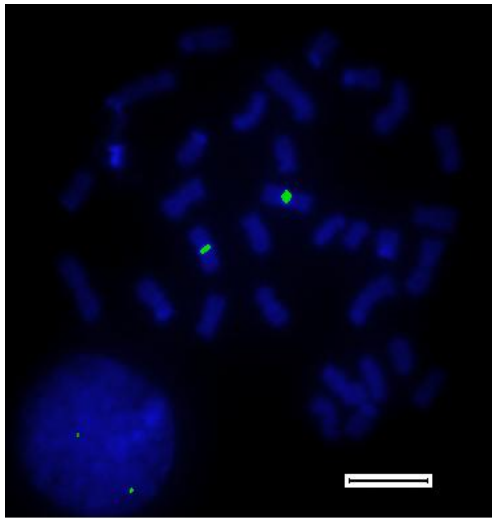
Karyotype: Giemsa staining



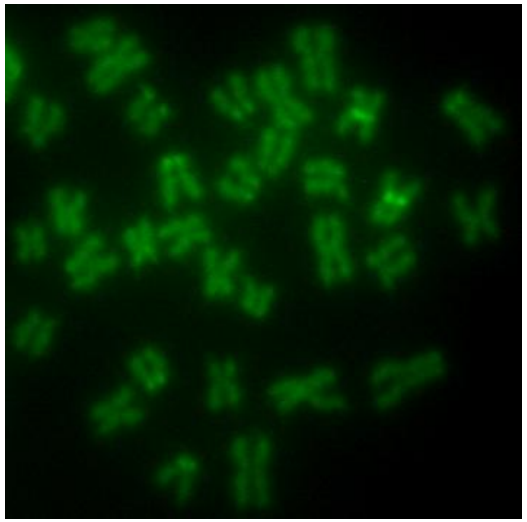
Channa punctata



NOR staining

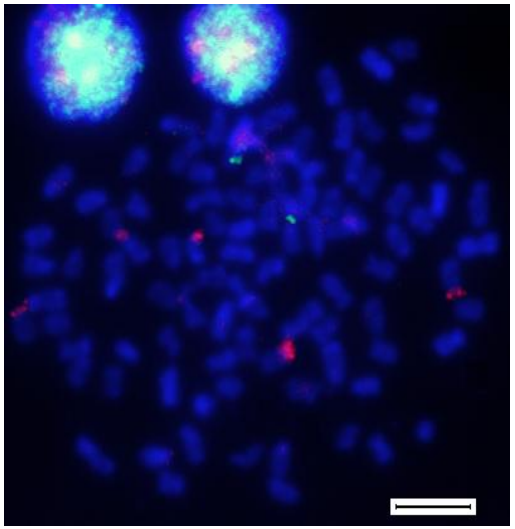


FISH (45S signal)

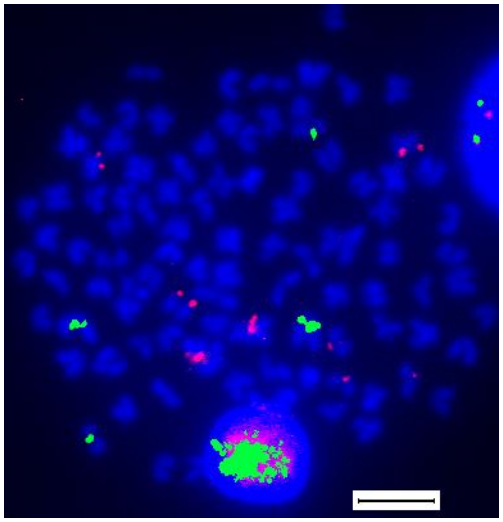


CMA_3 staining

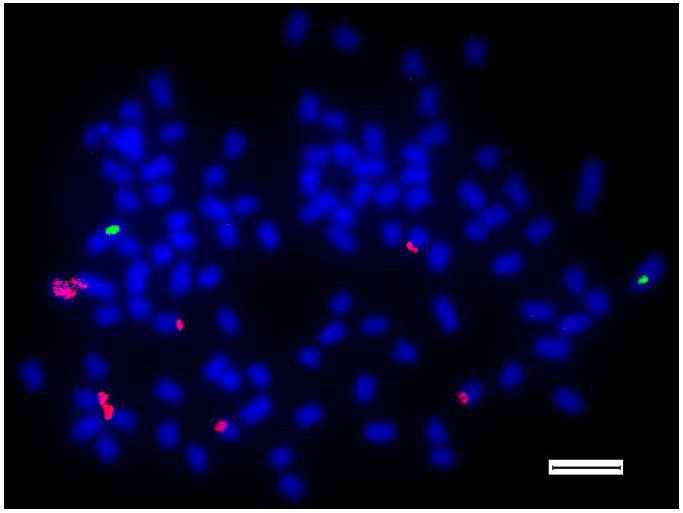
Karyotaxonomy for Stock/Population Identification: FISH in *Tor putitora*



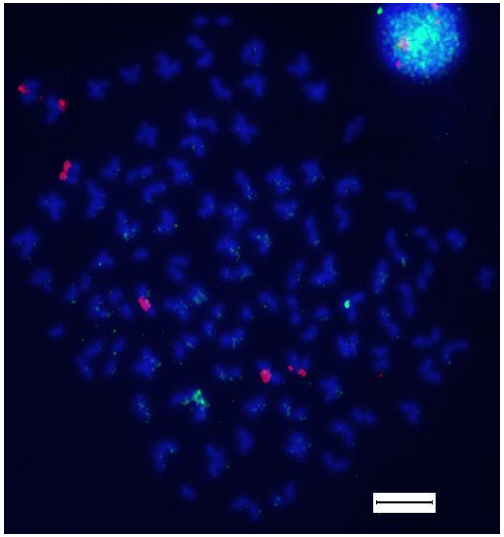
Satluj River



Kosi River



Alaknanda River



Lonavala Reservoir

All the populations showed Six 18S & two 5S signals except Kosi population

Kosi river population showed eight 18S & four 5S signals