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Modern Taxonomic Tools in Fish Identification

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SUMMARY

Morphological characters alone cannot be considered for the systematic classification of fishes. Millions of species on earth and only using the classical taxonomy cannot discriminate all the species. Taxonomists can identify around 0.01% of the total estimated 10 – 15 million species. Traditional/ conventional methods are although important but molecular evidence could be final or confirmatory evidence. Molecular Taxonomy considers the distribution and composition of chemical substances for the identification of any organisms. The genetic relationship can be established between the different taxa using molecular techniques. The study of an evolutionary relationship called phylogenetic is possible with the tools like DNA barcoding and DNA polymorphism. The result data are used to construct phylogenetic trees. The various modern tools such as karyo-taxonomy, chemotaxonomy, DNA barcoding and DNA polymorphism helps us by providing additional characters to the morphological character-based identification of fishes.

INTRODUCTION

Molecular taxonomy or Neosystematics is the classification of organisms based on the chemical substances' distribution and composition. Molecular techniques in biology have helped establish genetic relationships between the members of different taxonomic categories. Molecular phylogenetic - The study of evolutionary relationships among biological entities (individuals, populations, species, or higher taxa) by using a combination of molecular data (such as DNA and protein sequences, presence or absence of transposable elements, and gene-order data) and statistical techniques.

Demerits of Traditional Taxonomy

- **Phenotypic plasticity** (Ability of one genotype to produce more than one phenotype when exposed to different environments) in the characters employed for species recognition lead to incorrect identifications.
- Morphologically **cryptic species** (A biological process that results in a group of species that contain morphologically identical individuals but belong to different species) is difficult to differentiate.
- Juveniles, Young ones and the processed fishes are difficult to identify
- Traditional taxonomy requires high levels of expertise.
- Changes due to the evolution, ecology and environment and reproductively isolated population are difficult to identify with conventional taxonomy.

Methods:

- Karyo-taxonomy (Cytological)
- Protein-based analysis (Biochemical or Chemotaxonomy)
- DNA Barcoding
- DNA polymorphism

Karyo-Taxonomy (Cytological)

The branch of taxonomy that deals with the nucleus structure in various groups of organisms are called karyotaxonomy. This classification system helps in the identification of the phylogenetic relationship between organisms using the number, type and arrangements of chromosomes

The karyotype is characterized by the different morphological characters of chromosomes; diploid chromosome number (2n) and chromosome arms (NF). Karyotypes are prepared using standardized staining procedures that reveal characteristic structural features for each chromosome. The process of chromosome pairing and order of an organism, thus providing a genome-wide snapshot of an individual's chromosomes, is called karyotyping.

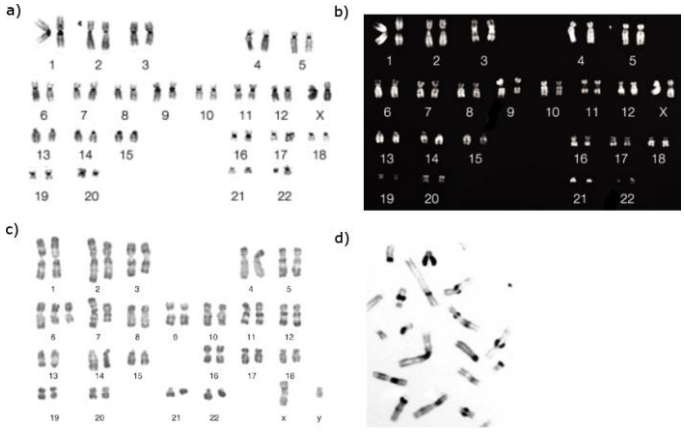

Figure 1. Karyotyping of chromosomes

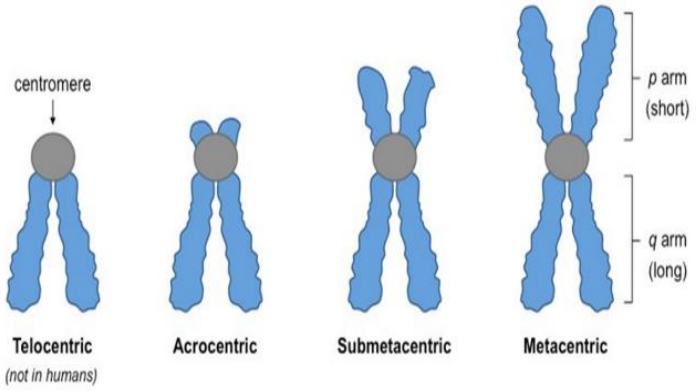

Figure2. Types of chromosomes

Structure of Chromosomes

According to the centromere position, a karyotype is characterized by the different morphological types of chromosomes, i.e. metacentric, submetacentric, subtelocentric or telocentric (acrocentric). Measurement of chromosomes that give an accurate location of the centromere position has contributed to an important gain of knowledge. These help in interspecific comparison of karyotypes and the identification of chromosomal rearrangement, an important or significant factor in evolution. The centromeric index, the arm ratio and the relative length of the chromosomes can be calculated using the arm length and position of the centromere.

Staining of Chromosomes:

Staining is responsible for the alternating dark and light bands on the chromosomes.

Objectives:

- For obtaining and understanding chromosome structures
- Understanding behaviour of chromosome
- Improving the reliability of banding

Chromosome staining- 4-5% Giemsa solution for the visualization of chromosomes. The karyotypic analysis in fishes is inherently complex due to a large number of small-sized chromosomes.

Chromosome banding methods

Variations in the longitudinal structure of the chromatids allow the banding in the chromosome, revealed by various techniques. Different banding techniques such as Q-band, C-band, Serial bands (G- and R- bands) and Fluorescent in situ hybridization (FISH) are available. Among these, C-banding is commonly used for fish species identification purposes.

Biochemical Taxonomy (Protein based analysis)

It is the method of biological classification based on similarities in the structures of certain compounds among the organisms being classified: proteins, amino acids and peptides are commonly used compounds for the biochemical taxonomy. Chemotaxonomy attempts to classify and identify organisms (originally plants) according to demonstrable differences and similarities in their biochemical compositions. Proteins are more reliable indicators of genetic relationships. As more closely controlled by the genes and less directly subject to natural selection than are anatomical features, Proteins are more conservative (i.e., more slowly evolving)

Principle

Varying levels of different proteins in different species may help differentiate species. Separating the proteins based on the charge and molecular mass is possible because proteins are made up of amino acids (electrically charged).

- Basic amino acids- arginine, histidine and lysine (positively charged)
- Acidic amino acids- aspartic acid and glutamic acid (negatively charged).
- "Isoelectric point" (pI) - the definite pH at which the net charge of the protein molecule is zero.
- The soluble proteins of the sarcoplasm are referred to as sarcoplasmic proteins. Among them, some albumins and so-called myogenic are the real water-soluble proteins. The genetic differences between the species are effectively studied using this group of proteins. On the other hand, salt soluble proteins such as myofibrils of the muscle fibre can also be used for chemotaxonomy.

Techniques

1. Electrophoresis
2. SDS-Polyacrylamide Gel Electrophoresis (PAGE)
3. Iso-Electric Focusing (IEF)

2-Dimensional Electrophoresis

Electrophoresis

The term electrophoresis comes from Greek and means "transport by electricity". Electrophoresis is the most common and important protein-based method.

PRINCIPLE: The basis of electrophoretic separation is that proteins of different net charges and different molecular sizes will migrate at different rates within an electric field. It is a very useful technique for separating cellular proteins and DNA.

SDS-Polyacrylamide Gel Electrophoresis (PAGE)

Seperation of proteins based on molecular mass. When protein molecules are dissolved in solutions of an anionic detergent (SDS), their charges are lost and a negative charge is formed due to SDS complexing with the protein. The uptake of detergent is the same per unit mass for all proteins, and in consequence, the mobility on electrophoresis is proportional to molecular mass. Separation based on the molecular mass of proteins thus provides an alternative method of analysing flesh proteins and fish species identification.

Iso-Electric Focusing (IEF)

In contrast to SDS electrophoresis, IEF is a method that separates molecules based on charge. Isoelectric focusing uses a polyacrylamide gel with a large pore containing polycarboxylic polyamine acids with a different isoelectric point. These form a stable pH gradient along with the gel in an electric field. Strong acid applied at the anode and strong base in the cathode contain and stabilize the gradient. Once a steady-state has been achieved, the gel is removed, fixed, stained, and washed for other polyacrylamide gels.

Dimensional Electrophoresis

This technique combines the techniques of IEF and polyacrylamide gel electrophoresis for the two-dimensional separation of proteins. This technique uses two independent properties of proteins, such as isoelectric focusing and molecular weight. The proteins are first separated by isoelectric focusing (this is the first dimension), separating proteins according to their charge (isoelectric point). Then, proteins are separated by SDS-PAG electrophoresis (the second dimension), separation based on Series of spots distributed throughout the polyacrylamide gel will be used to compare the species.

Sources and Extraction of Proteins

Protein solution can be prepared using body fluids such as plasma, serum and tissue proteins; Protein extract can be prepared from muscle, eyes and liver in distilled water and specific extraction buffer (10% sucrose solution). For larvae or small animals, it is recommended to use the whole animal and for Crustaceans, walking leg can be used without sacrificing the animal. After removing the animal, proteins begin to denature rapidly, so the tissue must be used immediately or stored deep-frozen. But at –18^{o}C, denaturation is relatively rapid and will produce an altered electrophoretic pattern.

DNA Barcoding

DNA barcoding is a method of species identification using a short section of DNA from a specific gene or genes. DNA barcoding is a large scale DNA sequence generation of a uniform DNA fragment for all the species. It is an emerging standard for identifying species and study global biodiversity using gene sequences. DNA analysis intended to identify a species rather than an individual is called DNA barcoding. Mitochondrial gene cytochrome c oxidase subunit I (COI) is the most suitable candidate gene for barcoding and it is being used as a global bio identification system for animals, including fish. Accurate and unambiguous identification of fish and fish products ranging from egg to adults is an indispensable effort to strengthen fish germplasm conservation and manage sustainable fisheries. DNA barcoding has made it possible to cut the time to identify a species from days to minutes. Useful for species identification, divergence analysis, provide support to taxonomist and molecular genetic analysis. DNA barcoding began in 2003 with the proposal that the organisms could be assigned to their correct species using a short gene sequence from a standardized position in the genome. In 2005, Consortium for the Barcode of Life (CBOL, www.barcoding.si.edu) launched the Fish Barcode of Life campaign (FISH-BOL; www.fishbol.org) to create a global reference library of all 30,000+ species of cartilaginous and bony fishes from marine, estuarine and freshwater ecosystems, one that is derived from voucher specimens with authoritative taxonomic identifications. The benefits of barcoding fishes include facilitating species identification for all potential users, including taxonomists.

Methodology

- Tissue sampling
- DNA extraction
- PCR amplification
- Sequencing reaction
- Sequencing
- Conversion of sequence to Barcode

Application

- Identification of fish, fillets, fins, fish eggs and fish larvae
- Identification of threatened, endangered & protected species
- Identification of prey items in stomach contents (food webs, ecosystem research).
- Identification of new/ cryptic species
- Disease vectors
- Agricultural pests
- Environmental indicators
- Drinking-Water quality control
- Biome Barcoding

Metabarcoding

The barcoding of DNA/RNA (or eDNA/eRNA) in a manner that allows for the simultaneous identification of many taxa within the same sample. The main difference between barcoding and metabarcoding is that metabarcoding does not focus on one specific organism but instead aims to determine species composition within a sample.

DNA Polymorphism- (Primer based methods)

DNA polymorphisms are the different DNA sequences among individuals, groups, or populations. Polymorphism at the DNA level includes a wide range of variations from single base pair change, many base pairs, and repeated sequences. (Place in the DNA sequence where there is a variation)

Types

Genomic variability can be present in many forms, including single nucleotide polymorphisms (SNPs), variable number of tandem repeats (VNTRs, e.g., mini- and microsatellites), transposable elements (e.g., Alu repeats), structural alterations, and copy number variations

Techniques

Different forms of DNA polymorphisms can be tracked using a variety of techniques;

Restriction Fragment Length Polymorphisms (RFLPs)

In RFLP analysis, a DNA sample is digested into fragments by one or more restriction enzymes, and the resulting restriction fragments are then separated by gel electrophoresis according to their size

Random amplification of polymorphic DNA (RAPD)

Type of polymerase chain reaction (PCR), but the segments of DNA that are amplified are random. RAPD creates several arbitrary, short primers (8–12 nucleotides), then proceeds with the PCR using a large template of genomic DNA, hoping that fragments will amplify

Amplified Fragment Length Polymorphism (AFLP)

PCR-based tool. AFLP uses restriction enzymes to digest genomic DNA, followed by ligation of adaptors to the sticky ends of the restriction fragments. A subset of the restriction fragments is then selected to be amplified. This selection is achieved by using primers complementary to the adaptor sequence, the restriction site sequence and a few nucleotides inside the restriction site fragments. The amplified fragments are separated and visualized on denaturing on agarose gel electrophoresis.

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