

TOWARDS RELIANCE ON MOLECULAR TAXONOMY FOR MARINE MAMMAL CONSERVATION

Interest in wildlife in general, and marine mammals in particular, has increased significantly in recent years, both among the general public and the scientific among communities. There has been a marked rise in the number of wildlife enthusiasts taking to educational and adventure expeditions to see marine mammals in their natural habitats. Simultaneously, there is also increasing awareness of the integral importance of marine mammals in healthy aquatic ecosystems, and of the growing threats that a variety of anthropogenic activities, such as destruction of habitats, fishery interactions (e.g. gill net fishery), illegal fishing methods and pollution challenge these animals and their environments. Research and education programmes should try to understand and highlight these threats and recommend appropriate steps to reduce or eliminate their impacts.

Accurate taxonomy is fundamental to all these activities, especially the conservation efforts on marine mammals; the units on which conservation is based are determined partly by population structure and ultimately by species designation. Imperfect taxonomy may result, at least as much as a lack of understanding of the population structure, in the loss of genetic variability, e.g. unwitting extinction of a species.

One major limitation in marine mammal taxonomy is the inadequate sample sizes. A series of adult animals are required for the documentation of geographic morphological variation and such series may take decades to accumulate in museums and research institutions, unless large-scale fishery mortality accelerates the process. The number of extant species of cetaceans (the largest group of marine mammals) remains debated. For example, about 87 species of whales, dolphins and porpoises are reported globally and 26 from the Indian EEZ and the contiguous seas, though it could be more than these numbers. There are yawning gaps in our present understanding of species status and geographic variation of cetaceans, which means that the list of

currently recognized species of cetaceans will probably undergo serious revisions.

Conventional Taxonomy

Characters such as ratio of the outer margin of the flipper to the total body length, colouration pattern, teeth count, comparative osteology, etc. are used conventionally to identify the cetaceans. It is important to study the available material in various museums and private collections before expanding the already reported number of species to a final inventory. Studying the archive materials, thus eliminating the possible repetition, can bring out unknown morphological details of a species. However, depending solely on conventional approach, such as examination of skeleton is more time consuming and expensive.

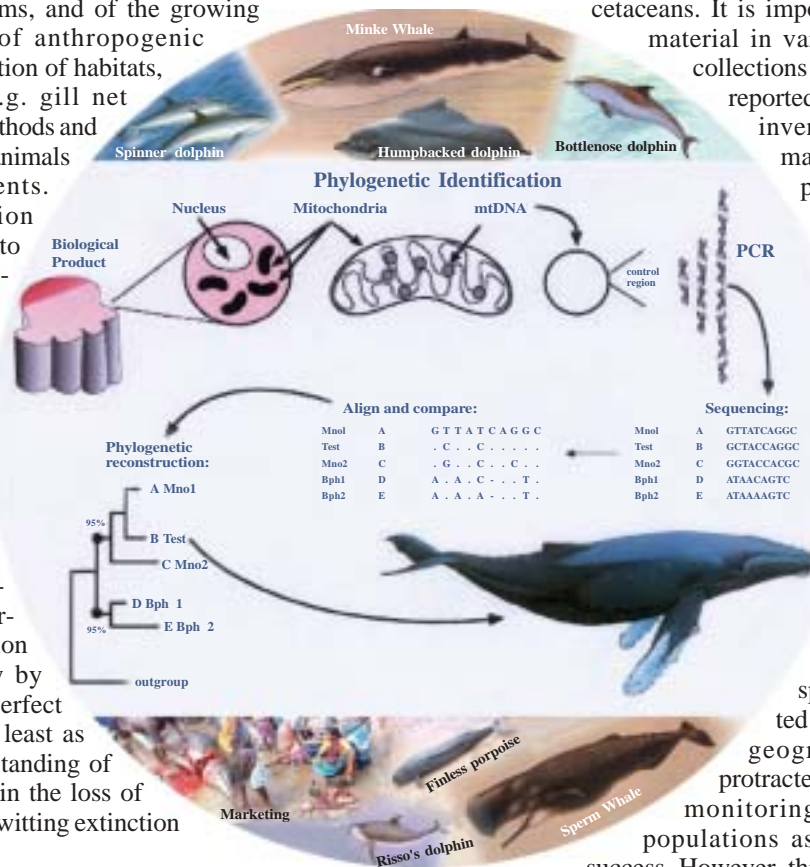
Photoidentification

Photographs of dorsal fins and flukes help in identification of individual cetaceans. This technique is useful for studying the school structure and species composition. A repeated photo-session from the same geographical location for a protracted period of time will help in monitoring resident and migrant populations as well as the reproductive success. However, this approach is cumbersome.

Molecular approach

Molecular taxonomy is not meant to be a critique of morphology-based taxonomy, but must be firmly anchored within the knowledge, concepts, techniques and infrastructure of traditional taxonomy. DNA-based taxonomy is especially relevant for cetaceans, because (i) they are very mobile and inaccessible organisms for which morphological, physiological and behavioural characters can be exceedingly difficult to score for population studies and (ii) their highly derived and specialized morphology reduces the utility of phenotypic data for assessing their phylogenetic position within mammals.

(Contd...)



The rapid advances in molecular techniques of the past few decades have led to significant contributions towards improving cetacean taxonomy. At higher taxonomic levels, the increasing case of generating useful molecular genetic data, notably DNA sequences, paralleled by theoretical advances and the development of computer programs, has stimulated reinvestigation of phylogenetic issues involving cetaceans. In some cases, results of these investigations have led to revisions of taxonomic relationships. Further, they can provide significant contributions to understand inter and intra-specific variations.

Specimens of marine mammals from which tissue samples are drawn are (1) beach-cast (2) accidentally caught in fishingnets and (3) live animals in the sea. For extraction of DNA, skin biopsy can be carried out from the first two sources, while biopsy dart is used to collect sloughed skin from wild ones. About 3 g of skin sample is collected from each individual, stored in 95% ethanol or frozen. The DNA is extracted from the tissue and appropriate regions (control region or cytochrome b gene) of mitochondrial DNA is amplified in PCR.

The nucleotide sequence of the amplicon (PCR product) is determined using automated sequencer. Thus obtained sequence, now referred to as “test”, is aligned and compared with the sequences from reference samples. Finally, the sample sequence is grouped, by phylogenetic reconstruction, with the most closely related reference sequences. The reconstruction is usually presented as a “tree”, with closely related sequences forming neighboring branches. This allows a hierarchical comparison to establish, first, the suborder and family derivation using a small number of reference sequences from a large number of species. A close relation, or match with a reference sequence provides evidence for identification of the species origin of the product. One or more “out groups” (i.e., distantly related species) are used to protect against misclassification error. Re-sampling (bootstrap)

procedures are used to indicate the relative degree of reliability or consistency of groupings among reference and test sequences.

The website www.dna-surveillance.auckland.ac.nz hosts a suite of analytical programs in a user-friendly interface that steps the user through the species identification procedure. The results can be cross checked with BLAST search from <http://www.ncbi.nlm.nih.gov/>.

Identification of cetacean products in the marketplace

For species protected by international regulations or threatened by overexploitation, molecular genetics provides a powerful tool for conservation – the forensic identification of commercial products and verification of trade records. Mitochondrial DNA regions amplified from the tissues using PCR and compared to known or ‘type’ samples can ensure the products sold in commercial markets belong to the species hunted legally under international agreements. This is particularly important since the products of protected or endangered species are often processed in ways that make visual identification difficult.

CMFRI initiatives in marine mammals molecular taxonomy

A project sponsored by the Department of Ocean Development (DOD) and entitled “Studies on marine mammals of Indian EEZ and the contiguous seas” has one of its objectives to inventorize the cetacean species from the region. Mitochondrial DNA (cytochrome b gene) sequences have already been generated from bottlenose dolphin (*Tursiops* sp), spinner dolphin (*Stenella longirostris*), Risso’s dolphin (*Grampus griseus*), common dolphin (*Delphinus* sp) and sperm whale (*Physeter macrocephalus*), aligned and phylogenetic tree constructed. Future work in this line would involve DNA-based identification of more species and individuals of cetaceans from Indian waters.

(Article contributed by Dr. P. Jayasankar, Senior Scientist, PNP Division)