Lead article

# Taxonomic identification of marine mammals – current research and approaches

P. Jayasankar*
ICAR-Central Marine Fisheries Research Institute, Kochi-682 018, Kerala

*E-mail: p.jayasankar@icar.gov.in

## Introduction

The term 'marine mammal' includes members of 5 different mammalian groups: cetaceans (whales, dolphins, and porpoises), sirenians (manatees and the dugong), pinnipeds (sea lions, the walrus, and seals), marine and sea otters, and the polar bear. They are warm-blooded animals which have undergone major adaptations that permit them to live in water. These involve the loss of hind limbs (cetaceans and sirenians), the adaptation of limbs for propulsion through water (pinnipeds), and the general streamlining of the body for hydrodynamic efficiency (all 3 groups). Structural modifications to the sea otters and the polar bear are less apparent in body form and they continue to closely resemble their terrestrial counterparts. While cetaceans and sirenians spend their entire lives in the water, other marine mammals come ashore for various reasons, at particular times in their life cycle. In recent years, there has been a marked rise in the number of wildlife enthusiasts taking to educational and adventure expeditions to see marine mammals up close in their natural habitats. There is also increasing awareness of the integral importance of marine mammals to 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 which challenge these animals and their environments. Research and education programmes should understand and clearly communicate these threats and recommend appropriate actions needed to reduce or eliminate their impacts.

## Current status of identification of marine mammal species

Accurate taxonomy is fundamental to the conservation efforts of living resources; 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. In cetaceans, morphological features are often subtle and difficult to compare because of the rarity of specimens or widespread distributions. 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. Thus identification of the geographical variants of recognized species of delphinids and phocoenids is difficult using the conventional approaches. Gaps in our present understanding of species status and geographic variation of cetaceans means that the list of currently recognized species of cetaceans will probably undergo serious revisions.

The order Cetacea comprises two extant sub-orders and one extinct sub-order. The extant sub-orders are Mysticeti- filter feeding (baleen whales) and Odontoceti (toothed whales) with at least 70 species, 40 genera, and 10 families. Both Mysticetes and odontocetes are thought to be descendants of Archaeocetes (Archaeoceti, ancient whales, known only from fossil records), an extinct sub-order. The number of extant species of cetaceans remains debated, ranging from 78 to 85.

With the recent consensus that recognizes three rather than one species of Right whale (*Eubalaena* sp), the total number of species comes to 85, and the number of subspecies is reduced to 41. Recently one special issue of a dedicated journal for marine mammal research (*Marine Mammal Science*, 2017, 33) focussed only on species, subspecies and populations of cetaceans. Marine mammals are identified through morphology-based, photo identification and molecular taxonomy approaches by researchers.

*Morphology based approach:* Cetacean specimens "in hand" can be identified by using the dichotomous keys to external features. Conventionally, 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 to identify the cetaceans. Skulls of many species are sufficiently similar that only examination of a full series of each can define reliable diagnostic features. Great variability in morphological characters of cetaceans is not uncommon. Sometimes it may only be possible to label an animals or group as "unidentified long-snouted dolphin" or "unidentified beaked whale", etc.

A study of the available material in various museums and private collections before expanding the already reported number of species to a final inventory can eliminate the possible repetitions and bring out unknown details of a species. The need for more of the world's cetacean collections in museums and other institutions to be catalogued and accessible in digital mode is highlighted. This effort is already underway in many major museums, but the smaller collections remain relatively unknown. To facilitate access and comparisons, catalogues should ultimately be linked, managed and the information standardized through a single centralized location with the following data: collection locality and date, age/sex class, material collected (including soft tissue samples), total length and photographs of external appearance and skull morphology.

*Photo identification:* Photographs of dorsal fins and flukes help in identification of individual cetaceans and this technique, known as photo-identification, 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. Identification of the species at sea is quite different from that of a dead animal on land. Even under ideal conditions, an observer often gets little more than a brief view of a splash, blow, dorsal fin, head, flipper, or back, often from a great distance. Rough weather, glare, fog, or other bad sighting conditions compound the problem. Many species appear similar to another, especially in the brief glimpses typical at sea and a fair amount of experience and expertise to master the technique of identifying free ranging marine mammals at sea is necessary.

Generally, sightings from the survey boats are initially identified as "possible" or "confirmed" or as "unidentified", usually for the animals far away. Photo and video documentation of these sightings help to confirm the identification with the assistance of experts later. Sixty eight per cent of individual cetaceans sighted during one souther ocean cruise could be identified to the species level (Jayasankar *et al*., 2007). Reports on vessel-based surveys to identify cetaceans based on their sightings are available from Maldives, Kerguelen islands, Mauritius, South China Sea, Mauritius to the Philippines, Indian Ocean, Seychelles, Caribbean Sea, Gulf of Mexico and Eastern Antarctica.

*Molecular taxonomy:* This approach must be firmly anchored within the knowledge, concepts, techniques and infrastructure of traditional taxonomy and 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. In general, molecular taxonomy outscores morphological taxonomy in the identification of groups showing little evolutionary differentiation, cryptic members of species complexes, members of closely related species that can only be identified at a particular life stage, inter-species hybrids, as well as in issues involving illegal fishing and marketing of endangered species. Illegal trade in animal/plant products is commonly practiced in some of the Asian countries, where they market some of the endangered species in the guise of ones approved by authorized bodies such as, the International Whaling Commission (IWC). Through a series of reports, IWC has published techniques and incidences of identification of market samples of cetaceans using DNA sequence analysis which has thus become a powerful tool for

conservation by identifying the source of samples thought to be derived from threatened or endangered species. Only minute amounts of DNA are required, allowing for remote sampling. It is possible to use hair, blood, feces, skin biopsies and sloughed skin as a DNA source and the PCR-based techniques are simple and rapid, making them practical for conservation and population studies. In cetaceans and dugongs, the technique can be effectively used in the forensic identification of commercial products, verification of trade records and also for identifying ambiguous beach-cast specimens.

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, these investigations have led to revisions of taxonomic relationships. Molecular genetics can also aid taxonomic understanding of inter and intra-specific variations for conservation and management purposes. Mitochondrial DNA (mtDNA) is well established and widely used tool for species identification and to a lesser extent, population identification. Mitochondria are structures within cells that convert the energy from food into a form which cells can use. Although most DNA is packaged in chromosomes within nucleus, mitochondria also have small amount of their own DNA. This genetic material known as mtDNA spans about 16,500 DNA building blocks (base pairs) representing a fraction of the total DNA in cells. MtDNA is often used in studies of marine mammals for a number of reasons including its high rate of evolution, maternal inheritance, low effective population size and lack of recombination. It has helped to define management units for the effective management of the exploitation of any species.

Of the total of about 37 genes and non-coding regions in the mtDNA, one gene (cytochrome *b*) and a non-coding segment (control region) are most commonly used for studies on marine mammals. This is based on the advantages of their rapid evolution rate and variability which would facilitate accurate delineation of species and detection of population differentiation. DNA sequences from the control region and cytochrome *b* are reconstructed to develop a "tree" which would give clue to the exact or possible identity of the species. Molecular identification of marine mammals can be done in two steps: (1) sequence similarity search under BLAST (Basic Local Alignment Search Tool) as implemented in GenBank (www.ncbi.nlm.nih.gov) (2) once it is confirmed that the tissue sample originates from a cetacean, the species identity is searched within *DNA Surveillance* (www.cebl.auckland.ac.nz:9000/). All sequences in *DNA Surveillance* are included only if the specimen had been expertly identified and diagnostic skeletal material or photographic records were collected. The purpose of checking the higher taxa of the unknown sample with BLAST search is important because if it does not belong to the order Cetacea, results of the phylogenetic identification could be misleading.

## Studies on marine mammals in India

The Ministry of Earth Sciences (MoES) had funded ICAR-CMFRI to study biology, trophodynamics, fisheries interaction, contaminant accumulation, molecular taxonomy and PCR-based sex identification of marine mammals from Indian coasts. This was followed by a genetic study of Irrawaddy dolphin in Chilka Lake supported by Chilika Development Authority (Jayasankar *et al*., 2011). Two works on marine mammal taxonomy have been published from India (Jayasankar and Anoop, 2010; Vivekandandan *et al*., 2010). Standardized PCR-based methods for gender identification of species of marine mammals as well as in forensic identification of commercial products for checking illegal trade of the meat of endangered and protect species were developed as a result. Three important advancements from the molecular taxonomy approach during implementation of MoES-funded project, which could not have been possible with conventional approaches alone were (i) Correction of misidentification of species due to external body coloration differences between juveniles and adults [a specific case of Pantropical spotted dolphin], (ii) many beach-cast baleen whales in different stages of deterioration could be identified using DNA, and (iii) Sex [gender] of all samples were identified using PCR. Peer reviewed research papers and reviews on molecular identification and sex identification of marine mammals from Indian seas were published during 2007-2014 (Table 1).

Table 1. Particulars of marine mammal species from Indian seas which were identified using mtDNA markers

| Species | Location | Number of individuals (n) | mtDNA gene | Reference |
|---|---|---|---|---|
| *Tursiops aduncus* | Vizhinjam, Kakinada & Chennai | 5 | Cytochrome b | Jayasankar *et al.*,2008 |
| *Stenella longirostris* | Kakinada & Chennai | 12 | Control region & Cytochrome b | |
| *Grampus griseus* | Chennai | 2 | Control region & Cytochrome b | Jayasankar, 2014 |
| *Physeter macrocephalus* | Chennai | 2 | Cytochrome b | |
| *Balaenoptera musculus* | Mandapam | 1 | Control region & Cytochrome b | |
| *Dugong dugon* | Mandapam | 1 | Control region & Cytochrome b | |
| *Stenella attenuata* | Chennai | 1 | Control region & Cytochrome b | |
| *Delphinus capensis* | Kakinada & Malpe | 3 | Cytochrome b | Jayasankar *et al.* 2008 |
| *Sousa chinensis* (later described as *S. plumbea*) | Gangoli & Mangalore | 2 | Control region & Cytochrome b | |
| *Neophocaena phocaenoides* | Gangoli, Malpe & Mangalore<br>Thiruvananthapuram | 7<br>1 | Control region & Cytochrome b<br>16S rRNA & COI | Jayasankar *et al.* 2008<br>George *et al*. 2011 |
| *Balaenoptera edeni* | Mandapam<br>Thiruvananthapuram | 1<br>1 | Control region & Cytochrome b<br>16S rRNA & COI | Jayasankar *et al*. 2007<br>George *et al*. 2011 |
| *Orcaella brevirostris* | Chilika Lake | 11 | Control region & Cytochrome b | Jayasankar *et al*., 2011 |

## *Neophocaena phocaenoides*

Finless porpoise (*N. phocaenoides*) is abundant in the west coast of India. This is the only representative of porpoises in Indian waters. Intraspecific genetic divergence is low when compared to some other dolphin species.

## *Stenella longirostris*

Spinner dolphin (*S. longirostris*) is likely to be the most abundant dolphin in Indian waters as the molecular study conducted by the present author indicates. However, the species also exhibited maximum intraspecific genetic divergence. The taxonomy of *Stenella* is a matter of ongoing debate, and presence of multiple subspecies could further complicate the scenario.

## *Tursiops aduncus*

Bottle nose dolphin (*T. aduncus*) was earlier mentioned as *T. truncatus* erroneously, and molecular study by the present author confirmed the species as *T. aduncus*. *T. truncatus* is larger than *T. aduncus* with a shorter beak. Certainly the species caught accidentally in gill nets is *T. aduncus*. However, among more oceanic species *T. truncatus* is likely to be present.

## *Delphinus capensis*

Previously misidentified as *Delphinus delphis* from Indian waters, the long beaked common dolphin was re-described as *D. capensis* based on molecular study conducted by the present author. Intraspecific genetic divergence was found to be high in this species. Some confusion in absolute identity still remains, because some haplotype were closely similar to *D. tropicalis*, although *tropicalis* is treated as a sub-species by some experts, which means the Indian species of common dolphin is very likely to be *D. capensis tropicalis*.

## New developments in molecular identification of marine mammals

Post 2014, the major technical advancement in molecular identification of marine mammals include application of DNA barcoding (COI, 16S rRNA), mass spectrometry (collagen peptide mass fingerprinting) and eDNA (droplet digital PCR). Next Gen Sequencing (NGS) has been more routinely applied to modern cetacean populations recovering full mitogenomes, genomic single nucleotide polymorphisms (SNPs), or even complete nuclear genomes to develop more nuanced models of their evolutionary systematics and population histories. Some of the current areas of molecular research on cetaceans globally are, DNA barcoding (Alfonsi *et al*., 2019), eDNA analysis (Baker *et al*., 2018), whole genome sequencing (Jia *et al*., 2019), mitogenomics (Cabrera *et al*., 2019) and molecular identification of market samples (Lee *et al*., 2019).

## Subspecies concept in marine mammal protection strategies

A species is a separately evolving lineage composed of a population or collection of populations. A subspecies is a population, or collection of populations, that appears to be a separately evolving lineage with discontinuities resulting from geography, ecological specialization, or other forces that restrict gene flow to the point that the population or collection of populations is diagnosably distinct (Taylor *et al*., 2017). Demographically Independent Population (DIP) means a sympatric group of individuals whose dynamics are more a consequence of births and deaths within the group (internal dynamics) than of immigration or émigration (external dynamics). DIP is an appropriate level of population structure for management objectives related to ecosystem function, like those of the U.S. Marine Mammal Protection Act (MMPA). Guidelines developed include reference to the need to provide information on the distributions of the

taxa or taxon and on sample locations, descriptions of life history, and comments on choice of genetic markers and analytical methods. Such guidelines would improve consistency in the field of taxonomy. "Quantitative standards" are developed to illustrate the magnitude of differentiation that warrants subspecies classification. These standards would facilitate improvement of the quality and transparency of arguments advanced on behalf of taxonomic proposals and that they be viewed as "living standards" that can evolve with experience and as knowledge grows. Genetic metrics, such as Nei's estimate of net divergence (dA) and per cent diagnosability perform best to categorize cetacean populations, subspecies and species except in recently diverged species (Rosel *et al*., 2017).

## Way forward

Molecular identification attempts of cetaceans of Indian seas have clearly indicated the need for studying more number of species and individuals; phylogenetic relationships to understand the evolution of different species; and genetic variation *vis-à-vis* global geographic distribution of different species for their biodiversity conservation plans. In India we are continually monitoring stranding of cetaceans and dugong along beaches and landing centres. It is recommended that in addition to morphometric measurements and pictures of the specimens, a little quantity of skin tissue extracted from the specimens with minimum degree of deterioration is preserved in alcohol and molecular identification with standard methods is done for the credibility in identification of beach-cast or stranded marine mammals. Further, it is essential to venture into stock assessment of these gentle giants in our seas using non-invasive techniques like eDNA analysis. This is even more important in the context of conforming to global ocean conservation efforts like Marine Mammal Protection Act (MMPA).

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