

REVIEW ON RESEARCH IN THE TAXONOMY AND SEX DETERMINATION OF WHALES, DOLPHINS AND PORPOISES (ORDER CETACEA)

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Abstract: Marine mammals, including dolphins, porpoise and whales are top predators and integral components of healthy aquatic ecosystem. They are facing growing threats of a variety of anthropogenic activities, which entail urgent need of research and education programmes to create awareness in the society for their protection and conservation. Accurate taxonomy is fundamental to conservation efforts, and imperfect taxonomy may result, at least as much as a lack of understanding of the population structure, in the loss of genetic variability. In cetaceans, morphological features are often subtle and difficult to compare because of the rarity of specimens or widespread distributions. The number of extant species of cetaceans remains debated. The yawning gaps in our present understanding of species status and geographic variation of cetaceans would probably imply on serious taxonomic revisions. Cetaceans are identified using morphology-based approach, photo identification and molecular taxonomy. DNA sequence analysis has become a powerful tool for conservation - identifying the source of samples thought to be derived from threatened or endangered species, thus enabling to identify the species even from a small piece of tissue sample, such as skin from the marketed product. Sex identification is of fundamental importance in the studies of population structure, social organization, distribution, behaviour or heavy metal accumulation in marine mammals. Molecular gender determination is essential in situations when only tissue samples are available or when sex-specific characters are either absent or difficult to observe. In India, marine mammal research has been restricted to reporting on their incidental catches in fishing nets or beach-cast samples. Ministry of Earth Sciences funded the first concerted attempt to study biology, trophodynamics, fisheries interaction, contaminant accumulation, biomarkers, molecular taxonomy and PCR-based sex identification of marine mammals from Indian coasts. Molecular identification of cetaceans of Indian seas has 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.

Key words: Marine mammals, species identification, mitochondrial DNA, molecular taxonomy, PCR-based sex identification, conservation

INTRODUCTION

In recent years there has been 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 challenge to these animals and their environments. Research and education programmes should try to properly understand

and more clearly communicate these threats and recommend appropriate steps to reduce or eliminate their impacts.

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 all warm-blooded animals

and have undergone major adaptations, which permit them to live in the water. The cetaceans and sirenians spend their entire lives in the water, while other marine mammals come ashore for various reasons, at particular times in their life cycle (most commonly to reproduce, moult, or rest). Major structural modifications to the bodies of cetaceans, sirenians, and pinnipeds 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).

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 (Baker *et al.*, 2004). 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 are difficult using the conventional approaches. 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.

The order Cetacea comprises two extant sub-orders and one extinct sub-order. The extant sub-orders are Mysticeti- filter feeding or (baleen whales) and Odontoceti (toothed whales) with at least 70 species, 40 genera, and 10 families. Both Mysticetes and Odontocets are thought to

be descendants of Archaeocetes (Archaeoceti, ancient whales, known only from fossil records), an extinct sub-order (Rice, 1984; Gingerich *et al.*, 2001; Reeves *et al.*, 2002). The number of extant species of cetaceans remains debated (Rice, 1998; Perrin, 2002a; Baker *et al.*, 2003). One report indicates about 78 species of cetaceans from world (Jefferson *et al.*, 1993), while another recognized 83 species of cetaceans, and 16 of these included from two to four subspecies (total: 42 subspecies). A list of IUCN consists of 84 species, including two Bryde's whales, the recently described *Mesoplodon perrini* (Dalebout *et al.*, 2002) and the resurrected *Mesoplodon traversii* in place of *M. bahamondi* (Van Helden *et al.*, 2002). Of the reported 84 living species of cetaceans, 16, including "stocks", "populations", or sub-species were assigned threatened status in 1996 (Reeves *et al.*, 2003, 2004). However, with the recent consensus that recognizes three rather than one species of right whale, the total number of species comes to 85 (Perrin, 2003), and the number of subspecies is reduced to 41.

APPROACHES FOR IDENTIFICATION OF CETACEANS

Cetacean systematics is rapidly changing for a variety of reasons, including advances in analytical techniques, application of molecular markers, and increase in the amount of material available and revisions are expected to continue at all levels (Milinkovitch *et al.*, 2002).

Morphology based approach

Cetacean specimens "in hand" can be identified by using the dichotomous keys to external features (Jefferson *et al.*, 1993). 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 (Rice, 1998). Skulls of many species are sufficiently similar that it will be necessary to examine a full series of each to define reliable diagnostic features. 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 details of a species.

More of the world's cetacean collections in museums and other institutions should be catalogued and made accessible through the internet (Reeves *et al.*, 2004). This effort is already underway by many major museums, but the contents of some 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.

Great variability in morphological characters of cetaceans is not uncommon. Sometimes it may only be possible to label an animal or group as "unidentified long-snouted dolphin" or "unidentified beaked whale", etc.

Photo identification

Photographs of dorsal fins and flukes help in identification of individual cetaceans. 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 at disposal 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, and this is often at 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. It needs fair amount of experience and expertise to master the technique of identifying free ranging marine mammals at sea.

Generally, sightings are initially identified as "possible" or "confirmed" or, usually for the animals far away from the vessel, "unidentified". Photo and video documentation of sightings would later help to confirm the identification with the assistance of experts. Sixty eight percent of individual cetaceans sighted during one southern ocean cruise could be identified to the species level (Jayasankar *et al.*, 2007c). Vessel-based surveys have been conducted to identify cetaceans based on their sightings in Maldives (Balance *et al.*, 2001; Anderson, 2005), Kerguelen islands (Borsa, 1997), Mauritius (Corbett, 1994), Indian Ocean sanctuary and the South China Sea, Mauritius to the Philippines (De Boer, 1999), Indian Ocean (Kasuya and Wada, 1991), Seychelles (Robineau, 1991), Caribbean sea and Gulf of Mexico (Jefferson and Lynn, 1994) and Eastern Antarctica (Thiele *et al.*, 2000).

Molecular taxonomy

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 (Tautz *et al.*, 2003). 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.

DNA sequence analysis has become a powerful tool for conservation - 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, faeces, skin biopsies and sloughed skin as a DNA source. PCR-based techniques technically are simple and rapid, making them practical for conservation and population studies. In cetaceans and dugong, the technique could be effectively used in the forensic identification of commercial products and verification of trade records and for identifying ambiguous beach-cast specimens. 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, the International Whaling Commission has brought out techniques and incidences of identification of market samples of cetaceans illegally traded by Japan and Korea. The DNA-based approach would help the conservationist to identify the species even from a small piece of tissue sample, such as skin from the marketed product.

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 (Arnason *et al.*, 1992; Milinkovitch, 1997). Molecular genetics can also provide significant contributions to taxonomic understanding of inter and intra-specific variations for conservation and management purposes (LeDuc *et al.*, 1999; Rosel *et al.*, 1999; Dizon *et al.*, 2000; Reeves *et al.*, 2004; Amaral *et al.*, 2007).

Mitochondrial DNA 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 (Hoelzel *et al.*, 1998). Understanding population structuring is important for the effective management of the exploitation of any species. Analysis of mitochondrial DNA (mtDNA) has become a standard method for investigating population structure because of the rapid rate of evolution and simpler interpretation of the haploid nature of this molecule relative to nuclear DNA, and has helped define management units of many exploited species of marine mammals (Helbig *et al.*, 1989; Stevens, 1989; Dozpm *et al.*, 1991; Hoelzel and Dover, 1991; Schaeff *et al.*, 1993).

Two regions of the mitochondrial genome most commonly used for studies on marine mammals are the mitochondrial control region and the cytochrome b gene. The mitochondrial control region is a non-coding segment of the mitochondrial genome that regulates the replication of this genome. The control region is commonly used due to its high level of variability (Brown *et al.*, 1986). The control region is the only major non-coding region and most rapidly evolving part of the mt genome (Upholt and Dawid, 1977; Cann *et al.*, 1984) making it particularly well suited for the study of intraspecific evolution. The combination of these characteristics makes the mt control region an ideal genetic marker for testing the hypothesis of long-term segregation of maternally directed feeding aggregations in an otherwise panmictic population. Cetacean mt control region has been reported to evolve at a lower rate than that of other mammals (Hoelze and Dover, 1991; Baker *et al.*, 1993). The cytochrome b gene has been used in numerous studies of phylogenetic relationships within mammals, and it is the gene for which the most sequence information from different mammalian species is available (Irwin *et al.*, 1991; Meyer, 1994; Johns and Avise, 1998). The sequence variability of cytochrome b makes it most useful for the comparison of species in

the same genus or the same family. The results obtained in many of the phylogenetic studies in which this gene has been used led to the proposition of new classification schemes that better reflected the phylogenetic relationships among the species studied (LeDuc *et al.*, 1999; Arnason *et al.*, 1995; Lara *et al.*, 1996).

Species level identification of marine mammals has relied primarily on the phylogenetic reconstruction of DNA sequences from the control region or cytochrome b gene of the mitochondrial (mt) genome. The control region of the mtDNA does not code for a protein or RNA and, in the absence of these constraints, accumulates mutational substitutions more rapidly than other regions. The cytochrome b gene a protein region of the mtDNA has also been used widely in species-level identification of marine mammals (Lento *et al.*, 1997).

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 was confirmed that the tissue sample was from a cetacean, the species identity was searched within DNA Surveillance (www.cebl.auckland.ac.nz:9000/). Most sequences in DNA Surveillance were 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. Details of these web-based sequence databases are given elsewhere (Jayasankar and Anoop, 2010).

SEX IDENTIFICATION

Sex identification is of fundamental importance in the studies of population structure, social organization, distribution, behaviour or heavy

metal accumulation in marine mammals (Gompper *et al.*, 1998; Hughes, 1998). For example, in heavy metal accumulation studies, males are preferred since lactating females would invariably pass the heavy metal contents to the baby, thus giving an erroneous level of concentration during testing. However, distinguishing the males and females among these animals is difficult due to the poor sexual dimorphism, especially during their free-ranging state (Gowans *et al.*, 2000). Direct anatomical evidence of an individual's sex comes only from a full-ventral inspection of its genital region and such opportunities are limited during field observations; unless the animal rolls and remains inverted at the surface (Clapham and Mayo, 1987) its genital region is visible only to an underwater observer, that too only at a very close range (Glockner, 1983).

Non-molecular methods of gender identification have proved difficult and often unreliable. By examining the carcass remains of stranded/beach-cast cetaceans, which are often at decomposition levels, accurate assessment of reproductive organs is not possible. Though the individual may be inferred to be female if it is observed near calf (Baker *et al.*, 1987; Slooten *et al.*, 1993; Knowlton *et al.*, 1994), such inferences are troublesome for some species, like the sperm whale, in which "babysitting" males are possible (Whitehead, 1996). Karyological identification of sex, though successful in some species of marine mammals, requires obtaining viable fibroblast tissue for cell culture, restricting its usefulness under many field conditions (Arnason *et al.*, 1985; Lambertsen *et al.*, 1988).

More recently, PCR amplification of sex-specific DNA fragments has been widely available for sex identification. Molecular gender determination is essential in situations when the animal is not present but tissues are available or when sex-specific characters are either absent or difficult to observe. Early molecular sexing techniques applied to cetaceans required

southern hybridization using probe derived from the human Y chromosome (Baker *et al.*, 1991) and amplification and then restriction digests of a fairly long fragment of the ZFX/ZFY genes (Palsboll *et al.*, 1992). Other techniques based on PCR amplification of sex-specific DNA fragments used genes Amg (Sullivan *et al.*, 1993), ZFX/ZFY (Gowans *et al.*, 2000; Aasen and Medrano, 1990; Berube and Palsboll, 1996; Shaw *et al.*, 2003; Morin *et al.*, 2005) and Sry (Richard *et al.*, 1994).

A widely practiced method has been amplification of Y-chromosome specific SRY locus amplified simultaneously with the homologous ZFX/ZFY genes on the X chromosome of females (ZFX) and XY chromosomes of males (ZFX/ZFY) as positive control for the absolute confirmation of sex. Females lack Y chromosome and the test is based on the absence of a SRY product in females. This method was successful in identifying sex of several mammalian groups (Aasen and Medrano, 1990; Fain and LeMay, 1995).

INDIAN SCENARIO

In the Indian seas, marine mammals are represented by two groups of marine mammals, cetaceans and sirenians. Latter is represented by a single species, dugong (*Dugong dugong*). Research on marine mammals in India has been restricted to reporting on their incidental catches in fishing nets or beach-cast samples. Spinner dolphin (*Stenella longirostris*), bottlenose dolphin (*Tursiops aduncus*), Indo pacific humpbacked dolphin (*Sousa chinensis*) and common dolphin (*Delphinus capensis*) are the commonly encountered delphinids and finless porpoise, the only known representative of phocoenids in India along with dugong. These species seem to be residents or regular visitors to the coastal areas, thereby facing higher risks of either entanglement in fishing nets other than the other offshore species. The annual mortality of cetaceans in gillnet fishery is about 1000-1500 (Yousuf *et al.*, 2008). Entanglement of cetaceans in other fishing gears such as trawls, purse

seines, shore seines and long-lines has also been reported. While the Indian Wildlife Protection Act of 1972 puts all marine mammals in Schedule I of the Act, very little has been done in ways to mitigate mortality. Dugong has a very narrow distribution and is considered endangered.

It is still unclear as to how many species of cetaceans exist in the Indian seas. In the absence of any dedicated survey to assess the abundance of cetaceans in Indian waters, we have no indication of their numbers; leave alone their population trends. Though the extant cetacean species number in Indian seas is suggested to be 25 (Kumaran, 2002, 2012; Vivekanandan and Jeyabaskaran, 2012), it could probably be more. Lack of adequate field keys and reliable inventory has resulted in several cases of misidentification. About 50% of the stranded baleen whales have not even been identified to the species.

At the Central Marine Fisheries Research Institute, under a research project sanctioned by the Ministry of Earth Sciences, the first major concerted attempt was made to study biology, trophodynamics, fisheries interaction, contaminant accumulation, biomarkers, molecular taxonomy and PCR-based sex identification of marine mammals from Indian coasts (Jayasankar and Anoop, 2010; Yousuf *et al.*, 2008; Jayasankar, 2003; Jayasankar and Anoop, 2003; Jayasankar *et al.*, 2006; Jayasankar *et al.*, 2007a, b, c; Jayasankar *et al.*, 2008a, b, c; Anoop *et al.*, 2008). The work on molecular taxonomy carried out under this project was an attempt to develop a database of genetic sequences for future cetacean research in addition to confirming the identity of delphinids and finless porpoise collected around India using standard genetic techniques and to make a comparison of Indian haplotypes with those of the corresponding species from other geographical seas. George *et al.* (2010) and Biju Kumar *et al.* (2012) used cytochrome oxidase 1 and cytochrome b mitochondrial genes for identifying marine mammals stranded along

Kerala coast. The smaller numbers analyzed in most of the cases would not probably resolve the species identity crisis; but could contribute for a comparison of the species from India with those of global occurrence.

A website (www.marinemammals.in) devoted to marine mammals of India, supported by the WDCS (Whale and Dolphin Conservation Society), provides preliminary information on identification of the species, stranding, marine mammal bibliography, etc.

Remarks on the results of molecular taxonomy study

Of the ten species of cetaceans identified using molecular taxonomy by the present author, nine were recorded by earlier workers from Indian seas, except *Delphinus capensis*, which was reported previously as *D. delphis* (Kumaran, 2002). Marine mammals in terms of number of species and individuals are abundant in the southwest coast of India, Gulf of Mannar and southern Sri Lanka. Though accurate estimates are not available, it appears that a few thousand dolphins and porpoise may die of non-targeted fishing every year (Yousuf *et al.*, 2008). For addressing all issues impacting the cetaceans around India, their unambiguous identification, inventory and cataloguing are essential. Several cases of misidentification of cetaceans committed by earlier Indian workers who solely depended on conventional tool of taxonomy has been brought to the notice – molecular approach can help address the species identity through standardized comparisons (Kumaran *et al.*, 2002).

The specimen of *S. attenuata* collected during the CMFRI study was apparently a juvenile, measuring 93 cm in total length. Initially it was misidentified as bottlenose dolphin in the field. Whale and Dolphin Conservation Society (WDCS) in its website have suggested that the Pantropical spotted dolphins are born without spots and that it could easily get muddled up with other species such as bottlenose dolphin. The specimens of

this species from far west Pacific, the Hawaiian form, are least spotted, nearly unspotted as adults (Dizon *et al.*, 1994). From its photograph and few body measurements, the species was confirmed as *S. attenuata* (William Perrin, Patricia Rosel, Susana Caballero, Richard LeDuc, personal communications). According to them the shape of the species was not that of bottlenose dolphin and the colour patterns around the head were consistent with *S. attenuata*. Molecular approach had ratified its species status.

As many as 11 haplotypes were observed in *S. longirostris* of Indian seas, indicating high genetic variability in the species. The taxonomy of *Stenella* is a matter of ongoing debate and presence of multiple subspecies of *S. longirostris* (Perrin, 1990; Perrin *et al.*, 1999) could further complicate the scenario. DNA Surveillance itself recommends caution on phylogeny-based molecular identification.

Evidence for the existence of two species, *T. truncatus* and *T. aduncus* from South Africa has been presented (Ross, 1997), but, after examining specimens from Australia, it was concluded that specimens from the two locations should be assigned to a single species, *T. truncatus* (Ross and Cockcroft, 1990). However, with the completion of extensive genetic analyses, and further morphological and ecological comparisons during the 1990s, a new consensus has emerged that recognizes two *Tursiops* species (Rice, 1998; Wang *et al.*, 1999, 2000a; Yang *et al.*, 2005; Charlton *et al.*, 2006). In the Chinese waters, the two bottlenose dolphin morphotypes/species were recommended to be treated as separate management units in making up conservation measures, though further study on the intraspecific structure using multiple molecular markers was suggested for effective conservation (Yang *et al.*, 2005). Based on several genetic markers, *T. aduncus* may be more closely related to *Stenella* and *Delphinus* species than to *T. truncatus* (LeDuc *et al.*, 1999; Natoli *et al.*, 2004).

What is clear, however, is the emerging worldwide picture that coastal bottlenose dolphins often have local fine scale population structure with unique regional patterns of genetic differentiation and morphology (Charlton *et al.*, 2006).

The earlier published studies from India have mentioned the bottlenose dolphin species as *Tursiops truncatus* (Sathasivam, 2004). However, it is now evident that the species of bottlenose dolphin which is often killed accidentally in the coastal gillnet fisheries is likely to be *T. aduncus*. *T. truncatus* is known to be larger than *T. aduncus* and has a shorter beak. All the three specimens collected in the study conducted by present authors showed closest genetic proximity to *T. aduncus*.

It was only in the early 1990s that the two forms of common dolphin, the long-beaked and short-beaked forms were clearly described as separate species on the basis of morphology and ecology (Heyning and Perrin, 1994) and genetics (Rosel *et al.*, 1994). While Rice (1998) recognized these two species, he also chose to recognize a very long-beaked morphotype as a third species, *D. tropicalis*, even while acknowledging that it “may yet be shown to intergrade” with the long-beaked common dolphin, *D. capensis*, in several areas. Status and relationships of the Indo-Pacific tropicalis form need further investigation (LeDuc *et al.*, 1999). On the basis of morphological comparisons, it was concluded that the tropicalis form should be regarded as a subspecies of *D. capensis* (Jefferson and Waerebeek, 2002). The long-beaked and/or the short-beaked forms should have originated independently in different ocean basins, though the relationship of the Indian Ocean *D. capensis* to the other forms is not clear (Mirol *et al.*, 2000).

Taxonomic status of common dolphin appears to be far from fully resolved, particularly that of its representatives in the Indian Ocean and Southeast Asia (Jefferson and Waerebeek, 2002). All the earlier workers have mentioned the species of

common dolphin from Indian seas as *Delphinus delphis* (Sathasivam, 2004). But the species encountered in the CMFRI study had a fairly long beak and based on the morphological features as well as mtDNA sequencing, is identified here as either *Delphinus capensis* or *D. tropicalis*. On the basis of morphological comparisons, the tropicalis form should be appeared to be a subspecies of *D. capensis* (Jefferson and Waerebeek, 2002). The Indian Ocean species is most likely to be *Delphinus capensis tropicalis* (T. A. Jefferson, personal communication). While one of the haplotypes identified by the present author had absolute genetic similarity with the one reported earlier (LeDuc *et al.*, 1999), the other one was extremely divergent (long branch) and in DNA Surveillance was placed in a cluster grouping two short-beaked common dolphins as well as one tropicalis form. It was decided to name this specimen as *Delphinus capensis* with an interrogation mark. Although the possibility of contamination of this sample is unlikely, sequencing of a nuclear pseudogene, which came about as a replication of cytochrome b cannot be ruled out (Mirol *et al.*, 2000).

The taxonomy and nomenclature of Sousa has long been in a state of confusion and entails clarification. Indopacific humpbacked dolphins are particularly vulnerable because of their near shore distribution, tendency to occur primarily in or near estuaries, and apparently low numbers. Their discontinuous occurrence and morphological variability across the range from East Africa to southern China and eastern Australia strongly suggest that multiple taxa should be recognized (Reeves *et al.*, 2004). Although many authorities have taken the “conservative” approach of regarding all the animals from southeastern Africa to southeastern Asia (including southern China) and Australia as a single polytypic species, *S. chinensis*, the Indo-Pacific humpback dolphin (Jefferson and Karczmarski, 2001), others have argued that

these animals should be divided into at least two species, one in the Indian Ocean sector (*S. plumbea*) and another in the Pacific Ocean sector (*S. chinensis*) (Rice, 1998; Ross *et al.*, 1994).

Preliminary results of genetic analysis have indicated that strong population structuring occurs in this genus, both within and across ocean basins (IWC, 2003a). Further, morphometric studies provided some support for the separation of *S. chinensis* and *S. plumbea*, although no formal taxonomic split was proposed, pending the results of ongoing molecular studies (Rosenbaum *et al.*, 2002; Jefferson and Waerebeek, 2004). In the CMFRI study, all the samples of Indopacific humpbacked dolphins were from the West coast of India; hence the possible genetic differences between the West and East coastal forms of this species could not be verified. Populations along the two coasts are reported to differ markedly in their body color and size of the dorsal hump (Sutaria and Jefferson, 2004).

Though currently considered monotypic, the genus *Neophocaena* may include two (or more) species, and at least some of these (putative) species occur in only one or a few countries. Greater emphasis should be placed on the inadequacy of sampling and further research might reveal species-level differences, particularly between the two principal morphotypes: *asiaeorientalis*-type with a narrow dorsal ridge and found primarily in temperate portions of the range, and *phocaenoides*-type with a wide dorsal ridge and found in more tropical areas (Jefferson, 2002a). The present author observed that CR sequences of two individuals of finless porpoise had 97% sequence similarity with *Neophocaena phocaenoides asiaeorientalis*. Extreme coastal distribution and vulnerability to gillnet entanglement, exposure to toxins, and other potential threats mean that improved clarity with regard to systematics would have important implications for conservation and management of the finless porpoise (Reeves *et al.*, 2004).

Remarks on the results of PCR-based sex identification

The sex determination method used by the present author for cetaceans and dugong is technically simple, requiring only PCR amplification and agarose gel electrophoresis. This technique is advantageous over the sex determination based on probe hybridization. It avoids the use of radioisotope, making it cheaper and less hazardous. Problems of failed or partial digestion associated with techniques relying on restriction digestion are obviated. The whole process, from the extraction of genomic DNA to visualization of amplification products would take approximately 9-12 h, as opposed to several days in the case of hybridization method. Amplification frequency was improved by using "HotStart" taq DNA polymerase, which remains inactive until the reaction mixture reaches higher temperature at 94°C after 1 min, thus providing an automatic hot start for the enzyme. Some studies have relied on nested PCR (reamplification from a PCR product) for gender determination (Palmirotta *et al.*, 1998), but nested-PCR procedures increase the risk of contaminants and more expensive.

The method was also reliable and yielded 82% success in 44 individuals of 11 species tested (Jayasankar *et al.*, 2008a). Every sample should produce at least one band and the absence of any amplification implies a failed PCR reaction. Testing the technique using samples of known sexes (determined by physical examination of stranded/ accidentally caught individuals) from ten cetaceans and dugong indicated that this sexing method was effective across a broad taxonomic range. The method can provide the secondary confirmation necessary for positive sex identification in marine mammal specimens, or a primary method where accurate field observation of gender is not possible.

In three cases, one each of spinner dolphin, sperm whale and Bryde's whale specimens, where

external sex determination was not possible, molecular sexing could be possible. In two cases, one each spinner dolphin and finless porpoise, PCR based method revealed erroneous sexing by external examination. However, in 8 individuals molecular sexing failed probably due to the highly deteriorated condition or non-availability of gDNA of the particular specimens. It is expected that the PCR-based gender identification method standardized by the present author (Jayasankar *et al.*, 2008a) would help in the studies of conservation, population structure and forensic issues of marine cetaceans and dugong.

CONCLUSIONS

The identity of many cetaceans, especially the delphinid species from Indian seas is as confusing as it is elsewhere. The Indian attempt mentioned here was restricted to only coastal collections, taken as fisheries by-catch. Some of the Indian haplotypes were comparable to those segregated far apart geographically; but not comparable to those in the same locality. This is perhaps because they are highly migratory and the segregation/aggregations are coupled with generations of migrations across the oceans. The first attempt on the molecular identification of cetaceans of Indian seas has 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 the biodiversity conservation plans of these vulnerable/endangered animals. Given the fact that even from a small piece of skin tissue of the animal, the species and sex can be identified will certainly have far reaching implications in the conservation and management of marine mammals.

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