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Molecular Identification of Delphinids and Finless Porpoise (Cetacea) from the Arabian Sea and Bay of Bengal

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Abstract

The exact number of extant delphinid species from seas around India is still debated and the lack of adequate field keys and reliable inventory has resulted in misidentification of several species. As a part of a project to develop a molecular taxonomy of cetaceans from this region, partial sequences of mtDNA cytochrome *b* were generated from accidentally caught/stranded delphinids and finless porpoise. Species were identified by phylogenetic reconstruction of sample sequences with the reference sequences available in portals GenBank (NCBI) and the web-based program *DNA Surveillance*. A comparison was made with the homologous sequences of corresponding species from other seas of the world. Our molecular investigations allowed us to identify five species of cetaceans from Indian coasts, including *Delphinus capensis*, previously reported as *D. delphis*. We detected unique haplotypes in Indo pacific humpbacked dolphin (*Sousa chinensis;* n = 2) and finless porpoise (*Neophocaena phocaenoides;* n = 12) from Indian coast. On the other hand, some haplotypes were shared with other regional populations in spinner dolphin (*Stenella longirostris;* n = 16) and bottlenose dolphin (*Tursiops aduncus;* n = 3). Common dolphins (*Delphinus capensis;* n = 2) had both unique and shared haplotypes including one highly divergent sequence.

Key words: Delphinids, finless porpoise, mitochondrial DNA, molecular taxonomy, haplotypes

Introduction

Taxonomy is fundamental to conservation efforts of marine mammals and the units on which conservation is based are determined largely by species designation. Ambiguous identification of species can lead to erroneous conclusions, such as loss of genetic variability and unwitting extinction of species. In cetaceans, morphological features are often subtle and difficult to compare because of the rarity of specimens or widespread distributions and regional variation (Reeves *et al.* 2004). Identifying the geographical variants of recognized species of delphinids and phocoenids is even more difficult using the conventional approaches and in this context molecular genetics can provide significant contributions to taxonomic understanding of inter and intra-

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specific variations for conservation and management purposes (Rosel *et al*. 1999, LeDuc *et al*. 1999, Dizon *et al*. 2000, Reeves *et al*. 2004, Amaral *et al*. 2007). At higher taxonomic levels, it has become possible to generate useful molecular genetic data, especially DNA sequences, supported by theoretical advances and computer programs, leading to reinvestigation of phylogenetic issues involving cetaceans (Milinkovitch *et al*. 2002).

DNA sequence analysis has become a powerful tool for conservation – particularly in 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. PCR-based techniques technically are simple and rapid, making them practical for conservation and population studies. In cetaceans, the technique is effective in the forensic identification of commercial products and verification of trade records as well as for identifying ambiguous beach-cast specimens (Reeves *et al*. 2004; Dalebout *et al*., 2007). Illegal trade in animal/plant products is commonly practiced in some of the Asian countries, where some of the endangered species are marketed in the guise of ones approved by authorized bodies such as, the International Whaling Commission (IWC) (Dizon *et al*. 2000).

The number of extant global species of cetaceans is debated (Rice 1998; Perrin *et al*. 2002; Baker *et al*. 2003). Cetacean systematics, particularly that of delphinids, is rapidly changing for a variety of reasons, including advances in analytical techniques, application of molecular markers, and increases in the amount of material available; and revisions are expected to continue at all levels (Milinkovitch *et al*. 2002).

Research on cetaceans 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. 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.

In Indian seas, however, it is unclear still as to how many species of cetaceans exist due to the absence of any dedicated survey to assess their abundance (Sathasivam 2004). Though the extant delphinid species number in Indian seas is estimated to be 13, it could probably be more (Kumaran 2002). Lack of adequate field keys and reliable inventory has resulted in several cases of misidentification. There has been no effort so far to use molecular tools for the identification of cetaceans from the Indian seas.

Against this background, the present study was carried out, with a view to generating species-diagnostic mitochondrial DNA (mtDNA) sequences for molecular identification of delphinids and finless porpoise from the Indian seas. Because of its small effective population size and its rapid rate of evolution compared to nuclear DNA, mtDNA has been the most widely used molecular marker in phylogenetic and population genetic analysis of marine mammals (Reeves *et al*. 2004). A number of mtDNA sequences of cetaceans are available in the two databases, GenBank (NCBI) and *DNA Surveillance*. With the use of these databases, molecular taxonomic identification of the species is possible from carcass of ambiguous stranded specimens or even from tissues of unknown samples.

The present study represents an initial attempt to develop a database of nucleotide sequences for future cetacean research in addition to confirming the identity of delphinids and finless porpoise collected around India using standard molecular techniques and to make a comparison of Indian haplotypes with those of the corresponding species from other geographical seas. The smaller numbers analyzed in most of the cases will not probably resolve the species identity crisis; but it is expected to contribute for a comparison of the species from India with those of global occurrence.

Material and methods

Sampling. The locations of marine mammal sample collection are shown in Fig.1 and particulars of the samples including the accession numbers of mtDNA cytochrome *b* (hereafter mentioned as CYB) partial sequences deposited in GenBank are given in Table 1. Skin samples were obtained from dolphins and por-

poises killed incidentally in coastal (or offshore) fisheries and identified initially as *Stenella longirostris* (n=16), *Tursiops aduncus* (n=3), *Delphinus capensis* (n=2), *Sousa chinensis* (n=2), and *Neophocaena phocaenoides* (n=12). The tissue was taken either from the dorsal fin and stored in 70% ethanol for subsequent analysis. In order to avoid contamination, sterilized forceps, blades and surgical gloves were used. Although skulls or skeletons of the specimens studied were not stored at any institution, photographs representing all the five species were made for identification purpose (Fig. 2).

TABLE 1. Particulars of the delphinid and finless porpoise samples examined during the present study.

Sl No.	Species	Place and date of sample collection	Sample code	GenBank accession numbers of CYB	Haplotype code used in the present study
1	*Tursiops aduncus*	Vizhinjam (5.11.04)	Viz1	DQ232769	IndTa1
2	*T. aduncus*	Chennai (4.10.04)	CHO4	DQ270184	IndTa1
3	*T. aduncus*	Chennai (12.10.04)	CHO8	EF203434	IndTa2
4	*Stenella longirostris*	Kakinada (20.09.04)	VRC/Dol/05	DQ270182	IndSl9
5	*S. longirostris*	Kakinada (20.09.04)	VRC/Dol/04	EF203445	IndSl5
6	*S. longirostris*	Kakinada (20.09.04)	VRC/Dol/06	EF057433	IndSl9
7	*S. longirostris*	Chennai (4.10.04)	CHO2	EF203446	IndSl2
8	*S. longirostris*	Chennai (4.10.04)	CHO3	EF203447	IndSl1
9	*S. longirostris*	Mangalore (8.9.04)	MNG 3	EF203448	IndSl3
10	*S. longirostris*	Chennai (4.10.04)	CH6	EF057434	IndSl11
11	*S. longirostris*	Chennai (4.10.04)	CHO7	DQ232770	IndSl10
12	*S. longirostris*	Chennai (26.10.04)	CH9	EF057436	IndSl8
13	*S. longirostris*	Chennai (26.10.04)	CH10	EF203449	IndSl2
14	*S. longirostris*	Chennai (26.10.04)	CH11	EF203450	IndSl3
15	*S. longirostris*	Chennai (26.10.04)	CH13	EF446614	IndSl2
16	*S. longirostris*	Chennai (26.10.04)	CH17	EF057437	IndSl7
17	*S. longirostris*	Chennai (26.10.04)	CH18	EF057438	IndSl6
18	*S. longirostris*	Chennai (26.10.04)	CH19	EF446613	IndSl4
19	*S. longirostris*	Cochin (15.9.07)	COK1	EU204619	IndSl3
20	*Delphinus capensis* (?)	Kakinada (23.08.04)	VRC/Dol/03	DQ320765	IndDc2
21	*D. capensis tropicalis*	Malpe (24.02.06)	MNG18	EF061405	IndDc1
22	*Sousa chinensis*	Gangoli (24.11.05)	MNG 4	DQ364689	IndSc1
23	*S. chinensis*	Mangalore (24.12.05)	MNG16	EF057445	IndSc1
24	*Neophocaena phocaenoides*	Gangoli (25.11.05)	MNG 5	EF203435	IndNp1
25	*N. phocaenoides*	Gangoli (25.11.05)	MNG6	EF203436	IndNp1
26	*N. phocaenoides*	Gangoli (25.11.05)	MNG 7	DQ364692	IndNp1
27	*N. phocaenoides*	Gangoli (25.11.05)	MNG 8	DQ364691	IndNp2
28	*N. phocaenoides*	Gangoli (25.11.05)	MNG 9	EF203437	IndNp1
29	*N. phocaenoides*	Gangoli (25.11.05)	MNG 10	EF203438	IndNp1
30	*N. phocaenoides*	Gangoli (25.11.05)	MNG 11	EF203439	IndNp1
31	*N. phocaenoides*	Gangoli (25.11.05)	MNG 12	EF203440	IndNp1
32	*N. phocaenoides*	Malpe (17.11.05)	MNG 13	EF203441	IndNp1
33	*N. phocaenoides*	Malpe (5.11.05)	MNG 14	EF203442	IndNp1
34	*N. phocaenoides*	Mangalore (1.12.05)	MNG 15	EF203443	IndNp1
35	*N. phocaenoides*	Mangalore (2.1.2006)	MNG 17	EF203444	IndNp1

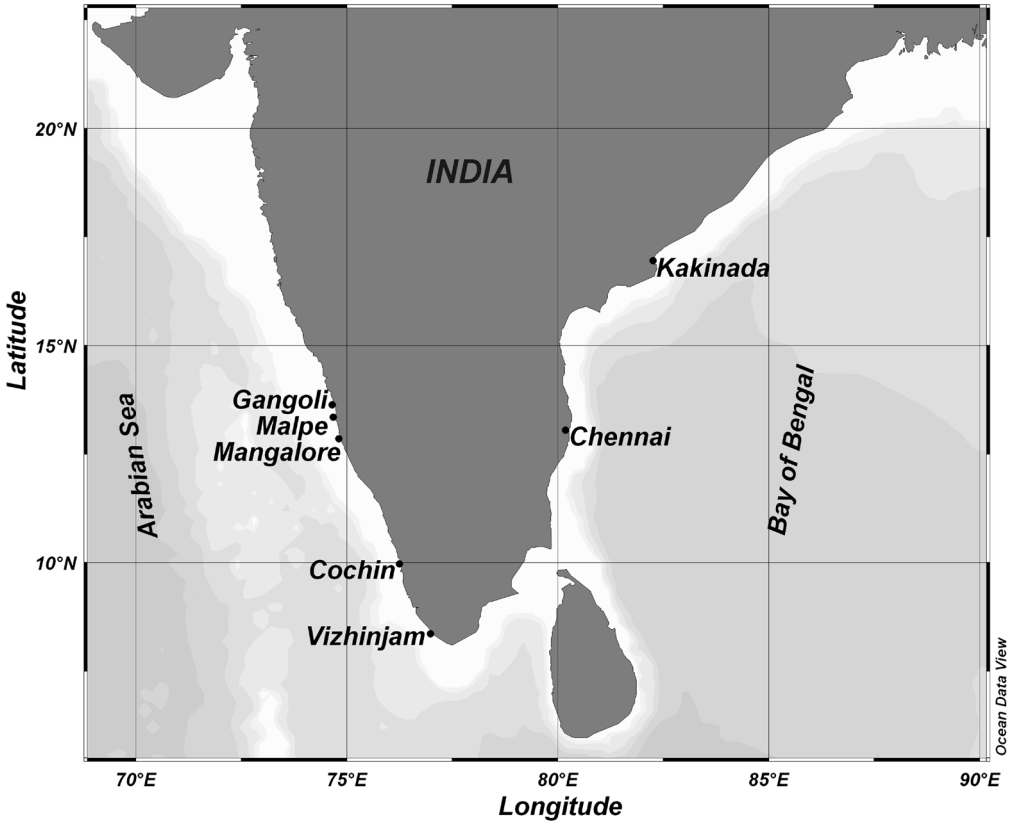

FIGURE 1. Locations where delphinids and finless porpoise were sampled in the present study

DNA Extraction, PCR and Sequence Analysis. Total genomic DNA was extracted using the standard phenol-chloroform method (Sambrook *et al*. 1989). Amplification reactions were performed on either PE24 (Applied Biosystems Inc.) or PTC100 (MJ Research) thermocycler in a total volume of 25 µl containing 10–100 ng of extracted genomic DNA template, 10 mM of Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 240 µM each of dATP, dTTP, dCTP and dGTP, 1 U of *Taq* DNA polymerase and 10–25 pM each of forward and reverse primers. The temperature profile for the amplifications was an initial denaturation (94°C) for 2 min followed by 35 cycles of 94°C for 1 min, 54^0 C for 2 min and 72°C for 1 min and a final extension of 72°C for 7 min. Primers for the mitochondrial DNA cytochrome *b* region used were from Palumbi (1996), namely GLUDG-L (5'-TGACTTGAARAACCAYCGTTG-3') and CB2-H (5'-CCCTCAGAATGATATTTGTCCTCA-3').

Quality of the PCR products was checked on 1.5% agarose gel. They were purified using the KT 62 Genei quick PCR purification kit. Cycle sequencing reaction was performed on a ABI 3100 PE automated capillary sequencer using the forward primer, reaction buffer and the fluorescently labeled dye terminators for required number of cycles at specific temperatures.

The sequences were first edited using BioEdit ver 7.0.5.3 (Hall 1999), aligned using the computer software Clustal W multiple alignment (Thompson *et al*. 1994) and corrected by eye. Primer and ambiguous sequences were deleted. CYB nucleotide sequences were translated to amino acid sequences using software Primer Premier ver 5.00 for submission in GenBank.

Species Identification. Morphological identification of the delphinids and finless porpoise was based on Rice (1998). Molecular identification was 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/), which contains a comprehensive database of mitochondrial DNA

sequences from mostly validated species by taxonomists. (Ross *et al*. 2003). Most sequences in this interactive portal were included only if the specimen had been expertly identified and diagnostic skeletal material or photographic records were collected (Dizon *et al*. 2000). 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. The sequences, after their confirmation, were submitted to GenBank.

Haplotype Comparisons. In order to perform haplotype comparisons, 7 homologous CYB sequences of spinner dolphin from GenBank were added to the sequences obtained in the present study. Similarly CYB sequences of bottlenose dolphin (n=4), common dolphin (n=7), Indo pacific humpbacked dolphin (n=3) and finless porpoise (n= 2) were also added from the GenBank for haplotype comparison.

FIGURE 2. Illustrations of the cetaceans sampled in this study. **a—***Stenella longirostris*: specimens CH02 & CH10 (haplotype code: IndSl2)**; b—***Stenella longirostris*: specimen Dol04 (haplotype code: IndSl5); **C—***Stenella longirostris*: specimens Dol05 & Dol06 (haplotype code: IndSl9); **d—***Tursiops aduncus*: specimen CH08 (haplotype code: IndTa2); **e—***Delphinus capensis* (?): specimen Dol03 (haplotype code: IndDc2); **f—***Sousa chinensis*: specimen MNG4 (haplotype code: IndSc1); **g—** *Neophocaena phocaenoides*: specimen MNG5 (haplotype code: IndNp1).

Results

MtDNA Sequence Products. Primers GLUDG-L/CB2-H generated robust PCR product of mtDNA CYB gene in all the five species with readable sequences ranging from 421 to 530bp.

Spinner dolphin (*Stenella longirostris*). Spinner dolphin was the most common species in the present collections, with 14 specimens coming from the east coast (Kakinada and Chennai) and two from the west coast (Mangalore and Cochin). We found 11 haplotypes, one of which (haplotype IndSl1) matched a reference sequence from the Timor Sea (GenBank AF084103) and another (haplotype IndSl2) matched a reference sequence from an unknown location (GenBank X92524; Arnason and Gullberg, 1996). Alignment with 7

TABLE 2. Variable sites from 374-bp of mtDNA CYB sequences of spinner dolphins determined in the present study in comparison with those of same species downloaded from GenBank. *n,* total number of individuals for each haplotype.

				Nucleotide positions																																
				1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3	3	*n*
				1	1	1	3	3	7	7	8	1	1	3	4	4	7	8	4	5	6	6	7	7	7	7	1	1	2	3	3	3	3	5		
				0	1	6	0	6	2	3	4	1	2	3	4	5	1	4	6	2	1	7	0	1	3	6	5	8	5	0	1	5	6	7		
Code	Individual GenBank Accession nos	Location	Reference																																	
TSSl1IndSl1	AF084103, EF203447	Timor Sea & India	LeDuc *et al.* (1999) & present study	A	A	G	A	C	C	T	C	T	T	A	C	T	T	G	C	C	T	T	C	C	G	C	T	G	C	A	A	T	C	C	2
PLSl1	AF084102	Philippines	LeDuc *et al.* (1999)	.	.	.	.	T	.	.	T	A	.	.	.	.	C	.	T	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	G	1
POSl1	AF084101	Eastern tropical Pacific	LeDuc *et al.* (1999)	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	1
GMSl1	AF084100	Gulf of Mexico	LeDuc *et al.* (1999)	.	.	.	.	T	.	.	T	A	.	.	.	.	C	.	T	.	.	.	T	.	.	.	A	.	.	.	.	.	.	.	.	1
CaSl1	X56292	unknown	Irwin *et al.* (1991)	.	.	.	C	.	.	.	T	C	C	.	A	A	.	.	.	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	1
CaSl2	X56293	unknown	Irwin *et al.* (1991)	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	1
CaSl3IndSl2	X92524,	unknown	Arnason and Gullberg (1996)	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	4
	EF446614, EF203449, EF203446	India	Present study																																	
IndSl3	EF203450, EF203448	India	Present study	.	.	.	.	T	.	.	T	A	.	.	.	.	C	.	T	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	2
IndSl4	EF446613	India	Present study	.	.	T	.	.	T	.	T	.	.	T	.	.	.	A	.	.	.	.	.	.	A	.	T	.	A	.	T	C	C	T	.	1
IndSl5	EF203445	India	Present study	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	C	.	.	A	.	.	A	.	.	.	.	.	.	1
IndSl6	EF057438	India	Present study	C	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	C	.	.	A	.	.	A	.	.	.	.	.	.	1
IndSl7	EF057437	India	Present study	.	C	.	.	.	.	C	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
IndSl8	EF057436	India	Present study	C	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	T	.	.	.	.	.	1
IndSl9	EF057433, DQ270182	India	Present study	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	2
IndSl10	DQ232770	India	Present study	.	.	.	.	.	T	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	T	.	.	.	.	.	1
IndSl11	EF057434	India	Present study	.	.	.	.	.	.	C	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1

sequences downloaded from GenBank showed 29 variable sites (22 transitions, 7 transversions and 2 both transition and transversion) (Table 2). Pictures of the individuals representing the three haplotype clusters obtained using *DNA Surveillance* (data not shown) are given in Figs 2a–c.

Bottlenose dolphin *(Tursiops aduncus)* (Fig. 2d)**.** Two haplotypes of bottlenose dolphin were identified, one of which was the same as that of a Japanese sample (AF425253). Alignment with five samples downloaded from GenBank showed 7 variable sites (5 transitions and 2 transversions) (Table 3).

TABLE 3. Variable sites from 346-bp of mtDNA CYB sequences of bottlenose dolphins determined in the present study in comparison with those of same species downloaded from GenBank. *n*, total number of individuals for each haplotype.

				Nucleotide positions							
				2 6	4 0	5 0	5 5	2 4 4	2 5 7	2 9 2	n
Code	Individual GenBank accession nos.	Location	Reference								
SATa1	AF084092	S Africa	Leduc *et al.* (1999)	G	A	T	C	G	T	T	1
JKTa1JaTa1	AF084091, AF425254	Jakarta, Indonesia & Japan: Western Kyushu	Leduc *et al.* (1999) Shirakihara *et al.* (2003)	.	.	C	T	A	C	C	2
JATa2IndTa1	AF425253,	Japan: Western Kyushu	Shirakihara *et al.* (2003)	.	.	C	T	.	C	C	3
	DQ270184, DQ232769	& India	Present study								
IndTa2	EF203434	India	Present study	T	C	C	T	.	C	C	1

Long-beaked common dolphin *(Delphinus capensis)* (Fig. 2e)**.** We identified 2 haplotypes, one of which was the same as that of an Indian Ocean specimen available in GenBank (AF084088). When compared to sequences downloaded from GenBank, 28 variable sites (14 transitions, 12 transversions and 2 transitions/transversions) were identified (Table 4). The haplotype IndDc2 of the present study exhibited maximum sequence divergence from the rest, even from the other Indian haplotype IndDc1 (5.9% divergence).

Indopacific humpbacked dolphin *(Sousa chinensis)* (Fig. 2f)**.** We detected one single haplotype for the two individuals studied. Using the sequences downloaded from GenBank, 15 variable sites (12 transitions and 3 transversions) were identified (Table 5). The Indian haplotype of *S. chinensis* was highly divergent from the South African and Hong Kong haplotypes.

Finless porpoise (*Neophocaena phocaenoides*) (Fig. 2g)**.** Finless porpoise was the second most abundant species collected during the present investigation with all the individuals obtained from the west coast of India. Two haplotypes were identified in the present study. When aligned with sequences from GenBank, only 5 variable sites (all five transitions) were identified (Table 6).

Discussion

Of the five species of delphinids identified using molecular taxonomy in the present study, four 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.*,

TABLE 4. Variable sites from 336-bp of mtDNA CYB sequences of long-beaked common dolphins determined in the present study in comparison with those of same species downloaded from GenBank. *n*, total number of individuals for each haplotype.

				Nucleotide positions																													
						1	2	4	6	6	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	3	3	3	3	*n*
						8	1	5	7	8	0	1	1	2	2	5	0	3	3	4	4	5	7	7	7	8	8	9	0	1	1	3	
				3	4						0	1	1	9	1	7	4	9	1	3	5	0	7	9	3	4	7	3	0	9	6		
Code	Individual GenBank accession nos.	Location	Reference																														
CADc1	AF084087, U02674	California	Leduc *et al.* (1999) Rosel *et al.* (1994)	C	A	A	C	A	A	G	C	A	A	T	G	A	T	A	G	C	T	C	A	G	C	A	C	C	T	A	A	2
CADc2	AF084086 ,U02675	California	Leduc *et al.* (1999) Rosel *et al.* (1994)	.	.	.	.	.	G	C	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	2
CNDc1	AY185135	China	Wang *et al**	T	G	.	.	G	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	T	.	.	.	1
CNDc2	AY185136	China	Wang *et al**	.	G	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	T	.	.	.	1
IndDc1 & IODt1	EF061405 AF084088	India & Indian Ocean	Present study Leduc *et al.* (1999)	.	G	.	T	.	G	.	T	.	.	C	.	.	.	.	.	T	.	.	.	A	.	.	.	T	.	.	.	2
IndDc2	DQ320765	India	Present study	.	T	C	.	.	G	.	T	C	T	C	A	C	C	T	C	T	A	A	C	C	T	C	.	T	C	G	C	1

*Unpublished GenBank reference

TABLE 5. Variable sites from 424-bp of mtDNA CYB sequences of Indopacific humpbacked dolphins determined in the present study in comparison with those of same species downloaded from GenBank. *n*, total number of individuals for each haplotype.

				Nucleotide positions																		
						1	1	1	2	3	3	3	4	4	4	4	4	4	
				4	7	0	1	8	0	2	5	8	1	2	2	2	2	2	*n*
				6	8	8	7	6	4	4	7	5	8	0	1	2	3	4	
Code	Individual GenBank accession nos.	Location	Reference																
HKSc1	AF084079	Hong Kong	Leduc *et al.* (1999)	G	T	G	C	T	C	G	G	G	T	C	T	G	A	G	1
SASc1	AF084080	South Africa	Leduc *et al.* (1999)	.	.	A	.	C	.	A	A	.	.	.	.	.	.	.	1
HKSc2	EF027031	Hong Kong	Caballero *et al.* (2007)	.	.	A	.	.	T	.	.	.	.	.	.	.	.	.	1
IndSc1	DQ364689, EF057445	India	Present study	A	C	A	A	C	.	A	.	A	A	T	C	T	G	A	2

2008). For addressing all issues impacting the cetaceans around India, their unambiguous identification, inventory and cataloguing are essential. Kumaran (2002) has pointed out several cases of misidentification of cetaceans committed by earlier Indian workers who solely depended on conventional tool of taxonomy – molecular approach can help address the species identity through standardized comparisons.

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.

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*. We have sighted *T. truncatus* in the oceanic waters off Indian coasts while undertaking many cruises (B.A., K.M.M.Y., V.V.A. and A.A.K.; data not shown). *T. truncatus* is larger than *T. aduncus* and has a shorter beak. All the three specimens collected in the present study showed closest genetic proximity to *T. aduncus*.

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 present 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*. Jefferson & Van Waerebeek (2002) concluded on the basis of morphological comparisons that the *tropicalis* form should be regarded as a subspecies of *D. capensis* and suggested that the present species is most likely to be *Delphinus capensis tropicalis* (T.A. Jefferson, pers. comm.). While one of the haplotypes in the present study had absolute genetic similarity with the one reported earlier by 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. We have 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).

TABLE 6. Variable sites from 373-bp of mtDNA CYB sequences of finless porpoise determined in the present study in comparison with those of the same species downloaded from GenBank. *n*,total number of individuals for each haplotype.

				Nucleotide positions					
				2	1	2	2	3	
				2	0	0	2	7	
				3	8	6	4	1	n
Code	Individual GenBank accessions	Location	Reference						
ChnNp1	AF334489, NPU09680	China	Hamilton *et al*. (2001) Rosel *et al*. (1995b)	T	C	C	C	G	2
IndNp1	EF203444, EF203443, EF203442, EF203441, EF203440, EF203439 EF203438, EF203437, EF203436 EF203435, DQ364692	India	Present study	C	T	T	T	A	11
IndNp2	DQ364691	India	Present study	C	T	.	T	A	1

The present samples of Indopacific humpbacked dolphins were all from the West coast of India, hence we could not verify the possible genetic differences between the West and East coastal forms of this species. Populations along the two coasts are reported to differ markedly in their body color and size of the dorsal hump (Sutaria and Jefferson 2004).

The identity of many delphinid species from Indian seas is as confusing as it is elsewhere. The present study 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. This first attempt on the molecular identification of delphinids and finless porpoise 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.

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