HABITAT ZONES AND VARIATIONS AT MITOCHONDRIAL GENOME LEVEL IN FRESH WATER DOLPHIN

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Abstract

Fresh water dolphins are present in geographically different areas of the world. Although they are the descendents of the single ancestor yet they have some genetic variabilities. The present study was conducted to discuss the characteristics and to analyze genomic differences among different fresh water dolphins.
Literature survey was done to find out major habitat zone of fresh water dolphins. The mitochondrial DNA sequence of dolphins was taken from GenBank. Mitochondrial nucleotide sequence alignment of dolphins was performed using bio-informatics tool Clustal W. Phylogenetic tree was constructed and percentage similarity was calculated among different species of fresh water dolphins.

On the basis of habitat, fresh water dolphins are divided into five major zones. Indus Dolphin mitochondrial genome indicated 99% homology with Ganges dolphin and 83% homology with Baiji Dolphin, 81% homology with the Franciscana Dolphin and 82% homology with the Boto Dolphin. The Baiji Dolphin has 85 % homology with the Boto Dolphin and 73% homology with Ganges Dolphin and the Franciscana Dolphin while the Boto Dolphin has 83 % homology with the Franciscana Dolphin. The Ganges Dolphin has 66 % homology with the Franciscana Dolphin. According to phylogenetic tree four species of fresh water dolphin were closely related while one was different.

There are genetic variations at mitochondrial genome level between Indus dolphin and other fresh water dolphins.

Introduction

Intensive work was conducted at molecular and morphological level to find the phylogenetic relationship among whales, dolphins and porpoises (Arnason and Best, 1991: Arnason and Gullberg, 1993, Arnason *et al,* 2004). These animals represent very important evolutionary transitions within vertebrates. The result of phylogenetic inquires showed key relationships among cetaceans. In addition, they also indicated relationship among fresh water dolphins of different geographical locations (Geisler and Sanders, 2003; Caballero *et al,* 2008).

The phylogenetic relationships among fresh water dolphins which were inferred by different studies are strongly contradictory due to different reason. Many evidences indicate that the traditional taxonomy of dolphins does not capture the diversity of the group and one clear example is the classification of Bottlenose dolphin into as few as one and as many as eight different species (Hershkovitz, 1966; Rice, 1998; Ross and Cockcroft, 1990). Recent analyses of bottlenose dolphins recognize two distinct species. So on the basis of results obtained from different studies the evolutionary history of dolphins is not completely resolved. One possibility of ambiguous results could be the methods utilized for research as the method may not capture the complex nature of DNA evolution (Verma *et al,* 2004). These days new tools have been developed that are reducing the chance of error and evolutionary relationships are calculated on the basis of several parameters. The use of mitochondrial DNA in phylogenetic analysis has further reduced the chance of error. In case of dolphins some researcher did the phylogenetic analysis only on the basis of part of mitochondrial sequence cytochrome b(Yang *et al,* 2002) but use of complete mitochondrial sequence give much better and reliable results.

In the present study initially we have given the details of habitat zone and morphology of fresh water dolphins and at the end phylogenetic analysis of complete mitochondrial genome of fresh water dolphins.

Materials and Methods

Literature review was carried out to find out characteristic and habitat zones of fresh water dolphins all over the world. The nucleotide sequences of their complete mitochondrial genomes were aligned by using different bioinformatics tools such as T-coffee and Clustal W version 1.83. Sequences were taken from the data which is present on GenBank (http://www.ncbi.nlm.nih.gov/)with accession number of, NC_005276, and NC_005277. NC_007629, NC_005275 and AY102537. These accession numbers correspond to *Inia geoffrensis* (Boto

Dolphin), *Pontoporia blainvillei* (Franciscana Dolphin), *Lipotes vexillifer* (Baiji Dolphin), *Platanista minor* (Indus River Dolphn), *Platanista ganetica* (Ganges Dolphin) respectively. Percentage similarity was calculated among different fresh water dolphins. Phylogenetic tree of fresh water dolphins was constructed using GeneBee Software Tree-Top phylogentic tree prediction (http://www.genebee.msu.su/services/phtree_reduced.html).

Results and Discussion

Habitat Zones of Fresh Water Dolphins

Platanista minor **(Indus River Dolphin):** Indus River Dolphin, (*Platanista minor*), is found in the Indus River of Pakistan only. It was used to live river system range, but now only found in the waters above the Kotri Barrage and below the Chasma, Islam Barrage, Trimmu and Sidhnai. Currently it exists only in the freshwater of Indus River. However, some paleontologists consider and believe that these dolphins might have evolved from marine-dwelling relatives that had eventually moved to estuaries and then rivers. These dolphins have the ability to swim on their sides and this adaptation enable them to exist in shallower water. Although they prefer water deeper than 3 meters. (Hamilton *et al*, 2001; Mann *et al*, 2000; Moreno, 2004)

Indus River dolphins sometimes are of light color on their underside but usually have some gray or brown color as of river. These have large visible teeth and have characteristically swollen beaks at the tip. Beaks are long, consisting of 20 % of the length of their bodies. In comparison to their ‘’beaks’’, their dorsal fins are small and reduced. Dolphins have large flippers and flukes with long and flexible necks to navigate effectively. Their ears are external and located below the eyes, but their eyes are small and can only see unclear and shadowy images. Female Indus river dolphins are larger than males (Moreno, 2004). It lives only in the Indus River system, while *Palatnista gagnetica* only inhabits the Ganges River System. These dolphins have the special ability to use their echolocation along with highly toothed, long snouts to look for animals including fish and invertebrates. (Moreno, 2004; Pilleri, 1979).

Lipotes vexillifer **(Baiji Dolphin):** The Chinese river dolphin is also a freshwater dolphin. This is regarded as one of the most endangered animals on the planet. This dolphin is also known as Yangtze River dolphin, ‘baiji’, white-flag dolphin, and white-fin dolphin. It has long beak that is slightly upturned and has 31-36 teeth on each jaw. Its dorsal fin is triangular and low and when it swims just below the surface of the murky Yangtze River, it looks like a light colored flag that is why, it is named as ‘’white-flag’’ dolphin. Its eyes are smaller than oceanic dolphins (Zhou, 1980).

There are no much studies conducted on baiji reproduction so very little is known about its reproduction. It is a consideration that it breeds in the first half of the year, with the peak calving season is between February and April. Gestation period of it is 10-11 months and it is considered that males mature at 40 year of age, while females at age of 6. After pregnancy of ten to eleven months, a single calf is born in spring. These calves weigh between 6 and 11 pounds and are about 3 feet long (Reeves *et al*, 2008).

It is different from Indus dolphin as it has different family that is lipotidae. There is very little information about its behavior, ecology and acoustics. It is an observation that they are usually present in group of two to six animals that sometime goes to aggregation of up to sixteen animals. It feeds on a large variety of fresh water fish species. These are shy of boats and when surfacing only the top of head, dorsal fin and a small part of back is exposed. There is very poor visibility in Yangtze river so baiji dolphin mainly depends on underwater sound for orientation and feeding. (Akamatsu *et al*, 1998; Reeves *et al*, 2008).

Inia geoffrensis **(Boto Dolphin):** The Boto dolphin is the largest of the river dolphins. Maximum length of the body of male is 255 cm and mass of 185 kg and smaller females reach 215 cm and 150 kg. They have large, wide and paddle-like flippers. It is widely distributed and can physically reach without going deep into marine waters that is why it is found almost everywhere. In Boto Reproduction often occurs year-round while with geographical distribution, seasonal peak varies.

The Amazon river dolphin is only present in fresh water. Boto Dolphin not only present in rivers but also in small channels and lakes and mainly concentrated at the mouth of rivers, below rapids and smaller channels running parallel to the main river during the high-water season (McGuire and Aliag, 2007).

Platanista ganetica (**Gangese Dolphin):** Gangese Dolphin is restricted to freshwater and mainly inhabits the Ganges and Indus rivers and many associated tributaries and associated lakes. There are two subspecies: *Platenista gangetica*, found in Nepal, eastern India, and Bangladesh in the Ganges, Karnaphuli, Meghna, Bramaputra, and Hooghly river system, and *Plantanista gangetuca*, found in Pakistan in the Indus River system (Moreno, 2003; Nowak, 2003).

Ganges River dolphin occupies freshwater river system in southern Asia. They are found in tributaries that pass through the hills and lowlands in Nepal. In addition they are sometimes in flood plains and areas of rivers

with heavy currents. These rivers dolphins prefer small islands, rivers bends and convergent tributaries, the areas that create eddy countercurrents (Moreno, 2003).

They can tolerate a wide difference in temperatures, some as cold 8^{o} C to warm waters above 33^{o}C as these animals occupies a vast area of river systems. They must surface every few minutes for air and inhabit depths from 3 to 9 meters. Ganges River dolphins locally migrate to tributaries in the monsoon season. In dry winter season they move back to larger river. During the monsoons freshwater flush out along southeastern coast of India, they move along the coast of the Bay of Bengal (Moreno, 2003).

Their teeths are long and sharp. On each side of the upper jaw there are 26 and 39 teeths and 26 to 35 on each side of lower jaw. In the old age the teeth eventually worn down and become flat. Unlike other dolphins, *Platanista ganetica* lack snout hairs (Moreno, 2003; Nowak, 2003; Reeves and Brownell, 1989; Wilson and Reeder, 2005).

It has been suggested from some unofficial records that adult females have a body length of 400 cm. At birth the average length is 70 cm and the average adult rarely exceeds 300 cm in length. The weight of adult is typically between 51 and 89 kg (Moreno, 2003; Nowak, 2003; Reeves and Brownell., 1989; Wilson and Reeder, 2005).

Pontoporia blainvillei **(Franciscana Dolphin):** The Franciscana dolphin is the only one of the five river dolphin species live in the marine environment. It is one of the smallest dolphins with a bulky head and an extremely long and narrow beak. Its color is lighter on the flanks and belly and brownish to dark gray on above part. Females are larger than males and have a body length ranging between 137-177 cm and males have between 121-158 cm. Females have weight up to 53kg and males have 43 kg (Crespo, 2009). They mature sexually at the age of 2 to 3 years. The length of calves is about 27 inches at birth and weigh 15 to 20 pounds. The adult males are 4.5 feet (1.3 m) and females are 4.6 feet in length. Gestation period in franciscana dlophin is 10.5 months and calves are nursed for 9 months.

Analyses of stomach contents showed that Franciscana consumes a wide variety of bottom-dwelling fish species (Reeves and Brownell, 1989). The main prey items are Sciaenid and engrailed fish. Shrimp are also reported to be consumed by Franciscana (Reyes, 1991).

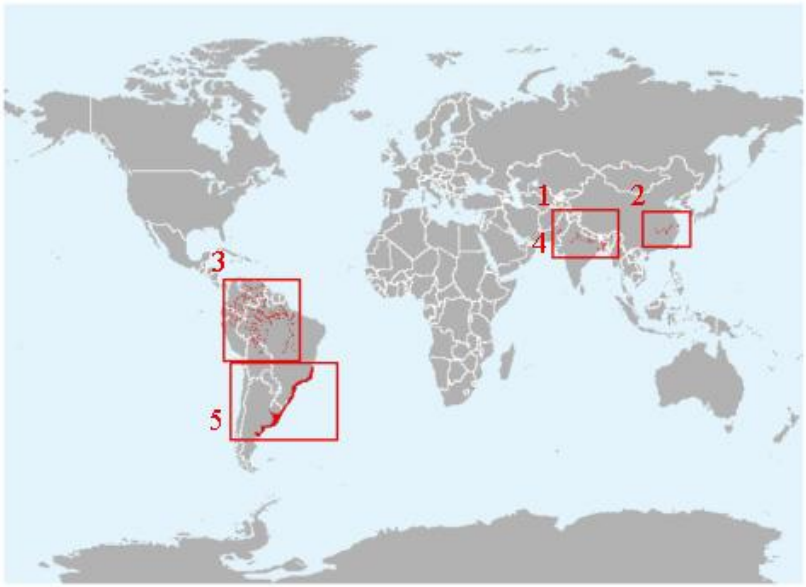

Fig 1. Distribution of fresh water dolphins in the world

1. Indus River Dolphin. **2**. Baiji Dolphin. **3**. Boto Dolphin. **4**. Gangese Dolphin. **5**. Franciscana Dolphin

Phylogenetic Analysis of Mitochondrial Genome: This study was undertaken to analyze the genetic variations at mitochondrial genome level between Indus dolphin and other fresh water dolphins. The sequence variability studies helped in better understanding of the evolutionary relationship among these dolphins.

When their mitochondrial genome sequences were aligned together using Clustal W for multiple sequence alignment, it was observed that Indus dolphin mitochondrial genome indicated 99 % homology with Ganges dolphin and 83 % homology with Baiji Dolhpin, 81 % homology with the Franciscana Dolphin and 82 % homology with the Boto Dolphin. The Baiji Dolphin has 85 % homology with the Boto Dolphin and 73 % homology with Ganges Dolphin and the Franciscana Dolphin while the Boto Dolphin has 83 % homology with the Franciscana Dolphin. The Ganges Dolphin has 66 % homology with the Franciscana Dolphin (Table 1).

Table 1. Percentage homology of fresh water dolphins with each other

	Homology of Indus Dolphin Mitochondrial genome with other dolphins			Homology of Baiji Dolphin Mitochondrial genome with other dolphins			Homology of Boto Dolphin Mitochondrial genome with other dolphins			Homology of Ganges Dolphin Mitochondrial genome with other dolphins	
1	Ganges Dolphin	99%	1	Boto Dolphin	85%	1	Baiji Dolphin	85%	1	Indus Dolphin	99%
2	Baiji Dolphin	83%	2	Indus Dolphin	83%	2	Franciscana Dolphin	83%	2	Baiji Dolphin	85%
3	Boto Dolphin	82%	3	Franciscana Dolphin	73%	3	Indus Dolphin	82%	3	Franciscana Dolphin	66%
4	Franciscana Dolphin	81%	4	Ganges Dolphin	73%	-		-	-		-

When we constructed the phylogentic tree, it shows that these species are very close to each other and have same decendants. It shows the evolutionary interrelations of different dolphin species. *P.minor, L.vexillifer, I. geoffrensis* and *P.blainvillei* are closely related as they are from same branche. But *P.gangetica* is not much related.

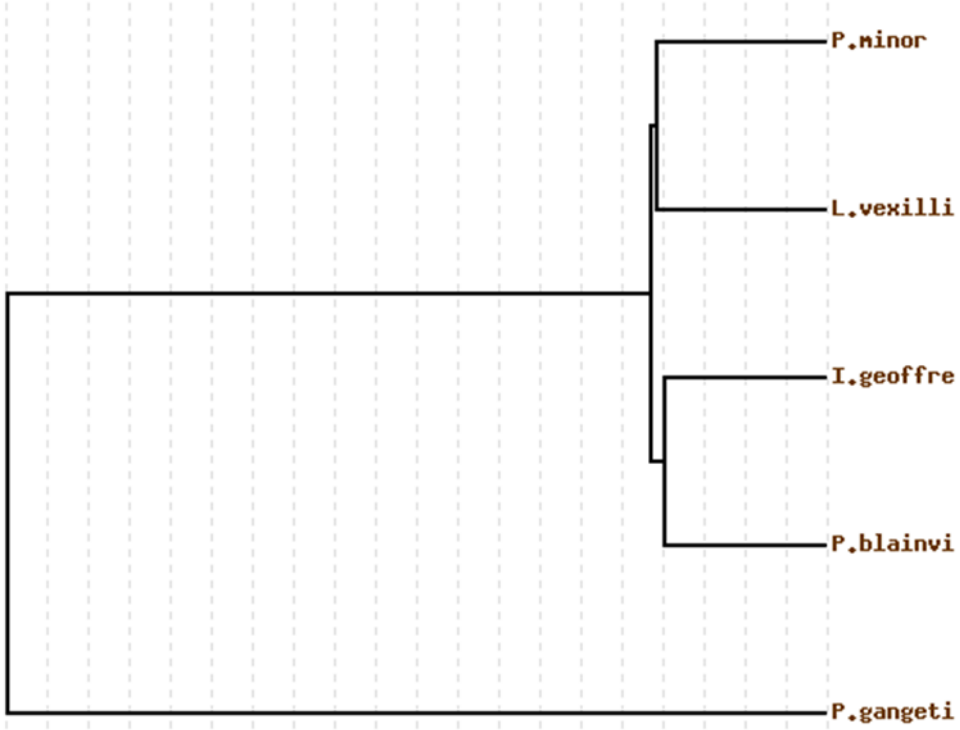

Fig. 2. Phylogenetic tree of different species of fresh water dolphins

A similar study was also carried out by a group of scientists in 2002 in which they used complete mitochondrial cytochrome-b gene sequences (Yang et al., 2002). Different studies showed very high sequence divergences among all river dolphin genera, suggesting a relatively longer period of separation time than those among other odontocete families. These species are same up to order level in classification system but differs in family, genus and species level. This difference indicates the difference at genetic level and these differences are due to the environmental conditions in the living zone of these fresh water dolphins.

Before 1970 Indus dolphin and Ganges dolphin were under category of same species but after 1970 these were designated as separate species due to differences in lipid composition and skull formation. After 1988 Indus Dolphin was designated as sub-species of Ganges dolphin but this should not be the sole basis for declaring sub-species because *Panthera leo persica* and *Panthera leo speleae* are two sub-species of Asiatic lions and they have a mitochondrial sequence homology of just 16 percent. In the same way the other fresh water dolphins have mitochondrial sequence homology range of 81 to 84 percent yet they are categorized under different genus and species level of classification. Also the habitats of these two species are very different from each other (Pilleri *et al*, 1982).

The fact that complete genome of Ganges dolphin has not been sequenced, therefore it is suggested that classification of Indus Dolphin as sub-species should be revised and a tedious research work combing with other

parameters such as anatomy, physiology, way of getting food, reproduction including sequence homology is required to eliminate the controversial classification of these dolphins. The approach to classify Indus Dolphin on the basis of complete mitochondrial genome could be very successful.

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