Reappraisal of the sub species hypothesis for *Gangetic dolphins* based on mitochondrial genomics

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Abstract

The Gangetic dolphin, *P. gangetica*, is one of the oldest known creatures, and its taxonomic status has been debated for over two centuries with no agreement. The clarification of the systematic relationships of *Platanista* taxa has been listed as among the highest priority for all cetaceans. Of the several taxonomic schemes proposed, all have been very limited and do not provide sufficient weight of evidence to defend any taxonomic classification. Consequently, to resolve this case, a new mitochondrial genome record for *P. gangetica gangetica* was created for quantitative evaluation of the Ganges and Indus River dolphin populations to estimate species boundaries. This has aided in the resolution of the taxonomic status of the Gangetic dolphin, *P. gangetica* by showing that the subspecies *P. gangetica gangetica* and *P. gangetica minor* are indistinguishable based on the molecular differences and do not follow the standards for molecular species boundaries. Also geographic historical reviews show that these two populations were one during the Miocene era, and that segregation occurred during the geosyncline effect of the Bay of Bengal. However, with the present set of evidences, we propose to abandon the subspecies hypothesis for these *P. gangetica* taxa.

Introduction

At present, two closely related but geographically separated subspecies of the Gangetic dolphin are recognized. These are the Ganges dolphin (*Platanista gangetica gangetica*) inhabiting the Ganges, Brahmaputra, and Karnaphuli river systems of India, Bangladesh, and Nepal, and the Indus River dolphin (*Platanista gangetica minor*) inhabiting the Indus River system of India and Pakistan. Over the past two centuries, there has long been debate on the true taxonomic status of these dolphins without a clear consensus being achieved.

A brief review of the historical attempts to taxonomically classify these creatures is as follows: First, the Ganges river dolphin was originally named *Delphinus gangetica* after being discovered and described in 1801 by William Roxburgh [1]. Later, however, Lesson [2] described the species as *Susu platanista*. In 1830 it was renamed again by Johann Wagler as *Platanista gangetica*. In a similar manner, the Indus River dolphin was named and described as *Platanista indi* by Blyth [3], but in 1976 was renamed *P. minor* due to discovery of an overlooked earlier description by Owen [4]. However, the first reported comparative analysis of the external morphology, skull morphology, and skeletal features of both the Ganges and Indus River dolphins found no evidence that would differentiate them as two species [5]. In the 1970s several other comparative studies were conducted, and these found evidence of morphological differentiation [6–8] that supported splitting these dolphins into two separate species. Nevertheless, Kasuya [8] abridged these two taxa into a single species containing two subspecies. Here, the Gangetic dolphin was designated as *P. gangetica gangetica* and the Indus dolphin was designated as *P. gangetica minor* along with the hope that these classification issues could be clarified through the use of new tools for taxonomic analysis in the future. To date, however, the taxonomic status of these two dolphin taxa has never been comprehensively reappraised, although it has been the subject of much speculation [9–10].

In addressing the changing taxonomic status of these dolphins, Rice [11] pointed out that most of the past studies were challenged by severe methodological flaws in terms of sampling, lack of statistical support and poor evidence to defend any particular taxonomic classification scheme. He concluded, however, that these taxa comprised a single species consisting of two subspecies, and this taxonomic classification has been used by the Society for Marine Mammalogy [12]. However, some authors have argued that the comparisons made for these taxa on various levels of morphology alone do not provide sufficient weight of evidence for a definitive classification scheme [13–23].

At the same time that the arguments over the sub-specific status for the Ganges and Indus dolphins have continued, these issues have also been brought to a higher priority by the need for a conservation policy [24]. As of now, both dolphins have seriously suffered from drastic declines in their populations across their entire ranges in both the rivers. In 1996 the IUCN changed their status from 'Vulnerable' to 'Endangered', in part because of events across the distribution of their ranges that severely affected their habitats and brought them to the verge of extinction. To resolve such controversies, especially for cases such as the Gangetic river dolphins, the use of alpha level taxonomy, combined with recognition of evolutionarily significant units within species, can be important components of conservation strategies directed towards the preservation of genetic diversity and evolutionary potential of these species.

The need to address challenges and limitations of resolving issues relating to the status of these *P. gangetica* species have prompted others to use molecular approaches [25–29]. Insights potentially gained from these approaches may also aid in reconstructing phylogenetic histories to delineate systematic relationships. In this study, we present the first quantitative evaluation of both the Ganges and Indus River dolphin and estimated species boundaries using a new set of complete mitochondrial genome data. Furthermore, our study also includes an analysis of the phylogenetic relationships among river dolphin clades as well and their relationships to other marine dolphins. The new data set generated through this mitochondrial genomics approach in combination with historical insights has advanced the understanding of the status of subspecies for *P. gangetica*.

Materials And Methods

Ethical statement

Tissue samples were obtained under permissions letter no. 4488, Dated 16.12.14 from the Principal Chief Conservator of Forests, Patna, Bihar (India). All methods were performed in accordance with the CPCSEA guidelines and regulations (http://cpcsea.nic.in/Content/55_1_GUIDELINES.aspx). We did not perform experimentation directly on live animals.

Sample collection, DNA extraction and amplification

Tissue samples from dead animals provided to us were washed and preserved in ethanol (95%), transported to the laboratory and stored at -50 °C until further processing. Genomic DNA was purified using the Promega wizard genomic DNA kit following manufacturer's instructions. Purified DNA was stored at -20 °C for downstream applications. For PCR amplification of the mitochondrial genome, two sets of primer were designed using Primer3 and published reference genome sequence information (Accession No. NC_005275.1) [30]. The complete mitochondrial genome was amplified using GoTaq Long PCR Master mix (Promega, Madison, USA) in two amplicons using newly designed primers (Supporting table S1). The reaction components were added following manufacturers' protocols, and thermal cycling conditions as follows: initial denaturation at 95 °C for 3 min, followed by 30 cycles at 94 °C for 30 s, annealing at 52-56 °C for 30 s, extension at 65 °C for 13 min, and a final extension at 72 °C for 10 min.

Cluster Generation and Sequencing

After quality checks, the libraries were loaded for cluster generation and sequencing on a NextSeq. Illumina platform (Illumina Inc. USA). Paired-end sequencing was used to have the template fragments sequenced in both the forward and reverse directions following manufacturer protocols.

De novo assembly and Annotation

High quality paired end data was generated denovo and assembled using CLC genomics workbench 6.0. Similarity searches were carried out against NCBI's Nucleotide (NT) database using BLASTN algorithm to identify scaffolds [31]. Scaffold #4 was used for annotation by the MITOS2 webserver because it showed significant similarity against the *P. minor* (AJ554058) reference mitochondrial genome [32]. Intergenic regions between the trnP and trnF genes was found to be 857 bp, and thus we suspected it as a control region. This was annotated using pairwise similarity searches (BLAST) against the control regions of reference mitochondrial genomes. Annotation of t-RNAs was confirmed using the MITOS 2 webserver and tRNAscan-SE v 2.0 [32-33]. The intergenic spacers and overlapping regions between genes were counted manually. The CG view webserver was used for preparing a circular map [34]. The relative synonymous codon usage (RSCU) of the protein coding genes was obtained using CodonW (Peden, n.d.).

Selection of taxa, Sequence alignment and Data partition

We selected data from 32 species of cetaceans as an in group and three species of Artiodactyla as an out group for rooting the phylogenetic tree (Supporting Table S2). For multiple sequence alignments, we used MAFFT version 7.273, which implements consistency based algorithms [35]. The L-INS-I algorithm was used for alignment. Partition Finder version 2.1.1 was used for data partition and choosing best

substitution model [36]. The model search for DNA sequences were set to “MrBayes”. The greedy algorithm was used, with branch lengths estimated, to search for the best-fit partitioning model. The branch lengths were set as linked.

Comparative mitogenomics

We compared the annotated mitochondrial genome of *P. gangetica gangetica* with mitochondrial genomes of 31 species (Supporting Table S2). Mitochondrial genomes were downloaded from NCBI refseq database [37]. The base composition of the mitogenomes in all taxa under study was calculated using MEGA 7.0 [38]. AT and GC asymmetries were calculated by $AT\ skew = (A - T\%)/(A + T\%)$ and $GC\ skew = (G - C\%)/(G + C\%)$ formula [39]. Rates of synonymous substitution (Ks), nonsynonymous substitutions (Ka) and ka/ks ratio were obtained using a computer program DnaSp ver. 4.0 [40].

Estimation of species boundary

Sequence divergence values within and among species were employed the Kimura two parameter (K2P) model [41] using analytical functions on MEGA 7.0. [38] for deciding species boundary as suggested by Hebert et al. [42]. A neighbor joining (NJ) tree based on K2P distance, nearest neighbor analysis (NN), and nucleotide composition values were also obtained using MEGA 7.0. The analysis of genetic distances was complemented by downloading of related sequences from GenBank [43].

Phylogenetic analysis

Phylogenetic relationships were constructed using the Bayesian inference method (BI) implemented through MrBayes v3.2.557 [44]. Here we used the GTR+ I+ G as an evolutionary model for 2 partitions and the HKY+ I+ G model for 1 partition. Four simultaneous Markov chains were run for 10 million generations, with tree sampling occurring every 1000 generations and a burn-in of 25% trees.

The Maximum Likelihood (ML) phylogeny was obtained using RAXML-HPC2 on the XSEDE (8.2.10) tool of the CIPRES Science gateway web portal based on the nucleotide sequences of the 13 protein-coding genes [45]. In the ML analyses, we used the GTRGAMMA for nucleotide sequences. For each analysis, 200 runs were conducted to find the highest-likelihood tree, followed by analysis of 1000 bootstrap replicates. NJ relationships were deduced with 1000 bootstrap in MEGA 7.0.

Results

Mitochondrial genome characteristics

Overview of the mitochondrial genome

This study reports on first annotated sequence of the mitochondrial genome of the Ganges river dolphin, *P. gangetica gangetica*. The sequence data obtained here have been deposited in GenBank under accession **MF 990206**. The annotated genome is double stranded molecule of 16563 bp (Fig. 1). It contains 37 genes which includes 13 protein coding genes (atp6, atp8, cytb, cox1 to cox3, and nad1 to nad6), two rRNA genes (rrnL, rrnS), 22 tRNAs and a D loop region (Table 1). Here we noticed 28 genes located on positive strand and nine genes on negative strand (Fig. 1) as well as two origins of replication region, OL (origin of “L” strand) which is of 31 bp and OH (origin of “H” strand) of 303 bp (Table 1).

tRNA Structure

Twenty two tRNA genes were observed which ranged from 66- 77 bp in size. Of these, 14 tRNAs were located on positive strand while eight were on the negative strand (Table 1 and Fig. 1). Also, all 22 of these tRNAs used unique antisense codons (Table 1).

Intergenic nucleotide arrangement

A total of 73 bp of non-coding intergenic nucleotides, ranging from 1 to 39 bp in size, were observed at 14 locations. The longest AT rich (61.53%) intergenic region of 39 bp was found between the cox1 and trnS2 genes. A total of 13 genes and one control region were found to be adjacent with no intergenic nucleotides. In addition, 10 genes were found to have overlaps ranging from 1 bp to 40 bp in length (Table 1). The largest overlap of intergenic nucleotides (40 bp) was observed between atp8 and atp6 while, the smallest overlaps (one bp) were noticed at four locations between OL-trnC; atp6-cox3; cox3-trnG and trnT–trnP respectively. Similarly, other gene overlaps were observed between trnI and trnQ (3 bp); nad2 and trnW (2 bp); nad4l and nad4 (7 bp) (Table 1).

Nucleotide composition

The nucleotide composition of the *P. gangetica gangetica* mitochondrial genome revealed that it is AT rich with strand asymmetry as reflected by AT and GC skew values. The AT skew value was found to be zero while the GC skew was -0.40. The nucleotide composition and GC percentage values of four river dolphins and 27 other marine cetacean species are depicted in Table 2.

Initiation and termination Codons

The initiation and termination codon bias for protein coding genes (PCG) was analysed and compared for four river dolphins and 27 other marine cetacean species (Table 3).

Codon usage

Among all protein coding genes, the Leu, Thr, Ile, Ser1, Pro, Ser2 amino acids were the most used whereas CUA, AUA, ACA and AUC were the most frequently used codons.

Gene order comparisons

The gene order predicted for *P. gangetica gangetica* was compared to other cetaceans. No significant changes in gene order were seen, but there were slight differences in some gene lengths (Table 1; Fig. 1).

Evolutionary rate of protein coding genes

To examine the evolutionary rate among the mitochondrial PCGs in cetaceans, values of non-synonymous substitutions (Ka), synonymous substitutions (Ks), and the ratio of Ka/Ks, (ω) were calculated for each PCG in the river dolphin species.

i. Evolutionary rate of PCGs of river dolphins and other cetaceans

The evolutionary rate was lowest (0.02) for the COX1 gene in the four river dolphin species. This can be compared the rate for the ND6 gene (0.32), which was highest (Fig. 2). Other cetacean species compared here showed the lowest evolutionary rate for the ND 5 gene (Fig. 2). Overall, all cetaceans have shown a relatively low evolutionary rate (<1), possibly suggesting that greater selection pressure, leading to the purifying selection, is acting on all PCGs.

Phylogenetic relationships

Comparative accounts

To deduce the phylogenetic relationships of *P. gangetica gangetica* in the river dolphins by clades, a comparative study was conducted by including previously published mitochondrial genomes of four river dolphin species and of 27 marine cetacean species (Supplementary Table S2).

Data partitioning resulted in three partitions as best suited for the evolutionary model. The first partition, GTR+I+G, included nine genes (NAD2, ATP8, ATP6, NAD4l, COB, NAD1, NAD4, NAD5 and NAD3). The second partition, GTR+I+G, included three genes (COX1, COX3 and COX2) while the third partition, HKY+I+G, included only a single gene ND6. Results of tests for tree topology are depicted in Table 4. Phylogenetic relationships of the river dolphins with other marine dolphin species was inferred using concatenated protein coding gene datasets, and this revealed that river dolphins are paraphyletic (Fig. 3). Also, the relationships of *P. gangetica gangetica* and four river dolphins revealed through analysis of the COI and CytB gene, is depicted in Fig. 4. This clearly resolves other river dolphin species but shows a mixed clade for *P. gangetica gangetica* and *P. gangetica minor*.

Comparison of genetic distance for species boundary

Genetic distances between *P. gangetica gangetica* and other four river dolphin species, as well as marine dolphins, were compared using various markers and genomic regions (Table 5). These values clearly differentiate all dolphin species (except *P. gangetica minor*) where intraspecific values were < 3%. Overall, this supports the applicability of mitochondrial genome analysis in detecting molecular differentiation between species. To further clarify species boundaries, a pairwise interspecies comparison was conducted for the four river dolphins (Table 6).

Discussion

Taxonomic assignments are of great importance for many reasons, yet their delimitation is often fraught with both conceptual and methodological difficulties [46]. The taxonomy of the Gangetic dolphins are one such example. Taxonomy issues for these dolphins have been debated for almost two centuries, largely because usable morphological characters as well as ambiguous fossil records were found to be largely confusing [14]. Resolution of such issues requires a comprehensive hypothesis that can be used to examine taxonomy through molecular and biogeographical explorations.

Over the past two decades, several evolutionary biologists have given considerable attention to studying the evolution of complete mitochondrial genome as a phylogenetic tool for systematic and comparative analysis and for resolving evolutionary relationships of a broad range of mammals and other organisms [47-49]. However, the necessary molecular data (nuclear and mitochondrial DNA sequences) to apply this approach to the Gangetic dolphins from India was lacking. The availability of such data on this species can serve as important genetic resource for advancing our knowledgebase about these and other taxa [13, 27].

To move in this direction, a new genetic resource of mitochondrial genome has been created here for *P. gangetica gangetica* from India. This species is similar to other cetaceans, particularly *P. gangetica minor*, in terms of several genomic features [50] (Table 1, 2 and 3). Using this genomic resource, the relationship of the river dolphin to the marine dolphin has been clarified based on BI and ML analysis

(Fig. 3). This revealed that river dolphins are paraphyletic in their origin, consistent to findings by others [13-15, 51-52] including past morphological studies noting that the Platanista are not closely related to the river dolphins [53, 16]. Also, as compared to other river dolphins, specifically the Ziphiidae, the Platanista show a basal position in the phylogeny (Fig. 3), indicating that *P. gangetica* has more ancestral characters than other river dolphins [54].

The evolutionary rate (ω) estimated here for the river dolphin species, in general, is substantially higher than that seen for marine dolphin species (Fig. 5). This is consistent with the suggestion that marine organisms are less diverged as compared to other aquatic and terrestrial taxa at the level of mitochondrial DNA, for ex. Bloom [55]. In the river dolphin, the COI gene showed the lowest evolutionary rate (0.03). In marine dolphins, the lowest rate was seen in the ND5 gene (0.01). This suggests that both genes can be potentially be used for in molecular taxonomic studies of differentiation of the species (Fig. 2) [56-57]. However, these findings also raised concern about the inferences made in previous studies about the river dolphin/marine dolphin relationships using genes like Cyt B that show higher evolutionary rates (>0.1) [58, 26-29] or other segments such as hyper variable D-loop regions or non-coding regions [58, 27].

In such cases, phylogenetic inferences may reflect soft polytomy as suggested for the case of the Gangetic dolphin [27]. Here, based on the lowest evolutionary rates (Fig. 2, Table 5 and 6), it can hypothesized that the river dolphin species can be best discriminated using both intraspecific and interspecific distances based on COI gene sequences. Marine dolphin species, however, can be discriminated using these same distances derived from the ND5 gene [57, 59-60]. These observations emphasize the importance of sequencing complete mitochondrial genomes to explore alternative hypotheses for molecular phylogenetics and species differentiation [61].

At the intraspecific level, the data in tables 5 & 6 show that taxonomic assignments for all river dolphin species could be resolved based on the criteria of a 3% threshold for species discrimination using molecular data as proposed by [42]. Here, these genes do not differentiate between *P. gangetica gangetica* and *P. gangetica minor* because genetic difference values of only 0.3% were observed, well within the species boundary suggested by [42]. The intraspecific distances at four genetic locus viz. the complete mitochondrial genome (1.4%), COI gene (0.34%), Cyt B (0.6%) and 13 protein coding genes (2.43%) were also all below this threshold value (Table 6). This clearly supports the idea that *P. gangetica gangetica* and *P. gangetica minor* are not two separate biological species, but represent instead a single biological species. These findings are highly relevant to suggestions by Anderson [5] and Kasuya [62]. Furthermore, the phylogenetic analysis supports the same conclusion with high statistical support (Fig. 4) confirming that *P. gangetica gangetica* and *P. gangetica minor* as also being indistinguishable. These results should help bring an end to the lengthy debate on species status of *P. gangetica* which has been ongoing for the past two centuries [1-12, 64-65]. Similar low intraspecific values have also been observed by others for these two taxa [14, 27], supporting the observations made here. In summary, it can be concluded that two populations of *P. gangetica* analysed here in fact belong to one biological species,

and the concept of subspecies proposed as by Kasuya [8] is refuted because the mitochondrial genomics do not support the sub species concept for this case [66].

The conclusion of the Committee on Taxonomy [12], accepting that the Gangetic dolphin *P. gangetica* is comprised of two subspecies, *P. gangetica gangetica* and *P. gangetica minor*, a valid parameter that might have been taken into consideration was the presence of two different river habitats (the Ganges and the Indus). The term subspecies was introduced by Eugenius Esper to represent the lowest possible taxonomic unit to enhance understanding of geographic varieties and speciation within animals [66-68]. However, the two *P. gangetica* populations (the Gangetic and Indus) studied here also do not show any morphological difference that may correlate with their evolutionary histories [69-70] or genetic differences to justify them being recognized as geographic varieties [67], except perhaps their unique habitats [68]. Thus our observations can add important insights into the debate on concept of subspecies [68, 71-74] since Linnaean taxonomy, including the identification of subspecies, has been predominantly based on morphological data and has operated under the frameworks of the morphological and biological species concepts [68, 75].

For these dolphins, although several studies have been undertaken, none can establish evidence consistent with either species concept [1–12, 63, 65-66]. It is well known that dependence on morphology to determine subspecies status can be challenging because morphological differences could simply be due to phenotypic plasticity and not unique evolutionary histories [76]. Additionally, as in the case of cryptic species, a lack of morphological difference does not necessarily indicate a lack of unique evolutionary histories [76-77]. Finally, since the use of the subspecies designation is rather subjective nature [76, 78-79], it does not seem to be appropriate to describe the two populations of *P. gangetica* as sub species simply based on geographical isolation [68], even though others have emphasized the prominence of geography in making this distinction [80-82].

For any further discussion of this issue, it will be necessary to consider the history of the origin of the Indus, Brahmaputra and the Ganges rivers. According to Pascoe [83] (1919), during the Eocene, within the Indian subcontinent, a gulf extended from Sind northwards to Afghanistan, then turned eastwards and south-eastwards through the Kohat and the Punjab extending a path to a great river, the head-waters of which consisted of the portion of the Brahmaputra flowing through Assam (Fig. 6). This river streamed west and north-westwards along the foot of the Himalayas as far as the North-West Punjab, where it turned southwards along a line of the modern Indus, and emptied to the Arabian Sea. This shows that the Assam Brahmaputra was once the origin of the river Indus, and it also means that the current two populations of *P. gangetica* both originated from one source- the Brahmaputra (Fig. 6). This is clear evidence that the Ganges and Indus populations became segregated during the geosyncline effect of the Bay of Bengal when these three river basins took their shape [83]. At the present, the two populations of the Gangetic dolphins still do not show any evidence of remarkable evolutionary adaptations in their morphologies [6-9]. This may be because, as an endothermic organism, the rate of evolution must be prohibitively low [84]. Furthermore, both the Ganges and Indus rivers are confined to a narrow latitudinal gradient (24° 45' 19.1282" to 25° 32' 15" North) and do not differ much in terms of the climatic

conditions, their origin, flow rate, basin width and depths, water availability, food and competition, etc. This might have allowed for similar selection pressures on both dolphin populations so that organisms inhabiting these two river systems would show similar evolutionary rates as has clearly been evidenced from mitochondrial genome data presented here (Tables 1, 2, 3 and Fig. 5) [85-90]. Accordingly we are proposing a hypothesis as depicted in Fig. 6, to apprise the sub species concept for the Gangetic dolphin.

Ball and Avise [91] have suggested that it is appropriate to define subspecies based on populations with major divisions in genetic diversity or that are phylogenetically distinct [92]. In our analysis, we rejected the molecular support to this subspecies concept when we conducted COI and Cytb gene based phylogenetic analysis for river dolphins, and we found that the two subspecies are phylogenetically undistinguishable and tightly cluster in a single clade with high boot strap support (Fig. 4 a & b). These finding are highly relevant to the arguments to define subspecies based on congruence amid data such as morphology, genetics, and ecology [75, 93-94]. Similarly, several studies have reported taxonomic non-consensus based on morphological versus molecular data [72-73, 76, 95] where reclassification of subspecies was undertaken [95-97, 70]. Several such examples have now prompted scientists to minimize the use of the subspecies taxonomic rank [80, 98], although there are others opposed this opinion [99].

Based on this, we are proposing to amend the taxonomic status of *P. gangetica*. Finally, although we are of opinion that the two *P. gangetica* populations are genetically indistinguishable and should not be described as subspecies, we do suggest that further scientific efforts be directed toward corroborating the biological species concept through breeding experimentations involving these two geo types to confirm the evolution of any reproductive isolation among these two populations. However, for the present, based on the evidence described here, we propose to abandon subspecies designation for *P. gangetica*.

Conclusion

We propose here a resolution of the debate occurring over two centuries on the taxonomic status of the Gangetic dolphin, *P. gangetica*, through the use of mitochondrial genomics. The study has provided a molecular basis to confirm that the subspecies *P. gangetica gangetica* and *P. gangetica minor*, recognized as geographic varieties, do not follow the criteria for species boundary and are in fact indistinguishable based on the molecular differences. Also, historical reviews show that these two populations were part of a single population during the Miocene era, and that they became segregated during the geosyncline effect of the Bay of Bengal when the river basins separated along with these dolphin populations. Further, through phylogenetic analysis it was established that river dolphins are paraphyletic and no relationship prevails between *P. gangetica* and other river dolphin species. Based on various comparisons and this historical review, we propose to abandon the subspecies designations used for *P. gangetica*.

Declarations

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Authors Contribution GDK, CDK, NP conceiving research idea, CJ collected samples and conducted laboratory experiments, GDK, CDK analysed data and GDK, CDK, BP wrote manuscript.

Competing interests: The authors do not have conflict of interest to declare.

References

- [1] Pilleri, G. William Roxburgh (1751-1815), Heinrich Julius Lebeck (†1801) and the discovery of the Ganges dolphin (*Platanista gangetica* Roxburgh, 1801). *Investigations on Cetacea*. 9, 11- 21 (1978). [2] Lesson, R. P. *Histoire naturelle générale et particulière des mammifères et des oiseaux découverts depuis 1788 jusqu'à nos jours*. 442pp (1828). [3] Blyth, E. On the Rorqual of the Indian Ocean, with notices of other cetals, and of the sirenia or marine pachyderms. *J Asiatic Soc Bengal*, 28, 481–498 (1859). [4] Owen, R. *Descriptive Catalogue of the Osteological Series Contained in the Museum of the Royal College of Surgeons. Volume II. Mammalia Placentalia*. Taylor and Francis, London. (1853). [5] Anderson, J. *Anatomical and Zoological Researches: Comprising an Account of Zoological Results of the Two Expeditions to Western Yunnan in 1868 and 1875; and a Monograph of the Two Cetacean Genera, Platanista and Orcella*. Bernard Quaritch, London, UK (1879). [6] Pilleri, G., Gühr, M. Differences observed in the skull of *Platanista gangetica* (Roxburgh, 1801) and *Platanista minor* (Blyth, 1859). *Investigations on Cetacea*. 3, 13-21 (1971). [7] Pilleri, G., Gühr, M. Osteological differences in the cervical vertebrae of *Platanista indi* and *gangetica*. *Invest Cetacea*. 7, 105–108 (1976a). [8] Kasuya, T. Some information on the growth of the Ganges dolphin with a comment on the Indus dolphin. *The Scientific Reports of the Whales Research Institute*. 24, 87–108 (1972). [9] Pilleri, G., Marcuzzi, G., Pilleri, O. Speciation in the *Platanistoidea*, systematic, zoogeographical and ecological observations on recent species. *Investigations on Cetacea*. 14, 15–46 (1982). [10] Reeves, R. R., Brownell, R. L. Jr. *Sussex Platanista gangetica* (Roxburgh, 1801) and *Platanista minor* Owen, 1853. Pp. 69-99 in SH Ridgway and RJ Harrison (eds.), *Handbook of Marine Mammals, Vol.4 River Dolphins and the Larger Toothed Whales*, Academic Press, London, UK, 430 pp 69–99 (1989). [11] Rice, D. W. *Marine mammals of the world: systematics and distribution*. The Society for Marine Mammalogy Special Publication. 4, 1-231 (1998). [12] Committee on Taxonomy. List of marine mammal species and subspecies. Society for Marine Mammalogy, <http://bit.ly/2dfBhY3> (2017). [13] Nikaido, M., Matsuno, F., Hamilton, H., Brownell, R. L., Jr., Cao, Y., Ding, W., Zuoyan, Z., Shedlock, A.M., Fordyce, R. E., Hasegawa, M., Okada, N. Retroposon analysis of major cetacean lineages: the monophyly of toothed whales and the paraphyly of river dolphins. *Proc Natl Acad Sci USA*. 1998, 7384– 7389 (2001). [14] Hamilton, H., Caballero, S., Collins, A. G., Brownell, J. Evolution of river dolphins. *Proc Royal Society B*:

Biol Sci. 268(1466), 549–556. <https://doi.org/10.1098/rspb.2000.1385> (2001). [15] Cassens, I., S. Vicario, V. H. Waddell, H. Balchowsky, D. Van Belle, W. Ding, C. Fan, R. S. Lai Mohan, P. C. Simoes-Lopes, R. Bastida, A. Meyer, M. J. Stanhope and M. C. Milinkovitch. Independent adaptation to riverine habitats allowed survival of ancient cetacean lineages. *Proc Nat Acad Sci.* 97, 11343–1134 (2000) [16] Messenger, S.L., McGuire, J.A. Morphology, molecules, and the phylogenetics of cetaceans. *Syst. Biol.* 47, 90– 124 (1998). [17] Arnason, U., Gullberg, A. Sequence analyses of the mitochondrial cytochrome b gene identify five primary evolutionary lineages of extant cetaceans. *Mol Biol Evol.* 13(2):407-17 (1996). [18] Fordyce, R. E., Barnes, L. G. The evolutionary history of whales and dolphins. *Annu Rev Earth Planet Sci* 22, 419– 455 (1994). [19] Messenger, S. Phylogenetic relationships of platanistoid river dolphins (Odontoceti, Cetacea): assessing the significance of fossil taxa. *Contributions in Marine Mammal Paleontology Honoring Frank Whitmore Jr. Proceedings of the San Diego Society of Natural History*:125-133 (1994). [20] Milinkovitch, M. C., Meyer, A., Powell, J. R. Phylogeny of all major groups of cetaceans based on DNA sequences from 3 mitochondrial genes. *Mol. Biol. Evol.* 11, 939-948 (1994) [21] Heyning, J. E. Comparative facial anatomy of beaked whales (Ziphiidae) and a systematic revision among the families of extant Odontoceti. *Contrib. Sci. Aat. Hist. Mus. LA County.* 405, 1-64 (1989). [22] Kaiya, Z. A project to translocate the Baiji, *Lipotes vexillifer*, from the main-stream of the Yangtze River to Tongling Baiji semi-nature reserve. *Aqu. Mammals*, 12(1), 21-24 (1986) [23] Kasuya, T. Systematic consideration of Recent toothed whales based on the morphology of the tympano-periotic bone. *Sci. Rep. Whales Res. Inst.* 24, 87-108 (1973). [24] Reeves, R. R., Perrin, W. F., Taylor, B. L., Baker, C. S., & Mesnick, S. L., (eds). Report of the Workshop on Shortcomings of Cetacean Taxonomy in Relation to Needs of Conservation and Management, April 30–May 2, 2004 La Jolla, California. NOAA Technical Memorandum NOAA-TM-NMFS-SWFSC-363, 1-94 (2004). [25] Yang, G., Zhou, K. A study on the molecular phylogeny of river dolphins. *Acta Theriol Sinica.* 19, 1-9 (1999). [26] Verma, S. K., Sinha, R. K., Singh, L. Phylogenetic position of *Platanista gangetica gangetica*: insights from the mitochondrial cytochrome b and nuclear inter photoreceptor retinoid-binding protein gene sequences. *Mol Phylog Evol.* 33, 280-288 (2004). [27] Braulik, G. T., Barnett, R., Odon, V., Islas-Villanueva, V., Hoelzel, A. R., Graves, J. A. One species or two? Vicariance, lineage divergence and low mtDNA diversity in geographically isolated populations of South Asian River dolphin. *J Mammal Evol.* 22, 111-120. <https://doi.org/10.1007/s10914-014-9265-6> (2015). [28] Ahrens, D., Fujisawa T., Krammer H. J., Eberle, J., Fabrizi, S., Vogler, A. P. Rarity and incomplete sampling in DNA-based species delimitation. *Systematic Biol.* 65, 478-494 (2016). [29] Reddy, Sushma., Rebecca, T. Kimball., Pandey, Akanksha., Peter, A. Hosner., Michaelj, Braun., Shannon, J., Hackett., Kin-lan, Han., John, Harshman., Christopher, J. Huddleston., Sarah, Kingston., Ben, D. Marks., Kathleen, J. Miglia., William, S. Moore., Frederick, H. Sheldon., Christopher, C. Witt., Tamaki, Yuri., Edward, L. Braun. Why do phylogenomic data sets yield conflicting trees? data type influences the avian tree of life more than taxon sampling, *Syst Biol.* 0(0), 1–23 (2017). [30] Untergasser, A., Nijveen, H., Rao, X., Bisseling, T., Geurts, R., Leunissen, J. A. Primer3Plus, an enhanced web interface to Primer3. *Nucleic Acids Res.* 2007 Jul;35 (Web Server issue): W71-4. doi: 10.1093/nar/gkm306. Epub 2007 May 7. [31] Johnson, M., Zaretskaya, I., Raytselis, Y., Merezuk, Y., McGinnis, S., Madden, T. L. NCBI BLAST: a better web interface. *Nucleic Acids Res.* 36, W5-W9 (2008) [32] Bernt, M., Donath, A., Jühling, F., Externbrink, F., Florentz, C., Fritzsch, G., Pütz, J., Middendorf, M., Stadler, P. F. MITOS: improved de novo metazoan mitochondrial genome annotation. *Mol*

Phylogenet Evol. 69(2), 313-319. doi: 10.1016/j.ympev.2012.08.023. (2013). [33] Lowe, T. M., Chan, P. P. tRNAscan-SE On-line: integrating search and context for analysis of transfer RNA genes. *Nucleic Acids Res.* 8(44), 54-57. doi: 10.1093/nar/gkw413 (2016). [34] Grant, J. R., Stothard, P. The CGView Server: a comparative genomics tool for circular genomes. *Nucleic Acids Res.* 36(Web Server issue), W181-4. doi: 10.1093/nar/gkn179. (2008). [35] Katoh, K., Standley, D. M. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol.* 30(4), 772-80. doi: 10.1093/molbev/mst010. (2013). [36] Lanfear, R., Brett C., Simon, Y. W. Ho, Stephane Guindon. PartitionFinder: Combined Selection of Partitioning Schemes and Substitution Models for Phylogenetic Analyses, *Mol. Biol. Evol.* 29(6), 1695–1701. <https://doi.org/10.1093/molbev/mss020> (2012) [37] Pruitt, K. D., Tatusova, T., Maglott, D. R. NCBI reference sequences (RefSeq): a curated non-redundant sequence database of genomes, transcripts and proteins. *Nucleic Acids Res.* 35(Database issue):D61-5. doi: 10.1093/nar/gkl842. Epub (2006). [38] Kumar, S., Stecher, G., Tamura, K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol Biol Evol.* 33(7):1870-4. doi: 10.1093/molbev/msw054. Epub (2016). [39] Hassanin, A., Léger, N., & Deutsch, J. Evidence for multiple reversals of asymmetric mutational constraints during the evolution of the mitochondrial genome of metazoa, and consequences for phylogenetic inferences. *Systematic biology*, 54(2), 277–298. <https://doi.org/10.1080/10635150590947843> (2005). [40] Librado, R., Rozas, J. Librado P, Rozas J. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, 25(11), 1451-1452. doi: 10.1093/bioinformatics/btp187. Epub (2009). [41] Kimura, M. A simple method for estimating evolutionary rate of basesubstitutions through comparative studies of nucleotide sequences. *J Mol Evol.* 16, 111–120 (1980). [42] Hebert, P. D. N., Cywinska, A., Ball, S. L., DeWaard, J. R. Biological identifications through DNA barcodes. *Proc Royal Society B: Biol Sci.* 270(1512), 313–321. <https://doi.org/10.1098/rspb.2002.2218> (2003). [43] Wiemers, M., Fiedler, K. Does the DNA barcoding gap exist? – a case study in blue butterflies (Lepidoptera: Lycaenidae). *Front Zool.* 4, 8 (2007). [44] Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A., Huelsenbeck, J. P. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst Biol.* 61(3), 539-42. doi: 10.1093/sysbio/sys029. (2012) [45] Miller, F., H. Osbahr, E. Boyd, F. Thomalla, S. Bharwani, G. Ziervogel, B. Walker, J. Birkmann, S. Van der Leeuw, J. Rockström, J. Hinkel, T. Downing, C. Folke, D. Nelson. Resilience and vulnerability: complementary or conflicting concepts?. *Ecology and Society* 15(3): 11. [online] URL: <http://www.ecologyandsociety.org/vol15/iss3/art11> (2010) [46] Thomson, S., Pyle, R. L., Ahyong, S. T. Alonso-Zarazaga, M. A., Ammirati, J., Araya, JF. et al., Taxonomy based on science is necessary for global conservation. *PLoS Biol.* 16(3), e2005075. <https://doi.org/10.1371/journal.pbio.2005075> (2018). [47] Arnason, U., Adegoke, J., Bodin, K., Born, E., Esa, Y., Gullberg, A., Nilsson, M., Short, R., Xu, X., Janke, A. Mammalian mitogenomic relationships and the root of the eutherian tree. *Proceedings of the National Academy of Sciences of the United States of America.* 99. 8151-6. 10.1073/pnas.102164299 (2002). [48] Brown, W., George, M. J., Wilson, A.C. Rapid evolution of animal mitochondrial DNA. *Proc Natl Acad Sci USA* 76:1967- 1971. *Proceedings of the National Academy of Sciences of the United States of America.* 76. 1967-71. 10.1073/pnas.76.4.1967. (1979). [49] Simon, C., Thomas, R., Buckley, F. F., James, B. Stewart, Beckenbach, A. T. *Ann. Rev. Ecol. Evol. Syst.* 37(1), 545-579 (2006). [50] Lee, Kyunglee., JunMo,

Lee., Hawsun, Sohn., Yuna, Cho., Young-Min, Choi., Hye, Kwon., Kim, Ji., Hyung, Kim., Dae, Gwin. Jeong. Complete mitochondrial genome of the Pacific white-sided dolphin *Lagenorhynchus obliquidens* (Cetacea: Delphinidae) *Conservation Genet Resour* (2018) 10, 201–204 (2018). [51] Steeman, M. E., Hebsgaard, M. B., Fordyce, R. E., Ho, S. Y., Rabosky, D. L., Nielsen, R., Rahbek, C., Glenner, H., Sørensen, M. V., & Willerslev, E. Radiation of extant cetaceans driven by restructuring of the oceans. *Systematic biology*, 58(6), 573–585. <https://doi.org/10.1093/sysbio/syp060> (2009). [52] Yan J., Zhou K., Yang G. Molecular phylogenetics of ‘river dolphins’ and the baiji mitochondrial genome. *Mol. Phylo- genet. Evol.* 37: 743–750. (2005). [53] Heyning, J. E. Comparative facial anatomy of beaked whales (Ziphiidae) and a systematic revision among the families of extant Odontoceti. *Nat. Hist. Mus. Los Angel. Cty. Contrib. Sci.* 405, 1- 64 (1989). [54] Kasuya, T. Systematic consideration of recent toothed whales based on the morphology of tympano-periotic bone. *Sci. Rep. Whales Res. Inst.* 25: 1- 103. (1973). [55] Bloom, D. D., Weir, J. T., Piller, K. R., Lovejoy, N. R. Do freshwater fishes diversify faster than marine fishes? A test using state dependent diversification analyses and molecular phylogenetics of new world silversides (Atherinopsidae). *Evolution*, 67(7), 2040–2057. <https://doi.org/10.1111/evo.12074> (2013). [56] Santos V., Tixier, M. S. Which molecular markers for assessing which taxonomic level? The case study of the mite family Phytoseiidae (Acari: Mesostigmata). *Cladistics*, 33: 251-267. <https://doi.org/10.1111/cla.12166> (2017). [57] Patwardhan, A., Ray, S., Roy, A. Molecular markers in phylogenetic studies: A Review. *J Phylogenetics Evol Biol.* 2, 131–140. doi:10.4172/2329-9002.1000131 (2014). [58] Hamilton, C. A., Formanowicz, D. R., Bond, J. E. Species delimitation and phylogeography of *Aphonopelma hentzi* (Araneae, Mygalomorphae, Theraphosidae): cryptic diversity in North American tarantulas. *PLoS One* 6: e26207 (2011). [59] Dabert, M. DNA markers in the phylogenetics of the Acari *Biol Lett* 43, (2) (2006). [60] Cruickshank, R. H. Molecular markers for the phylogenetics of mites and ticks. *Syst Appl Acaro.* 7(0), 3. <https://doi.org/10.11158/saa.7.1.1> (2002). [61] Oxusoff, L., Préa, P., Perez, Y. A complete logical approach to resolve the evolution and dynamics of mitochondrial genome in bilaterians. *PLoS ONE* 13(3), e0194334. <https://doi.org/10.1371/journal.pone.0194334> (2018). [62] Kasuya, T., A. K. M. A. Haqde. Some informations on the distribution and seasonal movement of the Ganges dolphin. *Sci. Rep. Whales Res. Inst.*, 24, 109-115 (1972). [63] Wagler, J. *Natürliches System der Amphibien, mit vorangehender Classification der Säugthiere und Vögel ein Beitrag zur vergleichenden Zoologie.* München, Stuttgart und Tübingen (1830). [64] Blyth E. On the great rorqual of the Indian Ocean, with notices of other cetals, and of the syrenia or marine pachyderms. *J. Asiatic Soc. Bengal* 28:481–498 (1859). [65] Pilleri, G. Preliminary analysis of the lipids present in the blubber of *Platanista indi* and *gangetica*. *Investigations on Cetacea*, 3, 51– 52 (1971). [66] Torstoma, Shannon. M., Kevin, L. Panglea., Bradley, J. Swanson. Shedding subspecies: The influence of genetics on reptile subspecies taxonomy, *Mol Phylogenetics Evol.* 76, 134-143 (2014). [67] Mayr, E. Speciation and macroevolution. *Evolution* 36, 1119–1132 (1982) [68] Braby, M. F., Eastwood, R., Murray, N. The subspecies concept in butterflies: Has its application in taxonomy and conservation biology outlived its usefulness? *Biol J Linn Soc.* <https://doi.org/10.1111/j.1095-8312.2012.01909.x> (2012). [69] Latch, E. K., Heffelfinger, JR., Fike, J. A., Rhodes, O. E. Specieswide phylogeography of North American mule deer (*Odocoileus hemionus*): cryptic glacial refugia and postglacial recolonization. *Mol Ecol.* 18, 1730–1745 (2009). [70] Hafner, M., Renwick, N., Brown, M., Mihailoviæ, A., Holoch, D., Lin, C., Pena, J. T. G., et al. RNA-ligase-dependent biases in

miRNA representation in deep-sequenced small RNA cDNA libraries. *RNA* (New York NY). 17(9), 1697–1712 (2011). [71] Vane-Wright, R.I. Evidence and identity in butterfly systematics. Pp. 477-513 in C. L. Boggs, W. B. Watt, and P. R. Ehrlich, eds. *Butterflies: ecology and evolution taking flight*. Univ. of Chicago Press, Chicago (2003). [72] Winker, K. Reuniting Phenotype and Genotype in Biodiversity Research. *BioScience*. 59(8), 657–665. <https://doi.org/10.1525/bio.2009.59.8.7> (2009). [73] Karberg, J. M., Gale, M. R. Exploration of meaningful subspecies definitions of the carnivorous Northern Pitcher Plant (*Sarracenia purpurea*), throughout its geographic distribution. *Cons Gene*. 11(6), 2369–2378. <https://doi.org/10.1007/s10592-010-0123-7> (2010). [74] Zink, R. M. The role of subspecies in obscuring avian biological diversity and misleading conservation policy. *Proc Royal Society B: Biol Sci*. 271(1539), 561–564. <https://doi.org/10.1098/rspb.2003.2617> (2004). [75] Padial, J. M., Miralles, A., De la Riva, I., Vences, M. The integrative future of taxonomy. *Fron Zool*. <https://doi.org/10.1186/1742-9994-7-16> (2010). [76] Vane-Wright, R. I., Tennent, W. J. Colour and size variation in *Junonia villida* (Lepidoptera, Nymphalidae): subspecies or phenotypic plasticity? *Syst Biodiv*. 9(4), 289–305 (2011). [77] Bickford, D., Lohman, D. J., Sodhi, N. S., Ng, P. K. L., Meier, R., Winker, K. et al. Cryptic species as a window on diversity and conservation. *Trends Ecol Evol*. <https://doi.org/10.1016/j.tree.2006.11.004> (2007). [78] Amadon, D. The seventy-five per cent rule for subspecies. *The Condor*. 51, 250–258 (1949). [79] Wilson, E. O., Brown, W. L. The subspecies concept and its taxonomic application. *Syst Zool*. 2, 97-110 (1953). [80] Mayr, E., Ashlock, P. D. *Principles of systematic zoology*. New York, McGraw-Hill. (1969). [81] Dobzhansky, T. *Genetics of the evolutionary process* (Vol. 139). New York: Columbia University Press (1970). [82] Cronin, M. A. The Preble's meadow jumping mouse: subjective subspecies, advocacy and management. *Ani Cons*. 10, 159–161 (2007). [83] Pascoe, E. H. Early History of the Indus, Brahma putra and Ganges. *Quart Joursi Geol Soc*. 75, 136 (1919). [84] Gillooly, J. F., Allen, A. P., West, G. B., Brown, J. H. The rate of DNA evolution: Effects of body size and temperature on the molecular clock. *PNAS, USA*. 102(1), 140–145. <https://doi.org/10.1073/pnas.0407735101> (2005). [85] Gary, G., Mittelbach., Douglas, W., Schemske., Howard, V., Cornell., Andrew, P., Allen., Jonathan, M., Brown., Mark, B., Bush., Susan, P., Harrison., Allen, H., Hurlbert., Nancy, Knowlton., Harilaos, A., Lessios., Christy, M., McCain., Amy, R., McCune., Lucinda, A., McDade., Mark, A., McPeck., Thomas, J., Near., Trevor, D., Price., Robert, E., Ricklefs., Kaustuv, Roy., Dov, F., Sax., Dolph, Schluter., James, M., Sobel., Michael, Turelli., *Evolution and the latitudinal diversity gradient: speciation, extinction and biogeography*. *Ecol Lett*. 10, 315–331 (2007). [86] Wallace, A. R. *Tropical Nature and Other Essays*. Macmillan, New York, (1878). [87] Dobzhansky, T. *Evolution in the tropics*. *Am Sci*. 38, 209–221 (1950). [88] Willig, M. R., Selcer, K. W. Bat species diversity gradients in the New World: a statistical assessment. *J Biogeo*. 16, 189–195 (1989). [89] Kaufman, D. M., Willig, M. R. Latitudinal patterns of mammalian species richness in the New World: the effect of sampling method and faunal group. *J Biogeo* 25, 795–805 (1998). [90] Stevens, R. D., Willig, M. R. Geographical ecology at the community level: Perspectives on the diversity of new world bats. *Ecology*. 83(2), 545–560. [https://doi.org/10.1890/0012-9658\(2002\)083\[0545:GEATCL\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2002)083[0545:GEATCL]2.0.CO;2) (2002). [91] Ball, R., Avise, M. J. C. Mitochondrial DNA phylogeographic differentiation among avian populations and the evolutionary significance of subspecies. *Auk*. 109, 626-63 (1992). [92] Crandall, K. A., Bininda-Emonds, ORP, Mace, G. M., Wayne, R. K. Considering evolutionary processes in conservation biology. *Tren Ecol Evol*. 15, 290–295 (2000). [93] Mayr, E. *Systematics and the origin of species*. Columbia University Press, New York (1942).

[94] Vignieri, S. N., Hallerman, E. M., Bergstrom, B. J., Hafner, D. J., Martin, A. P., Devers, P., Hitt, N. Mistaken view of taxonomic validity undermines conservation of an evolutionarily distinct mouse: A response to Ramey et al. (2005) *Animal Conservation*. <https://doi.org/10.1111/j.1469-1795.2006.00038.x> (2006). [95] Laurence, W.F., Carolina Useche, D., Shoo, L.P., Herzog, S.K., Kessler, M., Escobar, F., Brehm, G., Axmacher, J.C., Chen, I.-C., Gámez, L.A. Global warming, elevational ranges and the vulnerability of tropical biota. *Biol. Conserv.* 144,548–557.<http://dx.doi.org/10.1016/j.biocon.2010.10.010> (2011). [96] Johnson, E., Jarecki, J., Krasnow, M. Oxygen-dependent patterning of tracheal branching is mediated by the branchless FGF. *A Dros Res Conf.* 40, 139 (1999). [97] Cullingham, C. I., Pond, B. A., Kyle, C. J., Rees, E. E., Rosatte, R. C., White, B. N. Combining direct and indirect genetic methods to estimate dispersal for informing wildlife disease management decisions. *Mol Ecol.* 17(22), 4874–4886. <https://doi.org/10.1111/j.1365-294X.2008.03956.x> (2008). [98] Ryder, O. A. Species conservation and systematics: The dilemma of the subspecies. *Tre Ecol Evol.* 1, 9–10 (1986). [99] Gippoliti, S., Amori, G. The problem of subspecies and biased taxonomy in conservation lists: The case of mammals. *Folia Zoologica* (2007).

Tables

Please see the supplementary files section to view the tables.

Figures

Figure 1

Circular map of *Platanista gangetica gangetica* mitochondrial genome. Map shown in figure represents the location of genes and other mitochondrial genome features. Outer circle represents genes in positive (+)/Heavy strand, second inner circle part represents genes in negative (-)/Light strand, third inner circle represents GC content and fourth inner circle shows GC skewness. Transfer RNAs are shown in red; Ribosomal RNAs in pink; protein coding genes in blue, control region in gray, GC content in black, GC skew(+) in green and GC skew(-) in purple. OH represents origin of "H" strand replication and OL represents origin of "L" strand replication.

Figure 2

The values of non-synonymous substitutions (K_a), synonymous substitutions (K_s), and the ratio of K_a/K_s , (ω) for river dolphins

Figure 3

Box plot revealing the evolutionary rate of PCGs of river dolphins and other cetaceans.

Figure 4

Phylogenetic relationships of river dolphin with marine dolphin species was inferred from the concatenated protein coding gene datasets (BI method)

Figure 5

Phylogenetic relationship of River dolphin A. COI gene based NJ tree B. Cyt B gene based NJ tree

Figure 6

Correlation of species concept for the Gangetic River dolphin based on history of origin of the Brahmaputra, the Ganges and the Indus River during the geosyncline events

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