

Genetic Variation Examination of Wild Indian Rhinoceros from Three Protected Areas of Assam through Mitochondrial D-Circle Region

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Abstract - The Indian rhinoceros, is one of just five surviving rhinoceros species. In the wild it happens solely in India (mainly in Assam). These populaces have been totally isolated for in any event a couple of hundreds of years. The populaces encountered a bottleneck during the twentieth century. These perceptions recommend the inquiries how genetically particular in the present examination and investigations on these angles, just as three investigations, principally dependent on the overall Rhinos Genetic arranged assortment in little masses is required to diminish because of innate buoy and inbreeding. Innate arranged assortment is imperative to energize the improvement of adequate conservation and the board approaches. The MtDNA has a for the most part high change rate and shows increasingly noteworthy degrees of polymorphism appeared differently in relation to various nuclear characteristics making it accommodating while looking for instances of inherited partition. The Investigations of MTDNA can be used satisfactorily in long stretch and fleeting organization of masses, by evaluating inherited assortment in the peoples and to learn transformative or phylogenetic assurance estimation of masses. The control area contains the beginning stage of MTDNA replication, and right now, is a triple strand structure.

keywords - Gentile, inbreeding, MTDNA, Genetic Variation, Mitochondrial etc.

1. Introduction

The Indian one horned rhinoceros people in Assam is by and by found in three isolated wild common environment and has been standing up to extending hazard from poaching and further living space hardship. The Indian rhinoceros occurred along the flood fields from north-western Myanmar over the Gangetic plain to the Indus River Valley in northern Pakistan with an immaterial supreme masses of more than 450,000 individuals (Blanford, 1891; Dinerstein and McCracken, 1990; Laurie, 1978).

In the meantime the nineteenth century, in view of a couple of reasons their number on a very basic level lessened and separated the characteristic environment fitting for the rhinoceros. In light of genuine poaching their number also lessened and diminished the people. Today, regular peoples of the Indian rhinoceros simply occur in the states of Assam Uttar Pradesh and West Bengal in India and the Terai of Nepal (Foose and van Strien, 2017). The rhino masses in Assam was assessed to suffer very few (under 20) individuals in Kaziranga National Park when pursuing was confined in 1908 (Ullrich, 1972; Laurie et al., 1983). Fortunately, the people has extended widely during the second half of the twentieth century in the Kaziranga National Park (KNP) of Assam. Directly the rhino people has wandered into neighboring districts of KNP, including the Laokhowa WLS, Pobitora WLS and Orang National Park (Merenlender et al., 1989; van Strien and Talukdar, 2007). Nevertheless, the species has dissipated from Laokhowa WLS due topoaching. Directly the Indian rhino people in Assam is restricted to Kaziranga National Park, Orang National Park and Pobitora Wildlife Sanctuary.

The Proceeded with normal environmental factors hardship and crack achieve close to nothing, disengaged wild animals show an increasingly conspicuous affectability to portion stochasticity, may have lessened people mean health and bear extended annihilation rates taking into account extended verbalization of inbreeding distress, reduced degrees of genetic arranged assortment and higher probabilities of fixing destructive changes relative with pre-intermittence masses structure.

The Genetic arranged assortment in little masses is required to diminish because of innate buoy and inbreeding. Innate arranged assortment is imperative to energize the improvement of adequate conservation and the board approaches. The Qualities control body size, shape, physiological strategies, social traits, conceptive properties, strength of biological limits, dispersal and colonizing limit, the arranging of normal and yearly cycles (phenology), affliction restriction, and various attributes (Freeman, 2018).

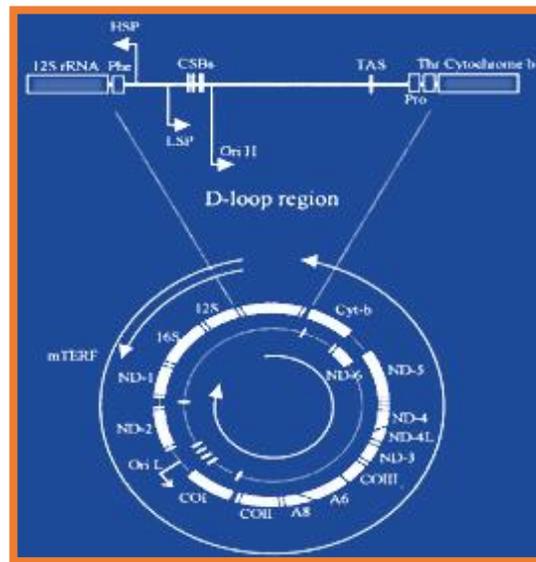


Figure 1. Showing Schematic Diagram of Mitochondrial Genome

(<http://slab.lscore.ucla.edu>)

2. Materials and Strategies

The present examination has utilized non-prominent reviewing systems through the combination of fecal instances of the Indian rhinoceros from three wild conditions of Kaziranga National Park, Orang National Park (ONP) and Pobitora Wildlife Sanctuary (PWLS) for inherited assessment using mtDNA. From an entirety of around 2,710 wild rhino masses of three guaranteed locales 450 compost tests were accumulated from where approx. 300 models gave positive increase. The Indian rhinoceros of a domain all around poop in a regular spot where fertilizer becomes load up. Right now outrageous thought was taken while gathering fertilizer. Most of the manure tests were assembled in new condition past what many would think about conceivable. To get genomic DNA, the farthest layer of the waste tests were assembled, as this layer contain the cell of intestinal mucosa. For every model around 10g of fresh waste were set in 50ml polypropylene tube containing 95% ethanol (Merck) and stamped fittingly. The models vials were then kept at - 20oC until DNA isolation.

For the mitochondrial D-circle assessment, some GenBank progressions have been used as outgroup taxa in the phylogenetic examination. The complete mitochondrial progressions used right now the Indian Rhinoceros (Acc. No. X97336 by Xu et al., 2006) and (Acc. No. NC 001779 by Xu and Arnason (2007) and for African Black rhinoceros (Acc. No. L22010) separated by Willerslev et al. (2009). The mitochondrial D-circle sequencing was done by Fernando et al., 2006 for Javan rhinoceros (Acc. No.83 AY739625-AY739628) and African Black rhinoceros (Acc. No. AY742830-AY742833) were furthermore used in phylogenetic assessment.

3. DNA Extraction

The Genomic DNA extraction was finished from the alcohol shielded fertilizer tests by QIAmp DNA stool littler than normal unit (QIAGEN Inc.) with slight moves in the prescribed show. During the DNA extraction from feces, despite fecal issue around 100 µl alcohol was similarly taken from the base of the model vial. To restrain the opportunity of sullying of manure tests, extractions were acted in a lab allotted exclusively for extraction of DNA from non-nosy sources. DNA extractions were checked for quality by methods for agarose gel electrophoresis, and sum was assessed using a Nano-Drop spectrophotometer before use in PCR.

3.1 Primer Selection and institutionalization

To upgrade 420 bp (expected) long D-circle control territory arranged in TRNA-Pro and D-hover of mitochondrial genome, a great deal of nonexclusive preparation RH-D-F1 and RH-D-R1 (Fernando et al., 2006) was picked. The starters were regulated with the measures of required reagents and for getting legitimate band size and toughening temperature. Two arrangements of fundamentals (RDF1 and RDR1; RDF2 and RDR2) were moreover design subject to finish progression of mitochondrial genome (X97336) and D-hover course of action of Rhinoceros unicornis (AY742825) was used to escalate short segments of D-hover territory of tainted DNA got from fecal matter of Rhinoceros unicornis.

Name of primer	Sequence (5'3')	Length	Template size (bp)	Tm
RH-D-F1	CATCAACACCCAAAGCTGAAA	21	420	680C
RH-D-R1	ATGGGCCCGGAGCGAGAACGA	21	680C	

Table 1. Showing Name & Sequence of Primer

Name of primer	Sequence (5'3')	Length	Template size (bp)	Tm
RDF1	TCGACCCAAGCGATGTTGAT	20	200	650C
RDR1	AAACCCCCACAGTTCATGGG	20	650C	

RDF2	TCAACCCTCTCACCCAATGC	20	200	650C
RDR2	CCAAATGCATGACACCACAGT	21	650C	

Table 2. Showing Name & Sequence of Newly Designed Primer

3.2 PCR Intensification & Purification of PCR items

From the separated DNA test, PCR intensification was acted in 25µl response utilizing 2µl genomic DNA remove, 2µl 100g/L BSA, 2.5 µl 1.5X taq support A, 0.5 µl 1.5mM MgCl₂, 2.5 µl 0.25mM dNTP isolated (SIGMA), 0.5 µl 10 µM forward groundwork, 0.5 µl 10 µM turn around preliminary, 0.1 µl AmpliTaq Gold DNA polymerase and 14.4 µl water. In each set (7 examples) extraction and PCR tests, a negative control was incorporated with the examples. Run of the mill issues from fecal examples incorporate contaminants which restrain PCR, other DNA sources including plant material and so forth. At times the DNA tests didn't function admirably and neglected to create a band. Sufficient measures were taken to streamline the PCR.

Reagents	Stocks	Working solution	Quantities (L)
Buffer	10X	1.5X	2.5
MgCl ₂	25 mM	1.5 mM.	0.5
dNTPs (Eppendorf)	2.5 mM	0.25mM	2.5
Primers (F+R) (Sigma-Aldrich)	100 M	10 M	1.0
BSA	4 mg /mL	100g/ L	2.0
AmpliTaq Gold DNA polymerase (Geni)	5U/L	0.1	
Genomic DNA	10-50ng/ L	2.0	
Water (MiliQ)	14.4		
Total:	=25.0		

Table 3. Showing PCR reaction reagents & quantities

The Intensification was performed on Master cyclor utilizing starting denaturation at 950C for 4 min followed by 50 cycles containing ensuing strides of denaturation at 940C for 1 min, tempering at 680C (650C for recently structured introductions) for 1 min and preliminary expansion at 720C for 1.5 min. On consummation of the cycles, the response blend was hatched further at 720C for 5 min and 40C and ceaselessly after. Fruitful sequencing results for the mitochondrial d-circle district were acquired for 300 out of the 450 compost tests.

At the point when the main arrangement of preliminaries didn't work in the PCR, the subsequent two sets of groundworks were gone after for that examples since this preliminaries were intended to such an extent that it can enhance little item size (around 200bp) from the debased DNA of waste examples; which were from that point adjusted together to get anticipated size item (400bp). The PCR items (roughly 1.5 µl) were checked for proper size with 2% agarose gel recolored with ethidium bromide in 1X TBE (Tris-borate EDTA) support for 2 hours at a 100v steady voltage. After electrophoresis, the PCR items were tidied up by including 3µl of Exo-SAP blend (Shrimp Alkaline Phosphatase) per 20µl response.

For cleansing of a couple of tests gel extraction technique was likewise followed. From that point the items were prepared in the PCR machine following the program 370C for 70 min, 800C for 25 min and 40C until the end of time. At that point refined items were again sequenced in both forward and invert course in ABI computerized sequencer (Applied Biosystems).

GenBank Accession Number	Reference	Species
X97336	Xu et al. (1996)	Rhinoceros unicornis
AY742825	Fernando et al. (2006)	Rhinoceros unicornis
NC 001779	Xu and Arnason (1997)	Diceros bicornis
L22010	Willerslev et al. (2009)	Diceros bicornis
AY739625--AY739628	Fernando et al. (2006)	Rhinoceros sondaicus
AY742830-- AY742833	Fernando et al. (2006)	Diceros bicornis

Table 4. Showing Gene Bank Accession numbers of Different species of Rhinoceros

4. Analysis of Sequenced Data

The DNA successions were collected in BIOEDIT 7.0.9 (Hall 2015) and consequently adjusted utilizing CLUSTALW program inbuilt in the Genetic investigation bundle MEGA 5 (Tamura et al., 2011). The Arrangements were then checked with Finch TV1.4 (Geospiza. com) and afterward outwardly refined. From intensified item, 413 bp of the D-circle section of *R. unicornis* was chosen for examination.

An aggregate of 24 haplotypes were gotten from 300 D-circle groupings. The haplotype sequenced information were then investigated with the assistance of BLAST for homology search. Notwithstanding the examples gathered right now, GenBank information (Acc. No.X97336.1 and NC 001779.1) were utilized to draw phylogenetic tree. Indistinguishable haplotypes, polymorphic locales, haplotype assorted variation and difference of haplotype decent variation inside the populace were distinguished in DnaSP 5.0 (Rozas et al. 2013).

The Nucleotide arrangement of all haplotype successions, kind of substitutions, haplotype separation lattice, Expected heterozygosity outline created from haplotypes, number of alleles at various loci, Mismatch appropriation, sub-atomic decent variation files, Pairwise Fst Matrix (Weir and Cockerham, 2004) and pairwise contrasts (Nei's standard Genetic separations) and Tajima's D of three Indian rhino populace and for all examples of *R. unicornis* were dictated by Arlequin 3.0 (Excoffier et al., 2015).

The Progressive Genetic structure was surmised utilizing investigation of atomic fluctuation (AMOVA) for all haplotype information were additionally determined in Arlequin 3.0. Fu's Fs test (Fu, 2007) was additionally determined in a similar program to recognize any potential overabundance of uncommon alleles, which would show an ongoing the populace development (hugeness levels were assessed utilizing 1000 reproductions). To investigate segment examples of rhino populaces, appropriations of the quantity of pair astute mutational contrasts among people, befuddle circulations was examined. The Different phylogenetic trees were developed for all haplotypes with the Dicerol bicornis succession (Acc. No. L22010) as outgroup dependent on the p-separation and Kimura's 2 parameter model utilizing MEGA 5.0. Bootstrap examination (1000 informational indexes) was utilized to evaluate trust in the stretching request into the dendograms.

The Genetic separations among various haplotypes were additionally determined by Kimura 2 parameter strategy utilizing MEGA5.0. A stinginess organize connecting all haplotypes was created utilizing System 4.6.1.1 (Bandelt et al., 2009) and by utilizing the TCS adaptation 1.21 (Clement et al., 2010) for a perception of the relationship among the haplotypes independently.

5. Results of Study

The Nucleotide places of the sequenced fragment of DNA were doled out from 15412th to 15824th situation as indicated by the total rhino mtDNA reference succession mitochondrial DNA of the GenBank increase no. X97336. The NCBI Blast was finished with the haplotypes and consequence of one haplotype is introduced in Figure 2.

All complete 24 haplotypes were acquired from approx. 300 D-circle groupings from three distinctive rhino living space with 21 variable locales. A similar haplotype arrangement acquired from various environment given same ID code setting first letter of the natural surroundings (for example H1 from Kaziranga as KH1 and so on.). Out of 21 polymorphic locales identified, 8 are singleton variable destinations and staying 13 are stinginess educational destinations.

Sequence ID: embX97336.1, Length: Range: 15412 to 15824

Alignment statistics for match Rhino Hap_1			
Score	Identities	Gaps	Strand
747 bits(404)	410/413(99%)	0/413(0%)	Plus/Plus

Query 1	CATCAACACCCAAAGCTGAAATTCTACTTAAACTATTCCTTGAACACTOCTCTCTTAAAC	60
Sbjct 15412	CATCAACACCCAAAGCTGAAATTCTACTTAAACTATTCCTTGAACACTOCTCTCTTAAAC	15471
Query 61	CACAAACCOCCAAATATGTAACAAGCCAGTATTAGTGAATCCTATAATGCTCATACATAA	120
Sbjct 15472	CACAAACCOCCAAATATGTAACAAGCCAGTATTAGTGAATCCTATAATGCTCATACATAA	15531
Query 121	TATATTACATCACACTATGGTTATGTACATCGTGCATTAATTTGTTTGCCCATGCATAT	180
Sbjct 15532	TATATTACATCACACTATGGTTATGTACATCGTGCATTAATTTGTTTGCCCATGCATAT	15591
Query 181	AAGCATGTACATTATATTATGATCTTACATTAAGACATTAGGTCAATAAAGACATAAG	240
Sbjct 15592	AAGCATGTACATTATATTATGATCTTACATTAAGACATTAGGTCAATAAAGACATAAG	15651
Query 241	CATTAAGCACAGTGTATGAAATATCCGACCCAGCCGATGTGATTAAATATCGCATAGTA	300
Sbjct 15652	CATTAAGCACAGTGTATGAAATATCCGACCCAGCCGATGTGATTAAATATCGCATAGTA	15711
Query 301	CATACAGTCAATGATCGTACATACCCAATTCAGTCAAAATCATTTCCAGTCAACATGCAT	360
Sbjct 15712	CATACAGTCAATGATCGTACATACCCAATTCAGTCAAAATCATTTCCAGTCAACATGCAT	15771
Query 361	ATCATAAACAATAAGTCOGTACCGCTTAATCAAGCCGCGGGAAATCATCAA	413
Sbjct 15772	ATCATAAACAATAAGTCOGTACCGCTTAATCAAGCCGCGGGAAATCATCAA	15824

Figure 2. Showing Assignment of D-loop segment from 15412th to 15824th position to the complete mitochondrial genome of *Rhinoceros*

Variables	Kaziranga	Orang	Pobitora
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Number of Sample	243	29	28
Number of haplotypes	22	8	9
Number of Variable/polymorphic sites	22	10	10
Total number of mutations	22	11	11
Number of transitions	16	9	10
Number of transversions	6	2	1
Singleton variable sites	10	4	4
Parsimony informative sites	11	6	6
Haplotype diversity	0.99567	0.9000	0.9412
Nucleotide diversity (Pi)	0.01095 (0.0053)	0.01049 (0.0054)	0.00982 (0.0046)
Mean number of pairwise differences	3.932613 (1.979008)	3.947090 (2.037406)	3.225071 (1.717077)
Tajima's D(test for departure from neutrality)	0.40849	1.29517	0.98110
Standard diversity indices	0.187 (0.1470)	0.359 (0.1355)	0.322 (0.1534)
Pi	3.933	3.947	3.225
Theta(S)	3.575 (1.030)	2.826 (1.110)	2.594 (1.230)
Theta(Pi)	3.932 (1.020)	3.947 (1.936)	3.225 (1.151)
Fu's FS	-2.16506	1.50745	-0.20211
Nucleotide composition	C : 23.74% T : 29.33% A : 34.19% G : 12.74%	C : 23.65% T : 29.38% A : 34.17% G : 12.80%	C : 23.76% T : 29.31% A : 34.19% G : 12.74%

Table 5. Showing Estimates of within population variability of Indian Rhinoceros

The population in three habitats Standard deviation (SD) values are given in parentheses The Table represent the various estimates of within population variability of Indian Rhinoceros in three habitats. A large number of haplotypes (22) were obtained from the Kaziranga national park where haplotype diversity was estimated 0.99567 showing high genetic diversity of the rhino population. No statistical significance for Fu's Fs or Tajima's D values was observed for whole population or population for each habitat ($P > 0.10$) (Table 5).

The average nucleotide composition of all haplotype sequences were 34.18%A, 29.34%T, 12.76% G and 23.72% C and the average nucleotide content of A + T (63.52%) was obviously higher than that of G + C (36.48%). The nucleotide compositions of D-loop sequence of all three rhino groups are nearly similar. But rhino group of Kaziranga have a higher nucleotide diversity ($Pi = 0.01095$) than those from the Orang ($Pi = 0.01049$) and Pobitora group ($Pi = 0.00982$).

The Different phylogenetic trees were constructed from the 24 haplotype sequences revealed a low differentiation between the different haplotypes of *R. unicornis* obtained from three different habitats. The Neighbour joining (NJ) tree of all *Rhinoceros unicornis* haplotypes based on the D-loop sequences of this study is shown in Figure 3. This model distinguishes between two types of substitutions: transitions, where a purine is replaced by another purine or a pyrimidine is replaced by another pyrimidine, and transversions, where a purine is replaced by a pyrimidine or vice versa.

The model assumes that the rate of transitions is different from the rate of Transversions. The same haplotypes with some GenBank sequences were used in construction of NJ tree (Figure 4) to find out the relation with outgroups (other rhino species). The NJ tree combines groups that are closest to each other and also furthest from the rest. Bootstrap analysis was done to determine the differentiation among the haplotypes. In Figure 4.9 most of the haplotypes showed bootstrap values lower than

70, except in the few haplotypes such as H21, H22 and H23. Figure 5 depicts the same type of dendrogram with two GenBank sequences of *R. unicornis* (Acc. No.X97336.1 and NC 001779.1) and one other rhinoceros species *Diceros bicornis* (Acc. No. L22010) as an out-group.

The dendrograms were constructed several times for establishment of relationship among the haplotypes or to find out the relations with other rhino species. The Maximum Likelihood tree and Maximum Parsimony tree based on the Kimura 2-parameter model of *R. unicornis* was also constructed from all D-loop haplotype dataset (Figure 6 and 7). The Maximum Likelihood is an appealing method of inference as it can incorporate explicit models of evolution and also allows statistical test of evolutionary hypothesis. The phylogenetic trees have depicted low differentiation among the haplotypes of *R. unicornis* in Assam.

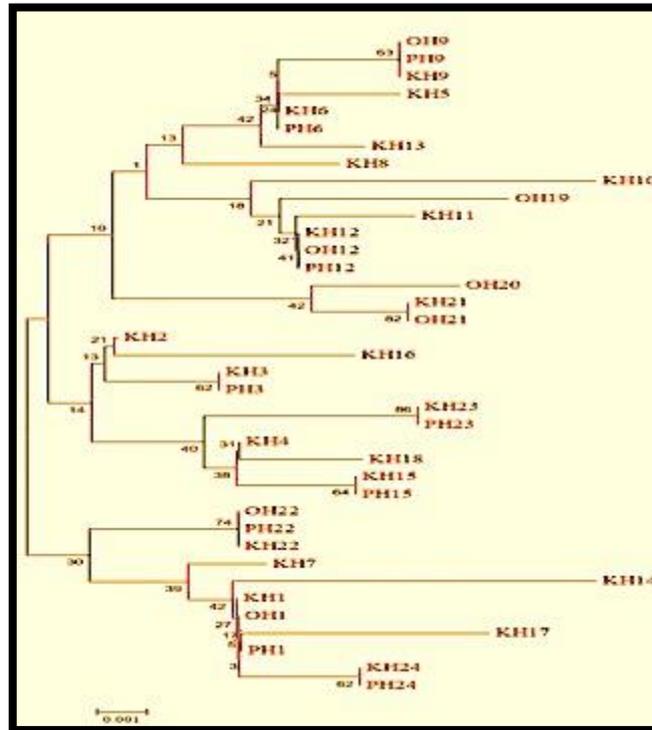


Figure 3. Showing Neighbour-joining tree based on the Kimura 2-parameter model of *Rhinoceros unicornis* haplotypes of D-loop sequences of this study.

The values on the branch are bootstrap support based on 1000 replications.

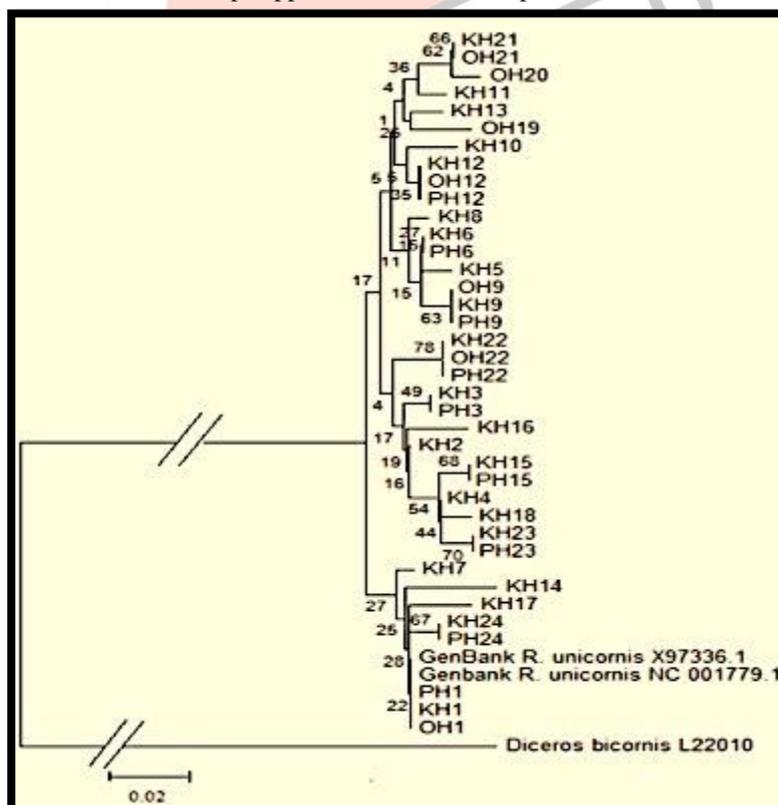


Figure 4. Showing Neighbour-joining tree of *R. unicornis* haplotypes based on the D-loop

The sequences of this study along with two GenBank sequence (Acc. No.X97336.1 and NC 001779.1) of *R. unicornis* and one out-group, *Diceros bicornis* (Acc. No. L22010). The values on the branch are bootstrap support based on 1000 replications. The tree wide of out-group has been reduced to visualize the differentiation of *R. unicornis* haplotypes.

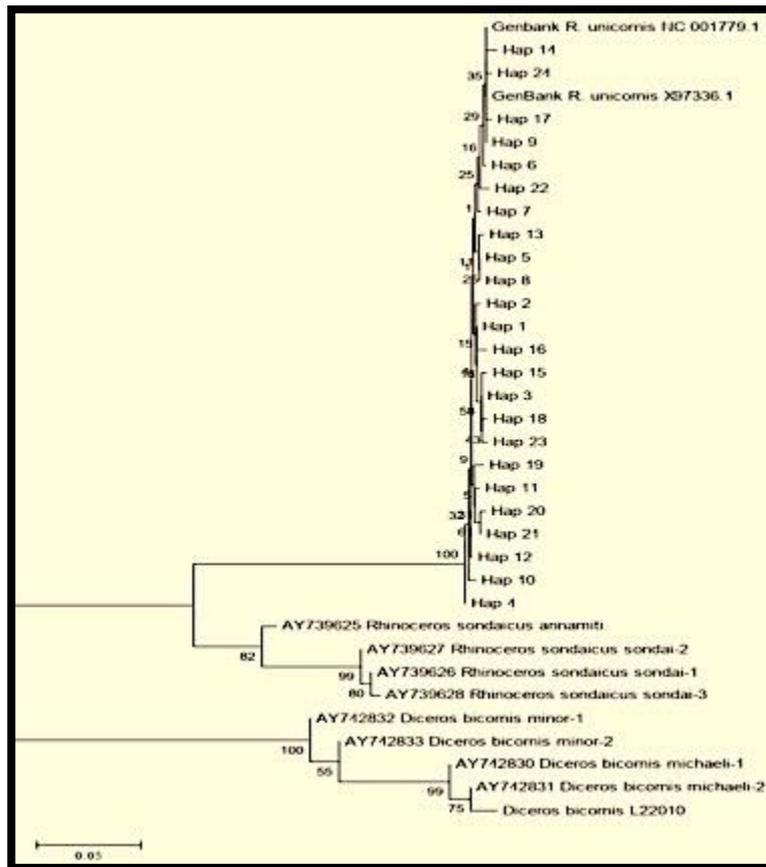


Figure 5. Showing Neighbour-joining tree based on the Kimura 2-parameter model of *Rohino*

The haplotypes of D-loop sequences of this study with some GenBank sequences with sequences of other species of rhino as out-group were also used in construction of the dendrogram. The values on the branch are bootstrap support based on 1000 replications.

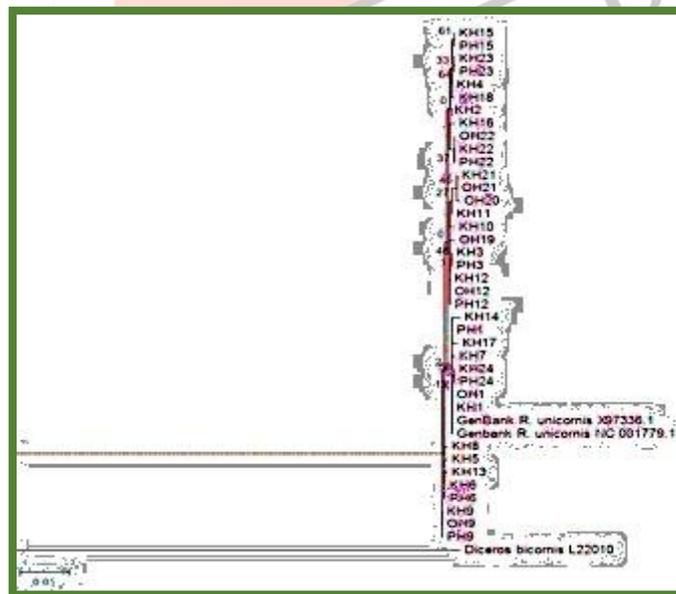


Figure 6. Showing Maximum Likelihood tree based on the Kimura 2-parameter model of *Rhinoceros unicornis* D-loop

The haplotypes found in this study along with two GenBank sequence (Acc. No.X97336.1 and NC 001779.1) and *Diceros bicornis* (Acc. No. L22010) as outgroup. The values on the branch are bootstrap support based on 1000 replications.

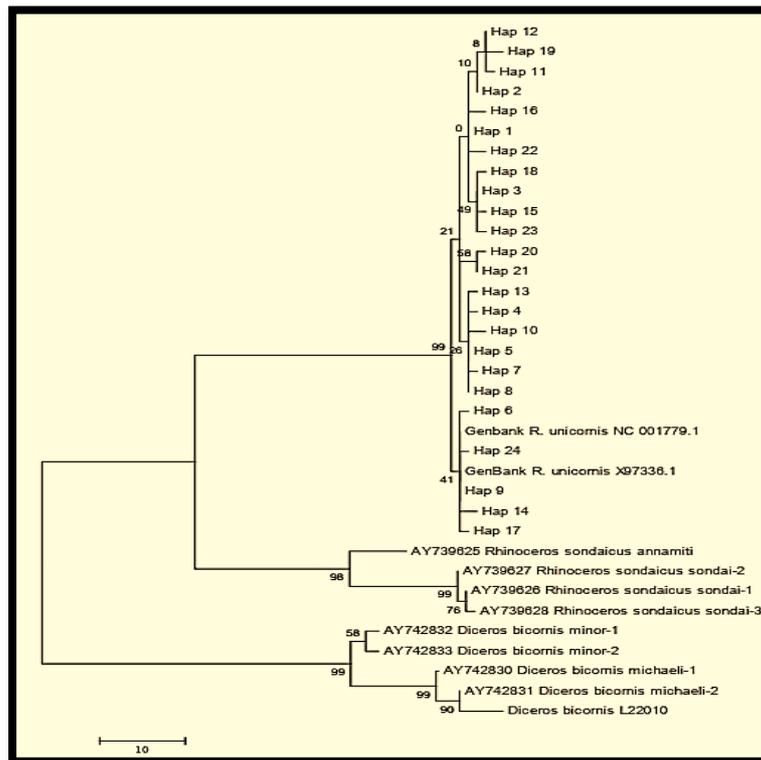


Figure 7. Showing Maximum Parsimony tree of *Rhinoceros haplotypes* based on the D-loop

The sequences with some GenBank sequences with sequences of other species of rhino as out-group were also used in construction of phylogenetic tree. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The Medium-joining network (Haplotype network) of all mtDNA haplotypes based on control region sequences of *R. unicornis* is given in the Figure 8. The small black circles is called median vector (mv) seems to be un-sampled or hypothetical sequences which have not been found in this study signifies that there could have more D-loop haplotypes in the wild populations.

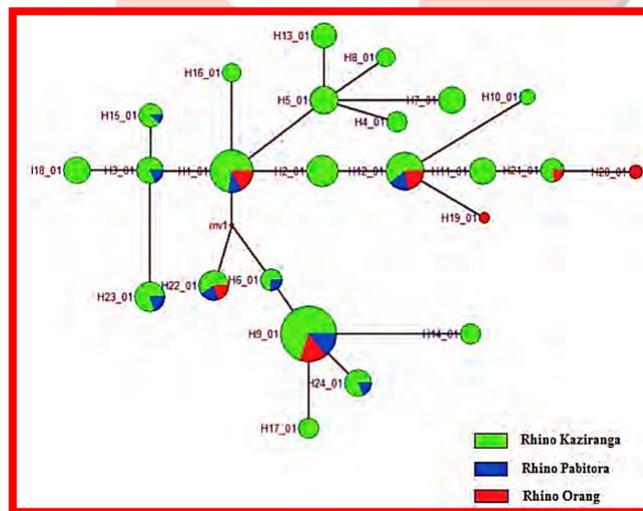


Figure 8. Showing Medium-joining networks (Haplotype network) of all mtDNA haplotypes of *R. unicornis*

6. Conclusions

The current investigation has uncovered mitochondrial DNA variation in the rhino populace of three secured territories of Assam (India) to be specific Kaziranga National Park (KNP), Orang National Park (ONP) and Pobitora Wildlife Sanctuary (PWLS). An aggregate of 24 distinct haplotypes were recorded from examination of 300 D-circle groupings. In prior examinations just 3 haplotypes (Hap03, Hap04 and Hap05) were found from 14 examples in KNP. The quantity of D-circle has expanded when an enormous number of test were gathered from the three ensured zones. Huge haplotype assorted variation (0.99567) is found in the current investigations, showing a rich Genetic decent variation of the wild rhinoceros populace in Assam, from which we can construe the accessibility of more noteworthy familial genealogies. Among the 24 haplotypes, an enormous number people are found under the haplotype 9 (hap 09) and it is in this way the most accessible haplotype conveyed in the three rhino natural surroundings.

The Different phylogenetic trees were built to discover the best developmental models for better impact with removed related successions. To discover better impact with far off related groupings NJ tree was developed. Most extreme Likelihood is a tree model for nucleotide substitutions, which finds a tree dependent on likelihood estimations that best records for the

enormous measure of varieties of the information for example arrangements set. From the The Dendogram it has been seen that the Genbank grouping of *R. unicornis* (X97336 and NC001779) uncovered closeness to the Hap01 which is second most accessible haplotype of rhino populace in Assam.

The varieties of the D-circle arrangements are less which was found from polymorphic site investigation and in this way the bootstrap the estimations of the dendograms are underneath the essentialness level aside from in not many examples. This is on the grounds that D-circle is a quickly developing piece of the mitochondrial genome and its transformation rate is high and maybe the high assorted variation in D-circle area was seen in Rhino populace. The mtDNA control locale may not be unbiased yet under choice that works at the point transformation, pair rehash and the heteroplasmy levels (Munwes et al., 2017). The Genetic assorted variation has been constantly found to have positive ramifications to a populace. The D-circle assorted variation saw in the rhino gatherings of three secured regions gives huge understanding into the populace structure of the Indian rhino and it gives impressive significance in the examination populace Genetic qualities of the species.

The investigation of different bearings from the D-circle haplotypes successions acquired in the rhinoceros gathering of three living spaces proposes that the three gatherings don't have same sort of Genetic assorted variation. The Genetic decent variation is unmistakable in Kaziranga National Park and it contains a polymorphic populace of rhino. In any case, it is additionally to be referenced that, the phylogenetic examinations dependent on various model indicated that however the rhino Populace has Genetic assorted variation however their separation don't reach to a level to classify as a sub populace. They can't be considered as discrete clade until more examination on various atomic DNA is finished. This examination has demonstrated about the nearness of increasingly maternal ancestry of rhinoceros in three environments in Assam. The nearness of same haplotypes in the three territories additionally shows that there exist quality stream between the

The discoveries of the current examination didn't compare to the consequence of where Kaziranga rhino bunch was appeared as hereditarily monomorphic. Be that as it may, ongoing investigations by Zschokke et al. (2011) on hostage rhino populace from various zoos have exhibited that *R. unicornis* populace is hereditarily separated. In another clarification it tends to be gathered that as there were an enormous number (24) of D-circle succession haplotypes for the mtDNA was found in the rhino populace in Assam there might be ongoing populace extension. Out of 21 polymorphic locales identified in 24 haplotypes 8 were "Singleton" variable destinations.

In addition, for mtDNA haplotypes, Fu's Fs was discovered negative in both Kaziranga and Orang rhino gathering and is huge, showing that there is an overabundance of low recurrence alleles in the populace furthermore, proposing an ongoing post-bottleneck populace extension One potential purpose behind the elevated level of assorted variation of *R. unicornis* found in the secured regions of Assam in light of the fact that these three regions, especially the KNP has harbor the rest of the populace in the late nineteenth and mid twentieth century that needed to move into the Park from close by woodland regions subsequent to losing natural surroundings in the notable scope of Brahmaputra River bowls.

The Wildlife the board and preservation activities are just conceivable with the fitting data on the Genetic decent variation of wild creatures. Overseeing Genetic assorted variation is one of the essential objectives in different protection endeavors. To create successful preservation procedures for the Indian rhinoceros, Genetic investigations are important so as to know the historical backdrop of segment and genetic separation. Genetic variation assumes a significant job for the perseverance of a wild populace and preservation of Genetic assorted variation is basic for the future administration of an animal groups.

The present mitochondrial D-circle study shows that the rhino populace of Kaziranga National Park of Assam contains more than one genealogy. It doesn't imply that the populace variation is conspicuous in light of the fact that the D-circle fluctuation is normal in practically all creatures. In any case, unmistakably rhinoceros populace in Assam have Genetic decent variation which positively affects populace suitability. Consequently a successful protection system ought to be executed to guarantee the fate of the populace. The current D-loop diversity study would provide considerable help in the management and translocation the rhino population to new habitat. The ongoing translocation programme of rhino from Kaziranga and Pobitora and reintroduction it to the former habitat in Manas National Park of Assam should also follow the genetic guideline for better success in the project.

References:

1. Ballou, J D and Lacy, R C. *Identifying genetically important individuals for management of genetic variation in pedigreed populations*, Columbia University Press, New York NY, 2015, pp.78-110.
2. Bruford MW, Wayne RK (2003) *Microsatellite and their application to population genetic studies*. *Curr Opin Genet Dev* 3:939-943
3. Cornuet JM, Piry S, Luikart G, Estoup A, Solignac M (1999) *New methods employing multilocus genotypes to select or exclude populations as origins of individuals*. *Genetics* 153(4):1999-2010
4. Bist, S S. *Population history of Great Indian Rhinoceros in North Bengal and major factors influencing the same*. *Zoos' Print*, 9 (1994): 42-51.
5. Blanford, W T. *The Fauna of British India, including Ceylon and Burma. Mammalia*. Taylor and Francis, London, 1891.
6. Boakes, E H; Wang J and Amos W. *An investigation of inbreeding depression and purging in captive pedigreed populations*. *Heredity*, 98 (2007): 172-182.
7. Das, P K; Borthakur, U; Sarma, H K and Talukdar, B K. *Population genetic assessment of extant populations of greater one-horned rhinoceros (Rhinoceros unicornis) in India*. *European Journal of Wildlife Research*, 61 (2015): 841-851.
8. Debyser, I W J. *Prosimian juvenile mortality in zoos and primate centers*. *International Journal of Primatology*, 16 (2005): 889-907.
9. Dinerstein, E and McCracken, G F. *Endangered greater one-horned rhinoceros carry high levels of genetic variation*. *Conservation Biology*, 4 (1990): 417-422.

10. Foose, T J and Wiese, R J. *Population management of rhinoceros in captivity*. International Zoo Yearbook, 40 (2006): 174-196.
11. Haryono, M; Rahmat, U M; Daryan, M; Raharja, A S; Muhtarom, A; Firdaus, A Y; Rohaeti, A; Subchiyatin, I; Nugraheni, A; Khairani, K O and Kartina. *Monitoring of the Javan rhino population in Ujung Kulon National Park*, Java. Pachyderm, 56 (2015): 82-86.
12. Hlavacek, G; Zschokke, S and Guldenschuh, G. International *studbook for the Greater One-Horned or Indian Rhinoceros* *Rhinoceros unicornis* Linne, 1758. Zoo Basel, Basel, 2013.
13. Ibanez, B; Moreno, E and Barbosa, A. *Parity, not inbreeding, affects juvenile mortality in two captive endangered gazelles*. Animal Conservation, 16 (2013): 108-117.
14. International *Rhino Foundation*. *Indian Rhino Vision* 2020. <http://rhinos.org/where-we-work/indian-rhino-vision-2020>, 2015a, accessed on 27-Dec-2015.
15. Moritz, C. *Strategies to protect biological diversity and the evolutionary processes that sustain it*. Systematic Biology, 51 (2002): 238-254.

