Consideration of genetic variation and evolutionary history in future conservation of Indian one-horned rhinoceros (*Rhinoceros unicornis*)

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Abstract:

The extant members of the Eurasian rhino species have experienced severe population and range declines through a combination of natural and anthropogenic factors since Pleistocene. The one-horned rhino is the only Asian species recovered from such strong population decline but most of their fragmented populations in India and Nepal are reaching carrying capacity. Implementation of any future reintroduction-based conservation efforts would greatly benefit from currently unavailable detailed genetic assessments and evolutionary history of these populations. We sequenced wild one-horned rhino mitogenome from all the extant populations (n=16 individuals) for the first time, identified the polymorphic sites and assessed genetic variation (2531bp mtDNA, n=111 individuals) across India. Results showed 30 unique rhino haplotypes distributed as three distinct genetic clades (F_{st} value 0.68-1) corresponding to the states of Assam (n=28 haplotypes), West Bengal and Uttar Pradesh (both monomorphic). Phylogenetic analyses suggest earlier coalescence of Assam (~0.5 Mya) followed by parallel divergence of West Bengal and Uttar Pradesh/Nepal (~0.06-0.05Mya), supported by the paleobiogeographic history of the Indian subcontinent. Combined together, we propose recognising three ‘Evolutionary Significant Units (ESUs)’ of Indian rhino. As recent assessments suggest further genetic isolations of Indian rhinos at local scales, future management efforts should focus on identifying genetically variable founder animals and consider periodic supplementation events while planning future rhino reintroduction programs in India. Such well-informed, multidisciplinary approach is the only way to ensure evolutionary, ecological and demographic stability of the species across its range.

Keywords: Megaherbivore, paleobiogeographic events, evolutionary significant units (ESUs), Rhinocerotidae family, reintroduction program, founder effect

1. Introduction

The members of Rhinocerotidae family were once one of the most diverse and widely distributed terrestrial herbivores with complex evolutionary history (Liu et al., 2021). By late Pleistocene, this family was reduced to only nine species (from more than 100 species) spread across Eurasia (seven species) and Africa (two species) (Wan and Zhang, 2017; Liu et al., 2021). Subsequently, early Holocene global warming (after Last Glacial Maxima) triggered their extinction in western Eurasia and southward movement of eastern Eurasian rhinos, leading to their distribution across Southeast Asia (Li et al., 2015; Wan and Zhang, 2017). Further, the range of all Eurasian rhino species (Javan, Sumatran and One-horned rhino) were affected by a combination of natural and anthropogenic factors during Pleistocene-Holocene transition period (0.015-0.009 million years ago (Mya)) (Alroy, 2011; Li et al., 2015; Lal, 2016; Mays et al., 2018; Liu et al., 2021), followed by recent events of exploitation of natural resources (during colonial era), industrialisation and poaching (since 17th century) (Menon 1996; Fernando et al., 2006; Das et al., 2015; Steiner et al., 2017). Population size of the most widely distributed Javan rhinos (during Holocene) (Antoine, 2012) were greatly reduced during human population expansion since 10,000 years ago (Li et al., 2015), whereas the Sumatran rhino populations became fragmented and isolated (since Holocene) due to submerged Sundaland corridors (late Pleistocene) (Mays et al., 2018). The one-horned rhinos faced climate-change driven habitat shrinkage in late Pleistocene (Patnaik, 2016). Currently the Javan and Sumatran rhinos are categorized as Critically Endangered (~60 Javan rhino- Margaryan et al., 2020 and <100 Sumatran rhinos- Steiner et al., 2017) and one-horned rhino as Vulnerable by IUCN (~3700 individual, Talukdar, 2021). Recovery of these species in their natural habitats requires deeper understanding of demography, ecology and genetics for appropriate conservation measures.

The one-horned rhino, being the only Asian species recovered from severe population decline in the past are critical for the evolutionary potential of this group. With a current population size of ~3700 individuals (increased from few hundred individuals in 1990s), it retains ~96% of the Asian rhino population (Steiner et al., 2017; Margaryan et al., 2020; Talukdar, 2021). As majority of the current one-horned rhino bearing areas in India and Nepal are reaching to their carrying capacities (Subedi et al., 2017; Jhala et al., 2021), future conservation efforts are directing towards reintroduction-based programmes. Detailed genetic assessment of the existing rhino populations is critical in this regard since strong historical demographic declines has led to loss of genetic variation in all rhino species (Black rhino- Moodley et al., 2017, White rhino- Moodley et al., 2018, Sumatran rhino- Mays et al., 2018 , Javan rhino- Margaryan et al., 2020). For example, Liu et al., (2021) suggested low population size and reduced genetic diversity across Rhinocerotidae family for an extended period of time. Similarly, mitogenome-based phylogeography reported low variations in both Sumatran (Steiner et al., 2017) and Javan (Margaryan et al., 2020) rhinos, but no such data is available for one-horned rhinos.

In this paper, we investigated the phylogeography and evolutionary history of one-horned rhinos in India (henceforth Indian rhino) as it harbours 83% (Kakati et al., 2021) of the global population of this species. We sequenced all the polymorphic sites in the Indian rhino mitogenome in 111 wild individuals surveyed across seven extant populations covering the states of Assam, West Bengal and Uttar Pradesh. Further, we identified the Evolutionary Significant Units (ESUs) in Indian rhinos and suggested appropriate conservation measures to secure the evolutionary potential of this species. We believe that the results will provide the most exhaustive genetic information for Indian rhinos that would be useful in future reintroduction and population management efforts.

2. Materials and Methods

2.1 Permission and ethical considerations

Data generated in this study is part of a collaborative programme titled “Implementing Rhino DNA Indexing System to counter rhino poaching threat and aid population management in India” (henceforth RhoDIS-India). Biological sampling from all the three rhino bearing states was permitted by Ministry of Environment, Forests and Climate Change (MoEF&CC), Government of India (Letter No. 4-22/2015/WL). Permission for dung sampling was provided by state forest departments of Assam (Letter No. A/GWL/RhoDIS/2017/913, 3653/WL/2W-525/2018, WL/FE.15/22), West Bengal (Letter No. 3967/WI/2W-525/2018) and Uttar Pradesh (Letter No. 1978/23-2-12 (G)). We have also received one tissue sample from Valmiki National Park, Bihar forest department assumed to be representing the wild rhinos of Nepal (Letter/no.-1296 dated 16.10.2020). No ethical permissions were required for tissues as they were collected from naturally dead rhinos as well as for dung samples.

2.2 Sample collection

In this study we have used 160 samples (72 tissues and 88 dung) covering all the seven rhino bearing parks of India (Fig. 1). The tissue samples of naturally dead rhino were provided by respective forest departments as part of RhoDIS-India protocol (2017-2021). Further dung collection was done to ensure spatial coverage for areas with no representative tissue samples. Rhino dung sampling can be challenging in the wild due to their use of communal latrine system (middens) (Laurie, 1982; Johnsingh and Manjrekar, 2016). In this study, sampling was conducted by intensive foot and vehicle surveys from already known midden sites across six rhino bearing parks (except Kaziranga NP). During sampling, only the fresh bolus from top of the midden was selected and swabbed twice with separate PBS-soaked sterile cotton swabs (Himedia, Mumbai, India). All samples were geo-tagged and transferred to laboratory in -20°C freezer till downstream processing. Park-wise sample details are provided in Table A1.

2.3 Laboratory work

2.3.1 DNA extraction

Tissue DNA was extracted using already established protocol for Indian rhino mentioned in Ghosh et al., (2021). For dung samples, a modified protocol from Biswas et al., (2019) was used. In brief, samples were digested overnight with a combination of 700 µl ATL and 65 µl Proteinase K (20mg/ml) at 56°C, followed by QIAamp DNA Tissue Kit (QIAGEN Inc., Hilden, Germany) protocol with adjusted volumes. DNA was eluted twice in 100 µl preheated (70 °C) 1X TE buffer and stored in -20°C freezer. Extraction negative was used for each set of extraction (n=23) to monitor possible contamination.

2.3.2 PCR amplification and sequencing

To assess genetic variation of the extant rhino populations, complete mitogenome data was generated for representative samples from each park (n=15, see Table A1 for details) and one from the Valmiki National Park (Bihar Forest Department). Mitogenome sequencing was performed using published 23 overlapping primers (Hassanin et al., 2009). For annealing temperature standardisation, gradient PCR was set in 10 µl reactions containing 4 µl of 2X Qiagen PCR buffer mix (QIAGEN Inc., Hilden, Germany), 1 µl of primer (3 µM), 2 µM BSA (4 mg/ml), 1.4 µl of RNase free water and 5 ng of rhino tissue DNA. PCR conditions included an initial denaturation (95 °C for 15 min); 35 cycles of denaturation (95 °C for 30 s), annealing (50-60 °C gradients for 40 s) and extension (72 °C for 40 s); followed by a final extension (72 °C for 10 min). During each set of reactions, PCR and extraction negatives were included to monitor contamination. Amplified products were visualized with 2% agarose gel, cleaned with Exonuclease (Thermo Scientific, Waltham, USA) and Shrimp Alkaline Phosphatase (Amresco, Solon, USA) mixture and sequenced bidirectionally in an ABI 3500XL bioanalyzer (Applied Biosystems). Out of these 23 primers, two did not show amplification in any samples. The remaining sequences (n=21 from 16 individuals) were

aligned with the available one-horned rhino mitogenome (Genbank: X97336, Xu et al., 1996) in Mega v7 (Kumar et al., 2016). Primers were designed manually in the flanking conserved regions adjacent to the gaps and sequences were generated from all the samples (n=16). The complete mitochondrial sequences (n=16) were aligned and manually screened to identify the segregating sites. Further, a total of 15 primers were designed to amplify all the polymorphic sites as <500 bp fragments to ensure higher success rate from fresh dung DNA. These primers were standardised following same protocol described above. For all field collected samples (tissue= 58 and dung=88) individual identification was performed using a panel of 14 microsatellites (described in Ghosh et al., 2021). After PCR amplification and genotyping of the markers, samples with <12 loci data were removed from downstream analysis. Further, genetic recaptures were removed and to ensure uniform representation we selected one sample from every area with clustered individuals. Sequence data (2531bp covering seven genes) was generated for the selected individuals to assess phylogeography patterns.

2.4 Analysis

2.4.1 Complete mitogenome annotation and comparative analysis

All rhino sequences (n=16) were aligned in Mega v7 to generate a complete mitogenome sequence and manually checked to identify any nucleotide ambiguities. Annotation was done using MITOS2 web (Bernt et al., 2013) and mitogenome map was created with OGDRAW (Greiner et al., 2019). To ascertain species-wise mitochondrial DNA diversity these sequences were aligned with already published rhino mitogenome sequences from *Diceros bicornis* (n=2, Genbank: FJ905814, NC012682 (Willerslev et al., 2009)), *Ceratotherium simum* (n=2, Genbank: Y07726, NC001808 (Xu et al., 1997)), *Dicerorhinus sumatrensis* (n=15, Genbank: MF066629-MFO66643 (Steiner et al., 2017) and *Rhinoceros sondaicus* (n=6, Genbank: FJ905815 (Willerslev et al., 2009), MK909142, MK909146, MK909148,

MK909149, MK909151 (Margaryan et al., 2020)). We calculated number of segregating sites (S), nucleotide (π) and haplotype diversity (Hd) using DnaSP v.5 (Librado and Rozas, 2009) for all genes in the mitogenome.

2.4.2 Genetic diversity in Indian rhinos

Population-wise basic indices of genetic variations (S, π and Hd) were calculated for concatenated sequence data (2531bp from seven genes) using DnaSP v.5 followed by a median joining (Bandelt et al., 1999) haplotype network constructed in PopART v. 1.7 (Leigh and Bryant, 2015). To ascertain any possible population structure a Bayesian approach implemented in BAPS v.5.3 was used as it considers linked loci data (Corander and Tang, 2007). Pairwise F_{st} and differential hierarchical AMOVA analysis was performed using Arlequin v. 3.0 (Excoffier et al., 2005) to confirm the pattern found in BAPS analysis.

2.4.3 Estimation of clade-specific divergence times

To identify the clades, Bayesian phylogeny was constructed with MrBayes v. 3.2.7 (Huelsenbeck and Ronquist, 2001) using 16 Indian rhino mitogenome and Javan rhino sequence (outgroup, as they are the sister clade of one-horned rhinoceros) (Harley et al., 2016). Analysis was conducted using GTR+G substitution model determined by jModelTest v2.1.3 (Darriba et al., 2012) (based on Akaike Information Criteria). The MCMC parameters included 2 runs of four chains each of 15 million generations with sampling after 1000 generations till split frequencies were below 0.01. Posterior probabilities were calculated for each node.

To estimate divergence among clades, rate of mutation for Indian rhino was calculated using BEAST v.2.3.6 (Drummond et al., 2007). Analysis was performed with five extant rhino mitogenome (without D-loop) (n=11 sequences, India=7, Java=1, Sumatra=1, White=1, Black=1) along with horse (*Equus caballus*, Genbank: NC001640), donkey (*Equus asinus*, Genbank: NC001788), Asiatic wild ass (*Equus hemionus*, Genbank: NC016061) and zebra

(*Equus zebra*, Genbank: NC018780) as outgroups. GTR+G substitution model was selected through jModelTest v2.1.3 for this multi-species data. Birth-death speciation was considered as tree prior (Ritchie et al., 2017; Steiner et al., 2017) along with uncorrelated relaxed log normal clock (Drummond et al., 2006; Steiner et al., 2017). During analysis, four established internal node calibration points (based on fossil records) with normal distribution priors were employed: (i) Caballine split (4 ± 0.5 million years ago (Mya)) (Eisenmann, 1992; Orlando et al., 2013); (ii) late Oligocene diversification of rhino groups (26 ± 3.5 Mya) (Hooijer, 1946); (iii) split of rhinoceros genus (3 ± 0.5 Mya) (Liu et al., 2021); (iv) origin of the perissodactyls (55 ± 3 Mya) (Prothero and Schoch, 1989; Benton and Donoghue, 2007). The first three calibration points were considered as monophyletic constraint (Drummond et al., 2006) as the last point includes both ingroup and outgroup taxa.

tMRCA (the Most Recent Common Ancestor) was inferred using the estimated mutation rate with lognormal distribution under strict molecular clock (intra species data, $n=16$) (Yoder and Yang, 2000; Ritchie et al., 2017). MCMC runs included 100 million generations, sampled at every 10,000 states with 10% burn-in. Data convergence was checked with Tracer v. 1.5 (Rambaut and Drummond, 2007) and the final tree (with maximum clade credibility) was estimated with TreeAnnotator (Helfrich et al., 2018) and visualised using FigTree v.1.4.2 (Rambaut, 2009).

3. Results

3.1 Rhino mitogenome data and comparative analyses

Sequencing with 23 primers (Table A2) generated 16828bp mitogenome (Figure A1) for wild Indian rhino ($n=16$, Genbank: MZ736693- MZ736708). Composition analysis revealed AT-skewed mitogenome with 13 protein coding genes, 22 tRNA, 2 ribosomal genes and a non-coding control region (Table A3). Comparative analyses with other rhino species (Figure A2) revealed that the Indian rhinos have low segregating sites ($S_{\text{Java}}=15514$, $S_{\text{Africa}}=10680$,

S_{Sumatra}=130, S_{India}=18) and nucleotide diversity ($\pi_{\text{Java}}=0.56$, $\pi_{\text{Africa}}=0.43$, $\pi_{\text{Sumatra}}=0.003$, $\pi_{\text{India}}=0.0005$) but high haplotype diversity (Hd_{Sumatra}=0.96, Hd_{India}=0.93, Hd_{Java}=0.91, Hd_{Africa}=0.67). Both African rhino species (white and black rhino) data were combined for this analyses as no intra-species variation was observed in the available data.

3.2 Phylogeography of wild Indian rhinos

Out of 15 primers designed to assess genetic variation, eight were finally used (Table A2) to amplify all 22 polymorphic sites (covering 2531bp sequence) of rhino mitogenome. This data was generated for additional 95 unique individuals (n= 56 tissue and 39 dung) (Genbank: MZ771364-MZ771458, MZ771459-MZ771553, MZ771554-MZ771648, MZ771649-MZ771743, MZ771744-MZ771838, MZ771839-MZ771933 and MZ771934-MZ772028). Remaining samples could not be used as they were genetic recaptures (n=13), individuals representing close geographic clusters (n=24) or low amplification success (n=12). Median joining network (n=111 individuals) showed a total of 30 haplotypes (h) across India. Majority of these haplotypes (93.3%, n=28) were from Assam whereas both West Bengal (one haplotype) and Uttar Pradesh (one haplotype) populations were found to be monomorphic (Fig. 2). The sequence from Bihar rhino individual was identical to the Uttar Pradesh population. Population-wise genetic variation indices (Table 1) showed overall highest values for KNP (n= 46; S=18, h=19, $\pi=0.0021$, Hd= 0.85), followed by MNP (n=12; S=14, h=6, $\pi=0.0023$, Hd= 0.89), ONP (n=12; S=9, h=6, $\pi=0.0016$, Hd= 0.89) and PWLS (10; S=2, h=3, $\pi=0.0002$, Hd= 0.51). Bayesian genetic clustering corroborated with the earlier pattern where samples from West Bengal and Uttar Pradesh formed distinct clusters whereas Assam showed geographically intermixed haplogroups (Fig. 2). The genetic differentiation (pairwise F_{st}) values among these three clusters were significantly high ranging from 0.68-1 (Table 2, indicating highly structured populations). The hierarchical AMOVA analysis using two separate groupings: a) seven populations and b) three states

showed higher within population (50%) and between group variance (45%) also indicating strong genetic structures in Indian rhinos (Table 2).

3.3 Divergence time of different rhino clades

The Bayesian phylogeny showed similar pattern of three clades: the West Bengal samples representing basal clade (Fig. 3, node G) followed by Assam (nodes C-E) and Uttar Pradesh (along with the Bihar sample) representing two sister clades (node F). Based on the calibrated root nodes and Indian rhino-specific mutation rate (1.2×10^{-4} mean rate of substitution per site per million years, Figure A3), tMRCA analysis suggested a divergence period spanning from 0.95 (HPD 1.36-0.81 Mya) to 0.05 (0.15-0.01 Mya) Mya (Fig. 3). Our results indicated the divergence of Indian rhinos ~ 0.95 Mya (node A, Fig. 3) corresponding to the emergence period of modern mammalian ancestors in the subcontinent (Lal, 2016; Patnaik, 2016). Next, the Assam population separated ~ 0.5 Mya (HPD 0.68-0.33 Mya, nodes B & C, Fig. 3) from the remaining clades. This is supported by reports of multiple rhino movements away from Assam (along Siwalik as well to Siva-Malayan region) during this period (van den Bergh et al., 2001; Patnaik, 2016). At population level, results suggest a relatively older coalescence of Assam ~ 0.19 Mya (HPD 0.3-0.07 Mya, node D & E, Fig. 3) compared to West Bengal and Uttar Pradesh (~ 0.05 Mya, HPD 0.15- 0.01 Mya, node F & G, Fig. 3). This period (0.12-0.01 Mya) is known for confinement of rhinoceros to the north and north-east of India due to monsoon intensification and grassland dominance (Patnaik, 2013, 2016; Lal, 2016).

4. Discussion

This study presents the most extensive mitochondrial DNA phylogeography of one-horned rhinos across its Indian distribution. Careful considerations involving mitogenome sequencing of representative samples across Indian rhino-bearing areas, identification of all polymorphic regions and their amplification from spatially-covered rhino samples helped us achieving accurate assessment of mtDNA variations. To the best of our knowledge, this is the

275 first report of wild Indian one-horned rhino mitogenome from all the extant populations.
 276 Despite relatively similar haplotype diversity of Asian rhinos (India-0.93 (16 samples),
 277 Sumatra-0.96 (15 samples), and Java-0.91 (6 samples), respectively), Indian rhino
 278 mitogenome showed much lower values for segregating sites and nucleotide diversity. Such
 279 mitogenome comparisons may be affected by limited sample size (in African rhinos,
 280 Moodley et al., 2017, 2018) or representation of historical genetic variations (in Javan rhinos,
 281 Margaryan et al., 2020). However, it was surprising to observe that despite similar historical
 282 demographic incidences (severe population decline due to habitat shrinkage (Patnaik, 2016;
 283 Mays et al., 2018) and anthropogenic pressures (Das et al., 2015; Steiner et al., 2017) Indian
 284 rhino retain much lower genetic variation than their Sumatran counterpart. This can be
 285 potentially attributed to recovery of the Indian species from extremely low founder
 286 population (as indicated by high H_d but low π) (Avice, 2000; da Silva et al., 2018).
 287 As expected, the phylogeography data (2531bp mtDNA, $n=16$ samples) revealed higher
 288 number of haplotypes than the mitogenome data ($n=30$ haplotypes) (due to large sample
 289 size). The only other study on one-horned rhino mtDNA variations (based on partial control
 290 region sequences, 428 bp) reported 10 haplotypes (Kaziranga National Park, India- 4 and
 291 Chitwan National Park, Nepal- 6, respectively) and moderate level of genetic difference (F_{st}
 292 value of 0.39 between them) (Zschokke et al., 2011). Careful scrutiny of our data revealed
 293 that all the polymorphic sites (or identified segregating sites) were found in fixed positions
 294 within one-horned rhino mitogenome (Table A4) across India. Given the fixed positions of
 295 these sites (and no new variations in the samples) and our sampling coverage it can be
 296 presumed with high confidence that all potential rhino mtDNA haplotypes from India have
 297 been reported in this study. This claim is also supported by the similar haplotype diversity
 298 values from the mitogenome and the phylogeography datasets (0.93 and 0.9, respectively).
 299 Our study also shows that the Indian rhinos have the highest number of haplotypes compared

to the other species/subspecies reported so far (Steiner et al., 2017, Moodley et al., 2017, 2018; Margaryan et al., 2020). The clustering analysis of the concatenated rhino sequences showed three distinct genetic clades (corresponding to the states of Assam, West Bengal and Uttar Pradesh) with high F_{st} value (0.68-1), corroborating with the haplotype network patterns. Mantel test (-0.83, $p=1$) confirmed that such strong genetic structuring is not due to isolation by distance pattern, but driven by lineage-specific evolutionary history (as suggested by AMOVA results). Such pattern of higher within population and between group variance (50% and 45% in Indian rhinos, respectively) has also been described in other species (barking deer- Martin et al., 2017, dog- Pang et al., 2009). Interestingly, we found that the sequence from the Bihar sample (representing samples from Nepal) was identical to the Uttar Pradesh sequences, including the state-specific SNPs. Though surprising, this pattern was expected as the founder animals of the reintroduced Uttar Pradesh population were sourced from Chitwan National Park, Nepal (four dominant breeding females) and Pobitora Wildlife Sanctuary of Assam (dominant breeding male) (Talukdar et al., 2012). Further comparison of 13 partial D-loop sequences from Chitwan National Park, Nepal (Zschokke et al., 2011) confirmed this pattern, indicating that the mtDNA signature of the Uttar Pradesh population belongs to Nepal. Given that the entire Uttar Pradesh rhino population showed only one haplotype, future studies need to evaluate the mtDNA variation in the Nepal population.

The phylogenetic analyses reconfirmed the relationship among the existing members of the Rhinocerotidae family (Xu et al., 1996; Steiner et al., 2011, 2017) and the genetic clades (similar to the phylogeographic pattern) within India. In the analyses, we used only the existing rhino species/subspecies data (Woolly rhinoceros sequence was not used) and the Sumatran and African rhino formed sister clades, separated from the Indian clade. However, within these reciprocal monophyletic clades in India the Assam population diverged first (0.5 Mya, Fig. 3) followed by the other two groups (West Bengal and Uttar Pradesh/Nepal),

diverging around similar time period (0.06-0.05 Mya, Fig. 3). The parallel divergence of the West Bengal and Uttar Pradesh is also supported by no shared genetic signatures between them (Fig. 2). The molecular dates were comparable to other published literature on rhino evolution (Lal, 2013; Patnaik, 2013, 2016) and supported by the paleobiogeographic history of the Indian subcontinent (Patnaik, 2013, 2016). For instance, the inward movement of rhinos from Assam along Siwalik (0.68-0.33 Mya, node B & C) coincides with drop in the sea level which facilitated movement of multiple genera (for example, *Elephas*, *Panthera*, *Rhino*, *Muntiacus* etc.) through Siva-Malayan route (van den Bergh et al., 2001; Vidya, 2015). Report of one-horned and Javan rhino co-existence in Bhutan ~0.56 Mya (Margaryan et al., 2020) provide further support of such movements. Finally, the coalescence time of the Indian clades corresponds to Holocene climatic optimum period known for monsoon intensification in north and north-east part of India resulting in range contraction for grassland dependent species (Lal, 2013; Patnaik, 2016; Kumar et al., 2017). We feel that our approach of using taxon-specific mutation rate and fossil data for node calibration has resulted in achieving such meaningful estimates of tMRCA. Future efforts should try to include molecular data from historical/ancient samples to tighten the variance associated with divergence estimates (Drummond et al., 2007). Overall, this approach reiterates the critical importance of large datasets (whole mitogenome from multiple individuals in this case), informative prior settings and its assessment with posterior outputs, taxon-specific mutation rate, node calibration points etc. for accurate tMRCA estimation (Yoder and Yang, 2000; Drummond et al., 2006; Subramanian et al., 2009; Knaus et al., 2011; Ritchie et al., 2017).

The spatially exhaustive sampling coverage and the patterns of population structure brings out some critical conservation perspectives for the Indian rhinos, particularly when compared with the other Asian counterparts. For example, the different divergence time and high genetic differentiation among the three populations of wild rhinos warrant a consideration of

possible subspecies designations for them. When looked carefully at the Sumatran rhino evolution, the three subspecies (designated based on morphological differences, Groves, 1967) shows very similar kind of divergence time history (Steiner et al., 2017) and much less genetic differentiation (0.098) than the Indian rhinos, providing strong argument for consideration of genetic subspecies for the three Indian populations. However, these populations are morphologically undistinguishable and interbreed among themselves (Dudhwa, Uttar Pradesh population is genetically mixed, Talukdar et al., 2012), and therefore would be meaningful to be recognised as ‘Evolutionary Significant Units (ESUs)’ as the current information emphasize their evolutionary histories. Based on the data, it is also important to point out that the conservation and management efforts need to equally focus for each of these populations and consider detailed understanding of their history. For example, the Uttar Pradesh and West Bengal population show state-specific monomorphic haplotypes representing unique but genetically depauperate populations. However, ever-continuing anthropogenic pressures and loss of habitat is further increasing the effects of fragmentation as seen in West Bengal where recent study showed strong genetic structure between the existing rhino populations (F_{st} value of 0.31 based on 11 microsatellite data) (Das et al., 2015). On the other hand, the reintroduced rhino population in Assam (Manas National Park) showed much higher mtDNA variation (six haplotypes), possibly due to periodic supplementation of individuals of varied genetic ancestry across different wild rhino populations (Talukdar et al., 2020). Careful management decisions in terms of selecting genetically variable founder animals, multiple reintroduction events etc. would be critical for future rhino reintroduction programs in India (Weeks et al., 2015; Jhala et al., 2021). Given that multiple reintroduction programs are planned as per the ‘National Conservation Strategy for the Indian One horned rhinoceros (*Rhinoceros unicornis*), Government of India, Ministry of Environment Forest and Climate Change, 2021’ objectives (in the states of Uttar Pradesh,

Bihar, West Bengal and Assam) in near future, the genetic signatures described in this study would be very helpful in selecting appropriate areas to identify founder animals.

5. Conclusion

The one-horned rhino was found throughout the Indo-Gangetic plains during the early 20th century (Rookmaaker, 1983; Rookmaaker et al., 2016) but faced drastic reductions in distribution and population size (including local extinctions) (Talukdar et al., 2008; Pant et al., 2019) and shown high extinction probability among the Indian megaherbivores (Karanth et al. 2010). However, strong conservation and management initiatives since late 1990s resulted in one of the most successful species recovery (increase in population size) in wild across the world (Menon, 1996; Rookmaaker et al., 2016; Pant et al., 2019). Despite a relatively long history of effective management efforts, information on genetic status of the species has not been used in translocation programmes (possibly due to lack of sufficient data). Rhino is a highly conservation-dependent species and any future translocation without inputs from the genetic data from the source populations may further impact the genetic status of the new habitats (as reported in case of Florida panther- Hostetler et al., 2010, Johnson et al., 2010; Cheetah- O'Brien et al., 2017; Gharial- Sharma et al. 2021). We present the first assessment of range-wide mitogenome diversity in Indian rhinos where we emphasize the importance of large data, spatial sampling coverage of populations and evolutionary history as fundamental information for future population reintroduction/ recovery programs. Our results are important for Indian rhino conservation because they suggest higher genetic diversity than earlier reported (Zschokke et al., 2011). However, the existing habitats are small, disjunct, isolated and reaching their respective carrying capacities (Pant et al., 2019; Jhala et al., 2021) and conservation options are becoming limited except establishing new habitats and translocation-driven population enhancement (Jhala et al., 2021). We believe that the genetic information provided here will assist in identifying

appropriate source populations and maintain adequate genetic diversity in the existing (and new) rhino populations, thereby ensuring evolutionary, ecological and demographic stability for their future survival.

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References

- Alroy, J., 2001. A multispecies overkill simulation of the end-Pleistocene megafaunal mass extinction. *Science* 292, 1893-1896.
- Antoine, P.O., 2012. Pleistocene and Holocene rhinocerotids (Mammalia, Perissodactyla) from the Indochinese Peninsula. *Comptes Rendus Palevol* 11, 159-168. doi:10.1016/j.crpv.2011.03.002.
- Avice J.C., 2000. *Phylogeography: the history and formation of species*. London: Harvard University Press.
- Bandelt, H., Forster, P., Rohl, A., 1999. Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* 16, 37-48. doi:10.1093/oxfordjournals.molbev.a026036.
- Benton, M.J., Donoghue, P.C.J., 2007. Paleontological evidence to date the tree of life. *Molecular Biology and Evolution* 24, 26–53. doi:10.1093/molbev/msl150.
- Bernt, M., Donath, A., Jühling, F., Externbrink, F., Florentz, C., Fritzsche, G., Pütz, J., Middendorf, M., Stadler, P. F., 2013. MITOS: Improved de novo metazoan mitochondrial genome annotation. *Molecular Phylogenetics and Evolution* 69, 313–319. <https://doi.org/10.1016/j.ympev.2012.08.023>.
- Biswas, S., Bhatt, S., Paul, S., Modi, S., Ghosh, T., Habib, B., Nigam, P., Talukdar, G., Pandav, B., Mondol, S., 2019. A practice faeces collection protocol for multidisciplinary research in wildlife science. *Current Science* 116, 11. doi: 10.18520/cs/v116/i11/1878-1885.
- Corander, J., & Tang, J., 2007. Bayesian analysis of population structure based on linked molecular information. *Math Biosciences* 205, 19-31. doi:10.1016/j.mbs.2006.09.015.
- Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. jModelTest 2: More models, new heuristics and parallel computing. *Nature Methods* 9, 772–772. <https://doi.org/10.1038/nmeth.2109>.

450 Das, P.K., Borthakur, U., Sarma, H.K., Talukdar, B.K., 2015. Population genetic assessment
451 of extant populations of greater one-horned rhinoceros (*Rhinoceros unicornis*) in India.
452 European Journal of Wildlife Research 61, 841–851. [https://doi.org/10.1007/s10344-015-](https://doi.org/10.1007/s10344-015-0960-2)
453 [0960-2](https://doi.org/10.1007/s10344-015-0960-2).

454 da Silva, M., Noll, F.B., e Castro, A.C.M-C., 2018. Phylogeographic analysis reveals high
455 genetic structure with uniform phenotypes in the paper wasp *Protonectarina sylveirae*
456 (Hymenoptera: Vespidae). PLoS ONE 13, e0194424.
457 <https://doi.org/10.1371/journal.pone.0194424>

458 Drummond, A.J., Ho, S.Y.W., Phillips, M.J., Rambaut, A., 2006. Relaxed phylogenetics and
459 dating with confidence. PLoS Biology 4, e88. <https://doi.org/10.1371/journal.pbio.0040088>.

460 Drummond, A.J., Rambaut, A., 2007. BEAST: Bayesian Evolutionary Analysis by Sampling
461 Trees. BMC Evolutionary Biology 7, 214. <https://doi.org/10.1186/147121487214>.

462 Eisenmann, V., 1992. Origins, dispersals, and migrations of *Equus* (Mammalia,
463 Perissodactyla). Courier Forschungsinstitut Senckenberg 153, 161–170.

464 Ellis, S., Talukdar, B.K., 2019 *Rhinoceros unicornis*: The IUCN Red List of Threatened
465 Species:e.T19496A18494149.
466 <https://doi.org/10.2305/IUCN.UK.20193.RLTS.T19496A18494149.en>.

467 Excoffier, Laval, L.G., Schneider, S., 2005. Arlequin ver. 3.0: An integrated software
468 package for population genetics data analysis. *Evolutionary Bioinformatics* 1, 47-50.

469 Fernando, P., Polet, G., Foad, N., Ng, L.S., Pastorini, J., Melnick, D.J., 2006. Genetic
470 diversity, phylogeny and conservation of the Javan rhinoceros (*Rhinoceros sondaicus*).
471 Conservation Genetics 7, 439-448. doi: 10.1007/s10592-006-9139-4.

472 Ghosh, T., Sharma, A., Mondol, S., 2021. Optimisation and application of a forensic
473 microsatellite panel to combat Greater-one horned rhinoceros (*Rhinoceros unicornis*)

474 poaching in India. Forensic Science International: Genetics 52, 102472.
 475 <https://doi.org/10.1016/j.fsigen.2021.102472>.

476 Greiner, S., Lehwark, P., Bock, R., 2019. Organellar Genome DRAW (OGDRAW) version
 477 1.3.1: Expanded toolkit for the graphical visualization of organellar genomes. Nucleic Acids
 478 Research 47, W59–W64. <https://doi.org/10.1093/nar/gkz238>.

479 Groves, C.P., 1967. The rhinoceroses of Southeast Asia. Säugetierkd Mitt 15,221–237.

480 Harley, E.H., de Waal, M., Murray, S., O’Ryan, C., 2016. Comparison of whole
 481 mitochondrial genome sequences of northern and southern white rhinoceroses
 482 (*Ceratotherium simum*): the conservation consequences of species definitions. Conservation
 483 Genetics 17, 1285-1291. doi:10.1007/s10592-016-0861-2.

484 Hassanin, A., Ropiquet, A., Couloux, A., Cruaud, C., 2009. Evolution of the mitochondrial
 485 genome in mammals living at high altitude: new insights from a study of the tribe Caprini
 486 (Bovidae, Antilopinae). Journal of Molecular Evolution 68, 293-310.
 487 <https://dx.doi.org/10.1007/s00239-009-9208-7>.

488 Helfrich, P., Rieb, E., Abrami, G., Lüking, A., Mehler, A., 2018. TREEANNOTATOR:
 489 Versatile Visual Annotation of Hierarchical Text Relations. In Proceedings of the 11th
 490 Edition of the Language Resources and Evaluation Conference, LREC 2018, Miyazaki,
 491 Japan.

492 Hooijer, D.A., 1946. Prehistoric and fossil rhinoceroses from the Malay Archipelago and
 493 India. Zoologische Mededelingen 26, 1–138.

494 Hostetler, J.A., Onorato, D.P., Nichols, J.D., Johnson, W.E., Roelke, M.E., O’Brien, S.J.,
 495 Jansen, D., Oli, M.K., 2010. Genetic introgression and the survival of Florida panther kittens.
 496 Biological conservation 143, 2789-2796. doi:10.1016/j.biocon.2010.07.028.

497 Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees.
 498 Bioinformatics 17, 754-755. <https://doi.org/10.1093/bioinformatics/17.8.754>.

499 Jhala, H.Y., Qureshi, Q., Jhala, Y.V., Black, S.A., 2021. Feasibility of reintroducing
500 grassland megaherbivores, the greater one-horned rhinoceros, and swamp buffalo within their
501 historic global range. *Scientific Reports* 11, 1-15. [https://doi.org/10.1038/s41598-021-83174-](https://doi.org/10.1038/s41598-021-83174-4)
502 [4](https://doi.org/10.1038/s41598-021-83174-4).

503 Johnsingh, A.J.T., Manjrekar, N., 2016., *Mammals of South Asia, Volume II*, Universities
504 Press, India.

505 Johnson, W.E., Onorato, D.P., Roelke, M.E., Land, D.E., Cunningham, M., Belden, C.,
506 McBride, R., Jansen, D., Lotz, M., Shindle, D., Howard, J., Wildt, D.E., Penfold, L.M.,
507 Hostetler, J.A., Oli, M.K., O'Brien, S.J., 2010. Genetic restoration of the Florida panther.
508 *Science* 329, 1641-1645. doi: 10.1126/science.1192891.

509 Kakati, P., Paine, S.K., Bhattacharjee, C.K., Bhattacharyya, C., Sharma, A., Phukan, D.,
510 Basu, A., 2021. Gut microbiome architecture of wild greater one-horned rhinoceros: a
511 vulnerable species from Kaziranga National Park, India. *Journal of genetics*, 100 (2), pp.1-7.
512 <https://doi.org/10.1007/s12041-021-01326-x>.

513 Karanth, K.K., Nichols, J.D., Karanth, K.U., Hines, J.E., Christensen Jr. N.L., 2010. The
514 shrinking ark: patterns of large mammal extinctions in India *Proceedings of the Royal*
515 *Society B* 277, pp. 1971-1979. doi:10.1098/rspb.2010.0171.

516 Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: Molecular Evolutionary Genetics
517 Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution* 33, 1870-1874.
518 doi:10.1093/molbev/msw054.

519 Kumar, A., Ghazi, M.G.U., Hussain, S.A., Bhatt, D. and Gupta, S.K., 2017. Mitochondrial
520 and nuclear DNA based genetic assessment indicated distinct variation and low genetic
521 exchange among the three subspecies of swamp deer (*Rucervus duvaucelii*). *Evolutionary*
522 *Biology*, 44(1), pp.31-42. doi: 10.1007/s11692-016-9387-2.

523 Knaus, B.J., Cronn, R., Liston, A., Pilgrim, K. and Schwartz, M.K., 2011. Mitochondrial
524 genome sequences illuminate maternal lineages of conservation concern in a rare carnivore.
525 BMC Ecology 11(1), pp.1-14.

526 Lal, P., 2016. Indica: A deep natural history of the Indian subcontinent, in: The Carnival of
527 Mammals, Random House India Pvt. Ltd., India, pp. 284-315.

528 Laurie, A., 1982. Behavioural ecology of the Greater one-horned rhinoceros (*Rhinoceros*
529 *unicornis*). Journal of Zoology 196, 307-341. [https://doi.org/10.1111/j.1469-](https://doi.org/10.1111/j.1469-7998.1982.tb03506.x)
530 [7998.1982.tb03506.x](https://doi.org/10.1111/j.1469-7998.1982.tb03506.x).

531 Leigh, J.W., Bryant, D., 2015. PopART: Full-feature software for haplotype network
532 construction. Methods in Ecology and Evolution 6, 1110-1116. [https://doi.org/10.1111/2041-](https://doi.org/10.1111/2041-210X.12410)
533 [210X.12410](https://doi.org/10.1111/2041-210X.12410).

534 Li, X., Jiang, G., Tian, H., Xu, L., Yan, C., Wang, Z., Wei, F., Zhang, Z., 2015. Human
535 impact and climate cooling caused range contraction of large mammals in China over the past
536 two millennia. Ecography 38, 74-82. doi: 10.1111/ecog.00795.

537 Librado, P., Rozas, J., 2009. DnaSP v5: A software for comprehensive analysis of DNA
538 polymorphism data. Bioinformatics 25, 1451-1452.
539 <https://doi.org/10.1093/bioinformatics/btp187>.

540 Liu, S., Westbury, M.V., Dussex, N., Mitchell, K.J., Sinding, M.H.S., Heintzman, P.D.,
541 Duchêne, D.A., Kapp, J.D., von Seth, J., Heiniger, H., Sánchez-Barreiro, F., 2021. Ancient
542 and modern genomes unravel the evolutionary history of the rhinoceros family. Cell 184, 1-
543 12. <https://doi.org/10.1016/j.cell.2021.07.032>.

544 Margaryan, A., Sinding, M.H.S., Liu, S., Vieira, F.G., Chan, Y.L., Nathan, S.K.S.S.,
545 Moodley, Y., Bruford, M.W., Gilbert, M.T.P., 2020. Recent mitochondrial lineage extinction
546 in the critically endangered Javan rhinoceros. Zoological Journal of the Linnean Society 190,
547 372-383. <https://doi.org/10.1093/zoolinnean/zlaa004>.

548 Martins, R.F., Fickel, J., Le, M., Van Nguyen, T., Nguyen, H.M., Timmins, R., Gan, H.M.,
549 Rovie-Ryan, J.J., Lenz, D., Förster, D.W. Wilting, A., 2017. Phylogeography of red muntjacs
550 reveals three distinct mitochondrial lineages. BMC Evolutionary Biology 17, pp.1-12. doi:
551 10.1186/s12862-017-0888-0.

552 Mays Jr, H.L., Hung, C.M., Shaner, P.J., Denvir, J., Justice, M., Yang, S.F., Roth, T.L.,
553 Oehler, D.A., Fan, J., Rekulapally, S., Primerano, D.A., 2018. Genomic analysis of
554 demographic history and ecological niche modeling in the endangered Sumatran rhinoceros
555 *Dicerorhinus sumatrensis*. Current Biology 28, 70-76.
556 <https://doi.org/10.1016/j.cub.2017.11.021>.

557 Menon, V., 1996. Under Siege: Poaching and Protection of Greater One-Horned
558 Rhinoceroses in India, TRAFFIC India. Available at
559 <https://portals.iucn.org/library/fr/node/7008>.

560 Moodley, Y., Russo, I.R.M., Dalton, D.L., Kotzé, A., Muya, S., Haubensak, P., Bálint, B.,
561 Munimanda, G.K., Deimel, C., Setzer, A., Dicks, K., 2017. Extinctions, genetic erosion and
562 conservation options for the black rhinoceros (*Diceros bicornis*). Scientific Reports 7, 41417.
563 doi: 10.1038/srep41417.

564 Moodley, Y., Russo, I.R.M., Dalton, D.L., Kotzé, A., Smith, S., Stejskal, J., Ryder, O.A.,
565 Hermes, R., Walzer, C., Bruford, M.W., 2018. Contrasting evolutionary history,
566 anthropogenic declines and genetic contact in the northern and southern white rhinoceros
567 (*Ceratotherium simum*). Proceedings of the Royal Society B 285, 20181567.
568 <https://doi.org/10.1098/rspb.2018.1567>.

569 O'Brien, S. J., Johnson, W. E., Driscoll, C. A., Dobrynin, P., & Marker, L., 2017.
570 Conservation genetics of the cheetah: lessons learned and new opportunities. Journal of
571 Heredity 671–677. doi:10.1093/jhered/esx047.

Orlando, L., Ginolhac, A., Zhang, G., Froese, D., Albrechtsen, A., Stiller, M., Schubert, M.,
Cappellini, E., Petersen, B., Moltke, I., Johnson, P.L.F., Fumagalli, M., Vilstrup, J.T.,
Raghavan, M., Korneliussen, T., Malaspinas, A-S., Vogt, J., Szklarczyk, D., Kelstrup, C.D.,
Vinther, J., Dolocan, A., Stenderup, J., Velazquez, A.M.V., Cahill, J., Rasmussen, M., Wang,
X., Min, G.D. Zazula, J., Seguin-Orlando, A., Mortensen, C., Magnussen, K., Thompson,
J.F., Weinstock, J., Gregersen, K., Røed, K.H., Eisenmann, V., Rubin, C.J., Miller, D.C.,
Antczak, D.F., Bertelsen, M.F., Brunak, S., Al-Rasheid, K.A.S., Ryder, O., Andersson, L.,
Mundy, J., Krogh, A., Gilbert, M.T.P., Kjær, K., Sicheritz-Ponten, T., Jensen, L.J., Olsen,
J.V., Hofreiter, M., Nielsen, R., Shapiro, B., Wang, J., Willerslev, E., 2013. Recalibrating
Equus evolution using the genome sequence of an early Middle Pleistocene horse. *Nature*
499, 74–78. doi:10.1038/nature12323.

Pang, J.F., Kluetsch, C., Zou, X.J., Zhang, A.B., Luo, L.Y., Angleby, H., Ardalan, A.,
Ekström, C., Skölleremo, A., Lundeberg, J., Matsumura, S., 2009. mtDNA data indicate a
single origin for dogs south of Yangtze River, less than 16,300 years ago, from numerous
wolves. *Molecular Biology and Evolution* 26, pp.2849-2864.

Pant, G., Maraseni, T., Apan, A., Allen, B. L., 2020. Trends and current state of research on
greater one-horned rhinoceros (*Rhinoceros unicornis*): A systematic review of the literature
over a period of 33 years (1985–2018). *Science of the Total Environment* 710, 136349.
<https://doi.org/10.1016/j.scitotenv.2019.136349>.

Patnaik, R., 2013. Indian Neogene Siwalik Mammalian Biostratigraphy. in: *Fossil Mammals*
of Asia, Columbia University Press, pp. 423-444.

Patnaik, R., 2016. Neogene–Quaternary Mammalian paleobiogeography of the Indian
Subcontinent: an appraisal. *Comptes Rendus Palevol* 15, 889-902.
<http://dx.doi.org/10.1016/j.crpv.2015.11.004>.

596 Prothero, D.R., Schoch, R.M., (Eds.). 1989. The evolution of Perissodactyls (No. 15). Oxford
597 University Press on Demand.

598 Rambaut, A. Drummond, A.J., 2007. Tracer. Available at: <http://beast.bio.ed.ac.uk/tracer>.

599 Rambaut, A., 2009. FigTree v1. 3.1. Available at: <http://tree.bio.ed.ac.uk/software/figtree/>.

600 Ritchie, A.M., Lo, N., Ho, S.Y.W., 2017. The Impact of the Tree Prior on Molecular Dating
601 of Data Sets Containing a Mixture of Inter- and Intraspecies Sampling. Systematic Biology
602 66, 413-425. <https://doi.org/10.1093/sysbio/syw095>.

603 Rookmaaker, L., 1983. Former distribution of the Indian rhinoceros (*Rhinoceros unicornis*)
604 in India and Pakistan. Journal of Bombay Natural History Society. 80, 55–563

605 Rookmaker, K., Sharma, A., Bose, J., Thapa, K., Dutta, D., Jeffries, B., Williams, A.C.,
606 Ghose, D., Gupta, M., Tornikoski, S., 2016. The Greater One-Horned Rhino: Past, Present
607 and Future. WWF, Gland, Switzerland, WWF.

608 Sharma, S.P., Ghazi, M.G., Katdare, S., Dasgupta, N., Mondol, S., Gupta, S.K., Hussain,
609 S.A., 2021. Microsatellite analysis reveals low genetic diversity in managed populations of
610 the critically endangered gharial (*Gavialis gangeticus*) in India. Scientific Reports 11, pp.1-
611 10. <https://doi.org/10.1038/s41598-021-85201-w>.

612 Singh, S.P., Sharma, A., Talukdar, B.K., 2012. Translocation of Rhinos within Assam: A
613 successful third round of the second phase of translocations under Indian Rhino Vision (IRV)
614 2020. Available at:
615 https://forest.assam.gov.in/sites/default/files/swf_utility_folder/departments/pccf_lipl_in_oid_4/menu/information_and_services/Assam%20Rhino%20Translocation%202nd%20Phase%20Round-3.pdf
616
617

618 Steiner, C.C., Ryder, O.A., 2011. Molecular phylogeny and evolution of the Perissodactyla.
619 Zoological Journal of the Linnean Society 163, 1289-1303. <https://doi.org/10.1111/j.1096-3642.2011.00752.x>.
620

Steiner, C.C., Houck, M.L., Ryder, O.A., 2017. Genetic Variation of complete mitochondrial genome sequences of the Sumatran rhinoceros (*Dicerorhinus sumatrensis*). Conservation Genetics 19, 397-408. doi:10.1007/s10592-017-1011-1.

Subedi, N., Lamichhane, B.R., Amin, R., Jnawali, S.R., Jhala, Y.V., 2017. Demography and viability of the largest population of greater one-horned rhinoceros in Nepal. Global Ecology and Conservation 12, 241-252. <https://doi.org/10.1016/j.gecco.2017.11.008>.

Subramanian, S., Denver, D.R., Millar, C.D., Heupink, T., Aschrafi, A., Emslie, S.D., Baroni, C. and Lambert, D.M., 2009. High mitogenomic evolutionary rates and time dependency. Trends in Genetics 2511, pp. 482-486. <https://doi.org/10.1016/j.tig.2009.09.005>.

Talukdar, B., 2021. Asian Rhino Specialist Group Chair report/Rapport du Groupe de Spécialistes du Rhinocéros d'Asie. Pachyderm 62, 29-34.

Talukdar, B.K., Bonal, B.S., Sharma, A., Sarma, K., Singh, S.P., 2020. Re-introduction of greater one-horned rhino in Manas National Park, Assam, India - under the Indian Rhino Vision 2020. Global Re-introduction Perspectives: 2013. Further case studies from around the globe, 164. Available at: https://www.researchgate.net/publication/346443488_Reintroduction_of_greater_one_horned_rhino_in_Manas_National_Park_Assam_India_-_under_Indian_Rhino_Vision_2020

Talukdar, B.K., Emslie, R., Bist, S.S., Choudhury, A., Ellis, S., Bonal, B.S., Malakar, M.C., Talukdar, B.N., Barua, M., 2008. Rhinoceros Unicornis: The IUCN Red List of Threatened Species. <http://www.iucnredlist.org/details/19496/0>.

Talukdar, B.K., Sharma, A., Guleria, H. Gupta, M., 2012. Dudhwa's Rhinos— A Plan for their Growth & Secured Future. doi: 10.13140/RG.2.2.10721.53601

van den Bergh, G.D., de Vos, J., Sondaar, P.Y., 2001. The Late Quaternary palaeogeography of mammal evolution in the Indonesian Archipelago. Palaeogeography, Palaeoclimatology, Palaeoecology 171, 385-408. [https://doi.org/10.1016/S0031-0182\(01\)00255-3](https://doi.org/10.1016/S0031-0182(01)00255-3).

646 Vidya, T.N.C., 2016. Evolutionary history and population genetic structure of Asian
647 elephants in India. *Indian Journal of History of Science*, 51(2), pp.391-405. doi:
648 10.16943/ijhs/2016/v51i2.2/48453.

649 Wan, X., Zhang, Z., 2017. Climate warming and humans played different roles in triggering
650 Late Quaternary extinctions in east and west Eurasia. *Proceedings of the Royal Society B*
651 284, 20162438. <http://dx.doi.org/10.1098/rspb.2016.2438> .

652 Weeks, A.R., Dorian, M., Thavornkanlapachai, R., Taylor, H.R., White, N.E., Weiser, E.L.,
653 Heinze., 2015. Conserving and enhancing genetic diversity in translocation programs,
654 *Advances in reintroduction biology of Australian and New Zealand Fauna*, CSIRO
655 Publishing, Melbourne, 127-140.

656 Willerslev, E., Gilbert, M.T.P., Binladen, J., Ho, S.Y.W., Campos, P.F., Ratan, A., Tomsho,
657 L.P., da Fonseca, R.R., Sher, A., Kuznetsova, T.V., Nowak-Kemp, M., Roth, T.L., Miller,
658 W., Schuster, S.C., 2009. Analysis of complete mitochondrial genomes from extinct and
659 extant rhinoceroses reveals lack of phylogenetic resolution. *BMC Ecology and Evolution* 9,
660 95. doi: 10.1186/1471-2148-9-95.

661 Xu, X., Janke, A., Arnason, U., 1996. The Complete Mitochondrial DNA Sequence of the
662 Greater Indian Rhinoceros, *Rhinoceros unicornis*, and the Phylogenetic Relationship
663 Carnivora, Perissodactyla, and Artiodactyla (+ Cetacea). *Molecular Biology and Evolution*
664 13, 1167-1173. doi: 10.1093/oxfordjournals.molbev.a025681.

665 Xu, X., Arnason, U., 1997. The Complete Mitochondrial DNA Sequence of the White
666 Rhinoceros, *Ceratotherium simum*, and Comparison with the mtDNA Sequence of the Indian
667 Rhinoceros, *Rhinoceros unicornis*. *Molecular Phylogenetics and Evolution* 7, 189-194. doi:
668 10.1006/mpev.1996.0385.

Yoder, A., Yang, Z., 2000. Estimation of primate speciation dates using local molecular clocks. *Molecular Biology and Evolution* 17, 1081–1090. <https://doi.org/10.1093/oxfordjournals.molbev.a026389>.

Zschokke, S., Armbruster, G. F., Ursenbacher, S., Baur, B., 2011. Genetic differences between the two remaining wild populations of the endangered Indian rhinoceros (*Rhinoceros unicornis*). *Biological conservation* 144, 2702-2709.

Figure legends

Figure 1: Map of the study area and distribution of the final samples used in this study (n=111). The top plate shows the position of the rhino-bearing parks across three Indian states (Uttar Pradesh, West Bengal and Assam). The reference sample of wild rhino received from the state of Bihar is also presented.

Figure 2: Representation of mtDNA variations and genetic structure in Indian rhinos based on 2531 bp concatenated sequence covering all polymorphic sites across seven genes. (a) Median joining network with park-level colour codes; (b) Haplotype frequencies at each of the sampled areas covering all variations (n=30 haplotypes); (c) Bayesian clustering shows monomorphism in Uttar Pradesh (with sample from Bihar, n=11) and West Bengal (n=20) populations and polymorphism in Assam (n=80).

Figure 3: Phylogenetic relationship and assessment of divergence time in Indian rhino populations. The left pane shows the basal clade of West Bengal samples (green) and sister clades representing Uttar Pradesh (blue) and Assam (red). Javan rhino sequence was used as outgroup. The posterior probability values (≥ 0.9) are shown in bold. The right pane indicates the divergence of Indian rhinos, where the Assam population diverged first (~0.5 Mya), followed by parallel divergence of West Bengal and Uttar Pradesh (0.06-0.05 Mya). Node-specific ages are marked (with posterior probability values ≥ 0.9). The major corroborating paleobiogeographical events are presented above.

Supplementary Fig.A1: Whole mitogenome organisation and annotation of *Rhinoceros unicornis*.

Supplementary Fig.A2: Gene-wise comparative analyses of polymorphism indices (S, Hd and π) for all extant rhino species.

Supplementary Fig.A3: Estimation of mitogenome mutation rate for Indian rhino using Caballine (Zebra, Donkey, Asiatic Wild Ass and Horse) as outgroup. Internal node calibration points are presented in italics (bold). Estimated node ages and branch mutation rates with available reference in literature is marked with * (Fernando et al., 2006; Steiner et al., 2011; Steiner et al., 2017; Liu et al., 2021).

Table legends

Table 1: mtDNA diversity indices of all seven rhino populations in India (n=110).

Table 2: Results of Pairwise genetic differentiation and hierarchical AMOVA test (Bihar sample considered under Uttar Pradesh clade).

Supplementary Table A1: Sample (both tissue and dung) and data details of Indian rhinos used in this study.

Supplementary Table A2: Details of the primers used in sequencing Indian rhino whole mitogenome and phylogeography data.

Supplementary Table A3: Mitogenome organization in *Rhinoceros unicornis*. Codons respective to each tRNA are mentioned in parenthesis.

Supplementary Table A4: Details of the variable sites based on concatenated sequence of 2531bp of Indian one horned rhino mtDNA haplotypes.

Table 1: mtDNA diversity indices of all seven rhino populations in India (n=110).

Protected areas	Sample size	Segregating sites (S)	Haplotypes (h)	Haplotype diversity (Hd)	Nucleotide diversity (π)
Kaziranga National Park, Assam	46	18	19	0.85	0.0021
Pobitora Wildlife Sanctuary, Assam	10	2	3	0.51	0.0002
Manas National Park, Assam	12	14	6	0.89	0.0023
Orang National Park, Assam	12	9	6	0.87	0.0016
Dudhwa National Park, Uttar Pradesh	10	0	1	0	0
Gorumara National Park, West Bengal	10	0	1	0	0
Jaldapara National Park, West Bengal	10	0	1	0	0
Total	110	22	30	0.89	0.0028

Table 2: Results of Pairwise genetic differentiation and hierarchical AMOVA test (Bihar sample considered under Uttar Pradesh clade).

Pairwise F _{st} among clades (*p<0.05)				
	Assam	Uttar Pradesh	West Bengal	
Assam	0			
Uttar Pradesh	0.68*	0		
West Bengal	0.73*	1.0*	0	
AMOVA test among three clades and seven populations				
Source of variation	d.f.	Sum of squares	Fixation index	Percentage of variation
Among groups	2	14.689	0.45 (F _{ct})	44.66
Among populations within groups	4	3.233	0.1 (F _{sc})	5.71
Within populations	104	31.267	0.50 (F _{st})	49.63
Total	110	49.189	0.60573

Supplementary Table A1: Sample (both tissue and dung) and data details of Indian rhinos used in this study.

State	Protected areas	Tissue collected	Tissue used for whole mitogenome data	Dung collected	Dung used for phylogeography
Uttar Pradesh	Dudhwa National Park	4	2	13	6
West Bengal	Gorumara National Park	2	2	18	8
	Jaldapara National Park	4	2	15	6
Assam	Manas National Park	3	1	22	9
	Pobitora Wildlife Sanctuary	9	2	5	1
	Orang National Park	3	3	15	9
	Kaziranga National Park	46	3	0	0
Bihar*	Valmiki National Park	1	1	NA	NA
Total		72	16	88	39

*Reference sample from non-rhino bearing area

Supplementary Table A2: Details of the primers used in sequencing Indian rhino whole mitogenome and phylogeography data.

S.No.	Primer	Sequence	Ta (°C)	Amplicon	Targeted genes	Polymorphic sites	Genbank accessions
1	P2*	F: CACTGAAAATGCCTAGATGAG	55	~950	tRNA ^{Phe} , rrnS, tRNA ^{Val}	0	MZ736693-MZ736708
		R: CTAGGTGTAAACTAGRTGCTT					
2	12S [#]	F: AGACATAAAAACGTTAGGTC	60	~350	rrnS, tRNA ^{Val}	0	
		R: GCTTTGGTTCAAAGTGGTCA					
3	P3*	F: GCACGCACACACCGCCCGTCAC	55	~600	rrnS, tRNA ^{Val} , rrnL	0	
		R: CGCTTTCTTAATTGRTGGCTGC					
4	P4*	F: AGCCTGGTGATAGCTGGTTGTCC	55	~650	rrnL	0	
		R: AAGCTCCATAGGGTCTTCTCGTC					
5	P5*	F: CCGTGCAAAGGTAGCATAATC	55	~800	rrnL, tRNA ^{Leu} , NADH1	0	
		R: CCTAGNACTTTTCGTTCTNACT					
6	P6*	F: GTGGCAGAGCCCGGTAATTG	55	~950	NADH1, tRNA ^{Ile}	0	
		R: TTACTCTATCAAAGTAACTC					
7	P7*	F: TYCGAGCATCHTAYCCHCGAT T	55	~800	NADH1, tRNA ^{Ile} , tRNA ^{Gln} , tRNA ^{Met} , NADH2	0	
		R: TGGTTTAGBCCBCCTCAKCCYCC					
8	P8*	F: CACTTYTGAGTNCCAGAAGT	55	~850	NADH2, tRNA ^{Trp} , tRNA ^{Ala}	0	
		R: TAGGGTRTTTAGCTGTTAAC					
9	P9*	F: AGACCAAGAGCCTTCAAAGC	55	~650	tRNA ^{Trp} , tRNA ^{Ala} , tRNA ^{Asn} , tRNA ^{Cys} , tRNA ^{Tyr} , COX1	0	
		R: GCTTCWACTATDGADGATGC					
10	P10*	F: GGNGGNTTYGGHAAAYTGACT	55	~750	COX1	1	
		R: GAARATRAAGCCTAGRGCTCA					

S.No.	Primer	Sequence	Ta (°C)	Amplicon	Targeted genes	Polymorphic sites	Genbank accessions
11	P11*	F: TTYACHGTHGGAATAGAYGT	55	~800	COX1, tRNA ^{Ser} , tRNA ^{Asp} , COX2	0	MZ736693-MZ736708
		R: GCRTCTTGRAANCCTARTTG					
12	P12*	F: CCCCCYAYWRYTGGTTTCAAGCCA	55	~500	tRNA ^{Asp} , COX2, tRNA ^{Lys} , ATP8	0	
		R: GTKGAYGTRTCTAGTTGYGGCAT					
13	P13*	F: CAATGCTCHGARATYTYGYGG	55	~950	COX2, tRNA ^{Lys} , ATP8, ATP6, COX3	1	
		R: GANARDGCTCCYGTDAGNGGT					
14	P14*	F: GCCTAYGTNTTYACYCTNCTAGT	55	~800	ATP6, COX3, tRNA ^{Gly} , NADH3	1	
		R: TGATTGGAAGTCARYTGTAC					
15	P16*	F: AGCYTGYGAAGCAGCACTAGG	55	~950	tRNA ^{Arg} , NADH4L, NADH4	0	
		R: GCKGTRGCTCCTATRTARCTTCA					
16	P17*	F: AGCTCHATYTGYYTHCGYCAA AC	55	~700	NADH4, tRNA ^{His} , tRNA ^{Ser} , tRNA ^{Leu}	1	
		R: CCAATTTTTTGGYTCCTAAGRCC					
17	P18*	F: CCGAAAAAGYAYGCAAGAACTGC	55	~750	tRNA ^{Ser} , tRNA ^{Leu} , NADH5	0	
		R: GCDGATTTTCCDGTKGCDGCTA					
18	P19*	F: GACGARCAAGYAGCHAAYACAGC	55	~700	NADH5	1	
		R: GTDAKTADDAGGGCTCAGGCG					
19	P20*	F: GGMAGCCTNGCNYTAACAGG	55	~600	NADH6	0	
		R: AGTTTAATGGDHTDGGDGATTG					
20	P21*	F: CCATAACTRTAYAAAGCHGCAA	55	~650	NADH6, tRNA ^{Glu} , CYTB	3	
		R: CCTCARAATGATATTTGKCCTCA					
21	P22*	F: CAGGMCTATTCCTRGCHATAAC	55	~950	CYTB, tRNA ^{Thr}	1\$21	

S.No.	Primer	Sequence	Ta (°C)	Amplicon	Targeted genes	Polymorphic sites	Genbank accessions
		R: CCCTTYTCTGGTTTACAAGACC					MZ736693-MZ736708
22	P23*	F: CATCGGACAAC TAGCATCTAT	55	~650	tRNA ^{Thr} , tRNA ^{Pro} , CR	10	
		R: CCTGAAGWAAGAACCAGATG					
23	CRintF2#	F: GATTAATATTGCATAGTA	50	~500	CYTB, tRNA-Thr, tRNA-Pro,CR	5(3\$23)	
	CRintR3#	R: AATAGTTAATAGAAAGGC					
24	CRF1#	F: CATGCCAGTATTAGTGAC	50	~450	tRNA ^{Phe} , CR	6\$23	
	CRR1#	R: CAAGGTGTTGTTAGCTAC					
Phylogeography Primers							
25	P10*	F: GGNGGNTTYGGHAAYTGACT	45	431	COX1	1	MZ771364-MZ771458
	COXR#	R: GTAGACTTCAAAATGACC					
26	ATP6#	F: TCTAATACACTTAATCGG	50	379	ATP6, COX3	2	MZ771934- MZ772028
		R: CTTGGAATGTGCTTTCAC					
27	NADH4#	F: CCAAACACCATGAAGTTA	50	262	NADH4	1	MZ771459- MZ771553
		R: GATCATGAGAATGATGAC					
28	NADH5#	F: AACAAATCTTCACACTTG	50	268	NADH5	1	MZ771554- MZ771648
		R: ATAGGGTGGTGATTGCTC					
29	CYTB1#	F: AAATCTCACCCACTAGTT	50	428	CYTB	2	MZ771649- MZ771743
		R: TTTGTAATGACTGTAGCT					
30	CYTB2#	F: CTACACGAAACAGGGTCC	50	338	CYTB	1	MZ771744 - MZ771838
		R: ATTGGCTTAGGGGTCGGA					
31	CRF1#	F: CATGCCAGTATTAGTGAC	50	281	CR	7	MZ771839- MZ771933

S.No.	Primer	Sequence	Ta (°C)	Amplicon	Targeted genes	Polymorphic sites	Genbank accessions
	CRintR2#	R: ATACGCATGTTGACTGGA					MZ771839- MZ771933
32	CRintF2#	F: GATTAATATTGCATAGTA	50	495	CYTB, tRNA-Thr, tRNA-Pro,CR	5	
	CRintR3#	R: AATAGTTAATAGAAAGGC					

*Designed by Hassanin et al. 2011

Designed in this study

§ Overlapping number of segregating sites with the primer name

Supplementary Table A3: Mitogenome organization in *Rhinoceros unicornis*. Codons respective to each tRNA are mentioned in parenthesis.

Genes	Position		Gene length	Spaces/ overlap	Strand	Codons	
	Start	Stop				Start	Stop
tRNA ^{Phe} (GAA)	1	69	69	0	H		
rrnS	70	1041	972	-1	H		
tRNA ^{Val} (TAC)	1041	1107	67	0	H		
rrnL	1108	2681	1574	3	H		
tRNA ^{Leu} (TAA)	2685	2759	75	2	H		
NADH1	2762	3718	957	-1	H	ATG	TAA
tRNA ^{Ile} (GAT)	3718	3786	69	-3	H		
tRNA ^{Gln} (TTG)	3784	3856	73	2	L		
tRNA ^{Met} (CAT)	3859	3927	69	0	H		
NADH2	3928	4971	1044	-2	H	ATA	TAG
tRNA ^{Trp} (TCA)	4970	5037	68	5	H		
tRNA ^{Ala} (TGC)	5043	5111	69	1	L		
tRNA ^{Asn} (GTT)	5113	5185	73	2	L		
OL	5188	5217	30	-1	H		
tRNA ^{Cys} (GCA)	5217	5282	66	0	L		
tRNA ^{Tyr} (GTA)	5283	5349	67	1	L		
COX1	5351	6895	1545	-3	H	ATG	TAA
tRNA ^{Ser} (TGA)	6893	6961	69	11	L		
tRNA ^{Asp} (GTC)	6973	7039	67	0	H		
COX2	7040	7723	684	3	H	ATG	TAA
tRNA ^{Lys} (TTT)	7727	7793	67	1	H		
ATP8	7795	7998	204	-43	H	ATG	TAA
ATP6	7956	8636	681	-1	H	ATG	TAA
COX3	8636	9420	785	-1	H	ATG	TAa
tRNA ^{Gly} (TCC)	9420	9488	69	-3	H		
NADH3	9486	9835	350	0	H	ATA	TAa
tRNA ^{Arg} (TCG)	9836	9905	70	0	H		
NADH4L	9906	10202	297	-7	H	ATG	TAA
NADH4	10196	11573	1378	0	H	ATG	Taa
tRNA ^{His} (GTG)	11574	11642	69	0	H		
tRNA ^{Ser} (GCT)	11643	11701	59	1	H		
tRNA ^{Leu} (TAG)	11703	11772	70	-9	H		
NADH5	11764	13593	1830	-17	H	ATA	TAA
NADH6	13577	14101	525	3	L	ATG	TAA
tRNA ^{Glu} (TTC)	14105	14173	69	4	L		
CYTB	14178	15317	1140	0	H	ATG	AGA
tRNA ^{Thr} (TGT)	15318	15386	69	1	H		
tRNA ^{Pro} (TGG)	15388	15453	66	268	L		
CR	15454	16828	1388	/	H		

Supplementary Table A4: Details of the variable sites based on concatenated sequence of 2531bp of Indian one horned rhino mtDNA haplotypes.

Clade	Gene		COX1	ATP6	COX3	NADH4	NADH5	CYTB			CR											
	Primers		P10F COXR	ATP6F ATP6R		NADH4F NADH4R	NADH5F NADH5R	CYTB1F CYTB1R		CYTB2F CYTB2R	CRF1 CRintR2							CRintF2 CRintR3				
	Position		5	8	8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
			9	5	7	1	2	4	4	4	5	5	5	5	5	5	5	5	5	5	5	
			7	2	9	2	4	2	5	8	3	5	6	6	6	7	7	7	8	8	8	
		8	0	4	6	8	8	5	8	2	5	0	8	8	0	3	7	3	6	6	8	
Haplotype	N				0	9	5	5	2	2	9	6	5	7	3	0	0	5	4	8	4	
Uttar Pradesh	H1	10	T	G	T	G	A	G	A	G	C	T	T	G	C	T	G	A	C	G	C	T
West Bengal	H2	20	*	*	*	A	*	A	G	A	T	*	C	*	T	*	A	*	*	*	T	C
Assam	H3	1	*	*	*	A	*	*	*	*	T	C	*	A	*	*	A	*	*	*	*	C
	H4	1	*	*	*	A	*	*	*	*	T	C	*	*	*	C	A	*	*	*	*	C
	H5	1	C	T	*	A	G	*	G	*	T	C	*	A	*	*	A	G	*	*	T	*
	H6	1	*	*	C	A	*	*	*	*	T	C	*	A	*	*	A	G	*	*	T	*
	H7	2	C	T	*	A	G	*	G	*	T	*	*	*	*	C	A	*	*	*	*	C
	H8	26	C	T	*	A	G	*	*	*	T	C	*	A	*	*	A	G	*	*	T	*
	H9	1	C	T	*	A	G	C	*	A	T	C	*	A	*	*	A	G	*	*	T	*
	H10	1	*	*	C	A	*	*	*	*	T	*	C	*	*	*	A	*	T	*	T	*
	H11	5	C	T	*	A	G	*	*	A	T	C	*	A	*	*	A	G	*	*	T	*
	H12	1	*	*	C	A	*	*	*	A	T	*	C	*	*	*	A	*	T	*	T	*
	H13	1	*	*	*	A	*	*	*	A	T	*	*	*	*	*	A	*	*	*	*	C
	H14	1	C	T	*	A	G	*	*	*	T	C	*	A	*	*	A	G	*	*	*	*
	H15	1	*	*	*	A	*	*	*	*	T	*	*	*	*	*	A	*	*	*	T	*
	H16	2	*	T	*	A	G	*	*	*	T	C	*	A	*	*	A	G	*	*	T	*
	H17	2	C	*	C	A	*	*	*	*	T	*	C	*	*	*	A	*	T	A	T	*
	H18	3	*	*	C	A	*	*	*	*	T	*	C	*	*	*	A	*	T	A	T	*
	H19	2	*	*	*	A	*	*	*	*	T	*	*	*	*	C	A	*	T	A	T	*
	H20	10	*	*	*	A	*	*	*	*	T	*	*	*	*	C	A	*	*	*	*	C
	H21	3	C	*	*	A	*	*	*	*	T	*	*	*	*	C	A	G	*	*	T	*
	H22	2	*	*	*	A	*	*	*	*	T	*	*	*	*	C	A	G	*	*	T	*
	H23	3	C	*	*	A	*	*	*	*	T	*	*	*	*	C	A	*	*	*	*	C
	H24	1	*	*	*	A	G	*	*	*	T	*	*	*	*	C	A	G	*	*	*	*
	H25	1	C	T	*	A	*	*	*	*	T	C	*	A	*	*	A	G	*	*	T	*
	H26	4	C	T	*	A	G	C	*	*	T	C	*	A	*	*	A	G	*	*	T	*
	H27	1	*	*	C	A	*	C	*	*	T	C	*	A	*	*	A	*	T	*	T	*
	H28	1	C	*	T	A	*	*	*	*	T	C	*	A	*	*	A	G	*	*	T	*
	H29	1	C	T	T	A	G	*	*	*	T	*	*	*	*	C	A	*	*	*	*	C
	H30	1	*	T	T	A	G	*	*	*	T	*	*	*	*	C	A	*	*	*	*	C

Positions of nucleotides corresponds to whole mitogenome of Indian one horned rhino

N number of samples

Red nucleotides represents the clade specific SNP