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Reduced genetic diversity in greater one-horned rhinoceros in Chitwan National Park: A new challenge and opportunity for rhino conservation in Nepal

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Abstract

Understanding the genetic diversity of a species is vital to improve the effectiveness of conservation management interventions. The greater one-horned rhinoceros (*Rhinoceros unicornis*) is one of the most iconic megaherbivores in South Asia but is classified as Vulnerable by the IUCN. The species is now broadly confined to two isolated populations in Chitwan National Park (CNP), Nepal and Northeast India, which are both constrained by poaching, and the loss or degradation of habitat. Since 2016, rhino mortalities have increased in CNP for unknown reasons. To assess the impact of any current or previous mortality events, we investigated the genetic diversity in the current CNP rhino population. We collected skin samples from 67 dead rhinos found in CNP and its buffer zone between 2012 and 2019. Fragments 428 bp of mitochondrial DNA D-loop were amplified from extracted DNA using PCR and sequenced to compare with complementary sequences derived from a previous study of CNP rhinos conducted during 1986-1987. A total of six haplotypes were detected in the older sample set with a haplotype diversity (Hd) of 0.38012 compared to only four haplotypes and a Hd of 0.3374 in our cohort. This decline in haplotype diversity was unexpected as the CNP rhino population has increased by 69% over the previous 27 years. In addition, we found the haplotypes were not equally distributed across CNP, with a greater variation detected in the eastern sector (4 haplotypes) compared to the west (1 haplotype). We recommend the CNP management authorities to enhance habitat management works to facilitate movement

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of GOHR alongside consider internal translocations within the park boundaries to promote breeding between the eastern and western subpopulations to disseminate and maintain genetic diversity throughout the population.

Keywords: Genetic diversity, Greater One-horned Rhinoceros, Haplotype, Mitochondrial DNA D-loop

1 Introduction

Threatened species with small populations are prone to low genetic diversity, which limits the ability of populations to adapt environmental changes (Frankham, 2003). Wild populations are often constrained by the availability of suitable habitat, with increasingly isolated subpopulations at greater risk from stochastic processes such as disease, inbreeding, extreme weather as well as longer term changes in climatic conditions. The capacity for a population to adapt to these changes is only possible if they maintain sufficient genetic diversity (Frankham et al., 2002). Genetic studies are therefore crucial for the design of conservation priorities to maximize the retention of genetic diversity of threatened species including rhinoceros (Goossens et al., 2013). In ancient times, greater one-horned rhinoceros (*Rhinoceros unicornis*, henceforth, GOHR) occupied an uninterrupted distribution along the flood plains from the Indo-Myanmar border across the Brahmaputra Valley and the Gangetic Plain Brahmaputra to as far as the Indus River Valley in northern Pakistan, with an estimated population of at least 450,000 individuals (Dinerstein and McCracken, 1990; Laurie, 1979; Thapa et al., 2013). Since then, rampant hunting, habitat degradation and fragmentation due to deforestation for agricultural land, unplanned land use for infrastructure development, extension of tea gardens, grassland and swamps restoration for human and livestock population and uncontrolled forest fire emerged as prominent reason for the disappearance of rhino from much of its historical range (Amin et al., 2006; Hoffman et al., 2011; Moss, 2001; Owen-Smith, 1992; Sinha and Sawarkar, 1991; Subedi et al., 2017). Currently, GOHR are confined to fragmented protected areas of India and Nepal with ca. 3,300 individuals (Talukdar, 2013). The species is currently categorized as vulnerable by the International Union for Conservation of Nature (IUCN) (Talukdar et al., 2008).

Chitwan National Park (CNP) possess the only founder population of GOHR in Nepal and has been used as the source for establishing two other managed meta-populations in the western part of the country. Prior to the eradication of malaria in the lowland terai of Nepal in 1950's, the national GOHR population numbered around 1,000 individuals. However, following the amelioration of malaria, the lowland opened up to human settlement causing massive deforestation and hunting of the GOHR reducing populations to just 100 individuals in 1960s (Laurie, 1979). In the years since, effective collective conservation efforts enabled a degree of population recovery to an estimated 605 individuals by 2015 (DNPWC, 2015). Similarly, GOHR poaching rate in CNP got diminished, however, annual mortality has still increased for unknown reasons causing serious concern for its future conservation (CNP, 2019).

The management plan for CNP and its buffer zone (2013-2017) and Thapa et al. (2013) emphasized the need for genetic research as an important part of the future GOHR conservation program. Until now, few studies have focused on the genetics of GOHR. Early research by Merenlender et al. (1989) detected no allozyme variation from three GOHR individuals from Assam, India. They

hypothesized that since the current Assam population had reportedly recovered from just 12 animals in 1908, this early and long-term demographic bottleneck may have been responsible for their low genetic diversity. The Chitwan GOHR population also faced a demographic bottleneck effect during 1960s declining to approximately 100 animals from 1966 to 1972. Dinerstein and McCracken (1990) conducted the first genetic diversity study of GOHR in Nepal based on 23 samples collected in CNP between 1986 and 1987. Using protein gel electrophoresis, they found high levels of genetic variation in the CNP population. However, Morales and Melnick (1994) examined the same samples using restriction site mapping of ribosomal genes of the mitochondrial DNA and found no genetic variation. It was later argued that the small sample size and the conservative nature of the allozyme surveyed might explain the lack of genetic variation (Zschokke et al., 2011). Zschokke et al. (2011) analyzed mitochondrial control region D-loop sequences of GOHR from CNP and Assam, eastern India. They analyzed 19 samples from CNP including eight samples which had been used by Dinerstein and McCracken (1990) collected in 1986–1987. The other samples were from captive animals (caught in Nepal between 1970 and 1997) and one from a museum specimen collected in Nepal in 1924. They identified ten haplotypes and a clear genetic differentiation between both populations: six haplotypes specific to the CNP population and four haplotypes specific to the Assam population. In this study, we sequenced the same mtDNA fragments as in Zschokke et al. (2011). Our objective was to measure the genetic diversity of current GOHR population in CNP to describe the spatial distribution of haplotypes in the park and assess temporal changes in genetic diversity. We anticipate that our genetic results will assist authorities to implement innovative and improved conservation and management interventions to maintain the genetic diversity of GOHR in the future.

2 Materials and Methods

Study Area

Designated in 1973, CNP is the first national protected area and is situated in southern inner Terai lowlands of central Nepal. The Park consists of a core area that lies N 27º 20' 19" E 83º 44' 50" and N27º 43' 16" and E84º 45' 03, and is surrounded by a buffer zone that lies between N 27º 28' 23", E 84º 77' 38" and N 27º 70' 38" and E 83º 83' 98". The altitude ranges from approximately 110 m in the river region to 850 m in the Churia hill range that lies to the south. The core area of the park is 952.63 km^2 with 729.37 km^2 as buffer zone. Listed as a world heritage site in 1984 by United Nations Educational, Scientific and Cultural Organization (UNESCO), the park is considered to be the last surviving example of the natural ecosystems of the lowland Terai region. CNP is divided into four management units: Eastern sector, Kasara sector, Madi sector and Western sector (Fig. 1). The park consists of dense Sal (*Shorea robusta*) forest with mosaic of grasslands and riverine forests. We referenced our study with 2015 GOHR survey data which revealed 102 rhinos were present in the Eastern sector, 218 in the Kasara sector, 196 in the Western sector and just four in the Madi sector (DNPWC 2015).

Sample collection

Skin samples weighing approximately 10 g were collected from dead rhinos found in the core and buffer zones of CNP between 2012 and 2019 and were preserved in 99% ethanol immediately while in the field. Samples were stored at ambient temperature at the CNP veterinary laboratory until extraction.

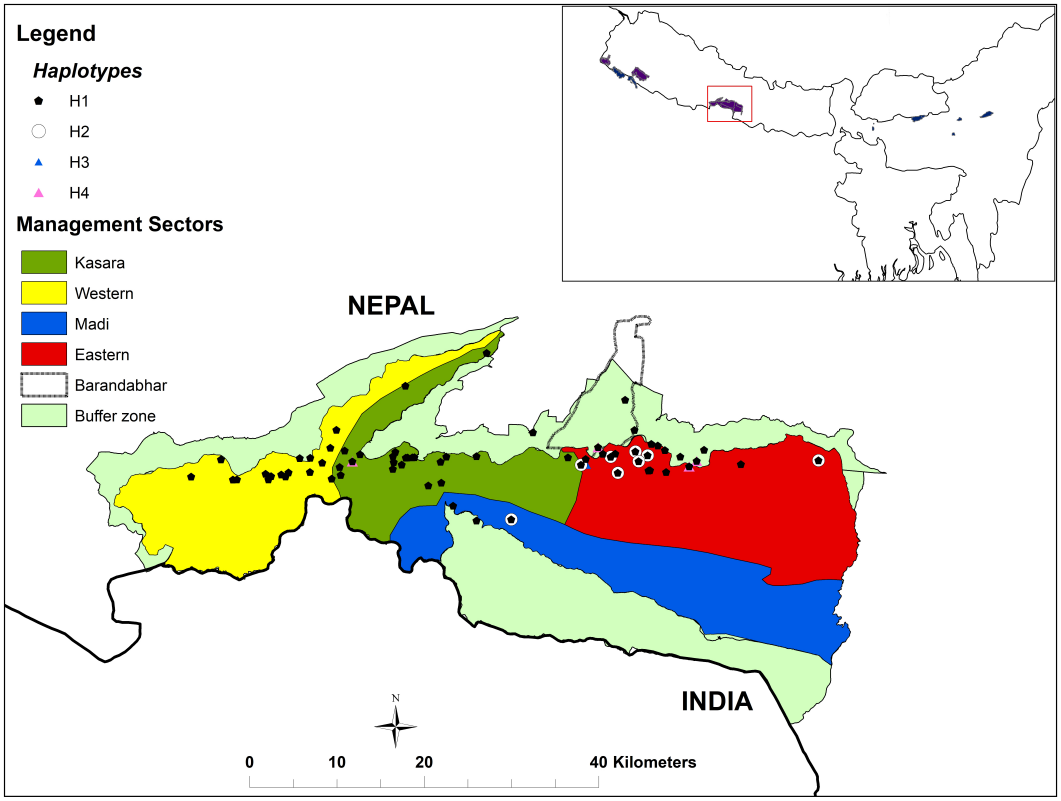

Figure 1. A map of CNP including core zone management units (Kasara = green, Western = yellow, Madi = blue, Eastern = red, buffer zone = olive and Barandabhar = hatched outline). Points represent sample collection locations, with haplotype identity indicated by colour as black diamonds (H1), white circles (H2), blue triangles (H3) and pink triangles (H4). The inset illustrates the location of the study area with reference to contemporary GOHR populations (dark blue).

DNA extraction

DNA was extracted from the tissue samples using DNeasy® Blood & Tissue kits (QIAGEN) following the manufacturer's protocol, with slight modification as described below. Approximately 25 mg of tissue was added to 180 μ Buffer ATL, to which 20 μl proteinase K (20mg/ml) was added and incubated overnight at 56°C until the tissue was completely lysed. The solution was placed in a spin column and incubated at room temperature for 1 min, and then centrifuged for 1 min at 6000 x g (8000 rpm) to elute the DNA. μ l of the eluted DNA was run on a 0.5% agarose gel electrophoresis to check its quality and quantity.

Mitochondrial D-loop sequence analysis

Polymerase chain reaction (PCR) was used to amplify a fragment of mitochondrial DNA (mtDNA) control region D-loop sequence (428 bp), using a primer pair (Rhino Forward: 5'-CGTGCATTAAATTGTTTGCC-3' and Rhino Reverse: 5'-ATACCAAATGCATGACACC-3') developed by Zschokke et al (2011). PCR was performed in 30 µl volume using 15 µl of Platinum™ II Hot-Start PCR Master Mix (2X) with 2.5 µl of DNA extract, 1.5 µl of each primer of 10 pmol concentration, 1.5 µl of bovine serum albumin and the remaining volume was adjusted by adding molecular grade water. Thermocycling conditions were set at 95℃ for 1 min for DNA denaturing, 52℃ for 1 min for primer annealing and 72℃ for 1.5 min for sequence extension. The thermocycling was repeated for 35 cycles and followed by a final extension at 72°C for 10 min. All PCRs were

conducted using Bio-Rad T100 (Bio-Rad). The PCR products were first checked for appropriate size with 2% agarose gel electrophoresis, then sequenced following bi-directional sequencing from ABI 3100 automated sequencer. Both the strands, forward and reverse strands were read from each sample. Sequences are deposited in GenBank (Acc.No. MW530801-MW530867).

The sequences were edited using Sequencher software (Ver. 5.4.5, Gene Codes Corporation). DNA SP 6.11 (Rozas et al., 2003) was used to construct haplotypes and calculate both haplotype and nucleotide diversity. Further, TCS v1.21 (Clement et al., 2000) was used to prepare parsimony network. In addition, to compare the spatial and temporal variation of genetic diversity, mtDNA control region sequences of GOHR from Zschokke et al. (2011) were extracted from NCBI GenBank (Accession number JF825390-JF825418).

3 Results

Mitochondrial D-Loop sequence analysis

In total, 428bp mtDNA D-loop sequences were obtained from 67 GOHR. From these sequences, seven polymorphic sites were found and four haplotypes were identified (H1-H4) (Appendix-I). Haplotype H1 was the most common haplotype which was found in 54 individuals, haplotype H2 was found in eight individuals, haplotype H3 was found in only one individual and haplotype H4 was found in four individuals. The haplotype diversity Hd was 0.3374 and the nucleotide diversity (Pi) was 0.00192. Haplotypes H1, H2 and H3 were found to be most similar to each other, differing by only one or two nucleotides whereas H4 was more distinct differing by five or six nucleotides from the others (Fig. 2).

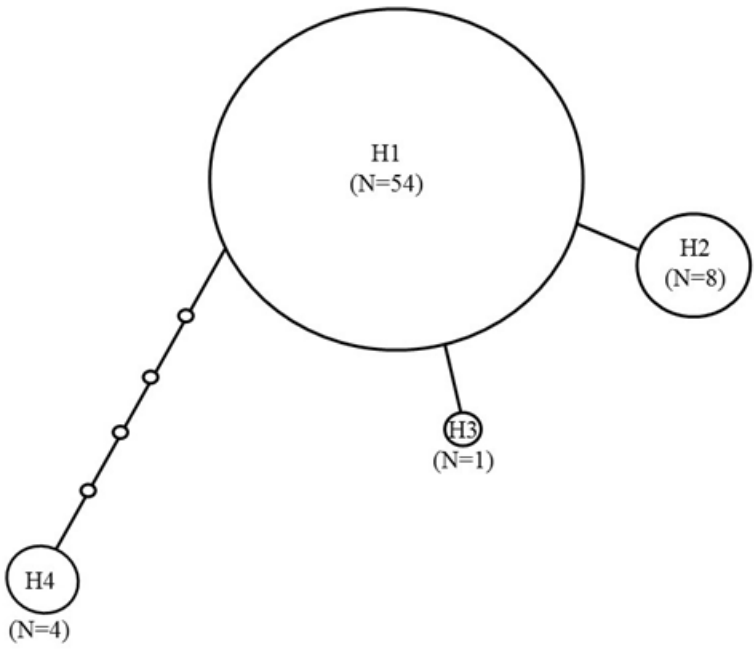

Figure 2. A parsimony network illustrating the relationship of mitochondrial D-loop sequence haplotypes identified in GOHR in CNP, Nepal (2012-19). Nodes illustrate individual nucleotides and are scaled to the number of individual rhinos identified with each haplotype (N).

Spatial and temporal variation of mtDNA haplotypes

The spatial distribution of haplotypes in CNP showed that they were not evenly distributed among sectors. All four haplotypes were found in the Eastern sector. In contrast, the other sectors containing only one or two haplotypes (Table 1, Fig. 1). For temporal variation of genetic diversity, we compared our findings with the earlier study that used comparable methods (Zschokke et al. 2011). Three haplotypes detected in this study were also identified by Zschokke et al. (2011) but the remaining haplotype has not been identified previously. Furthermore, Zschokke et al. (2011) found no overlap in the haplotype identity found in Assam and in the CNP GOHR population. However, haplotype H4 detected in this study had previously been detected in Assam by Zschokke et al. (2011) (Table 2). Despite a disparity in sample size, genetic diversity was lower in the contemporary sample set (four haplotypes, Hd =0.3374, n = 67) than in the earlier study (six haplotypes, Hd =0.38012, n = 19). Zschokke et al. (2011)'s Nepal samples came from different time periods: eight were collected in 1986-1987 from Chitwan, one was from a museum specimen (captured in 1924), and ten were collected from captive individuals originating from Chitwan. These individuals or their mothers respectively had been captured between 1970 and 1997 in Chitwan. Three of the haplotypes detected in our study were also detected by Zschokke et al. (2011)'s and the most common haplotype was the same in both studies (haplotype H1, equivalent to H7 in Zschokke et al.'s study). The frequency of the most common haplotype showed a tendency of increase though not significantly; in 1986-1987 samples, the frequency of this haplotype was 66.7% (6/9); in 1995-2004 samples, the frequency was 70% (7/10); in the 2012-2019 samples (this study), the frequency increased to 80.6% (54/67).

Table 1. Spatial distribution of mtDNA haplotypes detected in GOHR in each management sector of CNP, Nepal.

Sector	Estimated individuals*	Samples sequenced	Haplotype			
			H1	H2	H3	H4
Kasara	218	23	23	0	0	0
Western	196	18	17	0	0	1
Madi	4	2	1	1	0	0
Eastern	102	22	11	7	1	3
Shared region:						
Barandabhar (Kasara/ Eastern sector)	20	1	1	0	0	0
Rapti-Reu Dovan-Saili maili Khola (Kasara/ Western sector)	65	1	1	0	0	0
Total	**605**	**67**	**54**	**8**	**1**	**4**

*: Data from DNPWC (2015).

Table 2. The mtDNA control region D-loop sequence haplotypes of this study and Zschokke et al. (2011). Haplotypes in the same rows indicated the same sequences in both studies.

This study			Zschokke et al. (2011)		
Haplotype	No. of individuals	Source	Haplotype	No. of individuals	Source
			Zschokke-H1	1	Assam, India
			Zschokke-H2	1	Assam, India
			Zschokke-H3	3	Assam, India
H4	4	CNP*	Zschokke-H4	5	Assam, India
			Zschokke-H5	1	CNP, Nepal
			Zschokke-H6	1	CNP, Nepal
H1	54	CNP	Zschokke-H7	13	CNP, Nepal
			Zschokke-H8	1	CNP, Nepal
H2	8	CNP	Zschokke-H9	1	CNP, Nepal
			Zschokke-H10	2	CNP, Nepal
H3	1	CNP			

*: CNP: Chitwan National Park, Nepal.

4 Discussion

In this study, we used mtDNA D-loop sequences to estimate genetic diversity of GOHR in CNP. Our samples comprised 11% of the current population. We found that although the population size of GOHR in Nepal has increased by approximately 69% in the last 27 years (i.e. 358 & 605 GOHR individuals in 1988 and 2015 respectively in CNP), their genetic diversity did not increase accordingly. The haplotype diversity showed a slight decrease and the frequency of the most common haplotype increased slightly through time. This could be an indication of reduced genetic diversity of GOHR in Nepal through time. In addition, Zschokke et al. (2011) found that GOHR populations in Assam and Nepal were genetically distinct, with no mtDNA haplotype shared between Nepal and Assam GOHR populations. However, haplotype H4 of this study was also identified in Assam GOHR in Zschokke et al. (2011)'s study. This haplotype represented 6% of our samples, 50% of the Assam samples and was the most common haplotype found in Assam GOHR (Zschokke et., 2011). Both CNP and Assam (mainly in Kaziranga National Park) represent the two largest extant GOHR populations in the world. Given the long distance between the two places (ca. 850 km) and unsuitable habitats in between for this mega herbivore, it is unlikely that there is ongoing gene flow between CNP and Assam. Therefore, we argue that in the past, both populations might represent a single large population and the shared haplotype found in both regions reflects their historical connection.

We found that the mtDNA haplotypes are not evenly distributed across CNP with high genetic diversity found in the Eastern sector (Table 1). The tendency for GOHR to move between the management units (i.e. four different sectors) of CNP is unknown. However, previous studies have demonstrated significant seasonal movement of GOHRs within the Eastern sector, favoring the west of the sector in spring and moving to east during the monsoon (Laurie 1979). Currently, Kasara sector supports the highest number of GOHR individuals followed by the Western, Eastern and Madi sectors (DNPWC 2015) (See Table 1). The GOHR population in the Eastern sector is in rise increasing from 77 individuals in 2011 (Subedi et al., 2013) to 102 rhinos in 2015 (DNPWC, 2015). The recent increase of the GOHR population and the retention of haplotype diversity is positive within the Eastern sector which presents the sub-population as unique one while compared with the other sector. Elsewhere, the dominance of the H1 haplotype in the Kasara and Western sectors that hold the majority of GOHR is a cause for concern. Genetic diversity could be maintained

using a combination of strategies including management changes to halt the decline of GOHR in the Eastern sector, measures to encourage the movement of GOHR from the Eastern to the Kasara and Western sectors or vice-versa and the translocation of GOHR within the park.

Nevertheless, reduced genetic diversity represents a challenge for conservation but can be maintained with appropriate management intervention. Many rhino species are found with low genetic diversity. For instance, genetic diversity is now extremely low in both Sumatran rhinoceros (*Dicerorhinus sumatrensis*) and Javan rhinoceros (*Rhinoceros sondaicus*) (Goossens et al. 2013). Similarly, low genetic variation has also been measured in black rhinoceros (*Diceros bicornis*) and white rhinoceros (*Ceratotherium simum*) in Africa (Ashley et al. 1990; O'Ryan and Harley 1993; Van Coeverden de Groot et al. 2011). However, the results of genetic studies of black rhinos are not consistent throughout the African continent countries. For instance, in Kenya, while studying 12 subpopulations of black rhinos, the genetic diversity varied greatly among subpopulations (Muya et al. 2011). In white rhinos, Moodely et al. (2018) studied 217 southern white rhinos and 15 northern white rhinos, in which mitochondrial genetic study showed two haplotypes in southern white rhino and only one haplotype in northern white rhino. In this scenario, our GOHR genetic study's result doesn't present serious risk to the species instead reveals the necessity of high consideration of genetic aspect for future GOHR conservation programs.

Conservation Implications

The government of Nepal has prepared a GOHR conservation action plan (2017-2021) that is well-documented and incorporates wide consultations to outline detailed conservation actions to ensure the persistence of the species in Nepal (DNPWC, 2017). However, the current plan does not consider the genetic diversity of the population or prioritize strategies to maintain or improve genetic diversity in the long term. We recommend that genetic considerations be integrated into the existing action plan to ensure the maintenance of GOHR genetic diversity and prevent the extinction of this important population. Ignoring genetic factors may reduce the conservation program's objective in the long term (Frankham, 2010). Specifically, management of fragmented populations like that of the GOHR needs to consider how constrained gene flow and genetic drift in small population reduces overall diversity with resulting inbreeding to increasing extinction risks (Frankham, 2003). Furthermore, genetic results may be used together with ecological and behavioral data to define and manage viable populations of endangered species (Merenlender et al., 1989).

The decline in genetic diversity in our result emphasizes the need for a proper genetic improvement program in CNP through measures such as promoting GOHR movement within the protected area by developing better habitats alongside exploring opportunity for internal translocation that encourage local mixing of GOHR population. Translocation of GOHR from CNP to other protected areas in Nepal and India have been conducted between 1986-2017. In Nepal, 100 GOHR have been translocated to Bardia National Park and Shuklaphanta National Park in the west of the country to establish meta populations and reduce the threat of local extinction due to catastrophic events such as disease and poaching (Thapa et al., 2013). Similarly, four female GOHR were translocated from CNP to Duduwa National Park in Uttar Pradesh, India to supplement an existing population in order to increase its viability (Sale and Singh, 1987; Pluháček et al., 2007). Intriguingly, in Nepal several studies have proposed that CNP could contribute between five and 13 GOHR to Shuklaphanta National Park to increase the viability of the population there (Kafley et al., 2015, Subedi et al., 2017, Yonzon, 2003). However, as yet the internal translocation of GOHR within CNP has not been considered. Internal translocation would help to mix the population

of GOHR to promote gene flow within the park ultimately increasing or at least maintaining the genetic diversity of the population.

The introduction of genetic protocols prior to GOHR translocation to other locations is essential. Previously, more than 60% of GOHR translocated from CNP have originated from a single location within Kasara sector, more specifically Sukhibhar block (Bishnu Thapaliya, personal communication, May 31, 2020), which supports the highest number of rhinos (see DNPWC 2015 Technical report), but this strategy could be deleterious. Based on our results, it is likely that translocation of GOHR from this founder population would involve common rhino haplotypes that are over-represented in the founder population (i.e., haplotype H1 only). This could lead to reduced genetic diversity in the recipient populations. We recommend that a comprehensive plan and protocol be prepared prior to future translocations involving GOHR from different blocks and representing different haplotypes in a way that does not deplete the genetic diversity of the founder population but maximizes it in the recipient population. Nevertheless, this study is limited to mtDNA D-loop sequence analysis which provides an avenue for the in-depth study with advanced molecular markers in future. Further, integrated research on demographic, ecological and genetic aspects of GOHR, rhino genetic profiling for prosecution and capacity building of protected area officials with respect to conservation genetics is very important.

Conflict of interests

The authors declare that they have no conflict of interest.

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Appendix-I

Table 3. Details of GOHR samples used in this study

Sample ID	Collection date	Sex[1]	Location of collection	Latitude	Longitude	mtDNA haplotype
ARCT1	2016	M	Madi	27.50888	84.27129	H1
ARCT2	2016	M	Eastern	27.54458	84.49801	H1
ARCT3	2017	U	Western	27.53927	83.96721	H1
ARCT4	2017	M	Eastern	27.5543	84.48654	H2
ARCT5	2017	U	Eastern	27.56205	84.45947	H1
ARCT6	2017	U	Eastern	27.55851	84.40466	H3
ARCT7	2017	F	Eastern	27.55875	84.53489	H1
ARCT8	2017	U	Western	27.53621	84.01582	H1
ARCT9	2017	F	Eastern	27.55434	84.55384	H4
ARCT10	2017	F	Eastern	27.56203	84.44513	H1
ARCT11	2017	U	Kasara	27.55482	84.15444	H4
ARCT12	2015	F	Western	27.56886	84.12883	H1
ARCT13	2015	M	Kasara	27.54706	84.20128	H1
ARCT14	2012	F	Western	27.63283	84.2161	H1
ARCT15	2015	U	Kasara	27.58447	84.36405	H1
ARCT16	2015	M	Kasara	27.56225	84.16324	H1
ARCT17	2019	U	Kasara	27.56456	84.20432	H1
ARCT18	2016	U	Western	27.55358	84.11946	H1
ARCT19	2015	M	Eastern	27.56433	84.48281	H2
ARCT20	2015	U	Eastern	27.56575	84.56249	H1
ARCT21	2014	U	Western	27.56599	84.14544	H1

Sample ID	Collection date	Sex[1]	Location of collection	Latitude	Longitude	mtDNA haplotype
ARCT22	2016	M	Western	27.55886	84.22397	H1
ARCT23	2016	U	Western	27.58741	84.13589	H1
ARCT24	2016	M	Eastern	27.57206	84.5013	H1
ARCT25	2016	F	Western	27.66665	84.31065	H1
ARCT26	2017	F	Eastern	27.54298	84.51837	H1
ARCT27	2015	F	Eastern	27.56028	84.497	H2
ARCT28	2016	F	Western	27.53636	84.05702	H1
ARCT29	2016	M	Western	27.55849	84.10552	H1
ARCT30	2017	U	Kasara	27.5587	84.21855	H1
ARCT31	2016	U	Eastern	27.54236	84.46239	H2
ARCT32	2017	U	Eastern	27.58657	84.48189	H1
ARCT33	2017	M	Eastern	27.54873	84.54513	H4
ARCT34	2017	U	Western	27.54139	84.07194	H1
ARCT35	2016	M	Eastern	27.54494	84.49964	H1
ARCT36	2019	M	Eastern	27.55671	84.4252	H1
ARCT37	2019	M	Kasara	27.55144	84.21157	H1
ARCT38	2019	F	Western	27.53964	84.05997	H1
ARCT39	2019	F	Western	27.5439	84.10522	H1
ARCT40	2018	F	Kasara	27.56019	84.20146	H1
ARCT41	2017	F	Eastern	27.5703	84.50889	H1
ARCT42	2017	F	Western	27.55834	84.09326	H1
ARCT43	2017	M	Kasara	27.53627	84.02039	H1
ARCT44	2017	F	Madi	27.49466	84.33855	H2
ARCT45	2018	F	Eastern	27.55076	84.60509	H1

Sample ID	Collection date	Sex[1]	Location of collection	Latitude	Longitude	mtDNA haplotype
ARCT46	2018	M	Rapti-Reu Dovan-Sailimaili Khola[2]	27.53926	84.07678	H1
ARCT47	2019	M	Kasara	27.55901	84.22643	H1
ARCT48	2017	M	Kasara	27.55951	84.2634	H1
ARCT49	2018	F	Western	27.55699	84.002	H1
ARCT50	2018	U	Kasara	27.54899	84.13969	H1
ARCT51	2018	M	Western	27.54342	84.08036	H1
ARCT52	2018	F	Kasara	27.55407	84.20234	H1
ARCT53	2017	U	Kasara	27.54073	84.14082	H1
ARCT54	2017	M	Kasara	27.55858	84.21638	H1
ARCT55	2018	U	Kasara	27.49347	84.29817	H1
ARCT56	2018	U	Kasara	27.53707	84.13033	H1
ARCT57	2017	F	Eastern	27.55891	84.45422	H2
ARCT58	2018	F	Barandabhar[3]	27.61765	84.47122	H1
ARCT59	2018	F	Kasara	27.55997	84.29848	H1
ARCT60	2018	F	Eastern	27.56888	84.43966	H4
ARCT61	2017	M	Eastern	27.56552	84.51711	H1
ARCT62	2017	M	Eastern	27.55426	84.69524	H2
ARCT63	2019	F	Kasara	27.54211	84.05363	H1
ARCT64	2018	U	Kasara	27.53271	84.25755	H1
ARCT65	2019	M	Eastern	27.55079	84.41956	H2
ARCT66	2016	F	Kasara	27.55425	84.25677	H1
ARCT67	2016	F	Kasara	27.5298	84.24239	H1

Note: 1. M: male; F: female; U: undetermined. 2. Rapti-Reu Dovan-Sailimaili Khola region is the shared region of Kasara and Western sector. 3. Barandabhar region is the shared region of Kasara and Eastern sector.