

Additional file 1 Table S1: 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: CAATGCTCHGARATYTYGG	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: GCDGATTTCCDGTKGCDGCTA					
18	P19*	F: GACGARCAGAYGCHAAAYACAGC	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
22	P23*	R: CCCTTYTCTGGTTTACAAGACC	55	~650	tRNAThr, tRNAPro, CR	10	MZ736693-MZ736708
		F: CATCGGACAACACTAGCATCTAT					
		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	tRNAPhe, CR	6\$23	
	CRR1#	R: CAAGGTGTTGTTAGCTAC					
Phylogeography Primers							
25	P10*	F: GGNGGNTTYGGHAAAYTGACT	45	431	COX1	1	MZ771364-MZ771458
	COXR#	R: GTAGACTTCAAAATGACC					
26	ATP6#	F: TCTAATACTTAATCGG	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	6	
	CRintR3#	R: AATAGTTAATAGAAAGGC					

*Designed by Hassanin et al. 2011

Designed in this study

§ Overlapping number of segregating sites with the primer name

Additional file 1 Table S2: Sample (both tissue and dung) of Indian rhinos used in this study. A total of 111 individual rhino samples (72 tissue and 39 dung samples, respectively) were used in this study. Out of the 72 tissue samples, 16 samples representing different parks were used to generate the whole mitogenome data.

State	Protected areas	Total tissue samples ^a	Tissue used for whole mitogenome data ^b	Dung collected	Dung used for phylogeography ^c
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

^a Total number of tissue samples used in this study. Out of 72, 16 were used for mitogenome data generation as population-wise details given in next column.

^b Details of representative tissue samples to generate whole mitogenome data for identification of polymorphic sites across Indian rhino populations

^c Dung details of identified rhino individuals representing six parks to ensure spatial coverage for phylogeography analysis. In total 111 samples were used for this work consisting of 72 tissues and 39 dungs.

Additional file 1 Table S3: 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		

Additional file 1 Table S4: Comparative analysis results of genetic diversity indices among five extant rhino species

Mitochondrial genes	African rhino			Sumatran rhino			Javan rhino			Indian rhino		
	S	H _d	π	S	H _d	π	S	H _d	π	S	H _d	π
rrnS	51	0.67	0.0352	0	0	0	1	0.53	0.0006	0	0	0
rrnL	68	0.67	0.0289	7	0.85	0.0014	13	0.73	0.0037	0	0	0
ATP6	64	0.67	0.0627	5	0.69	0.6857	4	0.80	0.8000	1	0.50	0.5000
ATP8	12	0.67	0.0392	1	0.42	0.0021	2	0.60	0.0043	0	0	0
COX1	100	0.67	0.0431	12	0.84	0.0033	11	0.93	0.0029	1	0.50	0.0003
COX2	49	0.67	0.0478	2	0.51	0.0008	4	0.73	0.7333	0	0	0
COX3	69	0.67	0.0587	3	0.54	0.0008	3	0.60	0.0018	1	0.13	0.0002
CYTB	98	0.67	0.0573	14	0.88	0.0050	8	0.73	0.0036	3	0.57	0.0011
NADH1	55	0.67	0.0384	6	0.78	0.0027	4	0.73	0.0020	0	0	0
NADH2	78	0.67	0.0499	7	0.89	0.0022	6	0.73	0.0029	0	0	0
NADH3	28	0.67	0.0540	5	0.81	0.0054	1	0.33	0.0010	0	0	0
NADH4	108	0.67	0.0523	14	0.73	0.0042	7	0.80	0.0023	1	0.33	0.0002
NADH4L	27	0.67	0.0606	2	0.26	0.0009	2	0.53	0.0036	0	0	0
NADH5	144	0.67	0.0527	14	0.81	0.0032	11	0.73	0.0031	1	0.50	0.0003
NADH6	51	0.67	0.0648	1	0.42	0.0008	4	0.73	0.0042	0	0	0
tRNA	65	0.67	0.0290	7	0.82	0.0016	0	0	0	1	0.33	0.0002
CR	185	0.67	0.1395	31	0.93	0.0108	21	0.93	0.0118	9	0.85	0.0045

Additional file 1 Table S5: 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	ATP6F		NADH4F	NADH5F	CYTBF1	CYTBF2	CRF1					CRintF2									
			COXR	ATP6R		NADH4R	NADH5R	CYTBR1	CYTBR2	CRintR2					CRintR3									
	Position		5	8	8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Haplotype		N				0	9	5	5	2	2	9	6	5	7	3	0	0	6	5	4	8	4	
Uttar Pradesh	H1	10	T	G	T	G	A	G	A	G	C	T	T	G	C	T	G	A	T	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	*	C	*	*	*	T	*
	H16	2	*	T	*	A	G	*	*	*	T	C	*	A	*	*	A	G	*	*	*	T	*	
	H17	2	C	*	C	A	*	*	*	*	T	*	C	*	*	*	A	*	C	T	A	T	*	
	H18	3	*	*	C	A	*	*	*	*	T	*	C	*	*	*	A	*	C	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