Complete Mitogenome of Endangered and Endemic Nicobar Treeshrew (*Tupaia Nicobarica*) and Comparison with Other Scandentians

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Abstract

The Nicobar treeshrew (*Tupaia nicobarica*) is an endangered smaller mammal endemic to the Nicobar Island of the Andaman Sea, India regarded as an alternative experimental animal model in biomedical research. The present study aimed to assemble the first mitochondrial genome of *T. nicobarica* to elucidate evolutionary relationship. The structure and variation of the novel mitochondrial genome were analyzed and compared with other Scandentians. The complete mitogenome (17,164 bp) encodes 37 genes, including 13 protein-coding genes (PCGs), 22 transfer RNA (tRNAs), two ribosomal RNA (rRNAs), and one control region (CR). Most of the genes were encoded on majority strand, except nad6 and eight tRNAs. The nonsynonymous/synonymous ratio in all PCGs indicates strong negative selection among all Tupaiidae species. The comparative study of CRs revealed the occurrence of tandem repeats (CGTACA) found in *T. nicobarica*. The phylogenetic analyses (ML and BA) showed distinct clustering of *T. nicobarica* with high branch supports and depict a substantial divergence time (11.4 to 18.8 MYR) from the ancestor lineage of Tupaiidae. The 16S rRNA dataset corroborates the taxonomic rank of two subspecies of *T. nicobarica* from the Great and Little Nicobar Islands. The present study suggests the assembly of whole-genome to improve the understanding of evolutionary relationships of treeshrews and its implication in biomedical research.

Introduction

The biogeography of India is mainly classified into two categories, the mainland and groups of islands (Lakshadweep and Andaman-Nicobar). The Andaman and Nicobar (AN) archipelago was made up by submarine habitable mountains and constituted with 572 chain of islands located on the Bay of Bengal¹. The AN archipelago was formed due to collision between the Indian Plate and Eurasian Plate which commenced about 50 million years ago and continues today². Due to the unplugged landmass from the mainland of India and other diachronic makeup, this group of islands accommodates numerous unique elements of biodiversity³. Thus, these islands ecosystems were thought to be a trenchant biogeographic entity which can be evaluated to be a substantial model for evolutionary studies^{4,5}. The study on faunal diversity of AN archipelago has been started since 19th century, considering the large size or charismatic vertebrate fauna, including mammals, birds, and herpetofauna^{6–8}. Due to the remoteness and inaccessibility throughout the year, most of the regions of AN archipelago are sparsely explored. Remarkably, these oceanic islands also provide a suitable habitat for many smaller mammals like, treeshrews (order Scandentia), shrews (order Eulipotyphla), and rodents (order Rodentia)⁹.

The world treeshrew account for 23 species under four genera (*Anathana*, *Dendrogale*, *Ptilocercus* and *Tupaia*) of two families (Tupaiidae and Ptilocercidae) which are distributed in South Asia, Southeast Asia, and Southwest China^{10,11}. The mainland of India is known by two species, the Madras treeshrew, *Anathana ellioti* and the Northern treeshrew, *Tupaia belangeri*. However, the Nicobar treeshrew, *Tupaia nicobarica* is endemic to the Nicobar Islands¹². This species is categorized as 'Endangered' species in the IUCN Red List of Threatened Species and listed under 'Appendix II' in the Convention on International

Trade in Endangered Species of Wild Fauna and Flora (CITES)¹³. On the basis of geographical distribution, two subspecies have been recognized, viz., *T. nicobarica nicobarica* from the Great Nicobar Island and *T. nicobarica surda* from the Little Nicobar Island¹⁴. Later on, these subspecies status has been endorsed by certain researchers and hinted further molecular investigation required to corroborate their taxonomic status^{12,15,16}. Besides, the zoonotic disease has been originated or transmitted through different mammalian species and cause life threatening to human beings throughout the globe^{17,18}. The treeshrews (order Scandentia) species is evidenced to be a significant model for various human disorders like, depression, myopia, hepatitis B and C virus infections, and hepatocellular carcinoma^{19,20}. The molecular study and genome sequencing has been demonstrated the genetic basis of signaling pathways in nervous and immune systems of the Chinese treeshrew and evidenced as a potential model for biomedical research²¹.

The characterizations of complete mitogenomes are widely used genomics approaches for systematics studies and evolutionary research of wider group of taxa including mammals^{22–24}. So far, the complete mitogenome of five species, *Tupaia belangeri*, *Tupaia minor*, *Tupaia montana*, *Tupaia splendidula*, and *Tupaia tana* were generated from different geographical regions. The molecular studies were previously aimed to infer the phylogenetic and evolutionary relationship, genetic structure, and possible gene flow of Scandentia across their range distribution in Southeast Asia^{25,26}. In addition, the partial mitochondrial genes (12S rRNA, 16S rRNA, and Cytochrome b) were also utilized to elucidate the phylogenetic position and diversification of Tupaiidae species including *T. nicobarica*^{15,27,28}. However, the in-depth genetic information and structural motifs of *T. nicobarica* mitogenome is still anonymous to the scientific communities. To fill the gap of knowledge, the present study aimed to determine the complete mitogenome of *T. nicobarica* from AN archipelago, India. The comparative analyses were confronted to check the structure and variation within the Tupaiidae mitogenomes. The phylogenetic analyses and divergence time were estimated to infer the evolutionary relationship of *T. nicobarica* comparing with other Tupaiidae species. Further, an additional dataset of mitochondrial 16S rRNA was also constructed to clarify the taxonomic rank of the extant subspecies of *T. nicobarica* in the Great and Little Nicobar Island.

Materials And Methods

Sample collection and DNA extraction

The museum sample of *T. nicobarica* is vouchered at the National Zoological Collections of Zoological Survey of India, which was collected from the Galathia, Campbell bay (06.51 N 93.50 E). No treeshrew specimen was killed; hence no prior permission was required in the present study. The tissue sample was aseptically collected from the archival specimen with ample attention and stored in 70% ethanol for downstream molecular investigation. The collection locality map was prepared by the online platform (<https://noaa.maps.arcgis.com>). The tissue sample was homogenized with 1 ml buffer containing 0.32 M Sucrose, 1 mM EDTA, and 10 mM TrisHCl by the WiseTis HG-15 homogenizer. To remove the nuclei

and cell debris, the working mixture was centrifuged at 700 g for 5 min at 4°C. The supernatant was collected in 1.5 ml centrifuge tube and centrifuged at 12,000 g for 10 min at 4°C to precipitate the mitochondrial pellet. The pellet was re-suspended in 500 µl of buffer (50 mM TrisHCl, 25 mM of EDTA, 150 mM NaCl) and incubated at 56°C for 1-2 hr along with 20 µl of proteinase K (20 mg/ml). The mitochondrial DNA was extracted by using QIAamp DNA Investigator Kit (QIAGEN Inc.) and the eluted volume was reduced to 100 µl to increase the mtDNA concentration. The quality of the extracted mtDNA was checked through 1% agarose gel electrophoresis, and the concentration was quantified with a NANODROP 2000 spectrophotometer (Thermo Scientific).

Sequencing, assembly and annotation

The complete mitogenome sequence and assembly were carried out at PHIXGEN Pvt. Ltd. Gurugram, India (<http://www.phixgen.com>). The mitochondrial DNA (>100 ng) was used in Illumina TruSeq Nano DNA HT library preparation kit for library assembly (Illumina, Inc, USA). The mtDNA was fragmented by ultra-sonication and the A-tailed fragments were joined with the sequencing indexed adapters (Covaris M220, Covaris Inc., Woburn, MA, USA). The mtDNA fragments (450 bp) were selected through sample purification beads and enrich by polymerase chain reaction (PCR). The amplified PCR library was examined using Bioanalyzer 2100 (Agilent Technologies, Inc., Waldbronn, Germany) with high sensitivity DNA chips. Total >4 million raw reads were generated through Illumina NextSeq500 (150 X 2 chemistry) after obtaining the expected concentration (650.25 pg/µl) and mean peak size (465 bp) (Illumina, Inc, USA). To obtain the considerable sequencing depth (>75000X), the raw nucleotide reads were screened using cutadapt tool (<http://code.google.com/p/cutadapt/>) and trimmed with a cut-off of Phred quality scores of Q20. The high-quality reads were downsampled to 2 million using Seqtk (<https://github.com/lh3/seqtk>) and assembled using NOVOPlasty v2.6.7 using default parameters²⁹. The mitogenome of *T. belangeri* (accession no. NC_002521) was used as a reference seed sequence for assembly. The typical circular representation of the generated mitogenome of *T. nicobarica* was plotted by CGView Server (http://stothard.afns.ualberta.ca/cgview_server/) with default parameters³⁰. Further, the contig was subjected to confirmation by the MITOS v806 online webserver (<http://mitos.bioinf.uni-leipzig.de>). The direction and arrangements of PCGs, tRNAs, and rRNAs were confirmed through MITOS online server (<http://mitos.bioinf.uni-leipzig.de>)³¹. The start and stop codons of each PCG were assured through the Open Reading Frame Finder web tool (<https://www.ncbi.nlm.nih.gov/orffinder/>) on the basis of vertebrate mitochondrial genetic code and other publicly available reference sequences of Tupaiidae. The mitogenome was submitted to the GenBank database (Accession No. MW751815) using the NCBI Bankit submission tool.

Dataset construction and comparative analysis

On the basis of taxonomic classification, the mitogenomes of five Tupaiidae species were downloaded from GenBank and merged in the dataset for comparative analysis (Supplementary Table S1). The genome sizes and nucleotide compositions of all the studied species were calculated using MEGA X³². To calculate the base composition skew, we utilized previously known formula: $AT\ skew = (A - T)/(A + T)$,

GC skew = $(G - C)/(G + C)$ ³³. The overlapping regions and intergenic spacers of *T. nicobarica* and other Tupaiidae species mitogenomes were calculated manually. The pairwise test of the synonymous (Ks) and non-synonymous (Ka) substitutions were calculated between *T. nicobarica* and other Tupaiidae species using DnaSPv6.0³⁴. The comparative analysis of relative synonymous codon usage (RSCU) and relative abundance of amino acids were also calculated using MEGA X. The secondary structures of tRNA genes were affirmed by tRNAscan-SE Search Server 2.0 (<http://lowel.ab.ucsc.edu/tRNAscan-SE/>) and ARWEN 1.2^{35,36}. To speculate the putative domains and motif, the CR of *T. nicobarica* and other Tupaiidae species was screened from the database. The tandem repeats within the CR were predicted by the online Tandem Repeats Finder web tool (<https://tandem.bu.edu/trf/trf.html>)³⁷.

Phylogenetic analysis and divergence time estimation

To assess the phylogenetic relationship, the dataset was constructed with the representatives of Scandentia, Dermoptera, and Primates based on the previous literature¹⁵. The 13 PCGs of 14 mitogenomes were aligned and concatenated using TranslatorX (with MAFFT algorithm with L-INS-i strategy and GBlocks parameters) and SequenceMatrix v1.7.84537^{38,39}. The best fit model (GTR+I+G) was estimated by PartitionFinder 2 using lowest Bayesian information criterion (BIC) criterion⁴⁰ and the maximum-likelihood (ML) tree was constructed using the IQ-Tree web server with 1000 bootstrap support⁴¹. The estimation of divergence times among Tupaiidae species were calculated by Bayesian relaxed clock method in BEAST v2.4.7⁴². The GTR+ I+ G substitution model, empirical base frequencies, and relaxed uncorrelated log-normal clock with the Yule speciation model was applied as Tree prior. A total of four fossil calibration points were applied in the phylogeny to constraint the analysis as described in the previous study^{15,43-45}: (i) node A, 18 million years ago (MYA) log-normal prior as the minimum age of *Tupaia* based on the fossil of *T. miocenica*, (ii) node B, 23 MYA log-normal prior as the minimum age for the split between *Pygathrix* and *Hylobates*, (iii) node C, a normal prior for the primate outgroups with a mean of 77.5 MYA, and (iv) node D, the total tree height is considered as a normal prior with a mean of 90 MYA for Scandentia, Dermoptera, and Primates. Two independent Markov chain Monte Carlo (MCMC) runs were performed for 1,000,000 generations with 25% burn-in and trees sampled every 2000 generations. To combine these runs, log files were imported into LogCombiner of the BEAST package. The adequate MCMC mixing and convergence were estimated using the effective sample size (ESS) values (>200 in all parameters) in Tracer v.1.7.1⁴⁶. To reconstruct the Bayesian (BA) phylogeny, TreeAnnotator was used which is included in the BEAST package. The consensus tree was further visualized by FigTree 1.4.4 with a divergence time of 95% higher probability density (HPD)⁴⁷.

In addition, to check the taxonomic rank of two named subspecies, *T. nicobarica nicobarica* from the Great Nicobar Island and *T. nicobarica surda* from the Little Nicobar Island, an additional dataset of 16S rRNA gene was constructed, including 18 database sequences of 12 Tupaiidae species known from South and Southeast Asian countries (Supplementary Table S2). The 16S rRNA sequence of the Sunda

flying lemur, *Galeopterus variegatus* (Accession No. NC_004031) was used as an out-group in the second dataset.

Results And Discussion

Mitogenome structure and organization

The mitogenome (17,164 bp) of the endangered Nicobar treeshrew, *T. nicobarica* was determined in the present study (GenBank accession no. MW751815). The mitogenome contained 37 genes, comprising 13 PCGs, 22 tRNAs, 2 rRNAs, and a major non-coding CR. Among them, nine genes (nad6 and eight tRNAs) were placed on the negative strand, while the remaining 28 genes were placed on the positive strand (Table 1, Fig. 1). In the order Scandentia, the length of the Tupaiidae mitogenome varied from 16,183 bp (*T. montana*) to 17,164 bp (*T. nicobarica*). All Tupaiidae species showed the same gene arrangement as observed in typical vertebrate's mitogenome⁴⁸. The nucleotide composition of the *T. nicobarica* mitogenome was A+T biased (58.3%), as in all Tupaiidae species ranging from 58.3% (*T. nicobarica*) to 59.72% (*T. tana*) (Table 2). The AT skew and GC skew were 0.11 and -0.30 in the mitogenome of *T. nicobarica*. The comparative analysis showed that the AT skew ranged from 0.08 (*T. minor* and *T. tana*) to 0.11 (*T. nicobarica*) and the GC skew from -0.28 (*T. minor*, *T. montana*, *T. splendidula*, *T. tana*) to -0.30 (*T. nicobarica*) (Table 2). A total of 14 overlapping regions with a total length of 87 bp were identified in *T. nicobarica* mitogenome. The longest overlapping region (43 bp) was observed between the ATP synthase F0 subunit 8 (atp8) and ATP synthase F0 subunit 6 (atp6). Further, a total of 14 intergenic spacer regions with a total length of 68 bp were observed in *T. nicobarica* mitogenome with the longest region (33 bp) between tRNA-Asparagine (trnN) and tRNA-Cysteine (trnC) (Supplementary Table S3).

Protein-coding genes

The total length of PCGs was 11,410 bp in *T. nicobarica*, which represents 66.47% of the complete mitogenome. The nucleotide composition of the *T. nicobarica* PCGs was A+T biased (57.95%), as in all Tupaiidae species ranging from 57.95% (*T. nicobarica*) to 59.61% (*T. tana*) (Table 2). The AT skew and GC skew were 0.09 and -0.36 in the PCGs of *T. nicobarica* (Table 2). Most of the PCGs of *T. nicobarica* initiated with an ATG start codon; however, the ATC initiation codon was found in the NADH dehydrogenase subunit 2 (nad2), ATT in NADH dehydrogenase subunit 3 (nad3), ATA in NADH dehydrogenase subunit 5 (nad5). The TAG termination codon was used by six PCGs, TAA by four PCGs, AGA by Cytochrome oxidase subunit 1 (cox1), AGG by NADH dehydrogenase subunit 6 (nad6), and incomplete T(AA) by NADH dehydrogenase subunit 4 (nad4) respectively. The comparative study revealed that, most of the PCGs in other Tupaiidae species were initiated by ATG start codon and terminated by TAA stop codon (Supplementary Table S4). The analysis of mitogenome for detecting positive selection of PCGs assists to understand the influences of natural selection in evolution and protein function^{49,50}. The comparison of synonymous (Ks) and nonsynonymous (Ka) substitution rates in PCGs, witnessed for Darwinian selection and adaptive molecular evolution^{51,52}. It is reported that, for positive selection $Ka/Ks > 1$, for neutrality $Ka/Ks = 1$, and for negative selection $Ka/Ks < 1$ ⁵³. This

approach has the benefit to reveal the natural selection acting on PCGs. Thus, to investigate the evolutionary rates between homologous gene pairs, Ka/Ks substitutions were calculated and compared with six Tupaiidae species. The average Ka/Ks values of 13 PCGs varied from 0.006 (cox1) to 0.153 (atp8) and resulted in the following order: cox1<cox3<cox2<atp6<nad3<cytb<nad1<nad4<nad6<nad4l<nad5<nad2<atp8 (Supplementary Table S5, Supplementary Fig. S1). Most of the PCGs show Ka/Ks values of <1, which indicated a strong negative selection among the studied Tupaiidae species, that reflects natural selection works against deleterious mutations with negative selective coefficients as highlighted general patterns in other vertebrates. The comparative RSCU analysis indicated a significant fall in the frequency of GCG codon in Alanine (Ala) was observed in *T. nicobarica*, *T. montana*, *T. minor*, *T. splendidula*, and *T. tana*, except in *T. belangeri* with CCG in Proline (Pro) (Supplementary Fig. S2).

Ribosomal RNA and transfer RNA genes

The total length of two rRNA genes of *T. nicobarica* was 2,519 bp, compared to a range from 2,508 bp (*T. montana*) to 2,520 bp (*T. belangeri*) among other Tupaiidae species in the present dataset. The AT content within rRNA genes was 58.56%, while the AT and GC skew were 0.22 and -0.09 respectively observed in *T. nicobarica* rRNAs (Table 2). A total of 22 tRNAs were found in the *T. nicobarica* mitogenome with a total length of 1,497 bp. In other Tupaiidae species, the length of tRNAs varied from 1,493 bp (*T. minor*) to 1,564 bp (*T. belangeri*). The AT content within tRNA genes was 60.86%, while the AT and GC skew were 0.11 and -0.12, respectively observed in *T. nicobarica* tRNAs (Table 2). Most of the tRNA genes were predicted to be folded into classical cloverleaf structures, except trnS1 (without DHU stem and loop) and trnK (without DHU loop) (Supplementary Fig. S3). The conventional pairings (A=T and G≡C) were observed in most of the tRNAs bases⁵⁴; however, wobble base pairing was observed in the stem of 14 tRNAs (trnA, trnN, trnQ, trnE, trnC, trnG, trnL1, trnK, trnL2, trnP, trnS2, trnT, trnY, and trnW) (Supplementary Fig. S3). The wobble base pairing is a key feature of RNA structure and often substitutes the conventional base pairs due to thermodynamic stability. These characteristics play crucial functional roles in a wide range of phenomena⁵⁵. Thus, the comparisons of tRNAs secondary structures are crucial for inferring the structural and functional features of the mitogenomes⁵⁶.

Control regions

The CR of *T. nicobarica* was typically distributed with three functional domains: extended termination associated sequences (ETAS), central domain (CD), and the conserved sequence block (CSB), as observed in other mammalian mitochondrial CRs^{25,57}. Although, the ETAS and CSB domains contain varying numbers of tandem repeats, the CD domain consists with highly conserved sequences. Hence, the pattern of CR was varied among different mammals, including Tupaiidae (Scandentia). The total length of *T. nicobarica* CR was 1,757 bp, compared to a range of 778 bp (*T. splendidula*) to 1,757 bp (*T. nicobarica*) in the present dataset. In the *T. nicobarica* CR, the AT and GC skew was 0.12 and -0.30 (Table 2). The CR is also involved in the initiation of replication and is positioned between trnP and trnF for most of the Tupaiidae including *T. nicobarica*. The ETAS domain was divided into two regions: ETAS1 (60 bp)

and ETAS2 (67 bp), while the CSB domain was further divided into three regions: CSB1 (25 bp), CSB2 (17 bp), and CSB3 (18 bp). After CSB3, a six base pair (CGTACA) tandem repeats were found 60.3 times in *T. nicobarica*, while eight base pair (CACACATA) were found 23.8 times in *T. belangeri* (Fig. 2). Due to the short nucleotide length, no tandem repeats were found in other Tupaiidae species CRs. The structural features of CR play an important function in influencing transcription and replication in the mitochondrial genome^{58,59}. The present study evaluated the genetic features of CR among the studied Tupaiidae species mitogenomes including *T. nicobarica* that will be helpful to speculate the evolutionary pattern of this group.

Phylogenetic relationship

The phylogenetic position of Scandentia is repetitively examined within the eutherian tree. The treeshrews are widely considered as living fossils due to their approximating ancestral lineages with primates⁶⁰. Based on the anatomical evidence, Primates, Chiroptera, Dermoptera, and Scandentia were hypothetically within the superordinal clade Archonta without considering the paleontological or molecular evidence^{61,62}. Later on, the phylogenetic position of Scandentia has been studied based on the complete mitochondrial DNA sequences of wider group of taxa and corroborated a closer relationship with Lagomorpha^{24,25,63}. Further, multiple loci of mitochondrial genes has been assessed to check the phylogeny of treeshrews and diversification and the timescale of diversification in Southeast Asia^{15,27,64,65}. The present ML and BA phylogeny clearly discriminate *T. nicobarica* from other congeners by complete mitochondrial genome and congruent with earlier evolutionary hypotheses (Fig. 3, Supplementary Fig. S4). Further, using four calibration points from earlier studies, the present mitogenome-based dating analysis indicates that, the Tupaiidae species (Scandentia) were diverged from Primates and Dermoptera during the Cretaceous period (90 to 104.9 MYA). The studied Tupaiidae species, including *T. nicobarica* were diverged during the Pliocene to Miocene epoch (2.8 to 20.8 MYR). The endemic Nicobar treeshrew, *T. nicobarica* was diverged from the common ancestor lineages of other Tupaiidae species during the Miocene epoch (11.4 to 18.8 MYR) (Fig. 3). Further, based on 16S rRNA genes (1667 bp), we evaluated the status of two known subspecies of *T. nicobarica* from the Great and Little Nicobar Islands. The *T. nicobarica nicobarica* and *T. nicobarica surda* showed cohesive clustering in the BA tree as compared with other species (Fig. 4). Both the subspecies depicted 11 variable sites and maintained less genetic distance (0.7%) with each other. The 16S rRNA based topology showed a sister relationship of *T. nicobarica* with *T. javanica*, distributed in Sumatra and Java.

Biogeographic connection and conservation implication

Due to the volcanic eruption in Miocene–Pliocene and subsequent irregular land bridges during the Pleistocene, several small to large island ecosystems were built up in South and Southeast Asia. The South and Southeast Asian mainland and islands have been formed by the convergence of three major crustal units of the earth, the Eurasian, Indo-Australian, and Pacific plates². The tectonic drifts allowed multiple possibilities for animal dispersal and thus colonize many family members into the same or distant geographical distribution. Due to the adjacent biogeography realms, the biological connections

between the Indian mainland and Southeast Asia were well documented⁶⁶. However, the faunal diversity of the AN archipelago and their biotic networks is still anonymous in spectacular aspects. The bathymetric study evidenced that the well-developed seamounts have been detected on the Andaman seafloor, which extended up to Sumatra and Java Islands^{67,68}. Considering the skeletal variation, the treeshrew species showed intraspecific variation depending upon their distribution in mainland and island ecosystems⁶⁹. A recent molecular study also elucidates the biogeographic connection of smaller mammals in the AN archipelago with the Indo-Malayan and Sundaic realms³. The present mitogenome based phylogeny also manifested the close relationship of *T. nicobarica* with *T. minor*, *T. tana*, *T. splendidula*, and *T. montana* (distributed in Thailand, Peninsular and East Malaysia, Brunei Darussalam, Sumatra, and Indonesia) as compared with the widespread species *T. belangeri* known from South and Southeast Asia. Further, the single gene (16S rRNA) based phylogeny showed sister relationship of *T. nicobarica* with *T. javanica* (distributed in Sumatra and Java) as compared with other congeners.

Considering the conservation implication, the previous studies reported that, this arboreal mammal species confronted several threats due to the forest loss and fragmentation, and ongoing road construction from Galathia to Indira Point at the Great Nicobar Island⁷⁰. Although the species is listed on the IUCN with decreasing population trend, it has not yet listed in the Indian wildlife (Protection) Act, 1972. Other than a single ecology and behavior study and a nest record, no ample assessment has been approached so far^{12,71}.

Besides, the treeshrews species were considered as a significant model for studying hepatitis and influenza H1N1 viral infections^{19,20}. A recent study characterized the genome sequence to demonstrate the genetic basis of signaling pathways in nervous and immune systems of the Chinese treeshrew and evidenced as a potential model for biomedical research²¹. We propose the whole genome sequencing of *T. nicobarica* is essential for improving our ability to predict the signaling pathways linked with many pathogenic microorganisms as well as able to develop potential mitigations programs in advance. As the population of this treeshrew is confined to the insular habitats in the Nicobar Islands, we propose an integrated approach with taxonomy, ecology, and further molecular studies to save this endemic species before it reaches to the brink of extinction.

Declarations

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Declaration of Interest Statement

The authors declare that they have no competing interests.

Author Contributions

Conceptualization: S.K., V.K.; Data curation: S.K. M.K.; Formal analysis: S.K., A.P., D.S.; Funding acquisition: C.V., V.K.; Investigation: S.K., K.T.; Methodology: S.K. M.K., A.P., D.S.; Project administration: C.V., V.K.; Resources: C.V., V.K.; Software: S.K. K.T., A.P.; Supervision: C.V., V.K.; Validation: S.K., M.K.; Visualization: S.K., M.K., V.K.; Writing – original draft: S.K., M.K., A.P.; Writing – review & editing: S.K., M.K., V.K.

Data Availability

The following information was supplied regarding the accessibility of DNA sequences: The generated complete mitochondrial genome sequences of *Tupaia nicobarica* are deposited in GenBank of NCBI under accession number MW751815.

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Tables

Table 1: List of annotated mitochondrial genes of *Tupaia nicobarica*.

Gene	Direction	Location	Size (bp)	Anti-codon	Start codon	Stop codon	Intergenic Nucleotides
<i>trnF</i>	+	1-66	66	GAA	-	-	-1
<i>rrnS</i>	+	66-1013	948	-	-	-	4
<i>trnV</i>	+	1018-1084	67	TAC	-	-	0
<i>rrnL</i>	+	1085-2655	1571	-	-	-	0
<i>trnL2</i>	+	2656-2730	75	TAA	-	-	2
<i>nad1</i>	+	2733-3689	957	-	ATG	TAG	-2
<i>trnI</i>	+	3688-3756	69	GAT	-	-	-3
<i>trnQ</i>	-	3754-3824	71	TTG	-	-	-1
<i>trnM</i>	+	3824-3892	69	CAT	-	-	0
<i>nad2</i>	+	3893-4936	1044	-	ATC	TAG	-2
<i>trnW</i>	+	4935-5001	67	TCA	-	-	4
<i>trnA</i>	-	5006-5075	70	TGC	-	-	1
<i>trnN</i>	-	5077-5149	73	GTT	-	-	33
<i>trnC</i>	-	5183-5249	67	GCA	-	-	0
<i>trnY</i>	-	5250-5315	66	GTA	-	-	1
<i>cox1</i>	+	5317-6864	1548	-	ATG	AGA	-5
<i>trnS2</i>	-	6860-6928	69	TGA	-	-	4
<i>trnD</i>	+	6933-7001	69	GTC	-	-	0
<i>cox2</i>	+	7002-7685	684	-	ATG	TAG	1

<i>trnK</i>	+	7687-7750	64	TTT	-	-	2
<i>atp8</i>	+	7753-7956	204	-	ATG	TAA	-43
<i>atp6</i>	+	7914-8594	681	-	ATG	TAA	-1
<i>cox3</i>	+	8594-9379	786	-	ATG	TAA	-1
<i>trnG</i>	+	9379-9445	67	TCC	-	-	9
<i>nad3</i>	+	9455-9802	348	-	ATT	TAG	-10
<i>trnR</i>	+	9793-9858	66	TCG	-	-	1
<i>nad4l</i>	+	9860-10156	297	-	ATG	TAA	-7
<i>nad4</i>	+	10150-11527	1378	-	ATG	T(AA)	0
<i>trnH</i>	+	11528-11596	69	GTG	-	-	0
<i>trnS1</i>	+	11597-11655	59	GCT	-	-	-1
<i>trnL1</i>	+	11655-11724	70	TAG	-	-	-9
<i>nad5</i>	+	11716-13536	1821	-	ATA	TAG	2
<i>nad6</i>	-	13539-14060	522	-	ATG	AGG	0
<i>trnE</i>	-	14061-14128	68	TTC	-	-	3
<i>cob</i>	+	14132-15271	1140	-	ATG	TAG	-1
<i>trnT</i>	+	15271-15338	68	TGT	-	-	1
<i>trnP</i>	-	15340-15407	68	TGG	-	-	0
<i>CR</i>		15408-17164	1757	-	-	-	-

Table 2: Nucleotide composition of the mitochondrial genomes of different treeshrew mtDNA. The A+T biases of the complete mitogenome, PCGs, tRNAs, rRNA, and CRs were calculated by $AT-skew = (A - T) / (A + T)$ and $GC-skew = (G - C) / (G + C)$, respectively.

Species name	Size (bp)	A%	T%	G%	C%	G + C%	AT-skew	GC-skew
Complete mitogenome								
<i>T. nicobarica</i>	17164	32.56	25.74	14.54	27.13	41.70	0.11	-0.30
<i>T. belangeri</i>	16754	32.70	26.55	14.37	26.38	40.75	0.10	-0.29
<i>T. minor</i>	16187	32.06	26.83	14.77	26.32	41.09	0.08	-0.28
<i>T. montana</i>	16183	32.07	26.63	14.79	26.52	41.30	0.09	-0.28
<i>T. splendidula</i>	16189	32.33	26.72	14.54	26.17	40.71	0.09	-0.28
<i>T. tana</i>	16186	32.52	27.20	14.38	25.89	40.28	0.08	-0.28
Protein-coding genes (PCGs)								
<i>T. nicobarica</i>	11410	31.60	26.32	13.31	28.74	42.05	0.09	-0.36
<i>T. belangeri</i>	11394	30.50	28.30	14.30	26.91	41.21	0.04	-0.31
<i>T. minor</i>	11387	30.24	28.45	14.49	26.82	41.31	0.03	-0.30
<i>T. montana</i>	11389	30.25	28.22	14.49	27.04	41.53	0.03	-0.30
<i>T. splendidula</i>	11362	30.50	28.37	14.33	26.81	41.14	0.04	-0.30
<i>T. tana</i>	11389	30.89	28.72	14.00	26.39	40.39	0.04	-0.31
tRNA genes (tRNAs)								
<i>T. nicobarica</i>	1497	34.06	26.78	17.03	22.11	39.14	0.11	-0.12
<i>T. belangeri</i>	1564	31.91	29.35	20.33	18.41	38.75	0.04	0.05
<i>T. minor</i>	1493	30.94	30.01	20.76	18.29	39.05	0.02	0.06
<i>T. montana</i>	1496	31.15	29.95	20.52	18.38	38.90	0.02	0.05
<i>T. splendidula</i>	1496	31.75	30.21	20.12	17.91	38.03	0.02	0.06
<i>T. tana</i>	1497	32.00	29.86	19.97	18.17	38.14	0.03	0.05
rRNA genes (rRNAs)								
<i>T. nicobarica</i>	2519	35.84	22.70	18.69	22.74	41.44	0.22	-0.09
<i>T. belangeri</i>	2520	36.11	22.86	18.73	22.30	41.03	0.22	-0.09
<i>T. minor</i>	2516	35.41	23.21	18.64	22.73	41.38	0.21	-0.10
<i>T. montana</i>	2508	35.33	23.01	18.74	22.93	41.67	0.21	-0.10
<i>T. splendidula</i>	2514	35.52	23.31	18.70	22.47	41.17	0.21	-0.09
<i>T. tana</i>	2510	35.54	23.86	18.53	22.07	40.60	0.20	-0.09

Control regions (CRs)								
<i>T. nicobarica</i>	1757	33.23	25.72	14.34	26.63	40.97	0.12	-0.30
<i>T. belangeri</i>	1350	34.37	28.37	13.11	24.15	37.26	0.10	-0.30
<i>T. minor</i>	788	30.20	29.95	16.50	23.35	39.85	0.00	-0.17
<i>T. montana</i>	790	30.51	29.11	16.46	23.92	40.38	0.02	-0.18
<i>T. splendidula</i>	778	31.75	29.18	16.32	22.75	39.07	0.04	-0.16
<i>T. tana</i>	789	30.42	28.90	16.48	24.21	40.68	0.03	-0.19

Figures

Figure 1

The species photograph and mitochondrial genome of *T. nicobarica*. Protein-coding genes are marked by orcid color boxes, rRNA genes are marked by green color boxes, tRNA genes are marked by red color boxes, and control region is marked by grey color box. tRNAs are encoded according to their single-letter abbreviations. The GC content is plotted using a black sliding window; GC-skew is plotted using orange and blue color sliding windows as deviation from the average of the complete mitogenome. The figure was illustrated using CGView online server (<http://cgview.ca/>) with default parameters. The species

photograph taken by Govindarasu Gokulakrishnan and circular map was merged manually in Adobe Photoshop CS 8.0.

Figure 2

Comparison of nucleotide composition in different domains of control regions (CRs) and tandem repeats of six Tupaiidae species. The nucleotide compositions were compared through MEGAX software and the tandem repeats were predicted by the online Tandem Repeats Finder web tool (<https://tandem.bu.edu/trf/trf.html>). The figure was edited manually in Adobe Photoshop CS 8.0.

Figure 3

Bayesian phylogeny showed the molecular timescale for Tupaiidae species evolution compared with other Primates and Dermoptera species. Posterior values of branch lengths were represent by black digit along with each node. The divergence times (in MYR) were estimated by four calibration points (marked by green circle) with GTR+ I+ G substitution model and relaxed uncorrelated log-normal clock with the Yule speciation model in BEAST v2.4.7. Blue bars represent 95% highest probability density (HPD) around mean estimates of divergence times. The range of the estimated divergence times were marked by values in red along with each node. Geological time scale and Tupaiidae species photo (acquired from web) were edited manually in Adobe Photoshop CS 8.0.

Figure 4

Genetic status of two known subspecies of *T. nicobarica* based on 16S rRNA sequences. (A) Map showing the distribution of other comparative Tupaiidae species in the present phylogeny. Map prepared using QGIS 2.6.1 (<http://www.qgis.org>) and edited manually in Adobe Photoshop CS 8.0. (B) Bayesian Phylogeny (BA) Phylogeny showed distinct clustering of *T. nicobarica* subspecies and other Tupaiidae species. (C)

Distribution pattern of *T. nicobarica nicobarica* and *T. nicobarica surda* in the Great and Little Nicobar Island respectively.

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