Genetic characterization of rhesus macaque populations along the southeastern Qinghai-Tibetan plateau based on a mitochondrial ATP6 gene closely related to energy metabolism

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Abstract

Background Rhesus macaque (Macaca mulatta) is widely distributed in China, across different altitudes. The mitochondrial ATP6 gene,an ATPase subunit coding gene with fast evolution rate in the mitochondrial genome, plays an important role in the energy metabolism of animals, which may be a good molecular marker for studying the adaptive evolution of animals. Herein, we detected ATP6 genes of 334 rhesus macaques of 19 populations from multiple regions in China with an elevation span of 5-4000m, and mainly carried out the population genetic and evolutionary analysis in these macaques. Our aim is to explore the molecular mechanism of rhesus macaques in adapting to different environments, especially in high altitude extreme environments.

Results A total of 50 haplotypes were identified, and significant differences were found in haplotype sequences of rhesus macaque ATP6 gene at different elevations, especially in the high altitude haplotypes with multiple specific variation sites, leading to some region-specific haplotypes. Population genetic analysis showed that rhesus macaque had high genetic diversity (Pi =0.02332 ± 0.00226, Hd =0.802 ± 0.022 and K =14.982), and there was obvious genetic differentiation among different geographical populations.

Conclusions The results showed that the ATP6 gene had undergone adaptive evolution in the process of rhesus macaque adapting to different elevations, especially the high altitude environments. We also found that geographical isolation was an important factor in the genetic differentiation of rhesus macaque. Phylogenetic analysis showed that there were two subspecies of rhesus macaque in western Sichuan, namely, M. m. lasiotus and M. m. vestita , the former distributed in the western Sichuan region of the Yalong River Basin and the Qinghai-Tibet Plateau, while the latter distributed in the Dadu River Basin and northwest Sichuan. We speculated that the Daxueshan Mountains in western Sichuan was a critical geographical barrier for the differentiation of the two subspecies populations.

Background

Mitochondria existed in most eukaryotic cells and produced about 95% adenosine triphosphate by oxidative phosphorylation (1). In addition to acting as the energy factory of cells, mitochondria also participated in a variety of physiological processes to maintain cell survival and function, such as regulating the dynamic balance of Ca^{2+} concentration, apoptosis and immunoregulation (2–4). Mitochondrial DNA (mtDNA) contained 13 protein-coding genes (PCGs), the ATP6 gene encoded subunit 6 of the mitochondrial FiF0-ATP synthase (ATP-6), which is an inner membrane polypeptide of the F0 component and encoded within the mitochondrial genomes of all eukaryotic organisms currently tested (5). Furthermore, ATP-6 plays a critical role in the proton channel of the ATP synthase (complex V) (6), and is thought to be involved in ATP synthesis (7).

MtDNA was frequently used as a marker gene in molecular ecology in recent decades because of its relatively fast rate of variation and non-recombination, matrilineal inheritance (8–12). Because of its

important physiological function, mtDNA was usually considered to be selected by purifying selection (13). However,there had also reported of evidence of positive selection with mtDNA, although it might not be as common as purifying selection (14, 15). Mishmar et al. (16) suggested that ATP6 was the most variable gene among human mtDNA and probably resulted from the adaptation of Homo sapiens to the colder climate during the migration out of Africa. According to this adaptation theory, Mau et al. (17) compared the ATP6 gene in human and 42 primates, 25 adaptive amino acid residues were successfully identified. Selective analyses revealed significant evidence for positive selection in ATP6 gene of Galliform birds in three of the high-altitude lineages (18). In addition, by comparing Tibetan chicken with low altitude chicken (19), yak and low altitude Holstein cows (20), they both found that single nucleotide polymorphism (SNPs) of ATP6 genes were associated with altitude.

Rhesus macaque (Macaca mulatta) was the most widely distributed non-human primates in the world. It has been hypothesized that rhesus macaque diverged from a fascicularis-like ancestor that reached the mainland of Indo-China from Indonesia by approximately 1 million years ago (Ma) (21–23). Smith et al. (24) indicated that rhesus macaque in China and India reproductively isolated around the late Pleistocene. Hasan et al. (25) thought that Bangladesh might be the probable original homeland of rhesus monkeys. Wu et al. (26) suggested that Chinese rhesus macaque could be divided into eastern and western haplogroups, and Yunnan might be the first place for rhesus macaque to enter China.

At present, rhesus macaque geographical range extends from Afghanistan in western Asia to the Eastern China, with species occurring southward from the Himalayas and central China to the whole of South Asia, this range exceeds all other non-humans primates. In China, rhesus macaque was the only widely non-human primate distributed from low altitudes to high altitudes under natural conditions (27). Previous phylogenetic analyses of rhesus macaque had supported the division of rhesus macaque into Eastern and Western haplogroups, and the complex geomorphology in western China might be an important factor in promoting differentiation of rhesus macaque (24, 26). In this study, a total of 334 ATP6 genes of rhesus macaque from 19 populations were detected to analyze whether the correlation between genetic variation of ATP6 genes and environments. In this study, we put forward three hypotheses, 1) The mitochondrial ATP6 gene closely related to energy metabolism may be generated the corresponding adaptive evolution in the process of rhesus macaques adapting to different altitude environments, especially high-altitude extreme environments; 2) Previous studies on macaque genetic diversity were mostly based on non-protein coding genes as molecular markers, such as mitochondrial DNA control region and microsatellite DNA, etc. Here, how about the genetic diversity and differentiation of macaque based on mitochondrial DNA protein coding gene (ATP6)? 3) Which of the high mountains and large rivers produced by the uplift of the Qinghai-Tibet plateau may be the main geographical barriers to gene exchange among macaque populations?

Results

Haplotype profiles

All 334 samples were successfully amplified, the *ATP6* gene of Chinese rhesus macaque was 681 bp and 50 haplotypes were identified (GenBank accession numbers: MT039741-MT039790; Figure 1; additional file 1: table S1). Except for Hap1, Hap12 and Hap17, all other 47 haplotypes were unique to each population. Hap1 was widely distributed in high altitude populations and was the only haplotype detected in Xizang jiacha (XZJC), Yunnan jianchuan (YNJC) and Xizang leiwuqi (XZLWQ) populations, Hap1 also had the highest frequency of all haplotypes (accounting for 41.02% of all 334 samples). Hap12 was detected in Sichuan muli (SCML) and Sichuan panzhihua (PZH) populations (Hap12 was the only one haplotype detected in PZH). And Hap17 was detected in Sichuan seda (SCSD) and Sichuan xiaojin (SCXJ) populations. Seven haplotypes were detected in the Hainan lingshui (HNLS) population, which was the population with the highest number of haplotypes. Although Sichuan yajiang (SCYJ) had only five samples, four haplotypes were detected (*Hd* = 0.900±0.161, Table. 1).

We further analyzed *ATP6* haplotype sequences, total 586 conserved sites and 95 variable sites were detected, including 25 singleton sites and 70 parsimony informative sites (Figure 2A). The haplotype sequences were translated into amino acid sequences (Figure 2B) and a total of 36 amino acid mutation sites were detected. The variation of *ATP6* was significantly correlated with elevation. Haplotypes from high altitude populations were highly similar, especially 7 haplotypes from the Qinghai-Tibet Plateau (QTP), in addition to Hap3 detected M52L, the remaining six haplotypes detected M52F, and encoded the same amino acid sequence. In contrast, haplotypes of low altitude populations showed higher amino acid variation rate and some of the variation sites were shared interspecific: acid variant site M124S were detected in all haplotypes, M88M was detected in all haplotypes except for Hap35 and Hap36.

Genetic diversity

The haplotype diversity (*Hd*), nucleotide diversity (*Pi*), and the average number of nucleotide difference (*K*) were calculated to examine the genetic diversity of rhesus macaque populations (Table. 1). The overall *Pi*, *Hd*, and *K* values were 0.02332 ± 0.00226, 0.802 ± 0.022 and 14.982, respectively. Since only one haplotype was detected in XZJC、XZLWQ、XZGBJD and PZH, all three indices were 0. The *Hd* ranging from 0.100±0.088 (XZJD) to 0.900±0.161 (SCYJ). As for *Pi*, which was ranging from 0.00015±0.00041 (XZJD) to 0.00800±0.00163 (HN), and the *K* was ranging from 0.100 (XZJD) to 5.450 (HN). Tajima's D neutral test showed that except for SCML and GX populations, the test results of fourteen populations were not significant; conform to neutral mutations, reflecting these rhesus macaque populations sizes were relatively stable, and did not experience historically population contraction or expansion (XZJC, YNJC, XZLWQ and PZH did not conduct neutral test due to only one haplotype was detected). SCML (Tajima's D =-1.86311, $P<0.05$), indicated that SCML population had experienced population expansion, probably due to founder effect. GX (Tajima's D = 1.00927, $P < 0.05$), indicated population historical of contraction.

We calculated the pairwise gene flow (*Nm*), coefficient of differentiation (F-Statistics, F_{ST}) and genetic distance (*D*) among populations to reveal the differentiation populations (Additional file 2: Table S2 and additional file 3: Table S3). Results showed that within the most QTP populations, the pairwise *Nm* were

greater than 1, except for XZJD - QHYS (0.81100), QHYS - XZJC (0.49479) and YNJC - SCBY (0.78167). As for the low altitude populations, the pairwise *Nm* of PZH with XZJC, YNJC and XZLWQ were 0, and the maximum value was 1.20022 (SCXJ-SCSD)，indicating lower gene flow levels. The F_{ST} also got similar results, among populations from the QTP, only QHSY-XZJD, QHYS-XZGBJD, XZJC-XZGBJD, XZJC-QHYS, YNJC-XZGBJD and YNJC-QHYS were significant. For other rest populations, all the pairwise F_{ST} tests were significant, which indicated large genetic differentiation among these populations. As for *D*, the genetic distances among the QTP populations were 0 or 0.01, which indicating very low genetic distance. Gene flow test results showed that the *Nm* among PZH with XZJC, YNJC and XZLWQ were 0, however, including this three populations, the genetic distance among PZH with the QTP populations were low (0.004 - 0.007), while the genetic distance among other populations were higher, even SCML with a shared haplotype with PZH (*D* = 0.021).

Phylogenetic analysis

All 50 haplotypes of rhesus macaque *ATP6* genes yielded a well-resolved Bayesian (BI) tree (Figure 3) by using *M. thibetana* (EU294187) and *M. fascicularis* (FJ906803) as outgroups, which clustered into two major clades (clade A and B). Clade A could be further divided into three sub-clades (sub-clade Ⅰ, Ⅱ and Ⅲ). Sub-clade Ⅰ contained haplotypes from SCSD, Sichuan heishui (SCHS), SCXJ and Sichuan hanyuan (SCHY), sub-clade Ⅱ was consisted of Guizhou xishui (GZXS) and Sichuan gulin (SCGL), and sub-clade Ⅲ contained haplotypes from PZH, SCML, SCYJ and haplotypes from the QTP. Clade B contained haplotypes from Guangxi (GX), HNLS and he' nan (HN).

To further analyze the clustering and distribution of rhesus macaque *ATP6* gene, A Median-joining (MJ) network was diagrammed (Figure 4), which had an analogical topology to the BI tree. Consistent with the BI tree, Hap12 appeared to be the common ancestor of SCML, PZH, SCYJ and Tibetan plateau populations. Hap1 appeared to be at the center of haplotypes from the QTP, namely, the rest of the haplotypes from the QTP appeared to evolve from Hap1 radiation. Interestingly, the six haplotypes from HN were significantly divided into two branches, one branch contained Hap46 and Hap50, and the other contained Hap45, Hap47, Hap48 and Hap49, which were clustered with haplotypes from HNLS.

In this study, we used two combinations for analysis of molecular variance (AMOVA; Table. 2): according to the elevations of the sampling areas, we divided 19 populations into three groups of high, middle and low altitude populations (test 1); seven groups according to subspecies differentiation based on previous research(27) and phylogenetic results of this study (test 2) to speculate the most suitable isolation type. In test 1, variation among group proportioned 43.13% (P<0.01), while in test 2, variation among group proportioned 90.88% (P<0.01).

Population demography

To further analyze the population history of rhesus macaque, we performed a mismatch distribution analysis of seven population groups based on the results of phylogenetic tree (Figure 5). On the whole, the mismatch distribution of rhesus macaque was unimodal, indicating that rhesus macaque

experienced population expansion. Mismatch distribution of seven populations groups showed that except for HNLS (Figure 5F) and HN (Figure 5G), which were multimodal, the other five groups were unimodal, indicates that these two populations are stable.

Estimation of divergence time

Similar to previous studies (26, 28), our research of the divergence time among rhesus macaque populations (Figure 6) had also reached a phylogenetic tree that could be divided into eastern haplogroup (HNLS, HN and GX) and western haplogroup (populations from the QTP, Sichuan, Yunan and Guizhou). The divergence time of the eastern and western haplogroup was 1.07 Ma. The western haplogroup further differentiated into two sub-haplogroups and the divergence time among these two sub-haplogroups was 0.92Ma.

In relation to the divergence time between haplotypes and *ATP6* haplotypes, we focused on the divergence time of two time nodes, Hap1 and Hap12. It can be seen that the divergence time of Hap1 was only 0.04Ma. Hap12 was on the outermost side of the whole branch, which was consistent with the results of phylogenetic analysis, and the divergence time was 0.25Ma.

Discussion

Rhesus macaque was the most widely distributed non-human primates. It was widely distributed from tropical low altitude radiation to different elevations in China, especially in the high altitude areas of the QTP (29). In this process, rhesus macaque generated a variety of physiological and behavioral changes to adapt to different environments (30-33). This study aimed to explore the imprinting of different environmental effects on rhesus macaque mtDNA. We used nucleotide diversity (*Pi*), haplotype diversity (*Hd*) and average number of pairwise nucleotide differences (*K*) as indicators to measure genetic diversity, and used *Pi*=0.01 as the standard to measure the degree of variation (34, 35). These results were greater than the genetic diversity of rhesus macaque in Sichuan (36) or Taihang Mountain areas (37) analyzed by mitochondrial control region. However, the *Pi* within each population was less than 0.01, ranging 0.00015±0.00041 (XZJD) to 0.00800±0.00163 (HN); these results showed that the intraspecific difference of ATP6 gene was lower while the interspecific difference was higher in rhesus macaque populations. AMOVA came to a similar conclusion, we carried out the AMOVA of two clustering schemes, elevation distribution and geographic isolation model, no matter which test, the percentage of variation within populations was the lowest.

By contrasting the amino acid sequences, we found that there were large variations in *ATP6* gene distributed at different elevations. Generally, as the elevation increases, the *ATP6* amino acid sequence tends to be more conservative. We sampled a total of 156 samples from 7 populations (including YNJC, N = 39) from the QTP, and only 7 haplotypes were detected. Therefore, we speculated whether the rhesus macaque *ATP6* gene mutation was related to elevations. However, positive selection of *ATP6* did not detect relevant positive selection sites, and in AMOVA, the percentage of variation among groups was the

higher (90.88%, $P<0.01$) when divided into seven groups according to subspecies differentiation, instead of classifying it by elevations as we previously speculated.

Hap1 was widely distributed in the QTP and YNJC, and amino acid sequence analysis showed that the amino acid sequence of haplotype in the QTP were consistent with that of Hap1 except for Hap25 detected M52L > F. Therefore, we speculated that Hap1 was the haplotype evolved from the adaptability of rhesus macaque in the harsh natural environment of the QTP. However, the divergence time between Hap1 and Hap5 was only 0.04Ma, in addition, we performed a selective pressure analysis of the *ATP6* gene of rhesus macaque, and the results also did not support our conjecture, no significant positive selection sites were detected in Hap1. However, Hap1, or this branch of the phylogenetic tree, could be widely distributed in the QTP (altitude 3000-4000m) and YNJC(altitude 2500-3000m)，which was hundreds of kilometers away, thus, we still have reason to believe that Hap1 was a haplotype capable of adapting to harsh natural conditions at high altitudes. The reason why the test results were not significant might due to the short time of differentiation. Indirect evidence was that comparing the amino acid sequences of all 50 haplotypes, it could be found that the amino acid sequence of *ATP6* gene tends to be more conservative with the increase of altitude. The divergence time of Hap12 was consistent with the phylogenetic analysis: Hap12 and the haplotypes of the QTP were clustered into one branch, and the divergence time was 0.25Ma, which was much earlier than that of other haplotypes in the QTP. Therefore, we believed that Hap12 was the haplotype of the transition from western Sichuan to the QTP. Combined with the results of gene flow analysis, the level of gene flow of the QTP populations with external populations was lower; the widespread distribution of Hap1 might be the result of the drift of certain haplotype (e.g., Hap12).

For the calculation of genetic differentiation, we found that four populations located at the QTP (and YNJC), had a very low degree of genetic differentiation and might have frequent gene flow. Relatively, SCBY and QHYS have a higher degree of differentiation with the other five populations from the Qinghai-Tibet Plateau, which we speculated due to geographical isolation. For example, the Jinsha River, which was located at the junction of Tibet and Sichuan, might to some extent isolate the gene exchange between SCBY and other populations, while QHYS, located in southeastern Qinghai, may also be geographically isolated from other populations, meanwhile, the uplift of the QTP also promoted the isolation of rhesus macaque populations in this area (38).

In order to further analyze the geographic isolation mode among rhesus macaque populations, we focused on SCHY (located in the southeast of the Daxueshan mountains, Dadu river north bank), where located on the western edge of the Sichuan Basin, with an approximate straight-line distance to SCYJ and SCXJ. Obviously, SCHY was more closely related to the populations from northwestern Sichuan (SCSD, SCXJ and SCHS). This suggested that the Daxueshan Mountain and Dadu River, which located in northwestern Sichuan, might be important geographic barriers leading to the differentiation of rhesus macaque in western and northwestern Sichuan (36, 39). Based on this conclusion, and considering the strong migration ability of rhesus macaque, we further speculated that the YNJC population might be a

branch of the QTP rhesus macaque that migrates south along the Hengduan Mountains (e.g., the Nu River valley) (40).

Conclusions

In this study, the genetic diversity of rhesus macaque populations in southwest China was studied by using mitochondrial *ATP6* gene. It was found that 19 rhesus macaque populations had obvious phylogeography characteristics. The complex geomorphology in western Sichuan and the existence of the QTP might affect the population differentiation of rhesus macaque. We speculated the Daxueshan Mountains in western Sichuan might be an important factor leading to the differentiation of two subspecies of rhesus macaque.

It was worth noting that although rhesus macaque populations in southwest China showed high genetic diversity, the genetic diversity within each population was low, and the results of this study showed that low level of gene flow among rhesus macaque populations. We also noted that a single gene could not fully reveal the genetic characteristics of the rhesus macaque populations, so it was necessary to analyze the genetic diversity of the rhesus macaque populations based on the whole mitochondrial genome in the future. Considering that the gene communication activity of primates was mainly dominated by males, only maternal inheritance might also lead to the low interspecific gene flow in this study, and due to the genetic characteristics and limitations of mitochondrial DNA, by combing microsatellite and Y chromosome sex determination region (SRY) could be used to more profound analyze the genetic diversity of rhesus macaque populations.

Abbreviations

ATP6: ATP synthase F0 subunit 6; PCGs: Protein-coding genes; QTP: Qinghai-Tibet Plateau; SNPs: Single nucleotide polymorphism; Ma: Million years ago; bp: base pair; *Hd*: Haplotype diversity; *Pi*: Nucleotide diversity; *K*: Average number of nucleotide difference; *Nm*: Gene flow; F_{ST}: Coefficient of genetic differentiation (F-Statistics); D: Genetic distance; BI: Bayesian phylogenetic tree; AMOVA: Analysis of molecular variance; SRY: Y chromosome sex determination region; MJ: median-joining; MCMC: Markov Chain Monte Carlo.

Methods

Sample collection

Before sample collection, all the animal works were approved by the Institutional Animal Care and Use Committee of Sichuan Agricultural University (permit number SKY-S20171005). All field works were granted permission by the Administration for Wild Animal and Plant Protection and Nature Reserves and the Department of Forestry for the Tibet provincial region, as well as Hainan, Guangxi, Guizhou, Yunnan, Henan, Qinghai and Sichuan provinces.

We collected 334 wild Chinese rhesus macaque fecal samples from 19 regions of China, from June 2017 to October 2018. The sampling areas concentrated in the southeastern Qinghai-Tibetan Plateau (Figure 1): Jiangda (XZJD, N=20), Gongbujiangda (XZGBJD, N=34), Jiacha (XZJC, N=26), Leiwuqi (XZLWQ, N=7), Yushu (QHYS, N=12), Baiyu (SCBY, N=17), Yajiang (SCYJ, N=5), Muli (SCML, N=13), Jianchuan (YNJC, N=39) and Pangzhihua (PZH, N=20); Northwest Sichuan (East of the Daxueshan mountains): Seda (SCSD, N=18), Heishui (SCHS, N=19), Xiaojin (XCXJ, N=13) and Hanyuan (SCHY, N=15); Southeast Sichuan: Gulin (SCGL, N=10) and Xishui (GZXS). And samples from Guangxi (GX, N=22), Hainan (HNLS, N=15) and Henan (HN, N=19). In order to avoid repeated sampling, we collected fresh feces from identified individuals during the sampling process. Swabs from the surface of fecal samples were stored in the -80℃ cryogenic refrigerator until they were thawed for analysis.

DNA extraction and sequence amplification

Total DNA was extracted using QIamp PowerFeacal DNA Kit (Qiagen Hilden Germany), following the manufacturer instructions and detection using 1% agarose gel electrophoresis. Primer Premier 5.0 software and the complete *Macaca mulatta* mtDNA sequence (AY612638) were used to design the following pair of primers to amplify a 1204bp fragment comprises *ATP6* gene: forward primer: (5'-3') GCGTCTATTACGGACAATGCTC, reverse Primer (5'-3') GTGAAATCACATGACTAAGCCG (synthesized by Beijing Tsingke Biological Technology Co., Ltd., People's Republic of China). Polymerase chain reaction (PCR) amplification was performed in 20 μl volumes containing 10 μl 2× Es Taq MasterMix (Dye) (Beijing Cwbiotech Technology Co., Ltd., People's Republic of China), 0.6 μl (10 pmol/ml) of each primer, 1 μl (~ 25 ng) genomic DNA and ddH_2O to 20 μl, then amplification was carried out under the following thermocycler conditions: 95 °C for 8 min, 35 cycles of 94 °C for 50 sec denaturing, 58 °C for 50 sec annealing, and 72 °C for a 1min 40 sec extension. Successful amplification was detected using 1.2% agarose gel electrophoresis and then bidirectionally sequenced by TSINGKE (Chengdu) Biotechnology Co., Ltd.

Data analysis

In order to preclude the inclusion of NUMTs, we carried out a BLAST similarity search in NCBI to ensure that the sequenced fragment of DNA was our targeted sequence before sequence analysis. Sequences of *ATP6* gene were edited and aligned using the DNASTAR package (41). Identical haplotypes were collapsed using DAMBE (42). In addition, protein-coding nucleotide sequences were translated to amino acids using MEGA 7.0 to check for premature stop codons, and the pairwise genetic distance (*D*) among populations was calculated based on Kimura 2-parameter method (43). Nucleotide diversity (*Pi*), haplotype diversity (*Hd*), average number of nucleotide differences (*K*) and mismatch distribution were estimated by DnaSP 5.0 (44). Arlequin 3.5 (45) was used to calculate the pairwise gene flow (*Nm*) and the genetic differentiation coefficient (F_{ST}) among 19 populations of macaque and the level of differentiation (*Φst*) between populations was estimated by the AMOVA (Analysis of Molecular Variance) analysis method. Tajima's D neutrality tests in Arlequin v.3.5 were conducted to assess statistical significance over 10,000 permutations.

By using Tibetan macaque (*Macaca thibetana*, EU294187) and Cynomolgus monkey (*Macaca fascicularis*, FJ906803) *ATP6* gene sequences as outgroups, a Phylogenetic analysis was performed using the MrBayes3.1.2 (46). The evolutionary model was analyzed with jModeltest 0.1.1 (47). The best fitting evolutionary model was the TIM+G, with a proportion of invariable sites and rate variation. Four Markov Chain Monte Carlo (MCMC) were run for 1,000,000 generations at the same time, with sampling every 1000 generations. The first 250 aging samples and the last 250 aging samples were discarded to get tree files. Network 4.611 (48) was used to construct an unrooted median-joining (MJ) haplotype network diagram.

Divergence times were estimated using a Bayesian MCMC method implemented in BEAST 2.4.7 (49), which employed a strict molecular clock approach. A strict clock of haplogroup variation and a calibrated Yule model prior for branching rates were assumed. As a calibration point, we used the divergence time of 3.84 Ma (95% confidence interval = 2.66 – 5.03 Ma) for matrilines between *M. fascicularis* (FJ906803) and *M. thibetana* (EU294187) (26). Instead of using hard bounded calibration points, we relied on published dates and specified a normal distribution prior, with a mean of 3.84 Ma and a standard deviation of 0.5 Ma. Two replicates were run for 100 million generations while sampling trees and parameters every 1,000 generations. The adequacy of using a 50% burn-in to generate MCMC trees and for convergence of all parameters was assessed visually using TRACER 1.4.1. Tree file was converted using TreeAnnotator 1.7.5 and visualized using FigTree 1.4.0 (50).

Declarations

Ethics approval and consent to participate

All the animal works were approved by the Institutional Animal Care and Use Committee of Sichuan Agricultural University (permit number SKY-S20171005). All field work was granted permission by the Administration for Wild Animal and Plant Protection and Nature Reserves and the Department of Forestry for the Tibet provincial region, as well as Hainan, Guangxi, Guizhou, Yunnan, Henan, Qinghai and Sichuan provinces.

Consent to publish

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors report no conflict of interest.

Authors' contributions

XH, ZP and YY designed the experiment and wrote the first draft. ZK, JZ, LD, XM, NQ and ZM collected the fecal samples and performed preliminary preparation. All authors has helped in revision and approved the final manuscript.

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Tables

Table 1. *ATP6* nucleotide sequence polymorphism in 19 rhesus macaque populations.

Population	Sample size	Number of variable sites, Vs	Number of haplotypes	Nucleotide diversity, Pi ±SD, π	Haplotype diversity ±SD, h	Average number of pairwise nucleotide differences, K	Tajima's D
XZJD	20	1	2	0.00015±0.00041	0.100±0.088	0.100	-1.16439
XZGBJD	34	2	3	0.00055±0.00051	0.355±0.094	0.373	-0.47066
QHYS	12	1	2	0.00078±0.00049	0.530±0.076	0.530	1.38110
XZJC	26	0	1	0	0	0	--
YNJC	39	0	1	0	0	0	--
XZLWQ	7	0	1	0	0	0	--
SCBY	18	2	3	0.00131±0.00060	0.503±0.103	0.895	1.31765
SCYJ	5	8	4	0.00587±0.00199	0.900±0.161	4.000	0.29358
SCML	13	5	4	0.00113±0.00106	0.423±0.164	1.611	-1.86311*
SCSD	18	3	4	0.00107±0.00074	0.608±0.086	0.725	-0.46640
SCHS	19	2	3	0.00070±0.00059	0.444±0.124	0.690	-0.38718
SCXJ	13	3	4	0.00413±0.00082	0.718±0.089	0.974	0.02492
SCHY	15	2	1	0.00070±0.00045	0.496±0.092	0.476	1.12241
PZH	20	0	1	0	0	0	--
SCGL	10	1	2	0.00029±0.00052	0.200±0.154	0.200	-1.11173
GZXS	22	3	4	0.00182±0.00070	0.502±0.105	1.238	1.30474
HNLS	15	11	7	0.00366±0.00150	0.838±0.068	2.495	-1.01143
HN	19	15	6	0.00800±0.00163	0.854±0.039	5.450	1.00927
GX	9	8	4	0.00644±0.00153	0.806±0.089	4.389	1.00927*
Total	334	96	50	0.02416±0.00225	0.819±0.021	16.452	0.24772

Table 2. Result of analysis of molecular variance (AMOVA)

	Percentage of Variation (%)		
	Among-Groups	Among Populations within Groups	Within-Populations
Test 1. Three groups according to the altitude distribution of the sampling site.	43.13	53.31	4.55
Test 2. Seven groups according to Geographic isolation and subspecies distribution.	90.88	4.89	4.23

Figures

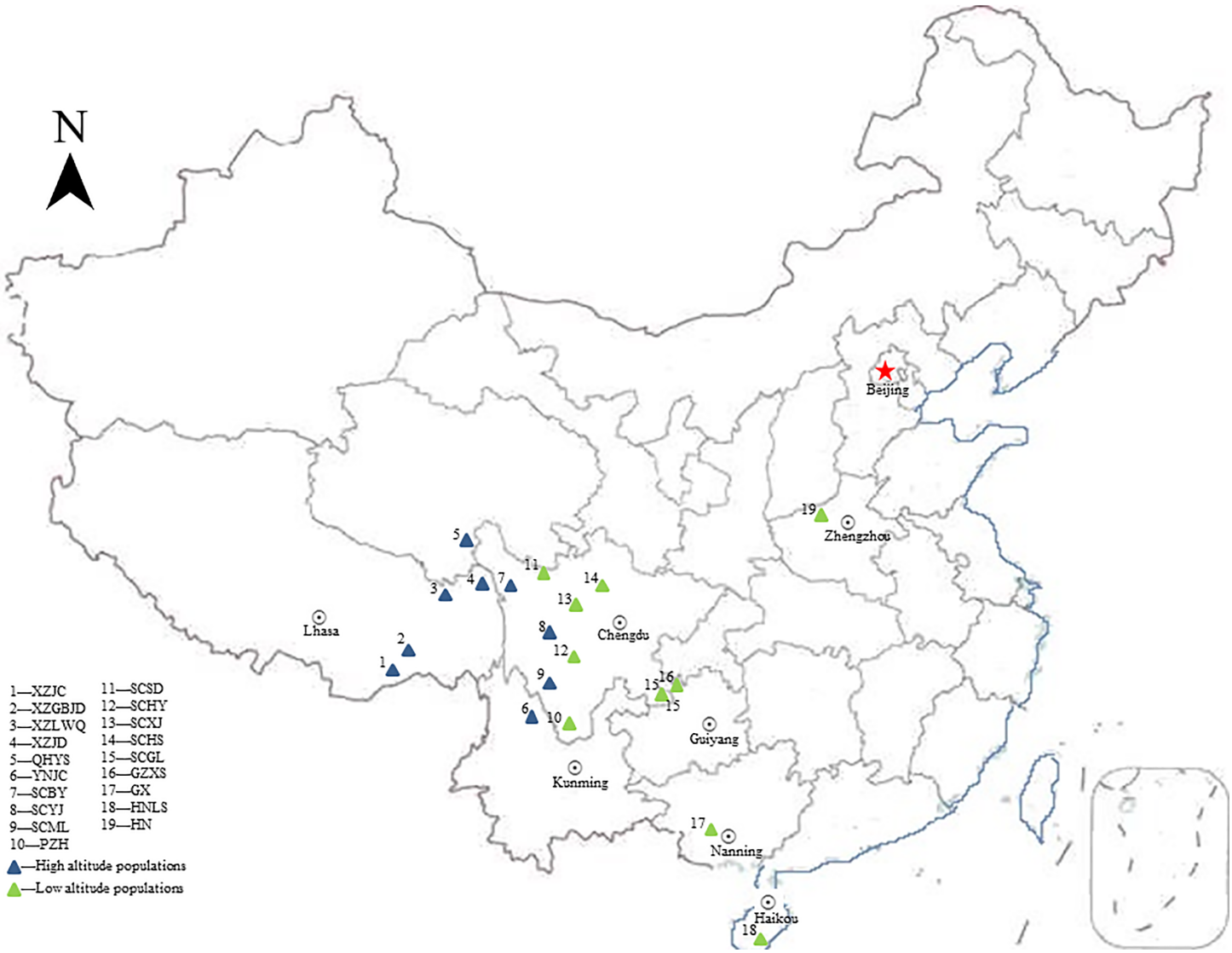

Figure 1

Distribution map of all collected samples. The base Chinese country-level vector map was download from National Geomatics Center (http://www.ngcc.cn, free access), and the collection sites were inserted by longitudinal and latitudinal coordinate. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

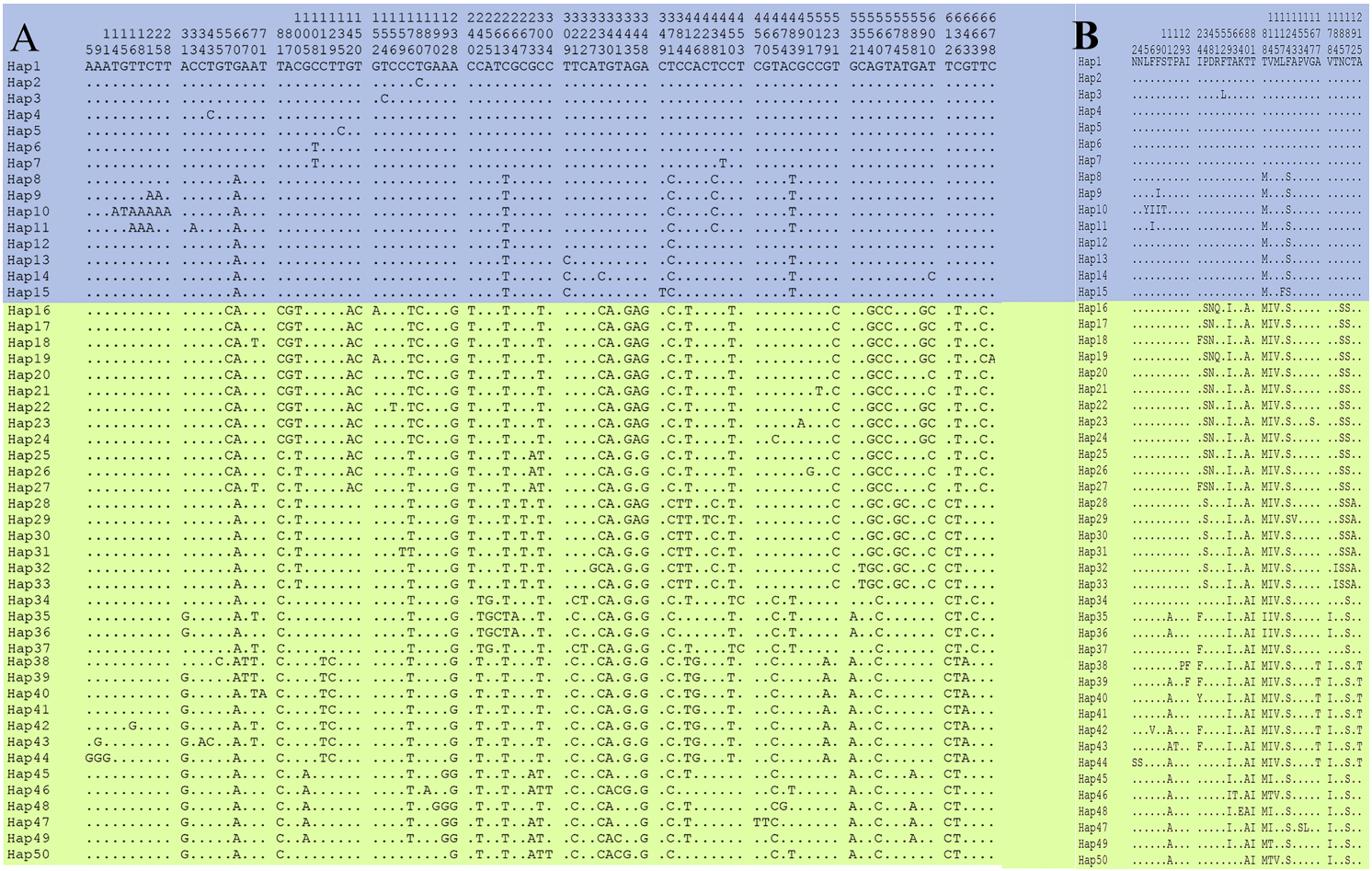

Figure 2

Variation sites of ATP6 gene haplotype sequences in rhesus macaques. (A) Nucleotide sequences, and (B) Amino acid sequences; Small dots indicated the same base or amino acid site as the first line. The grey highlighted haplotypes Hap1-15 are from the Qinghai-Tibet Plateau, while the green highlighted haplotypes Hap16-50 are from low-altitude populations.

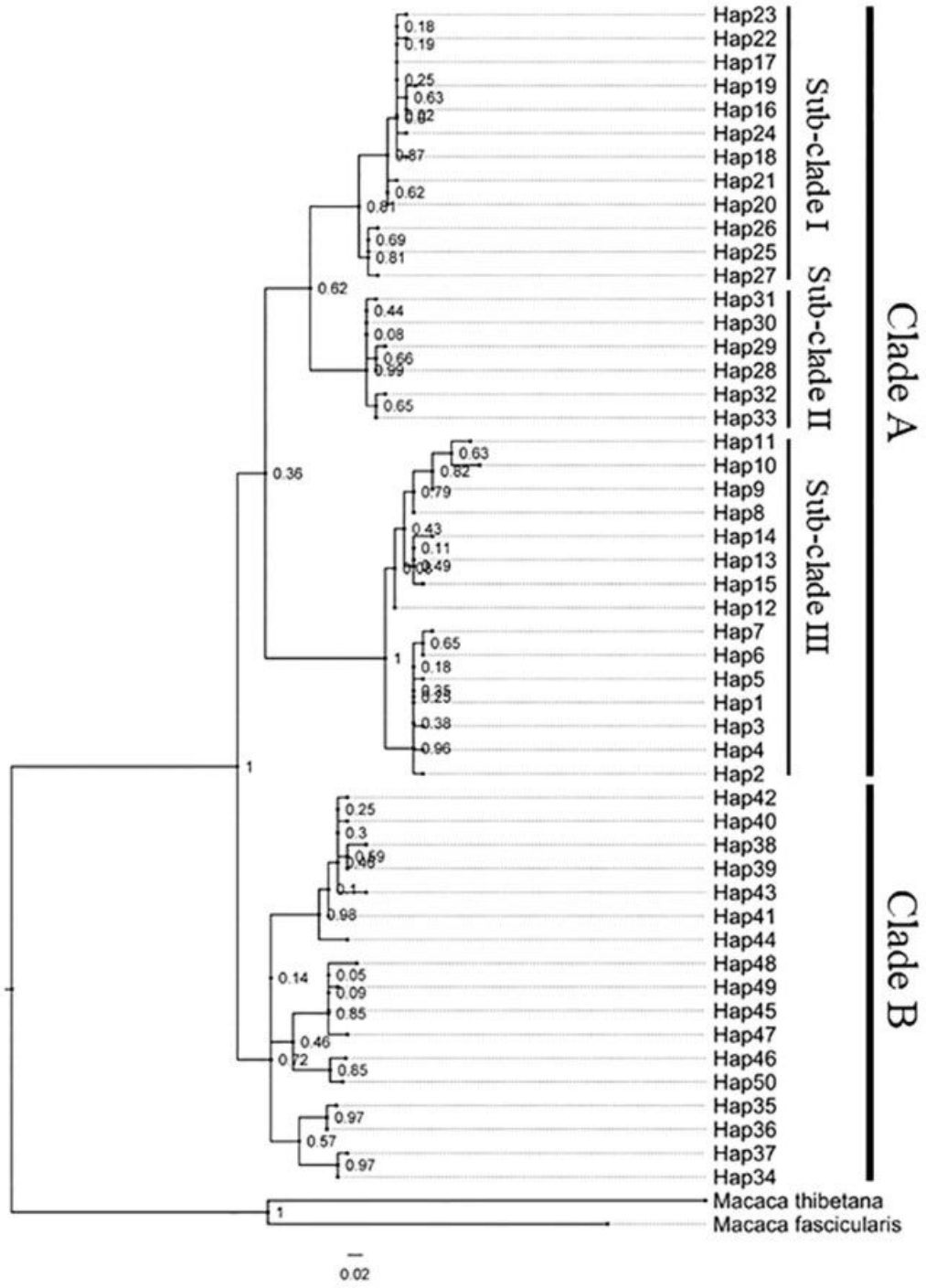

Figure 3

Bayesian phylogenetic tree of rhesus macaque mtDNA ATP6 genes. Numbers at the nodes were bootstrap values.

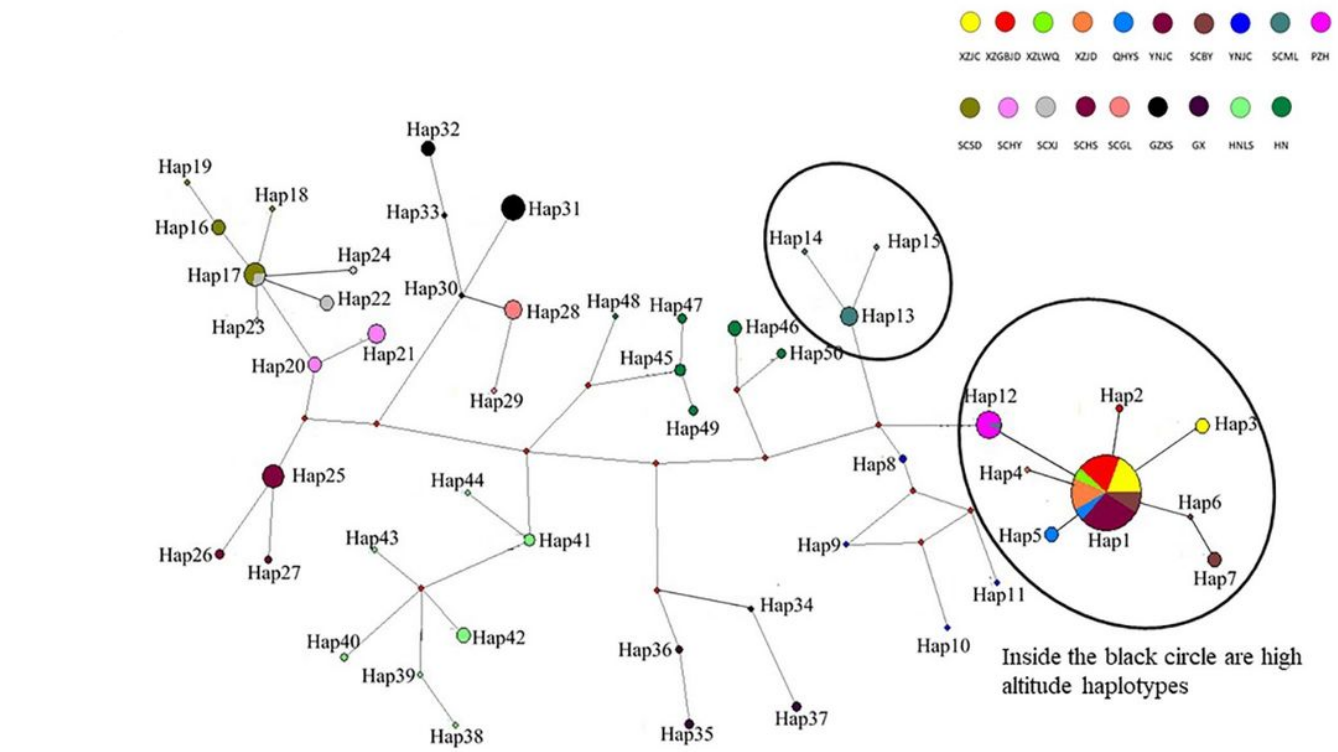

Figure 4

Median-joining (MJ) haplotype network diagram of 19 rhesus macaque populations. The color of the circle represents the haplotype of a specific region, and the size of the circle represents the sample size of a specific haplotype.

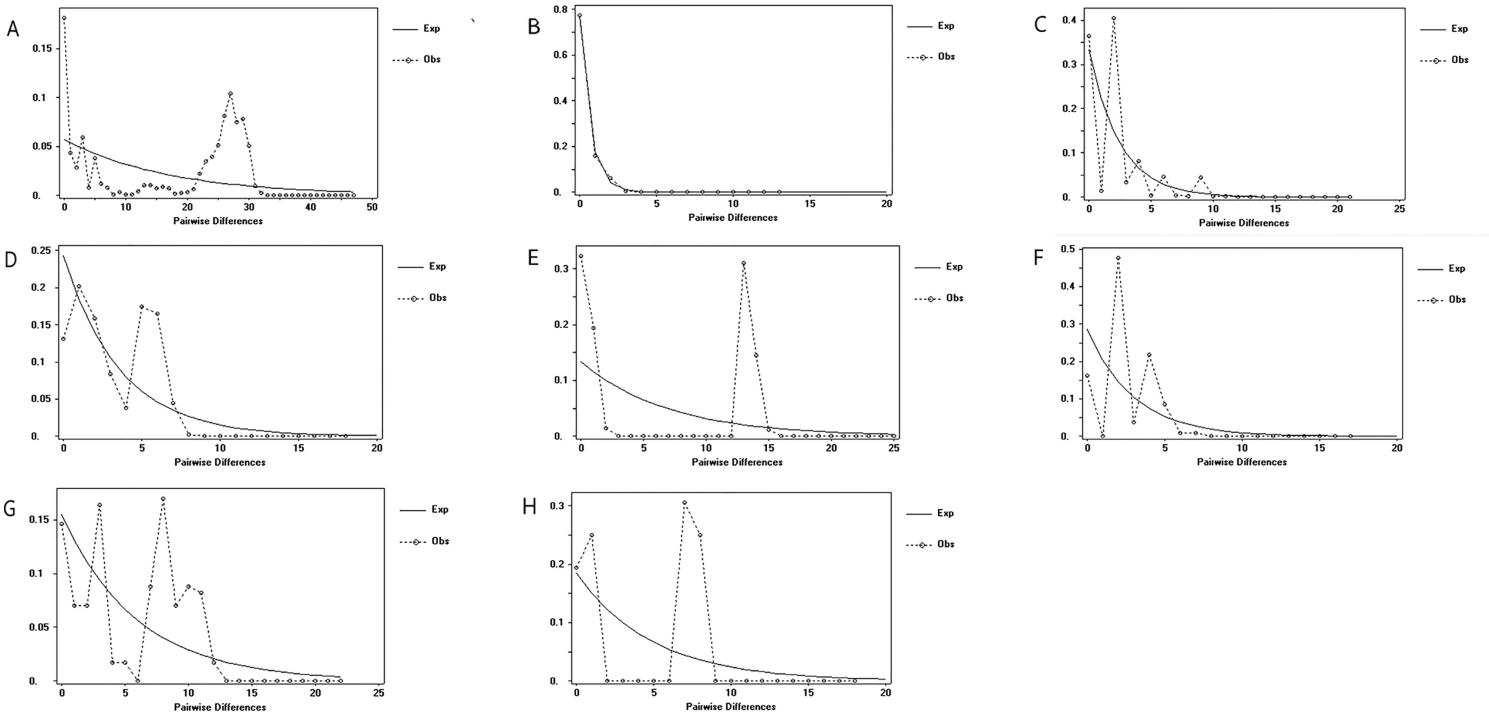

Figure 5

Mismatch distribution of seven rhesus macaque groups based on the phylogenetic analysis. A) Mismatch distribution of all macaques19 populations; B) including XZJD, XZGBJD, QHYS, YNJC, XZLWQ and SCBY; C) including PZH,SCML and SCYJ; D) including SCSD, SCXJ, SCHS and SCHY; E) including SCGL and GZXS; F) HNLS; G) HN and H) GX.

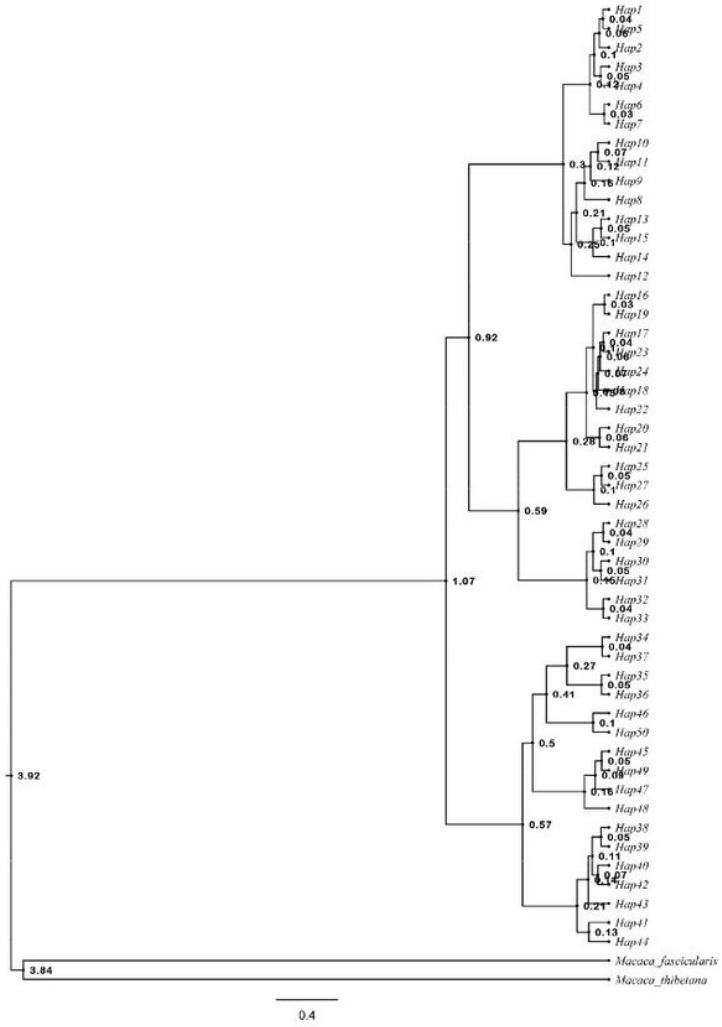

Figure 6

Divergence time of rhesus macaque mtDNA ATP6 haplotypes. Numbers at the nodes were divergence time between haplotypes (Million years ago, Ma)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile2.xlsx
- Additionalfile3.xlsx

- Additionalfile1.xlsx