

Evolutionary Antiquity of the Wanderoo Macaque (*Macaca silenus*): An Endangered Species of the Western Ghats

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Abstract

Lion Tailed Macaque is believed to be the forebear of all Asian macaques. Wanderoo macaque is endemic to the Western Ghats and has been observed in the regions of Karnataka, Kerala and Tamil Nadu. It draws the attention of scientists and researchers because of its K- strategy of populace growth and a unique characteristic, of evolution. This species has a very less populace and is also listed on the IUCN Red List of endangered species due to various anthropogenic activities. The Evolutionary tree helps in better understanding the behavioral personae of the macaque along with its familial history. A Phylogenetic tree has been constructed with Mega X software and the fallouts have been interpreted to apprehend the evolutionary antiquity of the macaque.

Key words: Lion tailed macaque, K- strategy, IUCN red list, Phylogenetic tree, Endangered species, Anthropogenic activities, Familial history, Western Ghats, Mega-X

Primates have evolved over 57-85/90 million years ago. One of the oldest known Nonhuman primates which very much bears a resemblance to a mammal is Plesiadapis. It was instigated in the woodlands of North America and Eurasia. This species subsisted during the Palaeocene epoch. Plesiadapis very much resembled a lemur and by no means had it had a resemblance like any other non-human primate from which hominids were meant to be evolved. Over an epoch of time, the evolution had brought a momentous change that the posterities of Plesiadapis also started to surmount the land and started scuttling onto open heaths to quest for nutriment and at the same passé evolution of the ever-loftier wits took place. This mammal was disinterred in France in the year 1877 after 15 years when Charles Darwin promulgated his tome "origin of species" where gigantic disputation still continued whether humans emerged from nonhuman primates. In distinction to the evidence of the petrification study, it is said that the precursor of this very own species subsisted in North America, possibly coexisting with dinosaurs and then slowly moved over to Western Europe by the way to Greenland [12].

The first mammal that paleontologists have acknowledged which confined primate-like physiognomies was Purgatorius a tiny mouse-sized creature that abided on earth during the cretaceous era, but this species looked more like a tree shrew rather than any other non-human primate. Purgatorius have certain connexions to that of a primate

dentition. Scientists have found the identical mouse-like Archicebus which lived 10 million years after Purgatorius as the first true primate and there is an even stronger postulate which wires the evidence. The Asian Archicebus subsisted around the North American region, such as Plesiadapis which was amended for an omnivorous diet that is more mystifying.

During the Eocene epoch, 55-35 million years ago lemur-like primates have concealed the woodlands around the world, nevertheless, the fossil indication is very gigantic. Most imperatives of these creatures were Notharctus which had simian traits such as the flat face, forward-facing eyes, flexible hands that abetted them in grasping branches, a sinuous backbone and very prominently bigger brains which were seen in any other vertebrate. Notharctus was the last living primate they existed in North America whose ancestors crossed Asia at the end of the Paleocene epoch. Similar to its characteristics is the European Darwinius that created an important subject that might be the earliest human ancestor, while many scientists snubbing this notion or weren't convinced with this fact.

Another such significant Eocene primate was the Asian Eosimias-" The Dawn Monkey", which is comparatively smaller than the Notharctus and Darwinius which weighed about 1-2 ounces maximum and this species very much looked like a Mesozoic mammal. Many experts have suggested that monkeys or non-Human primates have originated from Asia rather than Africa, though it is not approved or believed by

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some scientists. The Eocene period has also countersigned the North American *Smilodectes* and the *Necrolemur* which instigated in Western Europe and even the early pint-sized lemurs were abstractedly allied to modern lemurs and tarsiers.

Primate evolution would be sketchy without the brief depiction of a variety of prehistoric lemurs that once survived in the Indian Ocean Island of Madagascar off the East African coast. The Pleistocene epoch countersigned huge sized lemurs like *archaeoindris* which were about the size of a modern gorilla. Non-human primates such as *babakotia* and *paleopropithecus* that very much looked like sloths and also had similar physiognomies of sloths are existent but on a very gloomy note, these sloths like lemurs were condemned to extinction when the first human colonizers lived in Madagascar about 2000 years ago.

New world monkeys have evolved about 40 million years ago amid the Eocene epoch, while the discernment of old world and new world monkeys transpired about 25 million years later. The evolutionary history and the fossil almanac of the new world monkey are very unsubstantial. The preceding distinct species that has been discovered was *branisella* which extensively lived in South America between 30 and 25 million years ago. *Branisella* was a very diminutive species than the other new world monkey it had a flat nose with a prehensile tail that helped the species too actually to jump between two tree branches. A very good form of primate that is an intervening forerunner between old world monkey and new world monkey is *mesopithecus* a macaque with certain distinguished characteristics of apes foraged for shelter and food. Another possible example of showing strong acquaintance between the old-world monkey and new world primate is *oreopithecus* that dwelled in European island had also certain idiosyncrasy of apes but was also considered being true hominid.

During the Miocene epoch of about 23-25 million years ago, the apes and hominids lived in the wildernesses of Africa and Eurasia. The most distinctive ape of Africa is supposed to be *pliopithecus* which is believed to be descendants of modern-day gibbons. *Pliopithecus* have the status of non-hominid where its related apes are also set to be ancestors for humans and none of these species walked on two feet. Ape evolution is believed to occur in a greater range during the late Miocene period with the tree-dwelling *Dryopithecus*, *Gigantopithecus* and the *Sivapithecus* which are considered to be of the same genus unlike that of the *Ramapithecus*. *Sivapithecus* is an important ape because it is alleged to be the first ape that ventured down from the trees to the African grasslands an evolutionary transition that is believed to be occurring due to climate change [13].

The term *Macaca* is derived from Portuguese, which has actually originated from the West African fiot word (MA) Kaku, which generally means monkey. The majority of the macaque species are usually found in the Indian subcontinent predominantly in Southeast Asia and in Sundaland. Most of the species are arboreal species, but some species have adapted to semiterrestrial which have highly adapted to live on grounds [6].

The *Macaca* species are very unique among non-human primates for the range of adaptations, it has colonized from equatorial temperate ecosystems to evergreen forests, grasslands, human-modified environments and Deepwater continental islands. The genus *Macaca* has evolved over 10 million years ago in the course of the mid-Miocene epoch and lion-tailed macaques are said to be evolved around 3 million years ago. *Macaca* is the only widespread genera that have been diversified from North Africa to Japan and it is believed that 19 of the distinctive macaque species are recorded in Asia. the

silenus - *Sylvanus* has been recorded for its fragmented geographical distribution of which the 11 species of the same lineage are wide spreader because of early dispersal. The Barbary macaque is observed in the North African region, *Macaca silenus* is found in the evergreen forests of India whereas the Pig-Tailed macaque is found in the Indo Chinese region. The oldest known fossils that were recorded in Asia dated 3 mya where it was known to be dwelling in the Siwalik hills of Northern India [1].

Lion-tailed macaque has its very own unique place of the identity of Indian non-human primate, but certain anthropogenic activities have brought a very radical change of its very own population and endurance bringing it to a very deadly face endangered species. Wanderoo macaque is endemic to the Western Ghats and is found in the rainforest hills of Karnataka, Kerala and Tamil Nadu. This macaque species is an indicator of the health of the rainforests, making it a very imperative species not just for the conservation of its own but also conservation of other wildlife species existent in the region.

Other Indian macaque species such as the bonnet and rhesus macaque are habitat generalists because of their very own annual breeding cycles that help these macaques in terms of having a high population turnover rate because they tend to achieve sexual maturity at a very early state, such strategy is referred to as R strategy in behavioural biology, however, the liontailed macaque possess long inter-birth interval and delayed sexual maturity, which primes to low population turnover. This refers to a k strategy in terms of behavioural biology. Because of its very own distinguishing characteristics, it even makes scientists and behavioural biologists get to know about the ancestral history of lion-tailed macaque. In Valparai Tamil Nadu these species are witnessed nourishing on red coffee berries, a behaviour that was not detected in any other individuals of the western ghat region [3].

Charles Darwin in the year 1837 outlined the first phylogenetic tree since then analyzing and interpretation of the evolutionary history of the species had become a major part of biological studies [16]. The evolutionary tree helps one in understanding the evolution and close relatedness among the species. Phylogenetic trees do not only help in evolutionary studies but also aids in biodiversity studies such as measuring species richness, evaluating conservation strategies for endangered species, genetic studies et Cetra [7]. The phylogenetic analytics is usually depicted as a branching tree-like diagram which is the phylogenetic tree. In a molecular phylogenetic analysis by using a complete genome or a protein sequence evolutionary history of the particular species could be obtained [14].

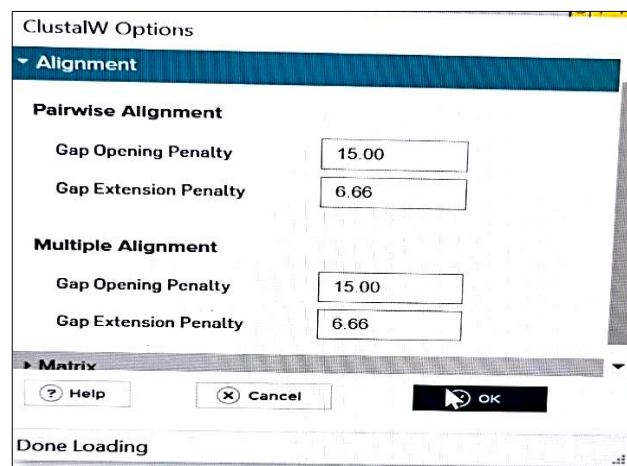


Fig 1 Alignment of nucleotide database by Clustal W program

MATERIALS AND METHODS

The phylogenetic analysis was investigated using MEGA- X Software (Molecular Evolutionary Genetics Analysis) which is computational courseware that conducts statistical premise of molecular evolution and for constructing phylogenetic trees. The nucleotide data were collected from NCBI (National Center for Biotechnology Information). The sequences were then poised in FASTA ("FAST-All") format and the nucleotide sequences were further scrutinized in BLAST (Basic Local Alignment Search Tool) format. The sequences that were inspected in BLAST format were copied to notepad and further study was carried out in MEGA-X software where the sequences were aligned with the help of the Clustal

W algorithm. Clustal w is a tool that is used for aligning multiple nucleotide or protein sequences, it also estimates the finest match for the designated sequences. The selected 26 sequences were further examined and with the tool model available in the software 'Find best DNA/Proteins model' option was selected, the substitution model was saved in the MS-Excel format and the phylogenetic tree was constructed with the help of the Maximum likelihood method and Tamura-Nei Model (Tamura 3-Parameter model), 100 bootstrap replicates were inferred to construct the tree. The ancestral states were also implied using the same procedures. Evolutionary relationships are commonly detected by means of cladograms or phylograms. Molecular Evolutionary Genetic Analysis is a statistical software tool that is used for constructing the phylogenetic tree.

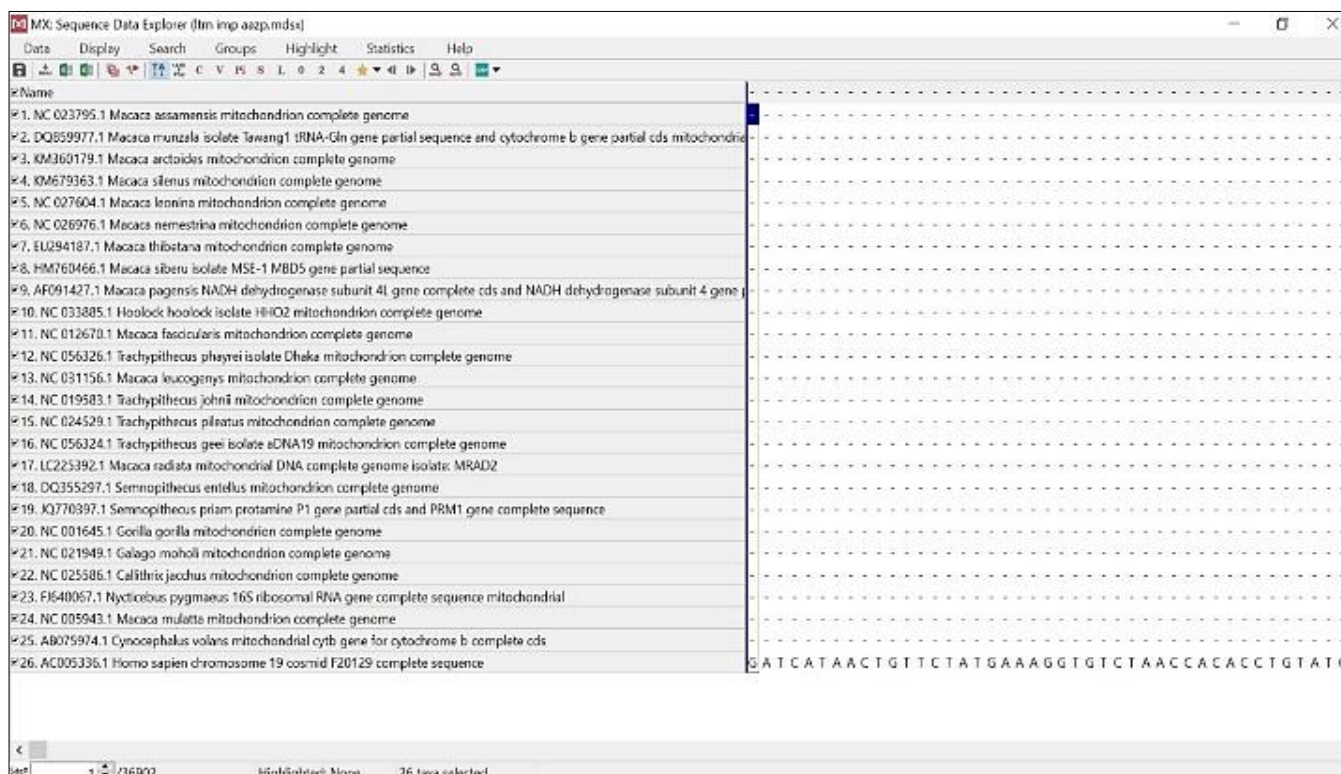


Fig 2 Sequences aligned by Clustal W programme for phylogenetic tree analysis

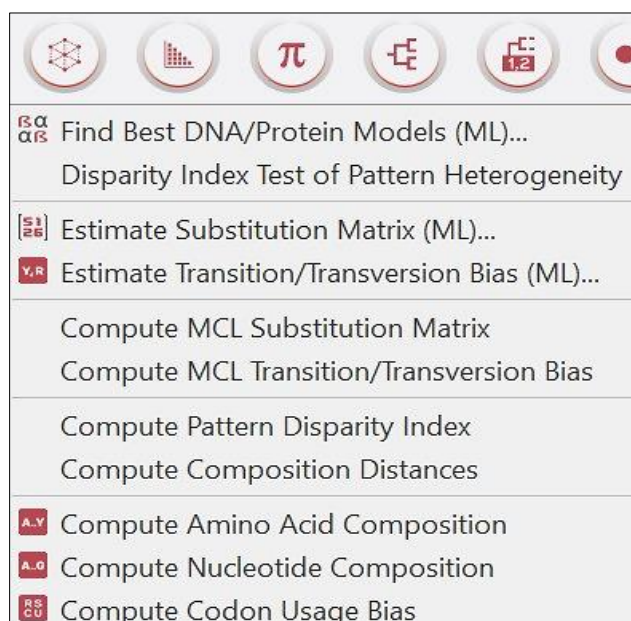


Fig 3 Alinement of sequences for carrying out the best nucleotide models for the construction of the phylogenetic tree

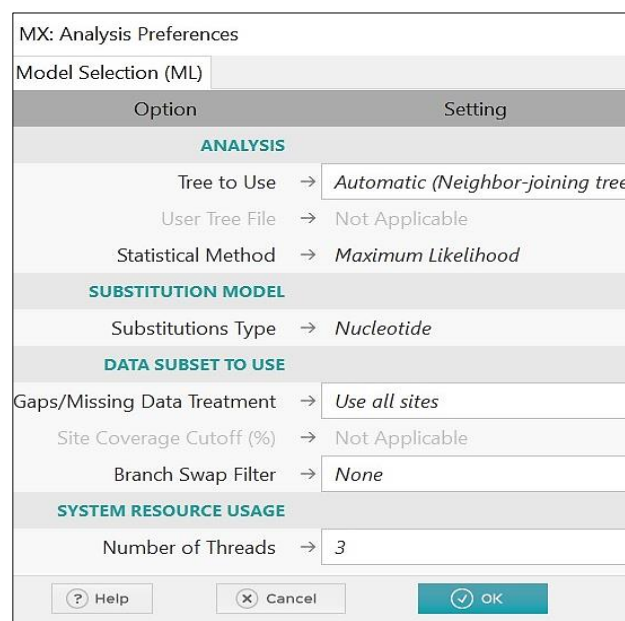


Fig 4 Model selection analysis for phylogenetic tree construction

Table. Maximum Likelihood fits of 24 different nucleotide substitution models																				
Model	Parameters	BIC	AICc	lnL	(+I)	(+G)	R	f(A)	f(T)	f(C)	f(G)	r(AT)	r(AC)	r(AG)	r(TA)	r(TC)	r(TG)	r(CA)	r(CT)	r(CG)
GTR+G+I	59	350647.279	350010.961	-174946.471	0.03	0.39	5.03	0.316	0.263	0.283	0.138	0.023	0.029	0.102	0.028	0.264	0.006	0.033	0.246	0.007
GTR+G	58	350701.239	350075.706	-174979.843	n/a	0.37	5.00	0.316	0.263	0.283	0.138	0.023	0.030	0.102	0.028	0.264	0.006	0.033	0.246	0.007
HKY+G+I	55	350819.359	350226.180	-175058.081	0.03	0.38	5.03	0.316	0.263	0.283	0.138	0.021	0.022	0.116	0.025	0.238	0.011	0.025	0.221	0.011
TN93+G+I	56	350849.030	350245.067	-175066.524	0.03	0.39	4.87	0.316	0.263	0.283	0.138	0.022	0.024	0.089	0.027	0.279	0.012	0.027	0.259	0.012
TN93+G	55	350984.835	350391.656	-175140.820	n/a	0.38	4.28	0.316	0.263	0.283	0.138	0.025	0.026	0.086	0.030	0.274	0.013	0.030	0.254	0.013
HKY+G	54	350988.059	350405.665	-175148.824	n/a	0.38	4.08	0.316	0.263	0.283	0.138	0.025	0.027	0.112	0.030	0.229	0.013	0.030	0.213	0.013
T92+G	52	353939.956	353379.131	-176637.558	n/a	0.41	4.17	0.290	0.290	0.210	0.210	0.027	0.020	0.171	0.027	0.171	0.020	0.027	0.235	0.020
T92+G+I	53	354013.880	353442.271	-176668.127	0.02	0.40	4.99	0.290	0.290	0.210	0.210	0.024	0.017	0.176	0.024	0.176	0.017	0.024	0.242	0.017
K2+G	51	355753.110	355203.071	-177550.528	n/a	0.41	4.09	0.250	0.250	0.250	0.250	0.025	0.025	0.201	0.025	0.201	0.025	0.025	0.201	0.025
K2+G+I	52	355856.023	355295.198	-177595.591	0.03	0.40	5.12	0.250	0.250	0.250	0.250	0.020	0.020	0.209	0.020	0.209	0.020	0.020	0.209	0.020
GTR+I	58	363153.865	362528.332	-181206.156	0.09	n/a	2.96	0.316	0.263	0.283	0.138	0.035	0.052	0.087	0.042	0.250	0.006	0.058	0.232	0.009
TN93+I	55	364302.270	363709.091	-181799.537	0.09	n/a	3.86	0.316	0.263	0.283	0.138	0.027	0.029	0.089	0.032	0.261	0.014	0.032	0.243	0.014
HKY+I	54	364626.024	364043.630	-181967.806	0.09	n/a	3.80	0.316	0.263	0.283	0.138	0.026	0.028	0.110	0.032	0.226	0.014	0.032	0.210	0.014
GTR	57	365132.978	364518.230	-182202.106	n/a	n/a	2.85	0.316	0.263	0.283	0.138	0.034	0.055	0.087	0.041	0.247	0.006	0.061	0.229	0.009
T92+I	52	366099.093	365538.269	-182717.127	0.09	n/a	3.89	0.290	0.290	0.210	0.210	0.029	0.021	0.168	0.029	0.168	0.021	0.029	0.231	0.021
TN93	54	366188.450	365606.056	-182749.020	n/a	n/a	3.31	0.316	0.263	0.283	0.138	0.030	0.032	0.089	0.036	0.249	0.016	0.036	0.232	0.016
HKY	53	366532.122	365960.513	-182927.248	n/a	n/a	3.26	0.316	0.263	0.283	0.138	0.030	0.032	0.107	0.036	0.219	0.016	0.036	0.203	0.016
T92	51	367539.411	366989.371	-183443.678	n/a	n/a	3.34	0.290	0.290	0.210	0.210	0.033	0.024	0.163	0.033	0.163	0.024	0.033	0.224	0.024
K2+I	51	367851.904	367301.865	-183599.925	0.10	n/a	3.99	0.250	0.250	0.250	0.250	0.025	0.025	0.200	0.025	0.200	0.025	0.025	0.200	0.025
K2	50	369452.181	368912.926	-184406.456	n/a	n/a	3.43	0.250	0.250	0.250	0.250	0.028	0.028	0.194	0.028	0.194	0.028	0.028	0.194	0.028
JC+G	50	372946.074	372406.820	-186153.403	n/a	0.54	0.50	0.250	0.250	0.250	0.250	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083
JC+G+I	51	372958.543	372408.504	-186153.244	0.01	0.56	0.50	0.250	0.250	0.250	0.250	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083
JC+I	50	382194.387	381655.132	-190777.559	0.10	n/a	0.50	0.250	0.250	0.250	0.250	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083
JC	49	383920.944	383392.474	-191647.230	n/a	n/a	0.50	0.250	0.250	0.250	0.250	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083

NOTE. – Models with the lowest BIC scores (Bayesian Information Criterion) are considered to describe the substitution pattern the best. For each model, AICc value (Akaike Information Criterion, corrected), Maximum Likelihood value (lnL), and the number of parameters (including branch lengths) are also presented [1]. Non-uniformity of evolutionary rates among sites may be modeled by using a discrete Gamma distribution (+G) with 5 rate categories and by assuming that a certain fraction of sites are evolutionarily invariable (+I). Whenever applicable, estimates of gamma shape parameter and/or the estimated fraction of invariant sites are shown. Assumed or estimated values of transition/transversion bias (R) are shown for each model, as well. They are followed by nucleotide frequencies (f) and rates of base substitutions (r) for each nucleotide pair. Relative values of instantaneous r should be considered when evaluating them. For simplicity, sum of r values is made equal to 1 for each model. For estimating ML values, a tree topology was automatically computed. This analysis involved 26 nucleotide sequences. There were a total of 36902 positions in the final dataset. Evolutionary analyses were conducted in MEGA X [2]

Abbreviations: TR: General Time Reversible; HKY: Hasegawa-Kishino-Yano; TN93: Tamura-Nei; T92: Tamura 3-parameter; K2: Kimura 2-parameter; JC: Jukes-Cantor./div>

Fig 5 Maximum likelihood fits of 24 different nucleotide substitution model

MX: Analysis Preferences	
Phylogeny Reconstruction	
Option	Setting
ANALYSIS	
Statistical Method	→ Maximum Likelihood
PHYLOGENY TEST	
Test of Phylogeny	→ Bootstrap method
No. of Bootstrap Replications	→ 100
SUBSTITUTION MODEL	
Substitutions Type	→ Nucleotide
Model/Method	→ Tamura-Nei model
RATES AND PATTERNS	
Rates among Sites	→ Uniform Rates
No of Discrete Gamma Categories	→ Not Applicable
DATA SUBSET TO USE	
Gaps/Missing Data Treatment	→ Use all sites
Site Coverage Cutoff (%)	→ Not Applicable
TREE INFERENCE OPTIONS	
ML Heuristic Method	→ Nearest-Neighbor-Interchange (NNI)
Initial Tree for ML	→ Make initial tree automatically (Default - NJ/BioNJ)
Initial Tree File	→ Not Applicable
Branch Swap Filter	→ None
SYSTEM RESOURCE USAGE	
Number of Threads	→ 3
<input type="button" value="Help"/> <input type="button" value="Cancel"/> <input type="button" value="OK"/>	

Fig 6 Construction of the phylogenetic tree using Tamura-Nei Model with 100 bootstrap replicates

RESULTS AND DISCUSSION

The evolutionary history was inferred by using the Maximum Likelihood method and Tamura 3-parameter model [16]. The tree with the highest log likelihood (-182717.12) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura 3 parameter model and then selecting the topology with a superior log likelihood value. The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 9.25% sites). This analysis involved 26 nucleotide sequences. There was a total of 36902 positions in the final dataset. Evolutionary analyses were conducted in MEGA X [9].

Ancestral states were inferred using the Maximum Likelihood method [10] and Tamura 3 parameter model [16]. The tree shows a set of possible nucleotides (states) at each ancestral node based on their inferred likelihood at site 1. The set of states at each node is ordered from most likely to least likely, excluding states with probabilities below 5%. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura 3 parameter model and then selecting the topology with a superior log-likelihood value. The rates among sites were treated as being invariant among sites (Invariant option). This analysis involved 26 nucleotide sequences. There was a total of 36902 positions in the final dataset. Evolutionary analyses were conducted in MEGA X [9].

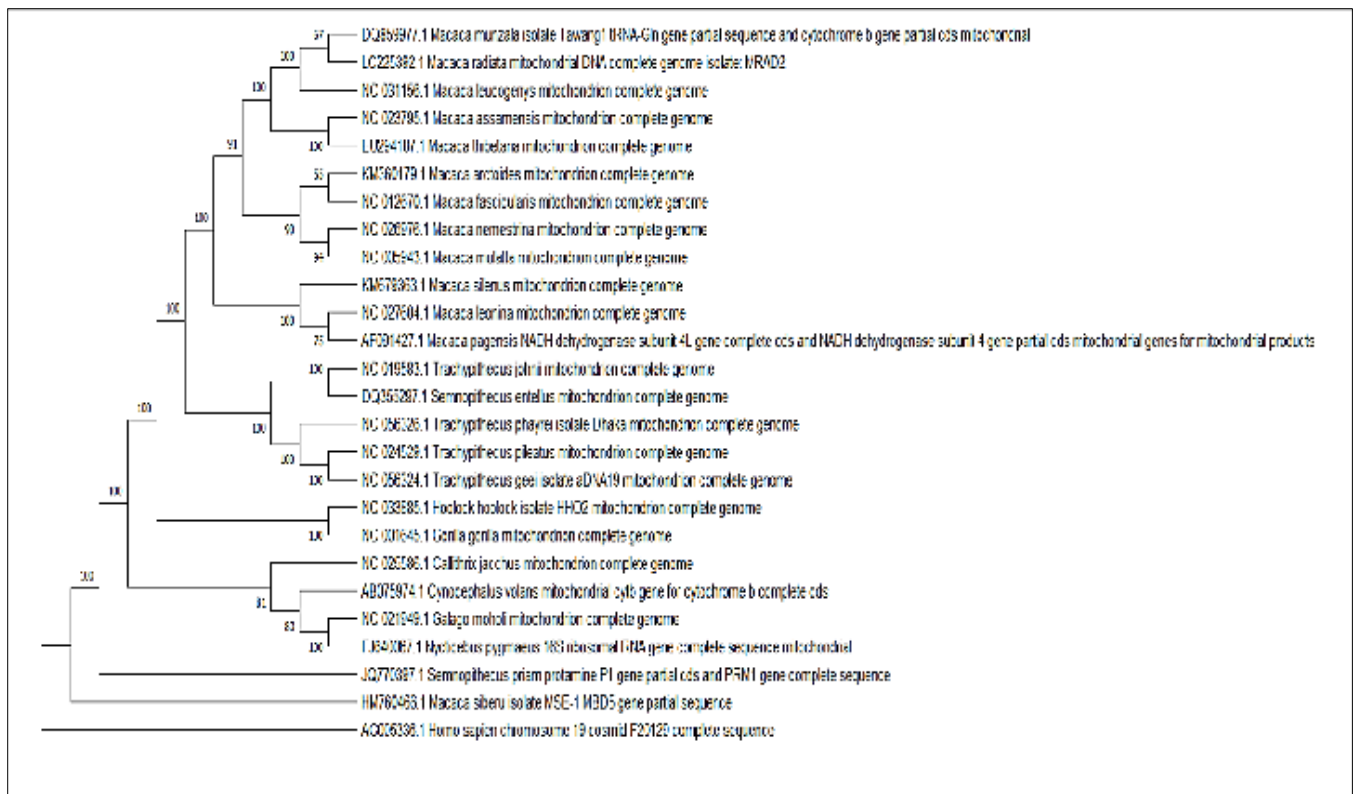


Fig 7 Evolutionary tree constructed by Maximum likelihood method and Tamura 3-parameter model

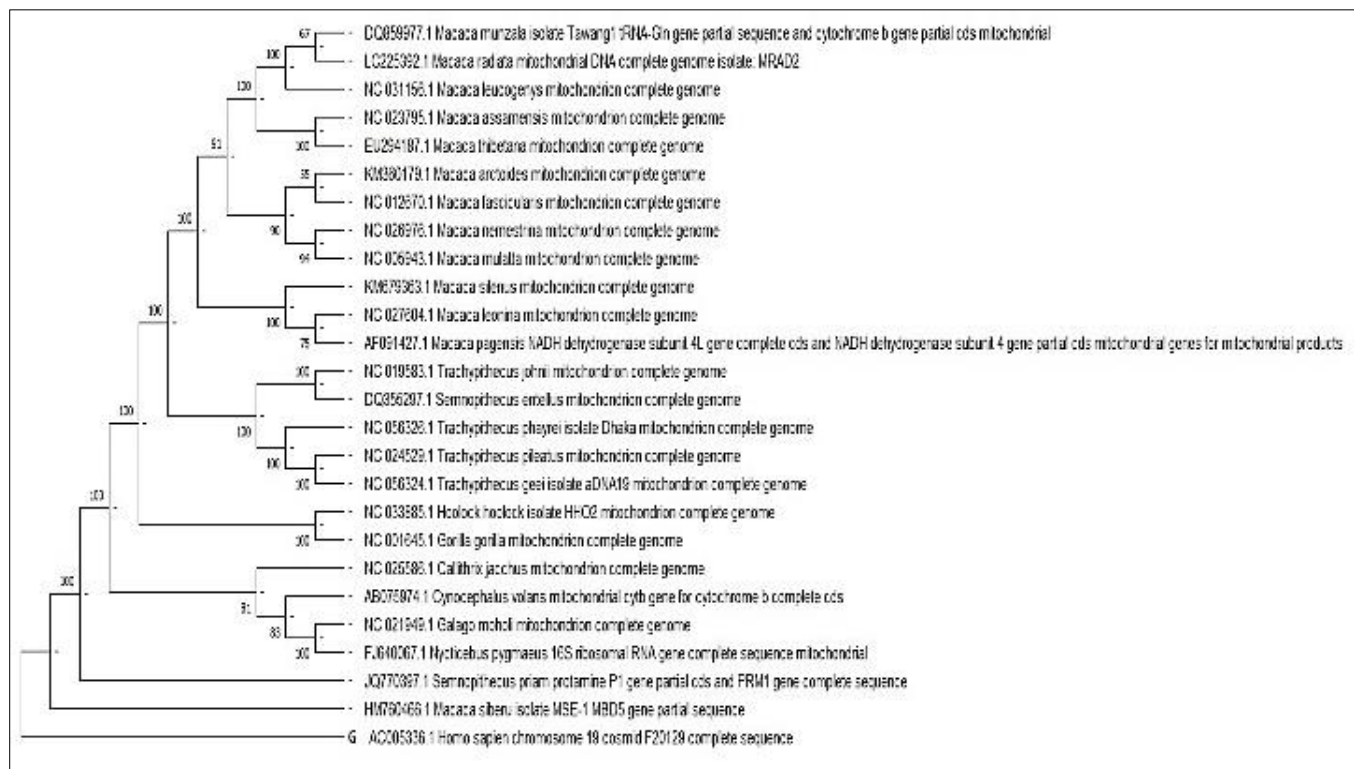


Fig 8 Ancestral states by Maximum Likelihood method and Tamura 3-parameter model

Macaca silenus is being diversified into *Macaca pagensis* and *Macaca leonina* (Northern Pig Tailed macaque), where *silenus* and *leonina* are believed to be sister taxa. Pagai macaque is an endangered species and it is endemic to Mentawai Island. Northern Pig-tailed macaque is an old-world monkey found in Bangladesh, India, Thailand, Vietnam and Myanmar. Northern and southern pig-tailed were once believed to be subspecies but later they are classified as individual species. The pigtailed macaques, pagai macaque and the lion-tailed macaque share a common ancestor, furthermore, a

detailed study of the macaques of both old world and new world macaque would give a better insight into the evolutionary history of the Lion Tailed macaque [11].

Macaca is the genus-group that are medium-sized cercopithecines, they are characterized by certain characteristics features such as moderately long snouts, high-crowned molar teeth with very low cusps and long third molars. Macaques share facial features with papionins which relatively have long faces. Macaques have occupied wider ranges of habitats in comparison with any other non-human primate

genus. Over the years macaques have adapted to various environmental conditions and variations in terms of morphology, physiology, reproductive biology, ecology, behaviour and genetics which indicates the complex evolution of the nonhuman primates [6].

The *Macaca* genus-group comprises 22 species and 37 taxa, it is believed to be one of the diverse species groups of old-world genera. The old world macaque genera consist of 3 monotypic groups with only one species each *M. sylvanus*, *M. arctoides* group and *M. fascicularis* group and four polytypic species groups containing several species *M. silenus*, *M. sulawesi*, *M. sinica* and *M. mulatta* group [2].

According to IUCN Red list the status among the selected 26 primate species 5 least concern, 6 vulnerable, 3 near threatened, 7 endangered, 2 critically endangered, 1 vulnerable threatened, 1 exact conservation status has not yet been determined and a homo sapiens sequence was included in the construction of the phylogenetic tree.

The lion-tailed macaque is a highly endangered species covered with long grey cheek whiskers. The wanderoo macaque is distinctively found in the Western Ghats of Southern India [6]. Macaques even though classified into many species' groups, but classifying the macaque species has been a matter of debate for about 50 years [2].

Macaca pagensis is the sister taxa of the Lion-Tailed macaque alongside the Northern pigtailed macaque. Pagai macaque is an old world monkey Cercopithecidae. *Macaca pagai* is also known as Pagai macaque or Bokkoi, this macaque is endemic to Mentawai islands of Sumatra. Unlike the wanderoo macaque, the Pagai macaque is also a critically endangered species because of its shrinking habitat. Bokkoi macaque was earlier considered to be the subspecies of the Siberut macaque but later both the species were differentiated into two different groups and they were formerly said to be the subspecies of the southern pig-tailed macaque. The Northern pig-tailed macaque is observed in tropical forests throughout southeast Asia, and are venerable from the southern pig-tailed macaque by the presence of diagonal lines [12].

CONCLUSION

A much-detailed evolutionary study on the old-world monkeys could give us an insight on the behavioral and on how the macaques diversified into sister taxa. The evolutionary history of Lion Tailed Macaque was analyzed using Maximum Likelihood Method, Tamura-Nei Model with 100 bootstrap replicate values. The results indicated that *Macaca silenus* has been diversified from *Macaca leonina*.

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