Building evidence for conservation globally

# Journal of Threatened Taxa

Open Access

10.11609/jott.2023.15.9.23827–23930
www.threatenedtaxa.org

26 September 2023 (Online & Print)
15(9): 23827–23930
ISSN 0974-7907 (Online)
ISSN 0974-7893 (Print)

ISSN 0974-7907 (Online); ISSN 0974-7893 (Print)

Publisher
**Wildlife Information Liaison Development Society**
www.wild.zooreach.org

Host
**Zoo Outreach Organization**
www.zooreach.org

43/2 Varadarajulu Nagar, 5th Street West, Ganapathy, Coimbatore, Tamil Nadu 641006, India
Registered Office: 3A2 Varadarajulu Nagar, FCI Road, Ganapathy, Coimbatore, Tamil Nadu 641006, India
Ph: +91 9385339863 | www.threatenedtaxa.org
Email: sanjay@threatenedtaxa.org



For Focus, Scope, Aims, and Policies, visit https://threatenedtaxa.org/index.php/JoTT/aims_scope
For Article Submission Guidelines, visit https://threatenedtaxa.org/index.php/JoTT/about/submissions
For Policies against Scientific Misconduct, visit https://threatenedtaxa.org/index.php/JoTT/policies_various

continued on the back inside cover

**Cover: Stripe-necked Mongoose *Urva vitticolla* in poster colours, adapted from photograph by Ashni Dhawale, by Pooja Ramdas Patil.**

Journal of Threatened Taxa | www.threatenedtaxa.org | 26 September 2023 | 15(9): 23827–23835
ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)
https://doi.org/10.11609/jott.8491.15.9.23827-23835
#8491 | Received 26 April 2023 | Final received 07 September 2023 | Finally accepted 11 September 2023
OPEN ACCESS
ARTICLE

# Phylogenetic insights on the delineation of Mysore and Malabar subspecies of the Grey Slender Loris *Loris lydekkerianus* in southern India

**Vinay Teja [1], Shivakumara Manu [2], Honnavalli N. Kumara [3] & Govindhaswamy Umapathy [4]**

[1,2,4] CSIR-Centre for Cellular and Molecular Biology, Uppal Road, Habsiguda, Hyderabad, Telangana 500007, India.
[3] Salim Ali Centre for Ornithology and Natural History, Anaikatty (POST), Coimbatore, Tamil Nadu 641108, India.
[1] vinay@ccmb.res.in, [2] smanu@ccmb.res.in, [3] honnavallik@gmail.com, [4] guma@ccmb.res.in (corresponding author)
[1,2] contributed equally.

**Abstract:** Slender lorises are a threatened genus of small and nocturnal strepsirrhine primates confined to India and Sri Lanka. The Grey Slender Loris *Loris lydekkerianus* is divided into several subspecies based on morphological variation and geographical distribution but not supported by molecular data. We investigated the phylogenetic divergence of two subspecies of the Grey Slender Loris in southern India: the Mysore Slender Loris *Loris lydekkerianus* ssp. *lydekkerianus* and the Malabar Slender Loris *Loris lydekkerianus* ssp. *malabaricus*. We generated whole genome shotgun sequence data and assembled the whole mitochondrial genomes of representative individuals from their distribution in southern India and compared them with publicly available mitogenomes of other lorises. We found that the Mysore and Malabar Slender Lorises vary by 2.09% in the COX1 and CYTB gene regions. Further, phylogenetic analysis of 13 protein-coding and two ribosomal RNA genes in the mitochondrial genome showed that the Mysore and Malabar Slender Lorises form distinct monophyletic clades that diverged about 1.049 million years ago, shortly after the divergence of Red Slender Loris *Loris tardigradus*. Considering this relatively high sequence variation and evolutionary divergence together with their already established morphological differences and geographically distinct habitats, we propose to recognize the Mysore and Malabar Slender Lorises as two distinct species *Loris lydekkerianus* and *Loris malabaricus*.

**Keywords:** Grey Slender Loris, Malabar Slender Loris, molecular dating, Mysore Slender Loris, phylogenetics.

**Editor:** K.A.I. Nekaris, Oxford Brookes University, Oxford, United Kingdom.

**Date of publication:** 26 September 2023 (online & print)

**Citation:** Teja, V., S. Manu, H.N. Kumara & G. Umapathy (2023). Phylogenetic insights on the delineation of Mysore and Malabar subspecies of the Grey Slender Loris *Loris lydekkerianus* in southern India. *Journal of Threatened Taxa* 15(9): 23827–23835. https://doi.org/10.11609/jott.8491.15.9.23827-23835



**Funding:** Council of Scientific and Industrial Research (CSIR) and Department of Biotechnology (DBT), Govt. of India.

**Competing interests:** The authors declare no competing interests.

**Author details:** Vinay Teja is a PhD student at CSIR-CCMB. He is interested in Bioinformatics and Molecular evolution of traits in primates.
Shivakumara Manu is a PhD student at CSIR-CCMB. He holds a Master's degree in Bioinformatics and Applied Biotechnology and is interested in solving biological problems through genomics and computational approaches.
Dr. Honnavalli N. Kumara is a wildlife biologist working as a Principal Scientist at SACON. His interest lies in understanding population dynamics, behavioural ecology, and conservation of primates, other mammals and birds.
Dr. Govindhaswamy Umapathy is a conservation biologist working as a Senior Principal Scientist and Group Leader at the LaCONES, CSIR-CCMB. His research interests include understanding species extinction in human dominated landscapes, genome sequencing and developing biotechnological tools for biodiversity conservation.

**Author contributions:** G.U. and H.N.K conceived the idea for this study and collected the samples. V.T. and S.M. analyzed the data and drafted the manuscript. All authors contributed to the revision of the manuscript and agreed to the final version of the manuscript.

For **Tamil abstract** see end of this article.

**Acknowledgements:** V.T. was supported by a CSIR PhD fellowship, S.M. was supported by a DBT-BINC PhD fellowship, and G.U. was supported by CSIR, Govt. of India. We thank Dr. Mihir Trivedi for providing useful suggestions for the phylogenetic analysis.

## INTRODUCTION

Slender lorises (genus *Loris*) are one of the two genera of extremely specialized nocturnal primates that inhabit India (Nekaris 2014). They belong to the family Lorisidae, which also includes Slow lorises, Pottos, and Angwantibos. Slender lorises are confined to India and Sri Lanka, where they inhabit dry to moist and lowland to montane forests (Singh et al. 2021). Slender lorises are characterized by their small size, long limbs, vestigial tail, large eyes, and slow locomotion. They are adapted for arboreal life, using their opposable thumbs and toes to grasp branches and their binocular vision to judge distances, visual acuity, precise hand-eye coordination and social communication. They feed mainly on insects but also consume fruits, flowers, gums, and other plant materials (Nekaris & Rasmussen 2003; Radhakrishna & Kumara 2010). They have a variety of vocalizations that may help them avoid predators and communicate with conspecifics (Radhakrishna & Singh 2002). Slender lorises are divided into two species: the Grey Slender Loris *Loris lydekkerianu*s found in southern India and Sri Lanka and the Red Slender Loris *Loris tardigradus* found only in Sri Lanka (Groves 2001). Both species show high phenotypic variation in fur color, body size, and cranial morphology, leading to the recognition of several subspecies, most of which are refuted by molecular studies (Nijman et al. 2020). The Mysore Slender Loris *Loris lydekkerianus* ssp. *lydekkerianus* (Image 1) and the Malabar Slender Loris *Loris lydekkerianus* ssp. *malabaricus* (Image 2), which live in the dry and wet forests of the Eastern and Western Ghats, respectively, are the two subspecies that have been recognized thus far in southern India (Kumara et al. 2013). There are several regions in their distribution where slender lorises face serious threats to their existence such as habitat loss due to deforestation and urbanization, electrocution on live wires, road accidents, pet trade, and illegal poaching for traditional medicine and black magic (Dittus et al. 2022; Gnanaolivu et al. 2022). The IUCN Red List of Threatened Species classifies the Mysore Slender Loris (Kumara et al. 2022a) and Malabar Slender Loris (Kumara et al. 2022b) as 'Near Threatened' and they are listed under Schedule I of the Indian Wild Life (Protection) Act, 1972. Recently, Tamil Nadu became the first Indian state to notify a sanctuary for slender lorises spanning 118.06 km$^2$, which is crucial for protecting their habitat and ensuring the survival of this unique primate species (Government of Tamil Nadu 2022).

The Mysore and Malabar subspecies of the Grey Slender Loris were delineated based on their morphological differences and geographic distribution (Groves 2001; Kumara et al. 2013). The Mysore Slender Loris is relatively larger (ca. 260 g) than Malabar Slender Loris (ca. 180 g) (Kumara et al. 2006). The Mysore Slender Loris has a grayish-brown coat and a prominent white stripe on its forehead, whereas the Malabar Slender Loris has a reddish-brown coat and a less distinct forehead stripe (Groves 2001; Kumara et al. 2006). The relative distribution of the two subspecies as well as their comparative densities and the extent of overlap between their distributions have been very well established (Kumara et al. 2013). The Mysore Slender Loris is found in the Eastern Ghats and eastern foothills of the southern Western Ghats, while the Malabar Slender Loris is confined to the western slope of the entire Western Ghats (Kumara et al. 2013). The Mysore Slender Loris prefers dry deciduous forests with moderate canopy cover and high tree density, while the Malabar Slender Loris prefers moist evergreen forests with high canopy cover and low tree density (Kumara et al. 2013). Their distributions overlap along the southern ridges of the Western Ghats, where hybridization may occur. While the diet of Mysore Slender Loris mostly consists of insects, plant material, and gum, the feeding behavior of the Malabar Slender Loris is not well studied (Radhakrishna & Kumara 2010). The reproductive biology and social system of the Mysore subspecies is influenced by factors such as seasonality, food availability, predation risk, and population density. It also has a seasonal breeding cycle that coincides with periods of high food availability (Radhakrishna & Singh 2004). No such information on the reproductive biology of the Malabar subspecies is available. Behavioral studies on lorises have always been more challenging than on relatively large, diurnal, and group-living primates such as macaques and langurs because they are nocturnal, small in size, and mostly semi-gregarious. Given the distinct habitat preferences and morphology of these two subspecies, understanding their evolutionary history and genetic differences is vital to address their conservation status and management issues.

Therefore, the main objective of this study is to investigate the phylogenetic relationship and genetic divergence between the Mysore and Malabar Slender Lorises in southern India. To achieve this, we sequenced and assembled the whole mitochondrial sequences from three representative samples. We aligned these sequences with the publicly available sequences of other lorises and constructed phylogenetic trees. We estimated the sequence divergence and divergence time between the two subspecies using phylogenetic analysis.


Image 1. Mysore Slender Loris *Loris lydekkerianus* ssp. *lydekkerianus.*


Image 2. Malabar Slender Loris *Loris lydekkerianus* ssp. *malabaricus.*

Our results support the morphological and geographical delineation of the Malabar and Mysore Slender Lorises and advocates for recognizing them as two distinct species. This study will contribute to the understanding of the biogeography and speciation processes of these threatened lorises and provide crucial insights for their conservation and management.

## MATERIALS AND METHODS

### Sample Collection, DNA extraction, and Sequencing

We followed the sample collection guidelines of the animal ethics committees of the CSIR-Centre for Cellular and Molecular Biology and Salim Ali Centre for Ornithology and Natural History. Necessary permissions for sample collection were obtained from the Central Zoo Authority of India, Ministry of Environment, Forests & Climate Change, Government of India, vide Ref. No. 9-2/2005-CZA(M) Vol III. Rescued lorises of known wild origin within the IUCN designated ranges (Figure 1) that were captive in Mysore and Hyderabad zoos were the sources of our samples. Blood samples were collected in EDTA vacutainers by qualified zoo veterinarians from three representative individuals of *Loris lydekkerianus* ssp. *lydekkerianus* (N = 2) and *Loris lydekkerianus* ssp. *malabaricus* (N = 1). We used the Qiagen DNeasy Blood and Tissue Kit to isolate the genomic DNA from the blood samples. We measured the quality and quantity of genomic DNA using Nanodrop and Qubit 4. We constructed whole genome libraries using the Truseq PCR-free library preparation kit according to Illumina's protocols. Briefly, 1 ug of genomic DNA was sheared to approximately 350 bp using the Covaris ultrasonicator. The fragmented DNA was then end-repaired and blunt-end ligated with sequencing adapters containing unique dual indices from IDT. The library was then size-selected using SPRI beads and verified on the Agilent fragment analyzer. The cleaned-up libraries were finally quantified in qPCR using the standards and Illumina adapter-specific primers from the Roche library quantification kit. Libraries having good concentration were pooled along with other samples and sequenced on the Illumina Novaseq 6000 platform for 300 cycles in paired-end mode.

### Mitochondrial genome assembly

We demultiplexed the base call files to separate the three samples with the dual-indexed barcodes using the BCL2FASTQ tool from Illumina. Raw reads were quality-filtered with a phred quality score threshold of 15 using FASTP v0.20 (Chen et al. 2018). We subsampled 10 million quality filtered reads to de novo assemble the circular mitochondrial genomes of all three samples using GetOrganelle v1.7.1 (Jin et al. 2020). We then annotated all the mitogenomes using MITOS2 (Bernt et al. 2013) with the Refseq 89 Metazoa reference mitochondrial database and the vertebrate mitochondrial genetic code. All the coding and non-coding genes were extracted from the mitochondrial genomes using the annotations.

### Sequence and Phylogenetic analyses

We aligned the full-length COX1 and CYTB genes of lorises using Clustal Omega with the "distmat" flag and calculated the pairwise distances between the sequences (Sievers & Higgins 2021). To build the phylogeny, along

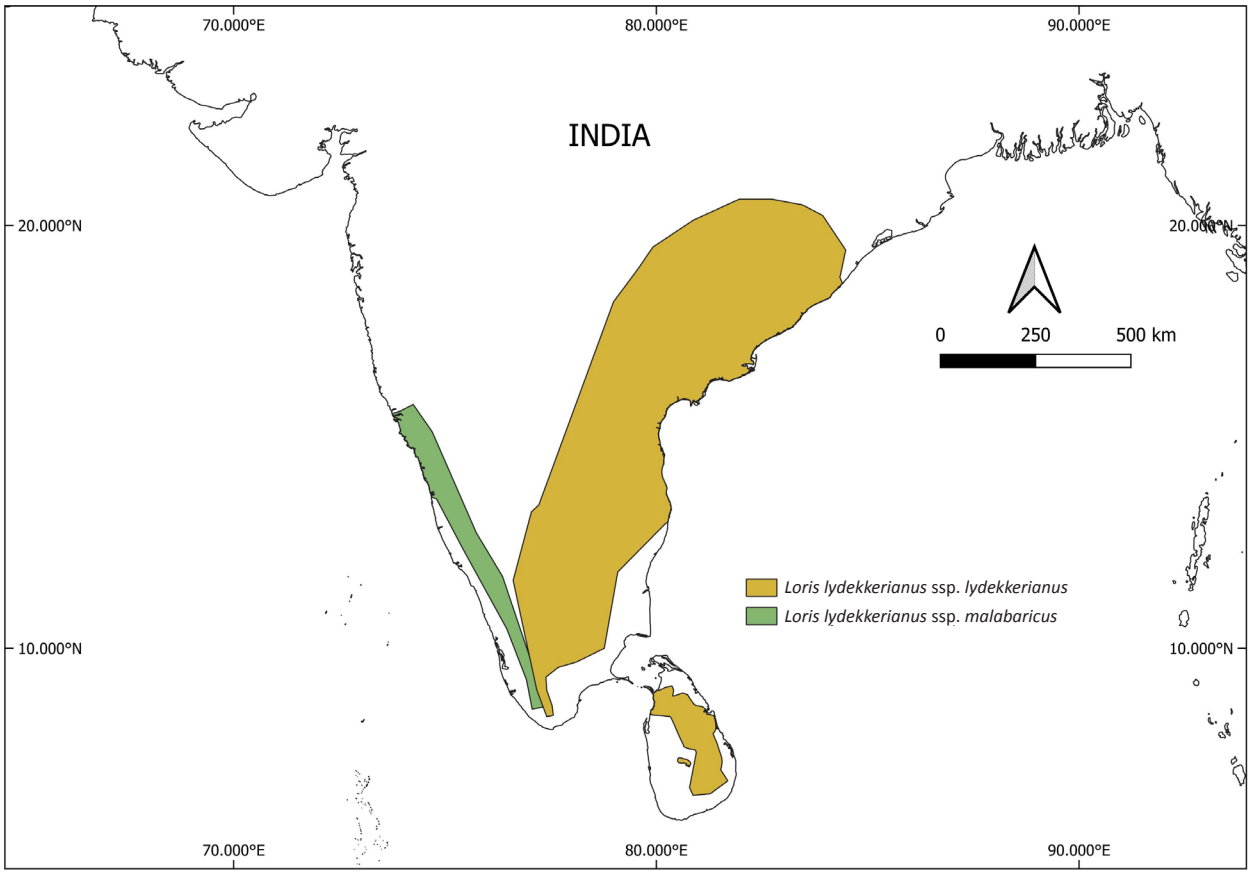


**Figure 1. IUCN Red List distribution range of the Grey Slender Loris subspecies in southern India and Sri Lanka.**

with our samples we used the NCBI RefSeq mitochondrial sequences from strepsirrhines namely, *Loris lydekkerianus*, *Loris tardigradus*, *Nycticebus coucang*, *Nycticebus bengalensis*, *Galago senegalensis*, and *Lemur catta*. We aligned the 13 protein-coding genes and two non-coding ribosomal RNA genes individually from the assembled mitochondrial genomes and reference sequences using the MUSCLE algorithm in MEGA7 (Kumar et al. 2016) and checked for the presence of any sequencing errors or frameshifts for codon position. We then concatenated all the gene alignments using MEGA7 (Kumar et al. 2016) and identified the optimum nucleotide substitution model for each partition based on the corrected Akaike information criterion (AICc) values using PartitionFinder2 (Lanfear et al. 2017) (Supplementary file 1). We built the maximum likelihood (ML) tree based using IQ-TREE (Minh et al. 2020) with 1000 times bootstrapping. The ML tree was visualized in Evolview v3 (Subramanian et al. 2019).

We utilized BEAST2.5 (Bouckaert et al. 2014) to create a divergence time tree using the same concatenated alignment of 13 coding and two non-coding genes from the complete mitochondrial genomes. We used the same partitioning scheme and substitution models identified by PartitionFinder2. We then chose two fossil calibration points:

1) We calibrated the crown node of Galagos with 38 mya based on the age of the fossil *Saharagalago misrensis* (PaleoDB collection 67706) (Seiffert et al. 2003). We applied a normal distribution at 40 Mya (SD = 0.04; 95% range: 36–43)

2) We calibrated the crown node of Slow Lorises with 13.82 mya based on the age of the fossil *Nycticebus linglom* (PaleoDB collection 48126) (Harrison 2010). We applied a normal distribution at 14 Mya (SD = 0.05; 95% range: 9–17)

For all the partitions, we created a relaxed lognormal clock and employed a birth-death process using prior distributions. To get to the final tree, we ran for 40 million generation runs, sampling every 2,000$^{th}$ generation using TreeAnnotator (Helfrich et al. 2018) with a 10% burn-in. We verified that all the ESS values were over 200 in Tracer 1.7 (Rambaut et al. 2018) and visualized the tree in FigTree v1.4.4 (Rambaut 2014).

## RESULTS

### Phylogenetic analyses support the morphological and geographical delineation of Mysore & Malabar Slender Lorises

To investigate the genetic differences between the two subspecies of slender loris in southern India, we first assembled three new circular mitochondrial genomes with an average length of 16,771 bp from two samples of the Mysore Slender Loris *Loris lydekkerianus* ssp. *lydekkerianus* and one sample of Malabar Slender Loris *Loris lydekkerianus* ssp. *malabaricus*. We annotated the mitochondrial genomes along with published reference sequences and obtained the full-length sequences of 13 protein-coding genes and two ribosomal RNA genes. To check the variation in the nucleotide sequence within the *Loris* genus, we estimated the pairwise sequence similarity in the COX1 and CYTB regions spanning 2,682 bp between all six Loris samples (Table 1). We observed the highest average sequence variation of 2.82% (S.D. 0.16) between the four sequences of the Grey Slender Loris *Loris lydekkerianus* and the two sequences of the Red Slender Loris *Loris tardigradus* as they belong to two different species within the *Loris* genus. While there was no sequence variation found within the two sequences of Red Slender Loris, there was considerable variation within the four sequences of Grey Slender Loris contributed by the difference between the two subspecies. We found about 2.09% (S.D 0.0) variation in the COX1 and CYTB sequences of the Mysore and Malabar Slender Lorises.

We then used phylogenetic analyses to understand the evolutionary relationships between the two subspecies. Along with our three samples, we included reference mitochondrial sequences from two species of slender lorises (*L. lydekkerianus*, *L. tardigradus*) and two species of slow lorises (*Nycticebus bengalensis*, *N. coucang*) along with galago and lemur as outgroups (Figure 2). The phylogenetic tree recapitulates the broad evolutionary relationships of slender lorises with slow lorises and the outgroups. It reveals an interesting pattern within the clade of slender lorises where the Mysore Slender Loris *L.l.* ssp. *lydekkerianus* clusters with the reference sequence of Grey Slender Loris to form a monophyletic clade and the Malabar Slender Loris *L.l.* ssp. *malabaricus* forms a separate monophyletic clade with very strong statistical support. We noted that the Malabar Slender Loris appears more closely related to the Red Slender Loris *L. tardigradus*, albeit with a very small branch length (Figure 2). To estimate the divergence time between the two subspecies and other lorises, we constructed a fossil-calibrated Bayesian tree (Figure 3). Our results suggest that the split between the Grey Slender Loris *L. lydekkerianus* and Red Slender Loris *L. tardigradus* occurred approximately 1.087 million years ago (mya). This was immediately followed by diversification of the Mysore Slender Loris *L.l.* ssp. *lydekkerianus* and Malabar Slender Loris *L.l.* ssp. *malabaricus* at around 1.049 mya (Posterior probability = 1) (Figure 3).

## DISCUSSION

Our results from the phylogenetic analyses based on the mitochondrial sequences show that the Mysore and Malabar Slender Lorises have significant genetic variation (2.09%) in the COX1 and CYTB genes and form distinct monophyletic clades in the phylogenetic tree that diverged a long time ago (1.049 mya), shortly after the divergence of Red Slender Loris from the Grey Slender Loris (1.087 mya). The observed sequence variation and divergence time between the Mysore and the Malabar Slender Lorises are surprisingly high, which is not very common between primate subspecies. Since they have been evolving independently for a

**Table 1. Sequence distance matrix of the *Loris* genus based on full-length cytochrome b and cytochrome oxidase 1 genes.**

| Sample | *Loris tardigradus-1* | *Loris tardigradus-2* | *Loris lydekkerianus malabaricus* | *Loris lydekkerianus lydekkerianus-1* | *Loris lydekkerianus lydekkerianus-2* | *Loris lydekkerianus-Ref* |
|---|---|---|---|---|---|---|
| *Loris tardigradus-1* | - | 0 | 2.58 | 2.82 | 2.95 | 2.9 |
| *Loris tardigradus-2* | 0 | - | 2.58 | 2.82 | 2.97 | 2.9 |
| *Loris lydekkerianus malabaricus* | 2.58 | 2.58 | - | 2.09 | 2.09 | 2.09 |
| *Loris lydekkerianus lydekkerianus-1* | 2.82 | 2.82 | 2.09 | - | 0.16 | 0.08 |
| *Loris lydekkerianus lydekkerianus-2* | 2.97 | 2.97 | 2.09 | 0.16 | - | 0.16 |
| *Loris lydekkerianus-Ref* | 2.9 | 2.9 | 2.09 | 0.08 | 0.16 | - |

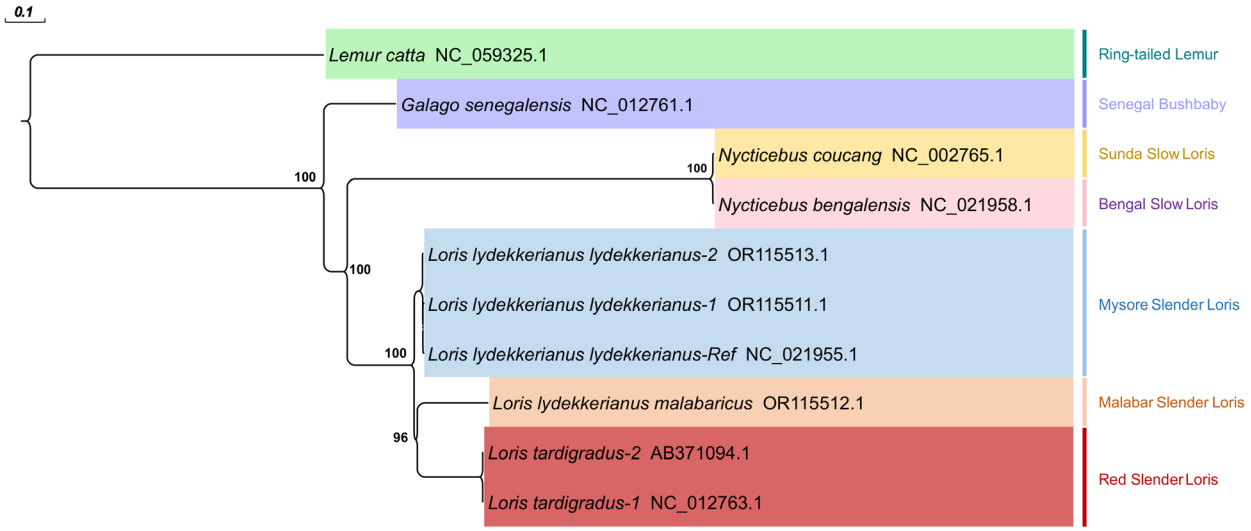


**Figure 2. Maximum likelihood tree based on 13 protein-coding and two ribosomal RNA genes from whole mitochondrial genomes. The bootstrap values are denoted at the nodes.**

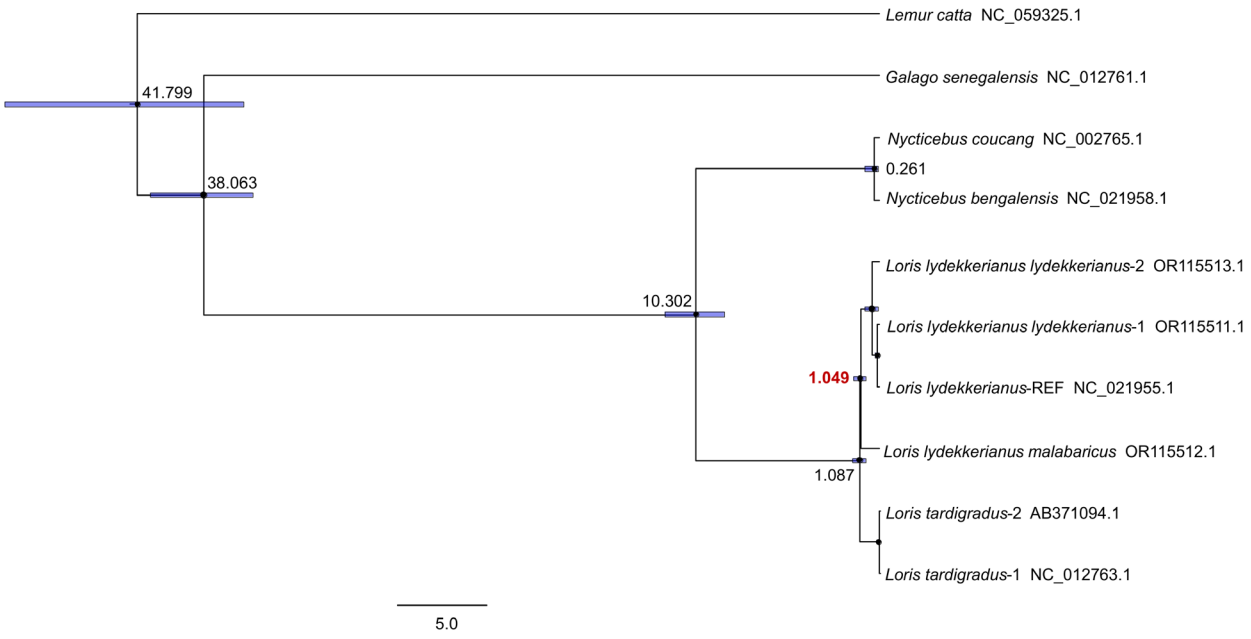


**Figure 3. Fossil-calibrated Bayesian inference tree based on 13 protein-coding and two ribosomal RNA genes from whole mitochondrial genomes showing the divergence time estimates at the nodes in Mya. Bars indicate 95% CI.**

long period comparable to the divergence time of their closest species (*Loris tardigradus*), the Mysore and Malabar Slender Lorises deserve independent recognition. Moreover, they occupy a geographically different landscape and unique habitat, where the Malabar Slender Loris occupies the wet zone of the Western Ghats, while the Mysore Slender Loris occupies the dry habitat of the eastern slope of the Western Ghats, dry forests of the Deccan plateau and Eastern Ghats (Kumara et al. 2006, 2009; 2013). They are also morphologically distinct, where the Malabar Slender Loris appears reddish in color and almost half the body size of the greyish colored Mysore Slender Loris (Kumara et al. 2006). Considering these significant differences in the morphological, geographical, and genetic factors, we propose to recognize the Mysore Slender Loris *Loris lydekkerianus* ssp. *lydekkerianus* and Malabar Slender Loris *Loris lydekkerianus* ssp. *malabaricus* as two distinct

species, *Loris lydekkerianus* and *Loris malabaricus*, respectively.

The divergence time estimates are supported by a number of molecular markers in the whole mitochondrial genome and is consistent with previous studies on the evolutionary history of this genus (Finstermeier et al. 2013). Several environmental, climatic, and geographical factors might have influenced the divergence of the Mysore and Malabar Slender Lorises about one million years ago. The mid-Pleistocene transition (1.25–0.7 Mya) was a time of dramatic climatic change and glaciation that influenced the environments and biogeography of Earth (Herbert 2023). The glaciation and interglaciation cycles affected the sea level, precipitation, temperature, vegetation, and habitat availability. The environmental conditions in India specifically during the Pleistocene were diverse and dynamic, ranging from deserts, tropical forests to grasslands (Morley & Morley 2022). The variability of monsoon coupled with expansion and contraction of forests due to glacial-interglacial cycles could have influenced availability of resources, fragmentation of habitats, and changes in forest cover promoting genetic differentiation and divergence of the Mysore and Malabar Slender Lorises.

Understanding the genetic structure and variation of species is crucial for the scientific management of threatened species and their eventual recovery. The findings of this study have important implications for the conservation and management of the slender lorises in India. With a clearer understanding of the genetic differences between the Mysore and Malabar Slender Lorises, it will be possible to more accurately identify and classify individual animals, which will in turn facilitate the development of an effective conservation breeding program. Such a program can be particularly beneficial for species like the slender loris that are threatened by habitat loss and fragmentation, and whose populations have been declining in recent years (Kumara et al. 2006, 2016).

The main drawback of this study is the limited sample size which we duly acknowledge. It is to be noted that the construction of whole mitochondrial genomes from WGS data for accurate molecular dating often requires good-quality DNA from animals of known geographic origin which is very difficult to obtain, especially for the Malabar Slender Loris. More samples from the Malabar Slender Loris could better resolve the phylogenetic tree and nuclear markers could also be used to confirm our findings and validate the species delimitation. Furthermore, sampling the individuals from the range edges and the overlapping ranges in the southern ridge of Western Ghats would provide more statistical power to establish the monophyly and identify any hybridization. It would also be prudent to include samples of the Mysore subspecies from Sri Lanka in future studies to fully comprehend the diversity and understand the evolutionary history of the slender lorises throughout its geographical range. Comprehensive genome sequencing of all the subspecies of slender loris would also help to understand the genomic basis of morphological differences and their adaptations to respective niches.

In conclusion, this study provides the first molecular evidence for the genetic divergence and distinctiveness of the Mysore and Malabar Slender Lorises. The sequence analysis, phylogenetic analyses, and molecular dating suggest that the Mysore and Malabar Slender Lorises are genetically distinct and have been evolving independently for a significant period. The high level of genetic divergence between them highlights the importance of preserving their genetic diversity and underscores the need for more efforts to conserve them in the wild. By considering the significant differences in the morphological, geographical, and genetic factors, we recommend to elevate *L.l.* ssp. *lydekkerianus* and *L.l.* ssp. *malabaricus* to the species level. We propose to recognize them as two distinct species *Loris lydekkerianus* and *Loris malabaricus* as each of them represents a unique evolutionary lineage and deserves separate recognition and protection. We advocate for further studies to validate the species delimitation with larger sample sizes and recommend for separate conservation measures and management actions to preserve their unique genetic diversity in the wild and captivity.

### Data Availability Statement

The three whole mitochondrial genome sequences generated in this study have been submitted to the NCBI database under the accessions OR115511, OR115512, and OR115513.

## REFERENCES

**Bernt, M., A. Donath, F. Jühling, F. Externbrink, C. Florentz, G. Fritzsch, J. Pütz, M. Middendorf & P.F. Stadler (2013).** MITOS: improved de novo metazoan mitochondrial genome annotation. *Molecular Phylogenetics and Evolution* 69(2): 313–319. https://doi.org/10.1016/j.ympev.2012.08.023

**Bouckaert, R., J. Heled, D. Kühnert, T. Vaughan, C.-H. Wu, D. Xie, M.A. Suchard, A. Rambaut & A.J. Drummond (2014).** BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS Computational Biology* 10(4): e1003537. https://doi.org/10.1371/journal.pcbi.1003537

**Chen, S., Y. Zhou, Y. Chen & J. Gu (2018).** fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* 34(17): i884–i890. https://doi.org/10.1093/bioinformatics/bty560

**Dittus, W., M. Singh, S.N. Gamage, H.N. Kumara, A. Kumar & K.A.I. Nekaris (2022).** *Loris lydekkerianus* (amended version of 2020 assessment). The IUCN Red List of Threatened Species 2022: e.T44722A217741551. Downloaded on 28 March 2023. https://doi.org/10.2305/IUCN.UK.2022-1.RLTS.T44722A217741551.en

**Finstermeier, K., D. Zinner, M. Brameier, M. Meyer, E. Kreuz, M. Hofreiter & C. Roos (2013).** A mitogenomic phylogeny of living primates. *PLoS ONE* 8(7): e69504. https://doi.org/10.1371/journal.pone.0069504

**Gnanaolivu, S.D., M. Campera, K.A.I. Nekaris, V. Nijman, R. Satish, S. Babu & M. Singh (2022).** Medicine, black magic and supernatural beings: Cultural rituals as a significant threat to slender lorises in India. *People and Nature* 4(4): 1007–1019. https://doi.org/10.1002/pan3.10336

**Government of Tamil Nadu (2022).** Notification of Kadavur Slender Loris Sanctuary in Karur and Dindigul districts of the Wild Life (Protection) Act, 1972. [G.O. Ms. No. 175, Environment, Climate Change and Forests (FR.5), 12th October 2022] No. II(2)/ECCF/809(a)/2022.

**Groves, C.P. (2001).** *Primate taxonomy*. Smithsonian Institution Press, Washington (DC), 350 pp.

**Harrison, T. (2010).** Later tertiary Lorisiformes, pp. 875–912. In: Werdelin, L. & W.J. Sanders (eds.). *Cenozoic Mammals of Africa*. University of California Press, California, 1008 pp.

**Helfrich, P., E. Rieb, G. Abrami, A. Lücking & A. Mehler (2018).** TreeAnnotator: Versatile Visual Annotation of Hierarchical Text Relations. In: *Proceedings of the Eleventh International Conference on Language Resources and Evaluation (LREC 2018)*. European Language Resources Association (ELRA), Miyazaki, Japan.

**Herbert, T.D. (2023).** The mid-Pleistocene climate transition. *Annual Review of Earth and Planetary Sciences* 51(1): 389–418. https://doi.org/10.1146/annurev-earth-032320-104209

**Jin, J.J., W.B. Yu, J.B. Yang, Y. Song, C.W. DePamphilis, T.S. Yi & D.Z. Li (2020).** GetOrganelle: a fast and versatile toolkit for accurate de novo assembly of organelle genomes. *Genome Biology* 21: 1–31. https://doi.org/10.1186/s13059-020-02154-5

**Kumar, S., G. Stecher & K. Tamura (2016).** MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33(7): 1870–1874. https://doi.org/10.1093/molbev/msw054

**Kumara, H.N., K.A.I. Nekaris & M. Singh (2022a).** *Loris lydekkerianus* ssp. *lydekkerianus*. The IUCN Red List of Threatened Species 2022: e.T44719A217742793. Downloaded on 28 March 2023. https://doi.org/10.2305/IUCN.UK.2022-1.RLTS.T44719A217742793.en

**Kumara, H.N., K.A.I. Nekaris & M. Singh (2022b).** *Loris lydekkerianus* ssp. *malabaricus* (amended version of 2020 assessment). The IUCN Red List of Threatened Species 2022: e.T44720A217744540. Downloaded on 28 March 2023. https://doi.org/10.2305/IUCN.UK.2022-1.RLTS.T44720A217744540.en

**Kumara, H.N., M. Singh & S. Kumar (2006).** Distribution, habitat correlates, and conservation of Loris lydekkerianus in Karnataka, India. *International Journal of Primatology* 27: 941–969. https://doi.org/10.1007/s10764-006-9054-z

**Kumara, H.N., M. Irfan-Ullah & S. Kumar (2009).** Mapping potential distribution of slender loris subspecies in peninsular India. *Endangered Species Research* 7: 29–38. https://doi.org/10.3354/esr00185

**Kumara, H.N., R. Sasi, S. Chandran & S. Radhakrishna (2016).** Distribution of the Grey Slender Loris (*Loris lyddekerianus* Cabrera, 1908) in Tamil Nadu, southern India. *Folia Primatologica* 87(5): 291–302. https://doi.org/10.1159/000452405

**Kumara, H.N., M. Singh, M. Irfan-Ullah & S. Kumar (2013).** Status, Distribution, and Conservation of Slender Lorises in India pp. 343–352. In: Masters, J., M. Gamba & F. Génin (eds.). *Leaping Ahead: Advances in Prosimian Biology*. Springer, New York, 410 pp.

**Lanfear, R., P.B. Frandsen, A.M. Wright, T. Senfeld & B. Calcott (2017).** PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution* 34(3): 772–773. https://doi.org/10.1093/molbev/msw260

**Minh, B.Q., H.A. Schmidt, O. Chernomor, D. Schrempf, M.D. Woodhams, A. von Haeseler & R. Lanfear (2020).** IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. *Molecular Biology and Evolution* 37(5): 1530–1534. https://doi.org/10.1093/molbev/msaa015

**Morley, R.J. & H.P. Morley (2022).** The prelude to the Holocene: tropical Asia during the Pleistocene, pp. 1–32. In: Kumaran, N. & P. Damodara (eds.). *Holocene Climate Change and Environment*. Elsevier, 661 pp.

**Nekaris, K.A.I. (2014).** Extreme primates: ecology and evolution of Asian lorises. *Evolutionary Anthropology: Issues, News, and Reviews* 23(5): 177–187. https://doi.org/10.1002/evan.21425

**Nekaris, K.A.I. & D.T. Rasmussen (2003).** Diet and feeding behavior of Mysore Slender Lorises. *International Journal of Primatology* 24(1): 33–46. https://doi.org/10.1023/A:1021442411455

**Nijman, V., T. Robbins, A.J. Maddock & A. Ang (2020).** Molecular phylogeny, taxonomy and conservation of slender Lorises. *Primate Conservation* 34: 143–151.

**Radhakrishna, S. & H.N. Kumara (2010).** Behavioural variation in the Mysore slender loris *Loris lydekkerianus lydekkerianus*. *Current Science* 99(9): 1226–1232.

**Radhakrishna, S. & M. Singh (2002).** Social behaviour of the slender loris (*Loris tardigradus lydekkerianus*). *Folia Primatologica* 73(4): 181–196. https://doi.org/10.1159/000065426

**Radhakrishna, S. & M. Singh (2004).** Reproductive biology of the slender loris (*Loris lydekkerianus lydekkerianus*). *Folia Primatologica* 75(1): 1–13. https://doi.org/10.1159/000073424

**Rambaut, A. (2014).** FigTree, a graphical viewer of phylogenetic trees. https://github.com/rambaut/figtree Accessed on 15 March 2023.

**Rambaut, A., A.J. Drummond, D. Xie, G. Baele & M.A. Suchard (2018).** Posterior summarization in Bayesian phylogenetics Using Tracer 1.7. *Systematic Biology* 67(5): 901–904. https://doi.org/10.1093/sysbio/syy032

**Seiffert, E.R., E.L. Simons & Y. Attia (2003).** Fossil evidence for an ancient divergence of lorises and galagos. *Nature* 422(6930): 421–424. https://doi.org/10.1038/nature01489

**Sievers, F. & D.G. Higgins (2021).** The Clustal Omega Multiple Alignment Package, pp. 3–16. In: Katoh, K. (ed.). *Multiple Sequence Alignment: Methods and Protocols*. Springer US, New York, 287 pp.

**Singh, M., M. Singh, H.N. Kumara, S. Kumar, S.D. Gnanaoliu & R. Sasi (2021).** A review of research on the distribution, ecology, behaviour, and conservation of the Slender Loris *Loris lydekkerianus* (Mammalia: Primates: Lorisidae) in India. *Journal of Threatened Taxa* 13(11): 19540–19552. https://doi.org/10.11609/jott.7562.13.11.19540-19552

**Subramanian, B., S. Gao, M.J. Lercher, S. Hu & W.H. Chen (2019).** Evolview v3: a webserver for visualization, annotation, and management of phylogenetic trees. *Nucleic Acids Research* 47(W1): W270–W275. https://doi.org/10.1093/nar/gkz357

**Supplementary file 1. Partitioning scheme and nucleotide substitution model selection output from PartitionFinder2.**

**Best partitioning scheme**

**Scheme lnL : -64454.10696411133**
**Scheme AICc : 129409.318896**
**Number of params : 246**
**Number of sites : 13594**
**Number of subsets : 33**

| Subset | Best Model | # sites | Partition names |
|---|---|---|---|
| 1 | GTR+I+X | 622 | NAD1_pos1, NAD4l_pos3, ATP6_pos1 |
| 2 | TRN+I+X | 533 | NAD1_pos2, ATP6_pos2 |
| 3 | HKY+X | 223 | ATP6_pos3 |
| 4 | HKY+X | 132 | ATP8_pos1, ATP8_pos2 |
| 5 | GTR+X | 317 | ATP8_pos3, COX3_pos3 |
| 6 | HKY+G+X | 368 | COB_pos1 |
| 7 | GTR+I+X | 368 | COB_pos2 |
| 8 | TRN+I+X | 368 | COB_pos3 |
| 9 | GTR+G+X | 496 | COX1_pos1 |
| 10 | HKY+G+X | 496 | COX1_pos2 |
| 11 | HKY+G+X | 496 | COX1_pos3 |
| 12 | K80 | 222 | COX2_pos1 |
| 13 | TRN+X | 222 | COX2_pos2 |
| 14 | TRN+I+X | 424 | NAD3_pos1, NAD4l_pos2, COX2_pos3 |
| 15 | JC | 251 | COX3_pos1 |
| 16 | HKY+X | 251 | COX3_pos2 |
| 17 | HKY+X | 310 | NAD1_pos3 |
| 18 | GTR+I+X | 340 | NAD2_pos1 |
| 19 | HKY+I+X | 340 | NAD2_pos2 |
| 20 | HKY+I+X | 339 | NAD2_pos3 |
| 21 | TRN+I+X | 111 | NAD3_pos2 |
| 22 | GTR+I+X | 201 | NAD4l_pos1, NAD3_pos3 |
| 23 | HKY+X | 457 | NAD4_pos1 |
| 24 | HKY+X | 456 | NAD4_pos2 |
| 25 | HKY+I+X | 456 | NAD4_pos3 |
| 26 | HKY+I+X | 596 | NAD5_pos1 |
| 27 | GTR+I+X | 596 | NAD5_pos2 |
| 28 | HKY+I+X | 596 | NAD5_pos3 |
| 29 | GTR+G+X | 181 | NAD6_pos1 |
| 30 | HKY+I+X | 180 | NAD6_pos2 |
| 31 | GTR+G+X | 180 | NAD6_pos3 |
| 32 | GTR+G+X | 1454 | rrns |
| 33 | GTR+I+X | 1012 | rrnl |

**Tamil:** மெலிந்த தேவாங்குகள், இந்தியா மற்றும் இலங்கையில் மட்டும் வாழும் இரவில் நடமாடுகிற சிறிய வகை ஈரமூக்கு கொண்ட முதனிகளில் ஒன்றான பேரினமாகும். இந்தப் பேரினம் தற்பொழுது அழிவுநிலைக்கு அச்சுறுத்தப்பட்டுள்ளது.. சாம்பல் நிற மெலிந்த தேவாங்கு (*Loris lydekkerianus*), உருவவியல் மாறுபாடு மற்றும் புவியியல் பரவல் ஆகியவற்றின் அடிப்படையில் பல துணை இனங்களாகப் பிரிக்கப்பட்டுள்ள போதும், இந்தப் பிரிவினை மூலக்கூறு சான்றுகளால் ஆதரிக்கப்படவில்லை. தென்னிந்தியாவில் வாழும் இந்த சாம்பல் நிற மெலிந்த தேவாங்குகளின் இரண்டு துணை இனங்கள்: அதாவது மைசூர் மெலிந்த தேவாங்கு (Loris lydekkerianus ssp. lydekkerianus) மற்றும் மலபார் மெலிந்த தேவாங்கு (*Loris lydekkerianus* ssp. *malabaricus*), பரிணாம வரலாற்றில் எப்பொழுது பிரிந்தன என்று ஆராய்ந்தோம். தென்னிந்தியாவில் வாழும் அவற்றின் புவியியல் தொகைகளில் இருந்து மாதிரிகள் எடுத்து, அவ்விலங்குகளின் முழு இழைமணி மரபணு தகவல்களை shotgun sequence எனப்படும் தகவல்களில் இருந்து ஒருங்கிணைத்து, பொது தரவுத்தளங்களில் கிடைக்கும் பிற தேவாங்குகளின் மரபணு தகவல்களுடன் ஒப்பிட்டோம். இதில் இருந்து மைசூர் மற்றும் மலபார் மெலிந்த தேவாங்குகளை ஒப்பிடுகையில், அவற்றின் இடையே COX1 மற்றும் CYTB மரபணு பகுதிகள் 2.09 சதவிகிதம் வேறுபடுவதை நாங்கள் கண்டறிந்தோம். மேலும், இழைமணி மரபணுவில் காணப்படும் 13 புரதங்கள் மற்றும் 2 ரைபோசோமல் RNAக்களைக் குறியிடக்கூடிய மரபணு தகவல்களை பகுப்பாய்வு செய்கையில், மைசூர் மற்றும் மலபார் மெலிந்த தேவாங்குகள், பரிணாம வரலாற்றில் சுமார் 10. 49 இலட்சம் ஆண்டுகளுக்கு முன்பு பிரிந்தன என விளங்கியது. இது சிவப்பு மெலிந்த தேவாங்கு (Loris tardigradus) பிரிந்த சற்று காலத்திற்குப் பின் நிகழ்ந்த நிகழ்வாகும். மரபணு தகவல்களில் உள்ள மாற்றங்கள் மற்றும் பரிணாம வேறுபாடுகளை கருத்தில் கொண்டு , அவற்றுடன் ஏற்கனவே நிறுவப்பட்ட உருவ வேறுபாடுகள் மற்றும் புவியியல் ரீதியாக தனித்துவமான வாழ்விடங்களை சேர்க்கையில், மைசூர் மற்றும் மலபார் மெலிந்த தேவாங்குகளளை *Loris lydekkerianus* மற்றும் *Loris malabaricus* என இரண்டு தனித்துவமான இனங்களாக அங்கீகரிக்க நாங்கள் முன்மொழிகிறோம்.

**Journal of Threatened Taxa** is indexed/abstracted in Bibliography of Systematic Mycology, Biological Abstracts, BIOSIS Previews, CAB Abstracts, EBSCO, Google Scholar, Index Copernicus, Index Fungorum, JournalSeek, National Academy of Agricultural Sciences, NewJour, OCLC WorldCat, SCOPUS, Stanford University Libraries, Virtual Library of Biology, Zoological Records.

NAAS rating (India) 5.64



Print copies of the Journal are available at cost. Write to:
The Managing Editor, JoTT,
c/o Wildlife Information Liaison Development Society,
43/2 Varadarajulu Nagar, 5th Street West, Ganapathy, Coimbatore,
Tamil Nadu 641006, India
ravi@threatenedtaxa.org

www.threatenedtaxa.org



**ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)**

**September 2023 | Vol. 15 | No. 9 | Pages: 23827–23930**
**Date of Publication: 26 September 2023 (Online & Print)**
**DOI: 10.11609/jott.2023.15.9.23837-23930**



**Publisher & Host**

Threatened Taxa