Rev. Inst. Flor. v. 34 n. 2 p. 172-181 dez. 2022
http://dx.doi.org/10.24278/2178-5031.202234206
ISSN impresso 0103-2674/on-line 2178-5031

THE RELATIONSHIP BETWEEN ECOLOGICAL, MORPHOLOGICAL AND GENETIC FACTORS AMONG LORIS SPECIES IN SRI LANKA[1]

A RELAÇÃO ENTRE FATORES ECOLÓGICOS, MORFOLÓGICOS E GENÉTICOS ENTRE AS ESPÉCIES DE LÓRIS NO SRI LANKA

Chamara HETTIARACHCHI [2,6]; Saman GAMAGE [3]; Faiz MARIKAR [4]; Shamali KUMARI [5]

ABSTRACT - The study on Loris species diversity in Sri Lanka was considered is based on environmental, morphological, and genetic factors. The distribution of the Loris species, according to genetics and morphology, was the focal point of this work. We have observed seven groups from 25 districts in Sri Lanka in the environmental analysis. When we compare the ecological factors of the Loris species in the study of distribution patterns, the genetic and morphological characteristics are considered for the occurrence of each species, and it also align with the environmental clustering style as seven clusters. Our results suggest that segregation plays a key role in the evolution of genetic structure within the Loris species populations under conditions of free movement. Given the monophyletic arrival of the Loris species, our study challenges the view that geographical barriers are strictly needed for genetic divergence. The study further raises the interesting prospect that social forces - such as social structure, feeding habits, and the breeding of *Loris* - might influence the genetic partitioning of the population.

Palavras-chave: Species occurrence; *Loris* distribution; Environment; Morphology; Genetics.

RESUMO - O estudo da diversidade de espécies de Lóris no Sri Lanka foi baseado em fatores ambientais, morfológicos e genéticos. A distribuição das espécies de Lóris, segundo genética e morfologia, foi o foco deste trabalho. Observamos sete grupos de 25 distritos do Sri Lanka na análise ambiental. Quando comparamos os fatores ecológicos das espécies de Lóris no estudo dos padrões de distribuição, as características genéticas e morfológicas são consideradas para a ocorrência de cada espécie, e também se alinham com o estilo de agrupamento ambiental como sete agrupamentos. Nossos resultados sugerem que a segregação desempenha um papel fundamental na evolução da estrutura genética dentro das populações das espécies de Lóris em condições de livre circulação. Dada a origem monofilética das espécies de Lóris, nosso estudo desafia a visão de que as barreiras geográficas são estritamente necessárias para a divergência genética. O estudo apresenta a perspectiva interessante de que forças sociais - como estrutura social, hábitos alimentares e reprodução de Lóris - podem influenciar a divisão genética da população.

Palavras-chave: Ocorrência de espécies; Distribuição de Lóris; Ambiente; Morfologia; Genética.

---

[1] Recebido para análise em 26.03.2022. Aceito para publicação em 10.10.2022. Publicado em 19.12.2022.

[2] Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba City, Japan

[3] University of Colombo, College House, 94 Kumaratunga Munidasa Mawatha, Colombo 00700, Sri Lanka.

[4] General Sir John Kotelawala Defence University, Kandawala Road, Ratmalana,10350, Sri Lanka

[5] The Open University of Sri Lanka, Nawala, Sri Lanka.

[6] Autor para correspondência: faiz@kdu.ac.lk

1 INTRODUCTION

The slender lorises (Suborder: Strepsirrhini, Family: Lorisidae, Genus: *Loris* É. Geoffroy Saint-Hilaire, 1796) are small nocturnal primates inhabiting India and Sri Lanka (Groves, 2001; Gamage et al., 2017). Based on museum specimens, Groves (2001) recognised 2 species of slender loris within Sri Lanka: *Loris tardigradus* (Linnaeus, 1758) and *Loris lydekkerianus* (Cabrera, 1908). A Sri Lankan, island-wide study conducted by Gamage et al. (2017) further confirmed that the classification of these two species needed to be reviewed. The biodiversity of this small island is rich due to its distinct climatic conditions. There are 4,000 flowering plants, reptiles, and mammals – one of the highest species densities in the region, as well as in the globe (Todua, 2018). Furthermore, several mammal species and vertebrates can be found in this island. This research study area is one of Asia's most biologically significant countries (Diaz and Yadvinder, 2022). The global importance of the country was listed as one of the 25 hotspots of biodiversity, as listed by Myers et al. (2000) and Brooks et al. (2002). The total land area consists of 64.740 km^2, making it the 25th largest island in the world (McElroy, 2003).

Ecological studies in Sri Lanka have produced a considerable knowledge about the habitat requirements for and threats to the Montane Slender *Loris tardigradus nycticeboides* (Gamage et al., 2017). Methods and channels capable of bringing this knowledge into the field of practical forestry and nature conservation are needed. The Montane Slender Loris, classified by the IUCN Red List as Endangered (Nekaris, 2008), is a primate inhabiting in the mountain region of central Sri Lanka (Gamage et al., 2017). Gamage et al. (2017) highlighted that the Montane Slender Loris is an extreme habitat specialist found only in the montane evergreen forests in the Nuwara-Eliya, Sri Lanka.

The distribution of species depend on the ecological differences between a region's contrasting habitats. The viable populations of endemic Lorises, as well as colonies of the other mammal species, are essentially confined to conserve themselves (Soberón and Peterson, 2005). All these factors are helpful to find a suitable habitat for a species and to be able to set the limits for the safety priorities (Peterson and Kluza, 2003). In Sri Lanka, the *Loris* species are at a high risk of extinction due to the lack of knowledge on how to conserve them (Mahanayakage, 2013; Hettiarachchi et al., 2018). Methods and channels are needed to provide this knowledge in the field of practical forestry and natural conservation.

Additionally, one study revealed that Loris's occupancy is closely related with altitude, canopy height, and canopy connectivity: the best habitat for Loris is 1600 to 2100 m height, with good canopy connectivity and high evergreen forest (height > 4 m) (Hettiarachchi et al., 2018). The study confirmed that *Loris* is one of the Sri Lanka's most rare and endangered species and identifying the most suitable habitats for the *Loris*'s protection is the key aspect of their conservation (Hettiarachchi et al., 2018).

Therefore, the main objectives of this study are to investigate the distribution patterns of *Loris* species; examine the presence/absence of *Loris* species based on environmental factors (altitude, precipitation, and forest climax), among the pairs of *Loris* species that share the same area in Sri Lanka; and compare with the genetic and morphological characters of the *Loris* species.

2 MATERIAL AND METHODS

Sri Lanka is located in the Indian Ocean, between 5° and 10° North latitude and between 79° and 82° East longitude, the temperature is warm and of tropical climate all year round (Figure 1). Samples were selected randomly from the places shown in the Figure 1. Climate data were obtained from National Oceanic and Atmospheric Administration (https://www.meteoblue.com) together with Switzerland's Basel University's weather service. Weather data was collected from 154 places in Sri Lanka for this study. The Climate Charts of Meteoblue 30 years (since 1990-2020) was considered for hourly weather model simulations. Terrain Data (the height and area of the districts) was "remotely" retrieved from the website, and data by satellite maps (http://www.distancesfrom.com) were collected. A total of three environmental variables (altitude, precipitation, and forest climax) were considered in this analysis.

Many of the wetland sites in Sri Lanka are today being recognized as dry zones. A total of 154 sites were surveyed in both wet and dry zone using occupancy monitoring technique of Mackenzie et al. (2003). Another survey was done by Nekaris and Jayawardene (2004) in 38 sites of *Loris* in the island. They used a broad reconnaissance survey technique, in which the observers followed preexisting trails and did both repeat and one-off transect surveys (White and Edwards, 2000; Nekaris and Jayawardene, 2004). GPS points were recorded for all *Loris* observations, and ArcGIS® and ArcMapTM version 10 was used to create the map (Figure 1).

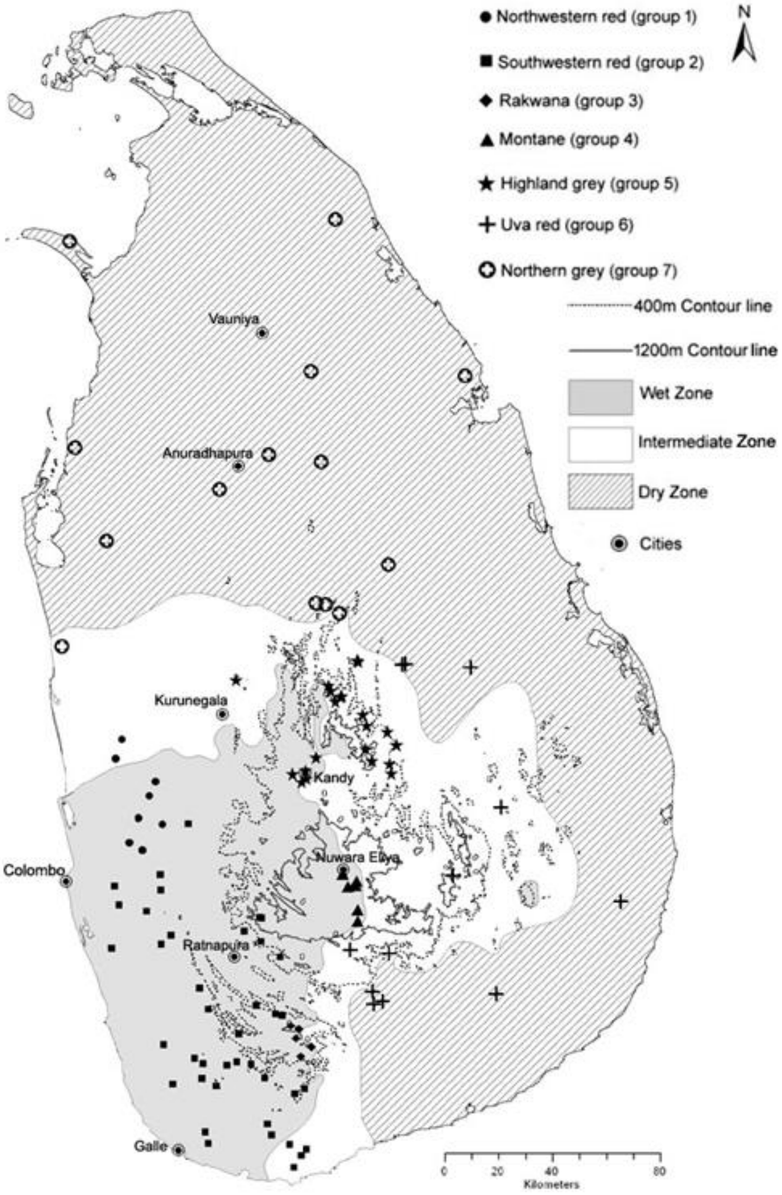

Figure 1. Distributions of the different slender loris groups in Sri Lanka and randomly selected sample from the locations.

Figura 1. Distribuições dos diferentes grupos de lóris-delgado no Sri Lanka e amostras selecionadas aleatoriamente dos locais.

Variation in the facial mask of the slender loris is especially useful in distinguishing between species. Thus, live animals and skins were grouped according to facial/coat features and area of origin. A guide was created for future research to reproduce or extend this study (Figure 2).

The research was carried out under the Department of Wildlife Conservation of Sri Lanka (permit number WL/3/2/1/9), guided and supervised by the National Research Council of the Wildlife Conservation Department. The animals were treated according to the policies and protocol of the American Society of Primatologists. Random sampling method was used in this study. Fifty-six wildlife *Loris* were caught in 7 different forest areas.

The *Loris* species for this study were obtained from Sri Lanka and they were found throughout 7 regions of the country: Group 1-Northwestern; Group 2-Southwestern; Group 3-Rakwana; Group 4-Montane; Group 5-Highland; Group 6- Uva; Group 7-Northern.

Blood samples were taken by puncturing the ear with a lancet; the blood was then collected in a paper filter. Each paper filter were left to airdry for 2 hours, after which they were stored in an envelope and in a portable desiccator until transport to the molecular laboratory. All the *Loris* were released to the environment after the blood samples were taken. The genomic DNA was extracted using DNeasy® Blood and Tissue Extraction Kit (QIAGEN Inc., Dusseldorf, Germany), following the manufacturer's protocol. The target 657-bp fragment of CO1 was amplified using M13-tailed cocktail primers (C_VF1LFt1 – C_VR1LRt1-Mammal cocktail; Ivanova et al., 2007) by polymerase chain reaction (PCR) using AccuPower® PCR PreMix (Bioneer Corp., Daejeon, Korea). The following thermal cycle parameters were used for 50 µL amplification reactions: initial denaturation for 5 min at 94 °C, followed by 34 cycles of 1 min at 94 °C for denaturing, 1 min at 45 °C for annealing, and 1 min at 72 °C for extension, with a subsequent final extension at 72 °C for 7 min. The PCR products were tested by electrophoresis on a 2% agarose gel. Only single, sharp, and clean bands were purified using a QIAquick® PCR purification kit (QIAGEN Inc., Dusseldorf, Germany) to be used in sequencing PCR reactions. After purification, the PCR products were sequenced in both directions by using the automated sequencer (ABI Prism 3730 XL DNA Analyzer) in BOWEI (PR of China, Nanjing) with the primers M13 forward 5'-TGTAAAACGACGGCCAGT-3' and M13 reverse 5'-CAGGAAACAGCTATGAC-3' (Messing, 1983). The resulting chromatograms were evaluated for miscalls and ambiguities and assembled into contigs using SeqManTM Pro (version 7.1.0, 2006; DNAstar® Inc., Madison, WI, USA). Successful amplification depends on DNA template quantity and quality. A total of 44 samples were amplified in this study.

CO1 sequences were aligned according to DNA sequences using MEGA X (Rambaut, 1996) and the data were analysed using the neighbour-joining method (NJ) and the maximum parsimony (MP) method (Felsenstein, 1981). Phylogenetic trees were built with the NJ and MP approach using RAxML v. 8.1.22 (Ronquist et al., 2003) and with the Bayesian approach using Mr Bayes v. 3.2.5 (Jansen et al., 2003). Table 1 shows the collection points, supporting document numbers, and GenBank accession numbers of each model. Indian *Loris* sights are not available in Genbank.

Ward Hierarchical Clustering method and the Euclidean Distance were employed to study the species' distribution and clustering patterns in each of the studied location in Sri Lanka. This analysis is distribution-free, and the results can be interpreted and reported as p-values, without reference to a statistic. Finally, Apriori algorithm was used for finding frequent itemsets in a dataset. The Apriori algorithm uses prior knowledge of frequent itemset properties. To improve the efficiency of the level-wise generation of frequent itemsets, the Apriori property was used to help reduce the search space. Using the R programming language, we have done the analysis to identify the most common series of *Loris* species in Sri Lanka.

3 RESULTS AND DISCUSSION

Seven groups were observed regarding environmental conditions. Furthermore, species distribution model was perfectly aligned, and it was fit to the model. We have already identified seven groups of the studied species (Figure 2). In this analysis, the environmental data types of compound data which is included to clarify the results. Clear differences were observed in the graph, the presence and absence of the creature were clustered according to weather conditions. Results firmly confirmed that weather-based analysis will cluster for all groups. We observed different variations in the face and fur features of the living population found in the field (Figure 3). A cluster analysis using the complete linkage method divided the 106 *Loris* specimens into seven clusters, which were sorted mostly, but not entirely, according to areas of distribution. Morphological analysis also classifies into seven such groups in this study.

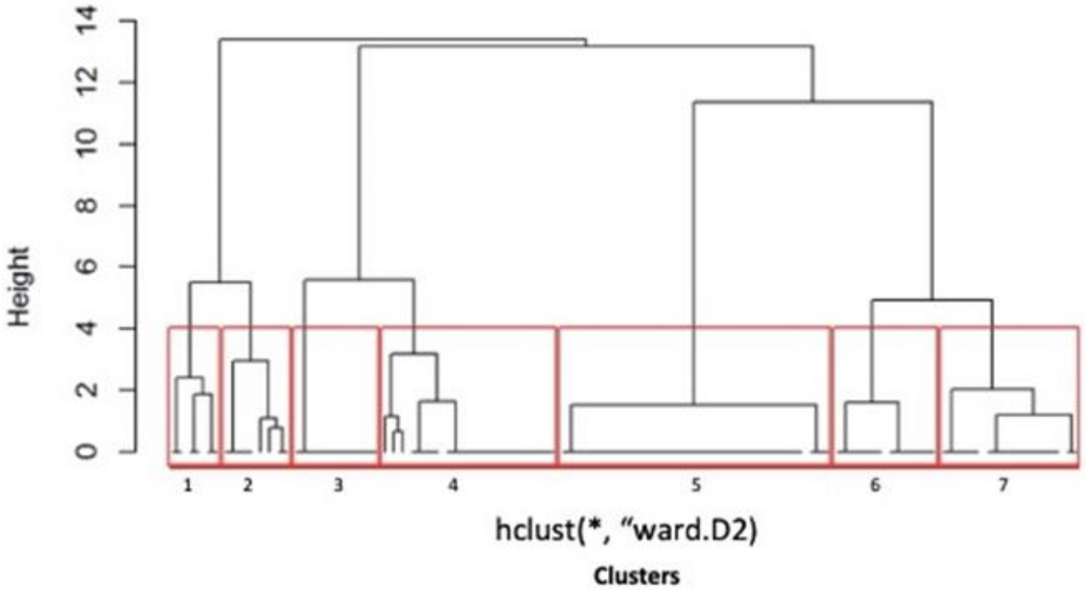

Figure 2. Cluster dendrogram for species composition in each location based on Ward's method.

Figura 2. Dendrograma de classificação para composição de espécies em cada local com base no método de Ward.

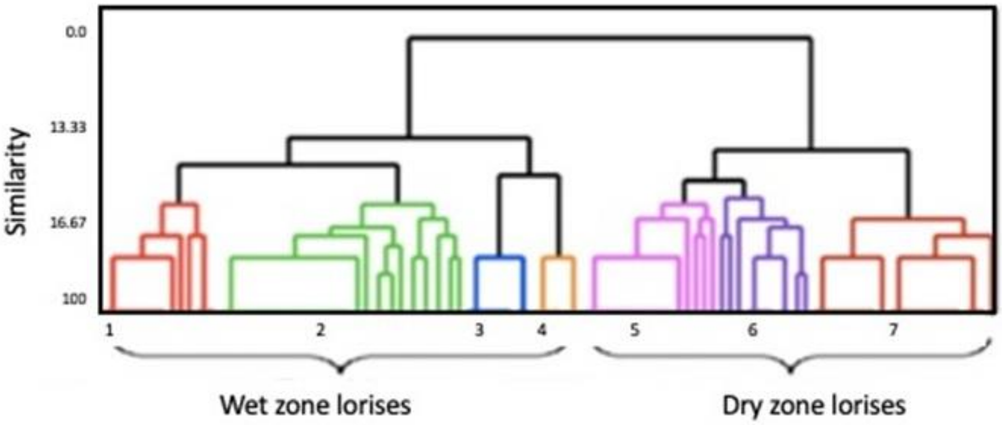

Figure 3. Dendrogram of complete linkage and Euclidean distance for the 23 features (facial and pelage) examined in 97 slender loris specimens, and photographs of nine live slender lorises were clearly separated into two clusters: the wet zone cluster (*Loris tardigradus*) and dry zone cluster (*Loris lydekkerianus*).

Figura 3. Dendrograma de ligação completa e distância euclidiana para as 23 características (facial e pelagem) examinadas em 97 espécimes de lóris-delgados, e fotografias de nove lóris-delgados vivos foram claramente separadas em dois grupos: o grupo de zona úmida (*Loris tardigradus*) e o grupo de zona seca (*Loris lydekkerianus*).

Table 1. Collection sites, voucher numbers, and GenBank accession numbers for each sample were used as given below.

Tabela 1. Locais de coleta, números de voucher e números de acesso do GenBank para cada amostra foram usados conforme abaixo.

Accession No Genebank*	**Species**	**Collected site**	**Lat/Long**	**Specimen No**
KX761807	*Loris tardigradu*s *parvus*	Kotakanda-Gampaha	7^0 13' 00"N,80^0 07' 10” E	SLCPL01
KX761808	*Loris tardigradu*s *parvus*	Mirigama-Gampaha1,	7^0 15' 42"N, 80^0 08' 46” E	SLCP 2015.06
KX761809	*Loris tardigradu*s *parvus*	Mirigama-Gampaha2,	7^0 15' 54"N,80^0 08' 39” E	SLCP 2015.17
KX761810	*Loris tardigradu*s	Ingiriya-Kalutara,	6^0 45' 20" N, 80^0 11' 30” E	SLCP 2015.09
KX761811	*Loris tardigradu*s	Yagirala-Kalutara,	6^0 25' 29” N,80^0 08' 53” E	SLCPL02
KX761812	*Loris tardigradu*s *tardigradu*s	Kalubovitiyana-Matara,	6^0 19' 19” N,80^0 24' 59” E	SLCPL03
KX761813	*Loris tardigradu*s *tardigradu*s	Oliyaga-Matara,	6^0 06' 01” N,80^0 31' 09” E	SLCPL04
KX761814	*Loris tardigradu*s *tardigradu*s	Kudawa-Sinharaja 1,	6^0 25' 41” N,80^0 25' 11” E	SLCP 2012.02
KX761815	*Loris tardigradu*s *tardigradu*s	Kudawa-Sinharaja 2	6^0 25' 21” N,80^0 25' 26” E	SLCPL05
KX761816	*Loris tardigradus nycticeboides*	Conical-NuwaraEliya,	6^0 55' 35” N,80^0 45' 59” E	SLCP 2015.18
KX761817	*Loris tardigradus nycticeboides*	Single-tree-Nuwara Eliya,	6^0 57' 04” N,80^0 45' 23” E	SLCPL06
KX761818	*Loris tardigradu*s *tardigradu*s	Ensalwatta-Deniyaya,	6^0 23' 36” N,80^0 35' 38” E	SLCPL07
KX761819	*Loris tardigradu*s *tardigradu*s	Morningside-Rathnapura,	6^0 24' 39” N,80^0 36' 22” E	SLCP 2015.08
KX761820	*Loris lydekkerianus grandis*	Mahalakotuwa- Knuckles,	7^0 31' 40” N,80^0 45' 51” E	SLCPL08
KX761821	*Loris lydekkerianus grandis*	Pitawala-Knuckles,	7^0 32' 30” N,80^0 45' 24” E	SLCP 2015.16
KX761822	*Loris lydekkerianus uva*	Lahugala-Ampara,	6^0 56' 01” N,81^0 43' 24” E	SLCPL09
KX761823	*Loris lydekkerianus uva*	Nilgala-Monaragala,	7^0 19' 36” N,81^0 26' 46” E	SLCP 2015.12
KX761824	*Loris lydekkerianus nordicus*	Mhintale-Anuradapura 1,	8^0 20' 58” N,80^0 30' 44” E	SLCPL10
KX761825	*Loris lydekkerianus nordicus*	Mhintale-Anuradapura 2,	8^0 20' 52” N,80^0 30' 36” E	SLCPL11
KX761826	*Loris lydekkerianus nordicus*	Talawa-Anuradapura,	8^0 16' 07” N,80^0 21' 12” E	SLCP 2015.14
KX761827	*Loris lydekkerianus nordicus*	Thambuthegama-Anuradapura,	8^0 12' 45” N,80^0 18' 40” E	SLCPL12
AB371094.1	*Loris tardigradu*s	Talawa -Anuradapura	8^0 16' 07” N,80^0 21' 12” E	SLCP 2008.13
KX761827.1	*Loris lydekkerianus*	Horton plains -NEliya	6^0 55' 35” N, 80^0 45' 59” E	SLCP 2008.16

*http://ncbi.nlm.nih.gov/nucleotides

In genetic study using the neighbour-joining (NJ) method, we chose the phylogenetic tree with the highest likelihood value as our best tree; its topology was congruent with the topology of the maximum parsimony (MP) tree (Figure 4 and Figure 5). The MP tree and the tree constructed by the NJ or ML method were very similar (node values of MP/NJ/ML are given in Figure 5). The monophyly of the genus *Loris* was well-supported (100% of bootstrap support). The concatenated and partitioned NJ and Bayesian trees showed similar topologies. In both analyses, there are several short internodes and polytomies; however, the clades of significance to Sri Lankan *Loris* were well supported. Also, it reflects that the genetic study can be classified in seven groups.

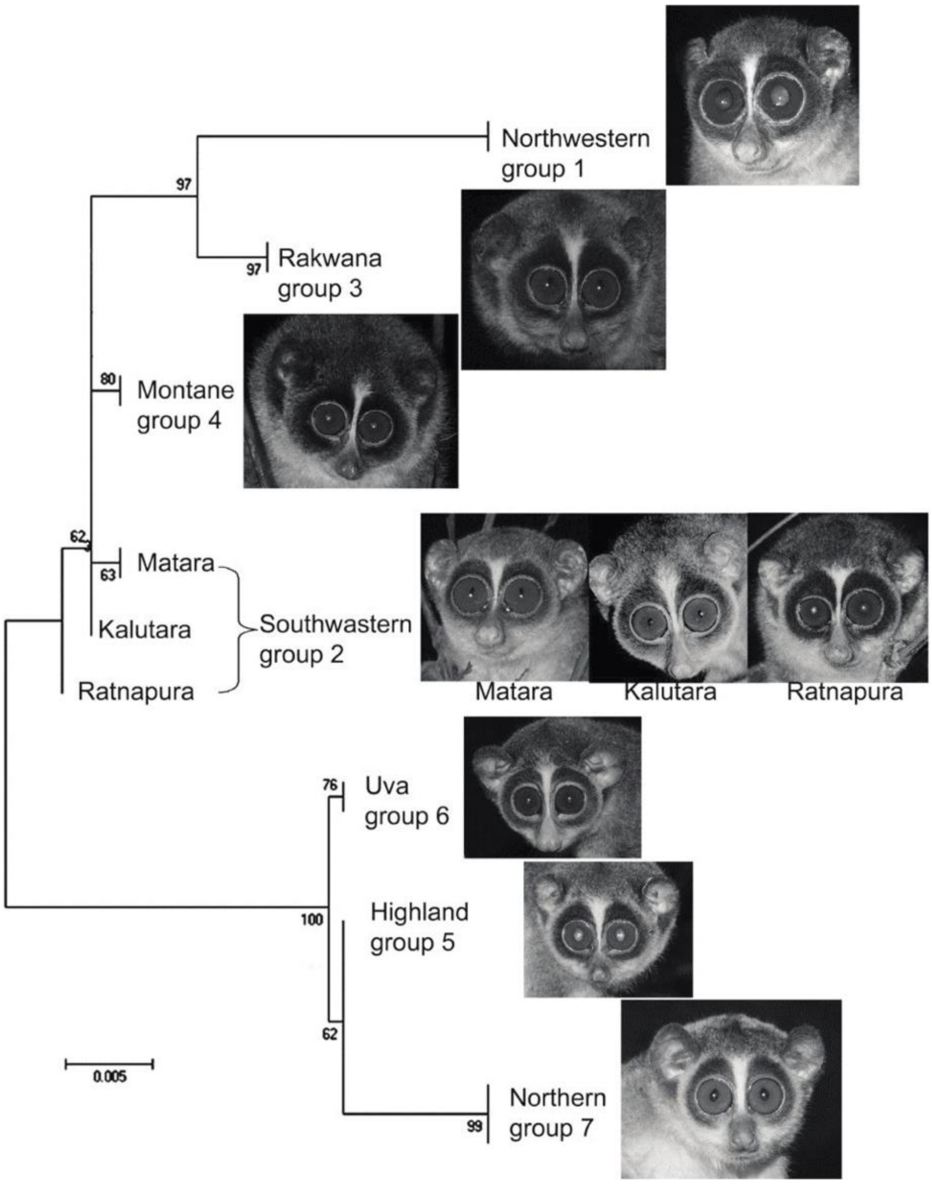

Figure 4. A neighbour-joining tree using 604 cytochrome c oxidase subunit I (CO1) sequences from 7 different slender loris (*Loris*) taxa found in Sri Lanka with their external appearance.

Figura 4. Árvore de proximidade usando 604 sequências de citocromo c oxidase subunidade I (CO1) de 7 diferentes táxons de lóris-delgados (*Loris*) encontrados no Sri Lanka com sua aparência externa.

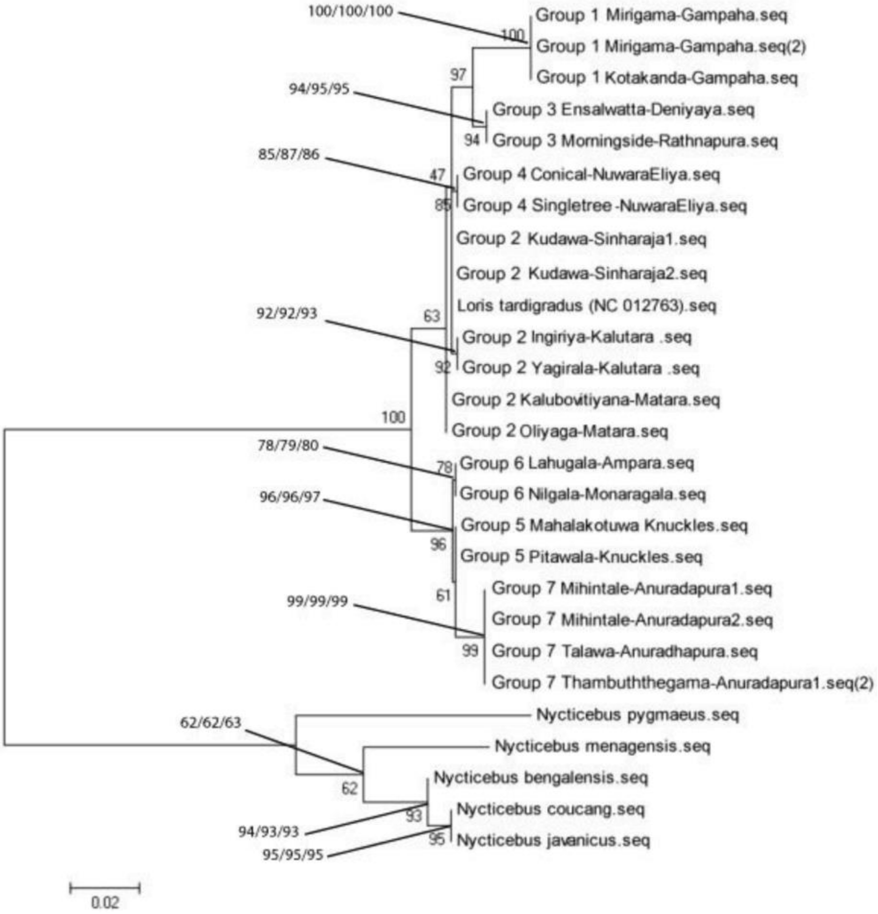

Figure 5. A neighbour-joining tree using 604 cytochrome C oxidase sub-unit I (CO1) sequences from 7 different slender loris (*Loris*) taxa, rooted using slow loris (*Nycticebus*) sequences deposited in the GenBank.

Figura 5. Árvore de proximidade usando 604 sequências de citocromo C oxidase subunidade I (CO1) de 7 táxons de lóris-delgados (*Loris*) diferentes, enraizadas usando sequências de loris-lentosorig (*Nycticebus*) depositadas no GenBank.

Beals smoothing is a multivariate transformation specially designed for data, on the presence/absence of species community, that contains noise and/or a lot of zeros. This transformation replaces the observed values of the target species with predictions of occurrence on the basis of its co-occurrences with the remaining species. In many applications, the transformed values are used as input for multivariate analyses. As Beals smoothing values provide a sense of "probability of occurrence," they have also been used for inference (Figure 6). Estimating the number of species that occur at each location is a simplistic way of estimating species richness for each location. In some cases, the number of individuals found may differ between locations. Since there may be a relation between the number of individuals counted in a location or sample and the taxa or species richness, rarefied species richness may be a more appropriate metric than total richness. Finally, we can conclude that all seven cluster in three different studies will align together.

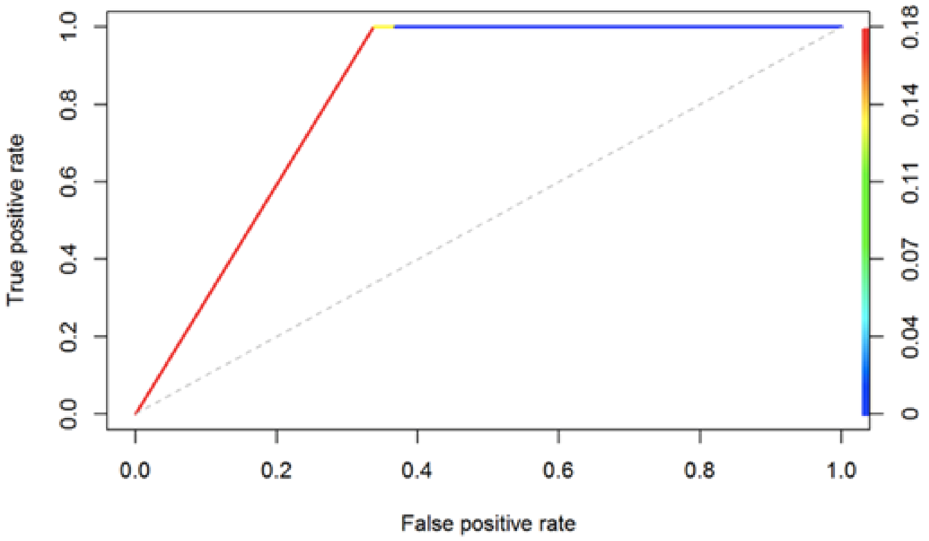

Figure 6. ROC curve checking the predictive ability.

Figura 6. Curva ROC para verificação da capacidade preditiva.

According to the habitat distribution models, the *Loris* is at risk of becoming an endangered species. The Wards method can account for environmental events and variables concerning the number of records of previous studies (Hoffman et al., 2008). This method proved to be more efficient than other modelling approaches (Ortega-Huerta and Townsend, 2008). The compact model with seven or more than one specific position uses environmental variations based on the creature's existence, which is assessed by the quality of the probability distribution of the Ward system and the ROC curve diagram (Figure 5). The three data sets from the seven clusters were generated. The current population of the Land Use stewardship plan, newly identified, prioritize topographical locations identified as natural habitats and recovery regions to be used as the most effective protection (Figure 3-Figure 6). Studies on the *Loris* population can help plan land use management, discover new populations, identify top-priority survey sites, and set priorities to restore its natural habitat for more effective conservation. More research is needed to determine whether the existing protected areas adequately cover the habitats for slender loris.

Our data show evidence of ecological, morphological, and genetic divergences to the *Loris* population, which may potentially lead to the emergence of a new species over time. Our analysis shows that a multitude of factors may play a role in ecological divergence, including some behavioural conditions that are specific to the system. In particular, the data constitute an example in which substantial effects of a competitor species on intra-specific evolutionary processes appears likely. Geographic isolation, on the other hand, seems to play only a small role. This study further indicates that it is easy to launch projects to conserve the *Loris* species in Sri Lanka according to the distributional pattern of *Loris* species and the environmental conditions. The study highlights that divergence processes are likely to be based on a variety of factors, and that little will be gained by exclusively adhering to a controversial debate about geographic speciation scenarios.

5 ACKNOWLEDGEMENTS

We thank the Director General of the Department of Wildlife Conservation (DWC) and the Research Committee, the Deputy Director of Research, and DWC staff for their support. Also, we extend our thanks to the Conservator General of Forests, the Conservator of Forests (Research and Education), and the Forest Department staff for their support.

REFERENCES

BROOKS, T.M. et al. Habitat loss and extinction in the hotspots of biodiversity. **Conservation Biology**, v. 16, n. 4, p. 909-923, 2002.

CABRERA, A. Sobre los loris, y en especial sobre la forma filipina. Boletín de la real Sociedad Española de {About lorises, and especially about form. lipine. Bulletin of the Royal Spanish History Society.} **Historia Natural**, v. 8, p. 135-139, 1908.

DÍAZ, S.; YADVINDER, M. Biodiversity: Concepts, Patterns, Trends, and Perspectives. **Annual Review of Environment and Resources**, v. 47, p. 120-129, 2022.

FELSENSTEIN, J. Evolutionary trees from DNA sequences: A maximum likelihood approach. **Journal of Molecular Evolution**, v. 17, p. 368–376, 1981.

GAMAGE, S.N. et al. The taxonomy, distribution, and conservation status of the slender loris (Primates, Lorisidae: Loris) in Sri Lanka. **Journal of Primate Conservation**, v. 30, p. 1-25, 2017.

GROVES, C.P. **Primate Taxonomy**. Smithsonian Institution Press: Washington, DC, USA, 2001. p. 25-89.

HETTIARACHCHI, C.J. et al. Habitat suitability model for the montane Slender Loris in the Hakgala strict nature reserve, Sri Lanka. **Asian Primates Journal**, v. 7, n. 1, p. 14-19, 2018.

HOFFMAN, J.D. et al. Predicting potential occurrence and spread of invasive plant species along the North Platte River, Nebraska. **Invasive Plant Science and Management**, v. 1, p. 359-367, 2008.

JANSEN, R. et al. A Bayesian networks approach for predicting protein-protein interactions from genomic data. **Science**, v. 302, p. 449-453, 2003.

MACKENZIE, D.I. Assessing site occupancy modelling as a tool for monitoring Mahoenui giant wet populations. DOC Science Internal Series Department of Conservation Press: Wellington, New Zealand, 2003. p. 145-160.

MAHANAYAKAGE, C.A. **An anthropogenic threat to Montane Slender Loris.** 2013. Doctoral dissertation (Dissertation for Diploma in Environment Management) - Faculty of Graduate Studies Press, University of Colombo, Sri Lanka.

MCELROY, J.L. Tourism development in small islands across the world. Geografiska Annaler: Series B. **Human Geography**, v. 85, n. 4, p. 231-242, 2003.

MYERS, N. et al. Biodiversity hotspots for conservation priorities. **Nature**, v. 403, n. 6772, p. 853, 2000.

NEKARIS, A. *Loris tardigradus* ssp. *nycticeboides*. The IUCN Red List of Threatened Species, 2008.

NEKARIS, K.A.I.; JAYAWARDENE, J. Survey of slender loris (Primates, Lorisidae Grey, 1821: Loris tardigradus Linnaeus, 1758 and Loris lydekkerianus Cabrera, 1908) in Sri Lanka. **Journal of Zoology**, v. 262, p. 327–338, 2004.

ORTEGA-HUERTA, M.; TOWNSEND, P. Modelling ecological niches and predicting geographic distributions: a test of six presence-only methods. **Revista Mexicana de Biodiversidad**, v. 70, p. 205-216, 2008.

PETERSON, A.T.; KLUZA, D.A. New distributional modelling approaches for gap analysis. In: **Animal Conservation Forum**. Cambridge: Cambridge University Press., 2003. p. 47-54.

RAMBAUT, A. Se-Al: Sequence Alignment Editor (Se-Al v2.0a11). 1996. Disponível em: <http://evolve.zoo.ox.ac.uk/>.

RONQUIST, F.; HUELSENBECK, J.P.; BAYES, E. Bayesian phylogenetic inference under mixed models. **Bioinformatics**, v. 19, p. 1572-1574, 2003.

SOBERON, J.; PETERSON, A.T**. Interpretation of models of fundamental ecological niches and species' distributional areas**. Oxford: Havana press, 2005. p. 132-180.

TODUA, V. Endemic Flora and Fauna of Georgia, Biodiversity, Peculiarities and Ecology. **Advances in Ecological and Environmental Research**, 2018. p.108-120.

WHITE, L.; EDWARDS, A. Methods for assessing the status of animal populations. In: L. WHITE, L.; EDWARDS, A. (Ed.), **Conservation Research in the African Rainforests**: A Technical Handbook, Wildlife Conservation Society Press, New York, 2000. p. 191-201.