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The role of primate-specific genes in the phenotypic evolution of lorises

Chunyan Chen[a,b], Yong Shao[c] (iD), Sheng Wang[c], Mingli Li[d], and Haifa Qiao[a,e,*]

[a]Shaanxi Key Laboratory of Acupuncture and Medicine, Shaanxi University of Chinese Medicine, Xixian New Area, Xianyang 712046, Shaanxi Province, China
[b]Traditional Chinese Medicine Master Research Institute, Shaanxi University of Chinese Medicine, Xixian New Area, Xianyang 712046, Shaanxi Province, China
[c]State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences, No.17 Longxin Road, Kunming 650223, China
[d]Department of Neuroscience, Yale University School of Medicine, 100 College Street, Suite C944, New Haven, CT 06510, USA
[e]College of Acupuncture and Tuina, Shaanxi University of Chinese Medicine, Xixian New Area, Xianyang 712046, Shaanxi Province, China

*Address correspondence to Haifa Qiao. E-mail: 1511006@sntcm.edu.cn.
Handling editor: Zu-Shi Huang

Pygmy lorises are arboreal primates primarily found in forest environments across Southeast Asia (Nekaris 2014). They have a diverse diet, including plant secretions, nectar, fruits, invertebrates, tree bark, and bird eggs. All 9 known species of pygmy lorises are listed as globally endangered species (Nekaris 2014). Pygmy lorises exhibit a range of unique phenotypic characteristics rarely seen among primates. Their metabolic rate is significantly lower than other similarly sized eutherian mammals, which is associated with the high concentrations of toxins and digestive inhibitors in their diet (Swapna et al. 2010; Starr and Nekaris 2013). Their muscle tissue is primarily composed of slow-twitch muscle fibers, which possess characteristics of low energy consumption and high endurance (Gyambibi and Lemelin 2013). This muscle composition differs from other mammals, resulting in the pygmy lorises' slow movement (Kimura et al. 1987). In addition, pygmy lorises are among the few primates that carry toxins. These toxins are primarily secreted by brachial glands and become activated upon mixing with saliva. Pygmy lorises deposit these toxins on their fur through grooming, serving as an effective defense mechanism (Nekaris et al. 2013). They possess detoxifying abilities to counteract toxins in both them and their food sources (Harris et al. 2021; Li et al. 2022). Furthermore, pygmy lorises demonstrate a rare ability for hibernation (Reinhardt 2019).

New genes typically refer to genomic loci that have recently emerged during evolution and are present only in specific species or lineages, hence also termed as species or lineage-specific genes (Kaessmann 2010; Long et al. 2013). New genes play a significant role in the phenotypic evolution of species or lineage (Zhang and Long 2014; VanKuren and Long 2018; Chen et al. 2022). The origin of most new genes is associated with gene duplication, and due to relaxed functional constraints or positive selection, these new genes often do not experience significant natural selection pressures (Zhang et al. 2010). In addition to gene duplication, de novo origination, where new genes evolve from previously non-coding sequences, is also a significant mechanism in the emergence of new genes (Zhang et al. 2019; An et al. 2023). Both processes contribute to the evolution of new and their roles in phenotypic diversity. Numerous studies have shown that primate-specific genes have crucial functions in the phenotypic evolution of primates (Zhang et al. 2012; Zhang and Long 2014; An et al. 2023). For instance, primate-specific genes original from duplicate or de novo are closely associated with the increase in brain volume, as well as advanced cognitive and social behaviors in primates (Zhang et al. 2012; Heide et al. 2020; An et al. 2023).
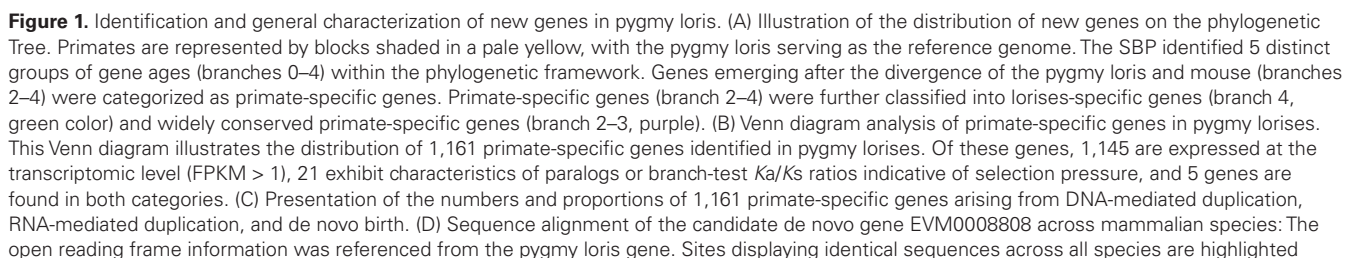
Pygmy lorises are primates with unique phenotypes, and the contribution of primate-specific genes to their phenotypic evolution remains unclear. In this study, we collected high-quality genomic data from published studies of primates such as humans and pygmy lorises (Supplementary Table S1), as well as non-primate mammals like sheep and mouse (Supplementary Table S1) to identify primate-specific genes (refers to new genes) of pygmy lorises. We also employed 8 pygmy loris tissues' transcriptomic data to explore the contribution of new genes to the evolution of specific phenotypes in lorises. Detailed materials and methods are described in the Supplementary Materials. Based on transcriptomic data, we manually corrected gene structures with identifiable errors in pygmy lorises (Supplementary Figure S1) and obtained 25,512 protein-coding gene structures. Using the synteny-based method (SBP) (Shao al. 2019) (Supplementary Table S2), we identified 3,934 primate-specific genes (branch 2–4) (Supplementary Table S3), further classified into lorises-specific genes (3,319 genes, branch 4) and widely conserved primate-specific genes (615 genes, branch 2–3) (Figure 1A). Analyzing primate-specific gene expression across 8 tissues using transcriptomic data revealed that only 1,145

Figure 1. Identification and general characterization of new genes in pygmy loris. (A) Illustration of the distribution of new genes on the phylogenetic Tree. Primates are represented by blocks shaded in a pale yellow, with the pygmy loris serving as the reference genome. The SBP identified 5 distinct groups of gene ages (branches 0–4) within the phylogenetic framework. Genes emerging after the divergence of the pygmy loris and mouse (branches 2–4) were categorized as primate-specific genes. Primate-specific genes (branch 2–4) were further classified into lorises-specific genes (branch 4, green color) and widely conserved primate-specific genes (branch 2–3, purple). (B) Venn diagram analysis of primate-specific genes in pygmy lorises. This Venn diagram illustrates the distribution of 1,161 primate-specific genes identified in pygmy lorises. Of these genes, 1,145 are expressed at the transcriptomic level (FPKM > 1), 21 exhibit characteristics of paralogs or branch-test Ka/Ks ratios indicative of selection pressure, and 5 genes are found in both categories. (C) Presentation of the numbers and proportions of 1,161 primate-specific genes arising from DNA-mediated duplication, RNA-mediated duplication, and de novo birth. (D) Sequence alignment of the candidate de novo gene EVM0008808 across mammalian species: The open reading frame information was referenced from the pygmy loris gene. Sites displaying identical sequences across all species are highlighted

(1,145/3,934 × 100% = 29%) (Supplementary Table S4) new genes were expressed in at least 1 tissue (FPKM > 1) (Figure 1B). Given our primary focus on determining whether new genes significantly contribute to the unique traits of pygmy lorises, we selected 1,161 new genes with transcriptomic expression evidence (FPKM > 1) or selection pressure evidence (Figure 1B) as a refined new gene set for in-depth analysis (Supplementary Table S5). During the paralog Ka/Ks analysis and the subsequent removal of outliers, in lorises-specific genes, we found 52 under positive selection ($Ka/Ks > 0.5$, q value < 0.05) and 4 under negative selection ($Ka/Ks < 0.5$, q value < 0.05) (Supplementary Table S6). In widely conserved primate-specific genes, we detected 10 under positive selection and 8 under negative selection. Comparative analysis revealed that lorises-specific genes had more potential positively selected genes (52/1,443) when compared with widely conserved primate-specific genes (10/651) (FET $P = 0.008$). Conversely, lorises-specific genes had fewer negatively selected genes (4/1,443) than widely conserved primate-specific genes (8/651) (FET $P = 0.01$). Validation with branch-test using ortholog genes confirmed significant positive selection signals in 5 lorises-specific genes and 1 widely conserved primate-specific gene ($Ka/Ks > 1$, $P < 0.05$), with no significant difference between the sets (FET $P = 0.67$). In addition, in the widely conserved primate-specific genes, 3 potential positive selection genes were found to be under significant negative selection pressure ($Ka/Ks < 1$, $P < 0.05$) in the branch-test analysis (Supplementary Table S6). A small fraction of all primate-specific genes experienced natural selection (21/2,094 × 100% = 1%) (Figure 1B). These findings suggest that lorises may have undergone more adaptive changes during evolution, likely due to their unique ecological niche, behavior, or physiology. The high number of negatively selected genes in widely conserved primate-specific genes suggests their involvement in critical biological pathways. In addition, most primate genes showed no selection signals, which could indicate ongoing evolution.

Of the gene set, the majority (90%) originated from DNA-mediated duplication events, comprising 840 (72%) DNA-mediated duplicates, 210 (18%) RNA-mediated duplicates (retrogenes) (Figure 1C), and 111 (10%) potential de novo genes (Figure 1C) (Supplementary Table S7). Figure 1D provides an illustrative example of a potential de novo gene (EVM0008808), showing the alignment with homologous sequences from other species and highlighting disrupted reading frames, which may represent novel coding regions specific to pygmy lorises. For duplicated genes, we retrieved paralogous genes in the pygmy loris and orthologs in other primates, using domain filtering for accuracy. We categorized paralog gene families based on copy numbers into 3 classes:

fewer than 5, between 5 and 10, and more than 10. Results showed that primate-specific genes are involved in 319 paralogous gene families, with the majority having fewer than 5 members (Figure 1E, Supplementary Table S8). Complex large gene families, such as olfactory receptors related gene family, included 200 primate-specific genes. Except for the olfactory receptor-related gene family, further analysis revealed that the proportion of lorises-specific and widely conserved primate-specific genes across the 3 gene family categories did not show significant differences (Figure 1F). We combined protein alignments and genomic synteny information to identify ortholog genes in 4 primates, detecting from 370 to 440 orthologs (Supplementary Table S9). Using the GTEx (Consortium 2015) database, we downloaded expression matrices for 54 human tissues and calculated the tissue-specific expression coefficient (τ). Most ortholog genes were broadly expressed, with 115 showing tissue-biased expression (Supplementary Figure S2A). Although testis, brain, and lymphocyte-biased genes were more prevalent, their proportions were still lower than background values, indicating that most orthologs have broad expression profiles (Supplementary Figure S2B). Given that many primate-specific genes are lorises-specific, strict criteria for reciprocal synteny and blastp homology in humans identified only 32 orthologs. This indicates that the analyzed ortholog genes are largely conserved across species, with relatively few being truly primate-specific, thus explaining the limited number of tissue-biased expression genes. Analyzing transcriptomic data from adult pygmy loris tissues revealed an age-dependent expression pattern, with younger genes exhibiting narrower and lower expression levels. Specifically, the median FPKM expression of primate-specific genes (branch 2–4) was <2 across 3 tissues, whereas the most conserved genes had a median FPKM expression > 2 across the 3 tissues and a median expression > 4 across all 8 tissues (Figure 1G,H). This observation consistence with previously reported expression patterns of new genes (Zhang et al. 2012; Shao et al. 2019; Chen et al. 2022).

To understand the role of new genes in loris phenotypic adaptations, we assessed tissue specificity indices (τ) using transcriptomic data, identifying tissue-biased expression genes (Supplementary Table S10). A significant proportion of new genes showed bias in spleen and heart expression compared to background levels across 8 tissues (Figure 2A). Stomach, liver, and tongue also exhibited higher proportions of new genes, though not statistically significant (Figure 2A, Supplementary Table S11–15). Gene ontology (GO) (Figure 2B, Supplementary Table S11–15) and KEGG pathway enrichment analyses (Supplementary Figure S3) focused on tissue-biased genes from spleen, liver, heart, tongue, and stomach. Such as, the top 5 enriched biological processes (BPs) of spleen-biased

in yellow. Premature termination codons and frame-disrupting indels are annotated in red. (E) Paralogous gene categories of primate-specific genes. Gene families are classified based on shared domains, grouping paralogous genes with the same domain into the same gene family. Gene families are categorized into 3 types based on the number of family members: those with fewer than 5 genes, those with 6–10 genes, and those with more than 10 genes. (F) Proportions of different primate-specific gene categories within each gene family category. These proportions are calculated by dividing the number of genes in each primate-specific gene category within a specific gene family category by the total number of genes in that primate-specific gene category, then multiplying by 100. (G) Violin plots illustrating expression profiles of primate-specific genes across different gene age groups. The median expression values, based on a logarithmic scale, are depicted across 8 tissues of adults pygmy loris. (H) Representation of the number of tissues where genes were expressed: The interquartile range is indicated by black bars, while the probability density of the data is depicted by the violin curve. The median value is denoted by a white dot. Genes were categorized into 3 gene age groups as defined previously. P values between age groups, calculated using Wilcoxon's test, are also provided. Group 0 denotes the oldest genes shared among mammals, while Group 1 encompasses genes predating the pygmy loris–mouse split. Groups 2–4 encompass primate-specific genes.
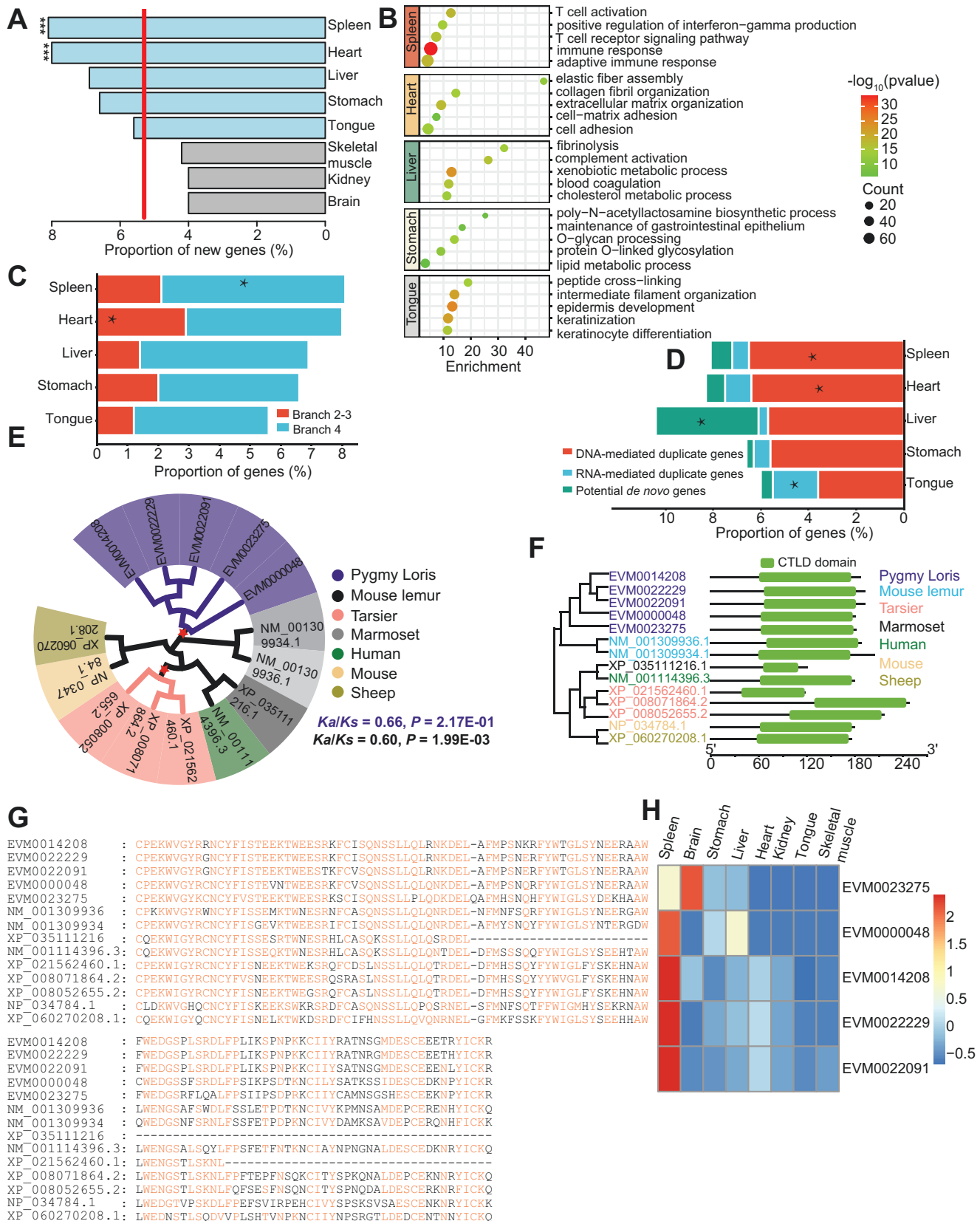
Figure 2. Proportion and function enrichment of primate-specific genes in different gene clusters. (A) Representation of the proportion of primate-specific genes within tissue-biased expression gene sets identified by the graded tissue specificity index τ. The genome-wide percentage of primate-specific genes is depicted by the horizontal red line. Modules with increased or decreased proportions of primate-specific genes are denoted by blue and gray blocks, respectively. Multi-testing adjusted P values, derived from comparing the percentage of primate-specific genes in each gene set with the significant level of primate-specific genes, are displayed above the respective boxes. *** indicates $P < 0.001$. (B) Summary of enrichment analysis results for the top 5 GO BPs terms in gene sets enriched with primate-specific genes. (C) Proportions of different primate-specific genes in 5

genes included immune response, T cell activation, inflammatory response, and positive regulation of interferon-gamma production (Figure 2B, Supplementary Table S11). The primary BP terms of liver-biased genes encompassed xenobiotic metabolic processes, complement activation, blood coagulation, and cholesterol metabolism (Figure 2B, Supplementary Table S12). The predominant BP terms of heart-biased genes involved extracellular matrix organization, collagen fibril organization, cell adhesion, elastic fiber assembly, and extracellular matrix adhesion (Figure 2B, Supplementary Table S13). Furthermore, we conducted additional analysis to examine the enrichment levels of tissue-biased genes with different ages and origin mechanisms in these tissues. The results revealed that the spleen exhibited enrichment of lorises-specific genes generated through DNA-level replication, whereas the heart displayed enrichment of widely conserved primate-specific genes produced via DNA-level replication (Figure 2C,D). The liver, stomach, and tongue showed no significant difference in the enrichment levels of lorises-specific genes and widely conserved primate-specific genes compared to the background values (Figure 2C) Interestingly, the liver exhibited significant enrichment of candidate *de novo* genes (Figure 2D), which may play important roles in liver metabolism and detoxification. On the other hand, the tongue demonstrated enrichment of primate-specific genes replicated at the RNA level, possibly associated with the physiological characteristics, dietary habits, or environmental adaptation of loris. In addition, we employed Mfuzz for expression pattern clustering, we identified 10 gene clusters (Supplementary Figure S4, Supplementary Table S16). These clusters exhibited high expression in the spleen, stomach, liver, tongue, and heart, consistent with tissue-specific coefficient τ results. The results have a high overlap between the identified tissue-biased genes and the Mfuzz results, the results from the tissue specificity indices (τ) are more rigorous (Supplementary Figure S5). These clusters 1, 3, 7, 8, and 10 are enriched with new genes (Supplementary Figure S6A). Functional enrichment analyses of these gene clusters revealed alignment with the biological functions of their predominantly expressed tissues (Supplementary Tables S17–21) (Supplementary Figures S6B and S7). For instance, the C7 gene cluster primarily shows liver-biased expression, and the top 5 GO BP terms relate to cytoplasmic translation, immune response, inflammatory response, and T cell activation, consistent with liver functions (Supplementary Figure S6B). Furthermore, for the Mfuzz analysis results, we also investigated the enrichment levels of new genes with different ages and origin mechanisms in 5 gene clusters. Different to the tissue-biased expression analysis results, significant enrichment of lorises-specific genes was observed in the liver (C7) and tongue (C8) high-expression gene clusters, while there was no significant difference in the proportion of

widely conserved primate-specific genes compared to the background values in the 5 gene clusters (Supplementary Figure S6C). Consistent with the tissue-biased expression results, the spleen (C1) and heart (C10) high-expression gene clusters exhibited enrichment of DNA-level duplicated primate-specific genes, whereas the tongue (C8) high-expression gene cluster showed significant enrichment of RNA-level duplicated primate-specific genes. In addition, the liver (C7) high-expression gene cluster exhibited significant enrichment of candidate *de novo* genes (Supplementary Figure S6D). These findings suggest that new genes originating through different mechanisms and at different times may contribute differently to the unique phenotypic traits of pygmy lorises. Our investigation suggests that the KLRD1 gene family likely plays an important role in various lorises' phenotypes, including detoxification, metabolism, and immune response. These genes serve as recognition molecules for T cells and natural killer cells, aiding immune cells in identifying and eliminating infected or cancerous cells (Nekaris et al. 2013; Li et al. 2022). Unlike humans, mouse, sheep, and marmosets, which possess only 1 gene copy, tarsiers, mouse lemurs, and pygmy lorises exhibit multiple gene copies, with pygmy lorises having up to 5 copies (Figure 2E). These additional gene copies have arisen through DNA-mediated replication, with 2 being primate-specific copies (Supplementary Table S22). Evolutionary analysis indicates that KLRD1 copies in pygmy lorises and other species are subject to different natural selection pressures. Despite the less pronounced positive selection of KLRD1 in pygmy lorises compared to the null hypothesis 0.5, suggesting the potential significance of the gene copies in pygmy lorises during evolution. Belonging to the CLECT superfamily (Figure 2F), KLRD1 possesses a C-type lectin-like domain (CTLD). Animal C-type lectins are involved in diverse BPs, including extracellular matrix organization, endocytosis, complement activation, pathogen recognition, and intercellular interactions. Sequence alignment of the CTLD domain reveals mutations in the 5 gene copies in pygmy lorises (Figure 2G), potentially leading to differentiated expression patterns (Figure 2H). Although the most conserved gene, EVM0023275, shows biased expression in spleen and brain, younger copies exhibit spleen-biased expression followed by liver-biased expression, indicating functional divergence. The spleen plays a role in immune function, hematopoiesis, blood storage, and metabolic regulation, the liver is crucial for metabolism, bile production, detoxification, coagulation, immune response, heat generation, and water-electrolyte balance. In brief, the KLRD1 gene family likely contributes significantly to specific lorises' phenotypes related to detoxification, metabolism, and immunity.

Lorises exhibit unique phenotypic traits, and their distinct genomic features may be closely related to their ecological niche and biological adaptability. Due to the important role

tissue-biased expression gene sets. The gene proportion is calculated by dividing the number of corresponding primate-specific genes in each gene set by the total number of genes in that gene set, then multiplying by 100. Significance levels were determined by Fisher testing the difference between the proportion of each type of primate-specific genes in each gene set and the proportion of primate-specific genes in all gene sets. (D) Proportions of primate-specific genes of different origin mechanisms in 5 gene sets. The gene proportion is calculated by dividing the number of primate-specific genes of each origin mechanism in each gene set by the total number of genes in that cluster, then multiplying by 100. Fisher testing was used to assess the difference between the proportion of primate-specific genes of each origin mechanism in each gene set and the proportion of primate-specific genes of each origin mechanism in all gene sets. The significant level of primate-specific genes are presented above the boxes. *$P < 0.05$, **$P < 0.01$. ***$P < 0.001$. (E) The copy number of KLRD1 in different species. Red pentagrams represent locations of gene family expansion. The purple Ka/Ks represents the selection pressure on the 5 KLRD1 copies in pygmy lorises, whereas the black Ka/Ks represents the selection pressure of KLRD1 sequences in other species. (F) Schematic representation of the distribution and length of the CTLD domain of KLRD1 across different species. (G) Sequence alignment of the CTLD domain across different species. Black amino acids represent sites with accumulated mutations, whereas orange sites are conserved sites. (H) Heatmap of the expression of the 5 KLRD1 copies in pygmy lorises.

of species- or lineage-specific genes in the phenotypic evolution of a species (Zhang et al. 2010; Shao et al. 2019; Heide et al. 2020). Thus, a comprehensive understanding of primate-specific genes can elucidate the biological mechanisms shaping loris evolution and adaptation. The accurate identification of species- or lineage-specific genes relies on high-quality genome data, such as Telomere-to-Telomere (T2T) assemblies and chromosome-level assemblies, which greatly facilitate the identification of new genes (Leng et al. 2024). Using high-quality genome assembly data from pygmy lorises and other species, we employed the SBP method to obtain a comprehensive and accurate primate-specific gene set. Through evolutionary and expression analyses, we observed that primate-specific genes in lorises exhibit patterns of expression and evolution likely to primate-specific genes in humans and lineage-specific genes in other mammals. Our research revealed that primate-specific genes are enriched in spleen, liver, heart, stomach, and tongue, which are integral to metabolic, detoxification, and immune processes. The functions of these tissues may be influenced by the contributions of primate-specific genes of different ages and origin mechanisms. Consequently, primate-specific genes likely play an important role in the distinctive phenotypic evolution of pygmy lorises. Specifically, the *KLRD1* gene family, characterized by spleen-biased and liver-biased expression, has experienced expansion within the pygmy loris genome. Members of this family are subjected to negative natural selection pressures, indicating potential significant roles in evolutionary processes. Given the physiological functions of the spleen and liver, the expansion of this gene family likely contributes substantially to the unique phenotypic traits associated with metabolism, detoxification, and immunity in pygmy lorises. In summary, our research revealed that primate-specific genes make significant contributions to the distinctive phenotypic traits of lorises. This study provides valuable genomic and biological data resources for loris research, enriching our understanding of their genetic characteristics and offering important references for related fields.

Funding

Authors' Contributions

C.C.Y. and Q.H.F. conceived the article and revised the manuscript. S.Y., W.S., and M.L. provided genomic data of pygmy lorises. C.C.Y. analyzed all data. C.C.Y. wrote the draft manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

Supplementary Material

Supplementary material can be found at https://academic.oup.com/cz.

References

An NA, Zhang J, Mo F, Luan XK, Tian L et al., 2023. De novo genes with an lncRNA origin encode unique human brain developmental functionality. *Nat Ecol Evol* **7**:264–278.

Chen CY, Yin Y, Li HR, Zhou BT, Zhou J et al., 2022. Ruminant-specific genes identified using high-quality genome data and their roles in rumen evolution. *Sci Bull (Beijing)* **67**:825–835.

Consortium G, 2015. Human genomics. The Genotype-Tissue Expression (GTEx) pilot analysis: Multitissue gene regulation in humans. *Science* **348**:648–660.

Gyambibi A, Lemelin P, 2013. Comparative and quantitative myology of the forearm and hand of prosimian primates. *Anat Rec (Hoboken)* **296**:1196–1206.

Harris RJ, Nekaris KA, Fry BG, 2021. Monkeying around with venom: An increased resistance to α-neurotoxins supports an evolutionary arms race between Afro-Asian primates and sympatric cobras. *BMC Biol* **19**:253.

Heide M, Haffner C, Murayama A, Kurotaki Y, Shinohara H et al., 2020. Human-specific ARHGAP11B increases size and folding of primate neocortex in the fetal marmoset. *Science* **369**:546–550.

Kaessmann H, 2010. Origins, evolution, and phenotypic impact of new genes. *Genome Res* **20**:1313–1326.

Kimura T, Kumakura H, Inokuchi S, Ishida H, 1987. Composition of muscle fibers in the slow loris, using the m. biceps brachii as an example. *Primates* **28**:525–532.

Leng L, Xu ZC, Hong BX, Zhao BB, Tian Y et al., 2024. Cepharanthine analogs mining and genomes of Stephania accelerate anti-coronavirus drug discovery. *Nat Commun* **15**:1537.

Li ML, Wang S, Xu PH, Tian HY, Bai MX et al., 2022. Functional genomics analysis reveals the evolutionary adaptation and demographic history of pygmy lorises. *Proc Natl Acad Sci USA* **119**:e2123030119.

Long MY, VanKuren NW, Chen SD, Vibranovski MD, 2013. New gene evolution: Little did we know. *Annu Rev Genet* **47**:307–333.

Nekaris KA, 2014. Extreme primates: Ecology and evolution of Asian lorises. *Evol Anthropol* **23**:177–187.

Nekaris KA, Moore RS, Rode EJ, Fry BG, 2013. Mad, bad and dangerous to know: The biochemistry, ecology and evolution of slow loris venom. *J Venom Anim Toxins Incl Trop Dis* **19**:21.

Reinhardt KD, 2019. *Ecophysiology of a Wild Nocturnal Primate, the Javan Slow Loris (Nycticebus javanicus)*. PhD dissertation, Oxford Brookes University, Oxford, United Kingdom.

Shao Y, Chen CY, Shen H, He BZ, Yu DQ et al., 2019. GenTree, an integrated resource for analyzing the evolution and function of primate-specific coding genes. *Genome Res* **29**:682–696.

Starr C, Nekaris KA, 2013. Obligate exudativory characterizes the diet of the pygmy slow loris *Nycticebus pygmaeus*. *Am J Primatol* **75**:1054–1061.

Swapna N, Radhakrishna S, Gupta AK, Kumar A, 2010. Exudativory in the Bengal slow loris (*Nycticebus bengalensis*) in Trishna Wildlife Sanctuary, Tripura, northeast India. *Am J Primatol* **72**:113–121.

VanKuren NW, Long MY, 2018. Gene duplicates resolving sexual conflict rapidly evolved essential gametogenesis functions. *Nat Ecol Evol* **2**:705–712.

Zhang L, Ren Y, Yang T, Li GW, Chen JH et al., 2019. Rapid evolution of protein diversity by de novo origination in Oryza. *Nat Ecol Evol* **3**:679–690.

Zhang YE, Landback P, Vibranovski M, Long MY, 2012. New genes expressed in human brains: Implications for annotating evolving genomes. *Bioessays* **34**:982–991.

Zhang YE, Long MY, 2014. New genes contribute to genetic and phenotypic novelties in human evolution. *Curr Opin Genet Dev* **29**:90–96.

Zhang YE, Vibranovski MD, Landback P, Marais GA, Long MY, 2010. Chromosomal redistribution of male-biased genes in mammalian evolution with two bursts of gene gain on the X chromosome. *PLoS Biol* **8**:e1000494.