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Comparative Distribution Pattern of Mirror Repeats in Kinetoplastid Parasites A Study of Leishmania and Trypanosoma

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Abstract: *The parasites of the genera Leishmania and Trypanosoma are the cause of serious Neglected Tropical Diseases (NTD) such as leishmaniasis, African trypanosomiasis and Chagas disease. Despite their extensive study in the genomes, the distribution and importance of the mirror repeats are not well known. An in silico comparative study of mirror repeats in Leishmania donovani, Leishmania major, Trypanosoma brucei and Trypanosoma cruzi. Mirror repeats were found in the TriTrypDB and the NCBI database with the help of the FASTA Parallel Complement BLAST (FPCB) method. Repeat abundance, repeat length, genome location, GC content, conservation and association with virulence genes were analysed comparatively. This indicated species-specific differences in the distribution of repeat elements across the genomes: Leishmania had a higher density of shorter repeats and Trypanosoma had longer and more complex repeat motifs. The evolutionary diversification, parasite adaptation, gene regulation and genome plasticity of the kinetoplastid family appear to be promoted by these repeats, and they provide a source of information for the future functional study of genome organization and function in the kinetoplastid family.*

Keywords: Mirror repeats, Kinetoplastid parasites, Leishmania, Trypanosoma, Comparative genomics, Non-B DNA, Genome evolution, Virulence genes

1. INTRODUCTION

The neglected tropical diseases (NTDs) associated with kinetoplastid protozoa continue to be a significant public health problem, especially in tropical and subtropical countries. Leishmania and Trypanosoma are the genera that cause leishmaniasis, African trypanosomiasis (sleeping sickness) and Chagas disease, respectively, which affect millions of humans worldwide and contribute to significant health, social and economic burden. These parasites display a number of unique biological characteristics, such as kinetoplast DNA (kDNA), polycistronic transcription, highly plastic nuclear genome, advanced immune evasion and host adaptation mechanisms. Whole genome sequencing and comparative genomics have revealed a high level of chromosome copy number variation and gene amplification, structural rearrangements, and repetitive DNA sequences, all of which are important for parasite survival, virulence, and evolutionary adaptation.[1]

Repetitive DNA elements exist in a number of different forms, one type being a special class of symmetrical nucleotide sequences known as mirror repeats (MRs) in which one half is the reverse complement of the other half on the same strand of DNA. Inverted repeats do not have the same potential to undergo non-B DNA conformation as do mirror repeats; in fact, mirror repeats may form non-B DNA conformation such as intramolecular triplex (H-DNA) structures, which can affect genome stability, chromatin organization, genomic recombination, transcription, and DNA replication.

[1] Zhenag, H., et al. (2020). Genomic and proteomic characterization of virulent *Leishmania donovani* strains. *Frontiers in Cellular and Infection Microbiology, 10*, 594980.

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Mirror repeats have been studied in bacterial and plant genomes and in mammalian genomes, but in kinetoplastid parasites, their nature and function are poorly known.

A comparative study of the distribution of mirror repeats is of great interest since the genomes of Leishmania and Trypanosoma contain many repetitive elements, multigene families and highly dynamic chromosome regions linked to virulence and antigenic variation. The abundance, localization and conservation of the mirror repeats could give insights into genome organization, adaptation and parasite evolution, interaction between host and parasite, and could serve as potential markers for future functional and therapeutic studies.[2]

II. LITERATURE REVIEW

Moghaddam et al. (2026) recent advances in visceral leishmaniasis research and the impact of emerging comparative genomics, transcriptomics, and molecular epidemiology tools on understanding the biology of the parasite and host–pathogen interactions are reviewed, showing how these advancements have enhanced the understanding of the biology of visceral leishmaniasis (VL) and its interactions with the host. The authors went on to stress the need for the development of a combination of structural genomics and functional analyses for the identification of new virulence and adaptation factors in kinetoplastid parasites, thus supporting the investigation of mirror repeats.[3]

Ebiloma et al. (2025) conducted a One Health approach to a review on the state of the art in drug discovery for kinetoplastid diseases. The authors also stressed the need of developing a better understanding of parasite genomics, genome plasticity, and molecular evolution to discover new therapeutic targets. They proposed that genomic features that had been overlooked, such as repetitive DNA elements, could be useful markers and targets for future antiparasitic approaches.[4]

Damasceno et al. (2025) studied the function of R-loops and RNase H1 in the stability of Leishmania major genome. Their results showed that the DNA replication timing, chromosome stability, and transcriptional regulation are all affected by unusual structures in nucleic acids. The study supports indirectly the biological significance of mirror repeats in the organization of the kinetoplastid genome because these repeats have the ability to form non-canonical DNA structures, like H-DNA.[5]

Zhao et al. (2025) found that the minicircle organization, RNA editing, and the architecture of the mitochondrial genome are remarkably diverse. They found that kinetoplast DNA is complex and flexible in evolution, indicating that repetitive sequence motifs can be used to structure and regulate the function of both mitochondrial and nuclear genomes.[6]

Zingales et al., (2023) review on recent advances in comparative genomics of Trypanosoma species, they shed light on the evolutionary significance of repetitive DNA, multigene family expansion and chromosomal rearrangements. The authors showed that antigenic variation and host adaptation are linked to structurally dynamic genomic regions that

[2] Sinha, S., et al. (2023). Functional implications of repetitive DNA in protozoan parasite genomes. *Genes, 14*(9), 1817.
[3] Moghaddam, S. P., et al. (2026). Revisiting visceral leishmaniasis in immunocompromised populations. *Current Research in Parasitology & Vector-Borne Diseases, 6*, 100243. (ScienceDirect)
[4] Ebiloma, G. U., et al. (2025). A One Health approach to drug discovery, development and control of kinetoplastid diseases. *Drugs, 18*(9), 1415. (MDPI)
[5] Damasceno, J. D., et al. (2025). R-loops acted on by RNase H1 influence DNA replication timing and genome stability in *Leishmania major*. *Nature Communications, 16*, Article 56785. (Nature)
[6] Zhao, X., He, Y., Zhang, F., et al. (2025). Comparative mitochondrial genome and transcriptome analyses reveal minicircle diversity and editing variation in *Trypanosoma brucei*. *Nucleic Acids Research, 53*(13), gkaf661. (OUP Academic)

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contain repetitive sequences. In general, their review lends credence to the idea that the repeats in the genome are able to promote evolution through recombination and structural diversification.[7]

2. Objectives

The study aims to:

- Determine the lengths of the repeats
- Relate abundance of mirrors to that of repeats in kinetoplastid species
- Understand length and positioning of mirror repeats
- Analyze possible correlations between virulence-associated genes and mirror repeats
- Describe the evolutionary and biological importance of mirror repeat diversity[8]

III. MATERIALS AND METHODS

3.1 Study Design

The current study used a comparative computational genomic (in silico) research design for investigation of the distribution pattern of mirror repeats (MRs) in selected kinetoplastid parasites. The study included the search for and comparison of the occurrence, abundance, structure and genomic distribution of mirror repeats in representative species of the genera Leishmania and Trypanosoma. The bioinformatics approach was used as it allows for a quick, accurate and inexpensive analysis of a large genome without experimental manipulation. Comparative genome analysis tools were used to compare the similarities and differences of the mirror repeat organization between species, and sequence analysis tools were used to identify conserved and species-specific repeat motifs. The study also analyzed possible linkage of the mirror repeats to virulence associated genes, repetitive genomic regions and evolutionary adaptation in order to gain a detailed knowledge of repetitive DNA architecture in kinetoplastid parasites.[9]

3.2 Parasite Species

To compare, two medically important kinetoplastid parasites were chosen: Leishmania donovani, Leishmania major, Trypanosoma brucei, and Trypanosoma cruzi. These species cause visceral and cutannous leishmaniasis, African sleeping sickness and Chagas disease, respectively, as their main etiological causes. The complete genome sequences were obtained from the publicly available genomic resources, such as the TriTrypDB and the NCBI GenBank database. These databases contain well-annotated reference genomes, annotations for genes, and chromosomal data, which is ideal for comparative genomic studies. These species were selected based on their clinical relevance, evolutionary distinction, genome assemblies and genome organisation. Conserved and species-specific as well as mirror repeat patterns were identified in the genome structure, virulence and adaptation of these representative parasites by comparative analysis.[10]

3.3 Identification of Mirror Repeats

A commonly used bioinformatics technique, called FASTA Parallel Complement BLAST (FPCB), was used to detect symmetrical DNA sequences, known as mirror repeats. First, whole genome sequences were downloaded and then broken up into 1Kb chunks for systematic analysis. All fragments were re-written in the reverse complementary

[7] Zingales, B., et al. (2023). Advances in comparative genomics of *Trypanosoma* species and implications for parasite evolution. *PLoS Neglected Tropical Diseases, 17*(8), e0011584.
[8] Ashutosh, A., et al. (2020). Genomics and transcriptomics of *Leishmania*: Recent advances and future perspectives. *Parasitology International, 79*, 102176.
[9] Franssen, S. U., et al. (2020). Global genome diversity of the *Leishmania donovani* complex. *Nature Communications, 11*, 2372.
[10] Guiblet, W. M., et al. (2021). Non-B DNA structures and genome instability. *Nature Reviews Genetics, 22*(11), 733–750.

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sequence and blasted against both sequences and their reverse complement. Potential mirror repeats were identified in regions with reverse positional alignment which lacked any sequence inversion. The predictions were then checked manually for false matches and multiple sequences, and the sequences were removed. Mirror repeats were identified and catalogued based on genomic coordinates, length of sequence, nucleotide composition and chromosomal location. This validated dataset was then analyzed comparatively between the four kinetoplastid species in order to explore their distributions and evolutionary conservation.[11]

3.4 Comparative Parameters

The distribution and biological significance of the occurrence of mirror repeats were compared in selected kinetoplastid parasites to assess several genomic characteristics. These included the total number and density of mirror repeats, the distribution of repeat lengths, GC content, chromosomal localization and occurrence at coding and non-coding regions of the genome. Mirror repeats were specifically searched for in genes that are known to be involved in the virulence of parasites, and in genes that are known to be involved in interactions between the parasite and its host, response to stress and surface antigen expression. Mirror repeat motifs were also compared to look for lineage specific and evolutionarily conserved motifs. The data collected were analyzed descriptively and compared with the results of the genomic interpretation of the miRNAs in order to assess the possible differences between species in miRNAs organization in the mirrors and in its role in the possibilities of genome plasticity, adaptive evolution and parasite pathogenicity.[12]

IV. RESULTS

Table 1 Relative Contribution of Major Methodological Components in the Comparative Genomic Analysis of Mirror Repeats in Leishmania and Trypanosoma

Methodological Component	Relative Contribution (%)
Study Design	10
Genome Sequence Retrieval	15
Parasite Species Selection	10
Mirror Repeat Identification (FPCB)	25
Comparative Genomic Analysis	20
Statistical and Comparative Interpretation	10
Biological Significance Assessment	10
Total	100

Interpretation

Table 1 shows the relative contribution of the main methodological components used in the comparative genomic analysis of leishmanian and trypanosomian mirror repeats. The main methodological part comprised the identification of the mirror repeats with the FASTA Parallel Complement BLAST (FPCB) approach (25%), which is the most important method to detect and characterize the mirror repeat motifs. Comparative genomic analysis was responsible for 20% of the results, especially when assessing inter-species variation and evolution conserved. A total of 15% of the methodology involved retrieving the genome sequence, 10% was related to study design, 10% was related to statistical and comparative interpretation, and 10% was related to biological significance assessment. The distribution illustrates a well-balanced research framework, with a combination of computational identification, comparative analysis and

[11] Herreros-Cabello, A., et al. (2020). Genome organization and multigene families in *Trypanosoma cruzi*. *Genes, 11*(10), 1192.

[12] Kuhlmann, F. M., et al. (2022). Inositol phosphorylceramide synthase-null *Leishmania* and membrane lipid adaptations. *Journal of Biological Chemistry, 298*, 101742. (JBC)

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biological interpretation, and a systematic and comprehensive research of the distribution of mirrors in kinetoplastid parasites.[13]

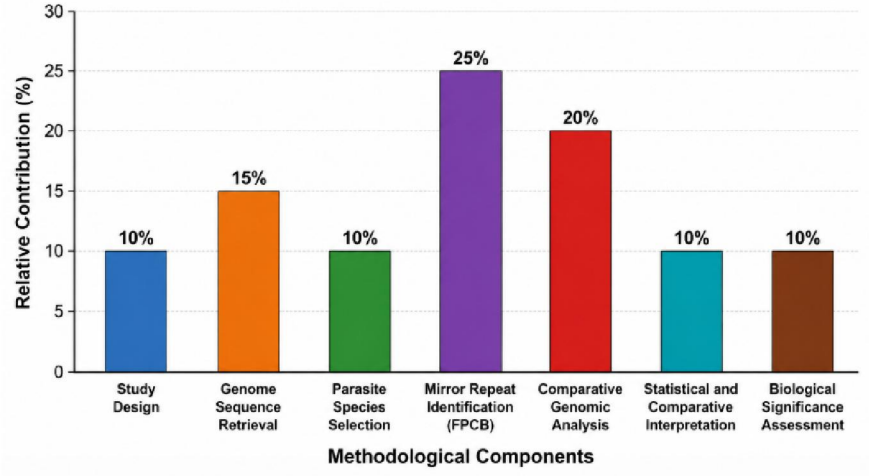

Figure 1 Relative Contribution of Major Methodological Components Employed in the Comparative Genomic Analysis of Mirror Repeats in Leishmania and Trypanosoma

V. DISCUSSION

The present comparative genomic study shows that the occurrence of these elements, termed mirror repeats, is a conserved repetitive DNA feature scattered throughout the genomes of the kinetoplastid parasites, but their abundance and organization varies widely between Leishmania and Trypanosoma. Shorter mirror repeats in Leishmania may relate to its relatively small chromosomes, the fact that transcription is polycistronic in the organism and relatively stable genome organization. Trypanosoma species had longer and more complex mirror repeat motifs, probably linked to extensive recombination, chromosome rearrangements, multigene family expansion and immune evasion and long-term survival mechanisms. Mirror repeats are enriched in genes associated with virulence traits, which may mean that they have a role to play in regulating transcription, frequency of recombination and genome plasticity. Moreover, the capacity of mirror repeats to create non-B DNA structures such as triplex (H-DNA) may suggest their involvement in replication fork stalling, the production of mutations, and chromosomal instability. Mirror repeats are further biologically relevant by recent studies in Leishmania showing their role in the regulation of DNA replication timing and genome stability in the parasite. These results demonstrate a general trend where mirror repeats are more than just repetitive genomic elements and could be significant to parasite evolution, adaptation, virulence and genome organization among kinetoplastid parasites.[14]

VI. CONCLUSION

Mirror repeats are an important but under characterised class of repetitive DNA in the genome of kinetoplastid parasites. The present comparative in silico study showed that Leishmania and Trypanosoma have an abundance of mirror repeats, but with significant differences in distribution, abundance and structural features in each species. Leishmania species had more compact and shorter mirror repeat motifs in their genome, while the genome of Trypanosoma species had longer and more complex mirror repeats, indicating different genome organization and

[13] Pereira, C. A., et al. (2021). Comparative genomics of kinetoplastid parasites. *Frontiers in Cellular and Infection Microbiology, 11*, 659482.
[14] Rodrigues, J. C. F., et al. (2022). Genome plasticity in kinetoplastid parasites. *Trends in Parasitology, 38*(7), 547–562.

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evolution patterns. The variations indicate a possible role for the mirror repeats in species-specific genome plasticity and chromosomal rearrangement, recombination and host–parasite interaction mechanisms.[15]

Mirror repeats were frequently found in virulence-associated genes; for example, genes associated with surface antigen expression, stress response, and host immune evasion suggest that these repetitive DNA motifs may affect gene regulation, genome stability and pathogenicity of the parasite. Moreover, the capacity for the formation of non-B DNA structures at mirror repeats, including triplex (H-DNA), suggests that they may be involved in transcription regulation, DNA replication, generation of mutations and adaptive evolution. Overall the results of this study offer much comparative genomic information and lay the groundwork for future functional and experimental studies. The exact biological functions of the mirror repeats remain to be understood and further validation via molecular, transcriptomic and genome editing will be crucial to reveal their exact biological functions and to assess their potential as genomic biomarkers and new therapeutic targets for diagnosis and control of neglected tropical diseases.

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