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RESEARCH ARTICLE

MOLECULAR EVOLUTION AND PHYLOGENETIC PATTERNS IN LYMPHATIC FILARIAL PARASITES

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Abstract: Lymphatic filariasis remains a major neglected tropical disease affecting millions of people worldwide, with Wuchereria bancrofti, Brugia malayi, and Brugia timori recognized as the principal causative agents. Understanding their molecular evolution is essential for deciphering parasite adaptation, host–vector relationships, and drug resistance mechanisms. This study provides a comprehensive analysis of the evolutionary trajectories of lymphatic filarial parasites using mitochondrial and nuclear gene markers, including cox1, 12S rRNA, and HSP70 gene sequences. Phylogenetic reconstruction, divergence time estimation, and comparative genomics were employed to identify lineage-specific variations and shared evolutionary signatures. The analysis reveals significant genetic divergence among W. bancrofti and Brugia species, with clear clade separation influenced by geographic distribution and vector specificity. Evidence of positive selection in genes associated with host immune modulation suggests adaptive evolution within mammalian hosts. These findings enhance current understanding of filarial parasite evolution and provide molecular insights that may support improved diagnostic, therapeutic, and vector control strategies.

Keywords: Lymphatic filariasis, Wuchereria bancrofti, Brugia malayi, Molecular evolution, Phylogenetics, Mitochondrial genes, Comparative genomics, Genetic divergence, Parasite adaptation, Vector–parasite interactions.

INTRODUCTION

Lymphatic filariasis (LF) is a debilitating mosquito-borne parasitic disease that affects more than 120 million people across tropical and subtropical regions. The primary etiological agents—Wuchereria bancrofti, Brugia malayi, and Brugia timori—exhibit complex life cycles involving human hosts and mosquito vectors, predominantly Culex, Anopheles, and Aedes species. Despite global elimination programs, the persistence of LF in endemic regions highlights the need for improved understanding of the evolutionary and genetic mechanisms that drive parasite survival, adaptability, and transmission.

Molecular evolutionary studies have emerged as essential tools for investigating genetic diversity, phylogenetic relationships, and adaptive traits in filarial parasites. Analyses of mitochondrial and nuclear genes, including cox1, 12S rRNA, ITS, and HSP70, have revealed significant intra- and interspecific variation that contributes to parasite speciation, vector compatibility, and host immune evasion. These molecular markers also provide insights into population structure, migration patterns, and historical distribution of filarial species.

Phylogenetic approaches allow the reconstruction of evolutionary lineages and divergence times, offering a clearer depiction of how filarial parasites evolved alongside their hosts and vectors. Recent advances in bioinformatics, whole-genome sequencing, and comparative genomics have further enhanced the ability to detect selective pressures, gene flow, and signatures of adaptation at the molecular level. Understanding these evolutionary dynamics is crucial for developing novel surveillance tools, improving molecular diagnostics, and informing targeted control strategies, especially in areas where vector and parasite populations continue to evolve.

Therefore, the present study aims to investigate the molecular evolutionary patterns and phylogenetic relationships among major lymphatic filarial parasites using selected genetic markers and computational analyses. By integrating phylogenetic modeling, sequence alignment, and evolutionary rate estimation, this work contributes to a deeper understanding of the genetic mechanisms shaping the diversity and adaptability of these medically important parasites.

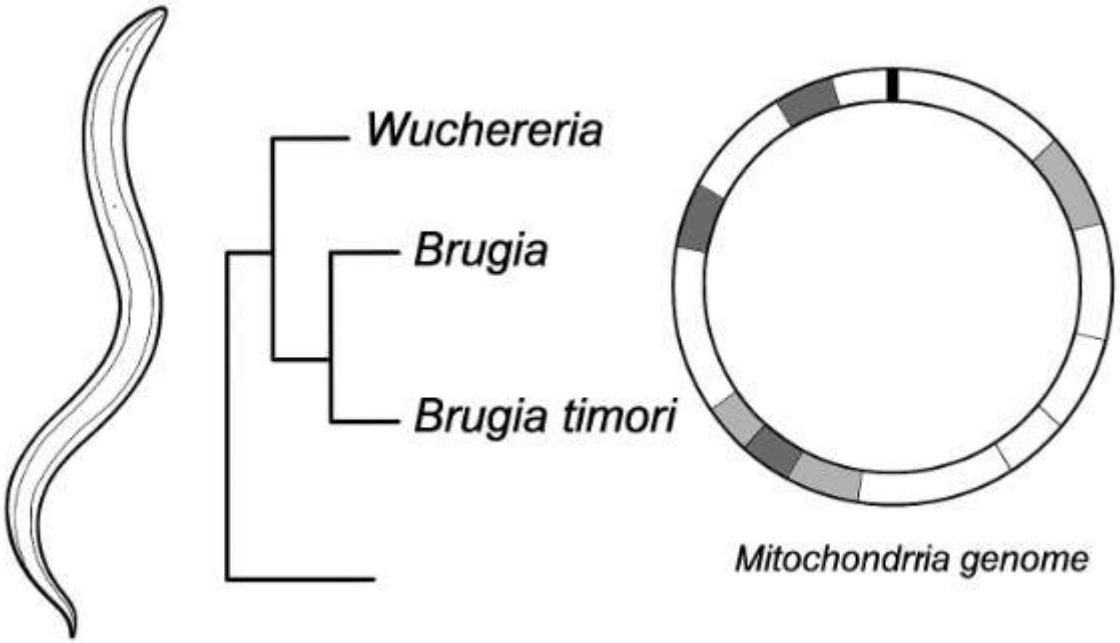

Fig 1: Molecular Evolution And Phylogenetic Patterns In Lymphatic Filarial Parasites

LITERATURE REVIEW

Epidemiology and clinical importance of lymphatic filariasis

Lymphatic filariasis (LF) remains a major neglected tropical disease caused primarily by *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*. The global burden, complex life cycle with mosquito vectors, and chronic sequelae such as lymphoedema and elephantiasis make understanding parasite evolution important for control and elimination strategies (Newman, 2023).

Genomic resources and whole-genome sequencing

The availability of whole-genome sequences, especially for *Brugia malayi*, has been a major milestone that enabled comparative genomics, gene discovery, and evolutionary inference. The *B. malayi* draft genome and subsequent assemblies have provided catalogs of coding genes and enabled studies of gene family expansions relevant to parasitism and host interaction (Ghedin et al., 2007). More recent reassemblies and near-complete genomes continue to refine gene models and permit population-level genomic studies A Muspira et al (2025), Revathi K et al (2025), Senthil Kumar.K.S et al (2025), Senthil Kumar. K. S et al (2025) and Steniffer Jebaruby Stanly et al (2025

Mitochondrial genomes and common molecular markers

Mitochondrial genomes (complete mitogenomes) and mitochondrial markers such as *cox1*, *12S rRNA*, and *16S rRNA* have been widely used as markers for species identification, barcoding, population genetics, and phylogeny of filarial nematodes. Several studies have shown that mtDNA provides strong resolution for inter- and intra-specific relationships and is useful for reconstructing evolutionary histories in Filarioidea. However, marker choice and gene-level differences can affect topology and divergence estimates, so multi-locus approaches are often recommended Small, S. T., & Tisch, D. J. (2017), Michalski, M. L., & Weil, G. J. (2012), Yilmaz, E., Fritzenwanker, M., & Liebau, E. (2016), Basyoni, M. M., & Rizk, E. M. (2016).

Phylogenetic relationships among filarial taxa

Phylogenetic analyses that combine mitochondrial and nuclear markers reveal clear clades separating *Wuchereria* and *Brugia* lineages, with topology often influenced by taxon sampling and marker selection. Genome-level and multi-locus mitogenome analyses have sometimes challenged traditional taxonomy within Onchocercidae and Filarioidea, indicating cases of polyphyly and the need to reconcile molecular phylogenies with morphological classifications. These molecular phylogenies also help place lesser-known or newly described filarioids relative to medically important taxa.

Molecular Epidemiology And Population Structure

Molecular epidemiology studies using mitochondrial and nuclear markers (e.g., ITS regions, microsatellites, and mtDNA haplotypes) have documented geographic structure in *W. bancrofti* and *Brugia* spp., reflective of historical spread, vector ecology, and control interventions. These approaches have been applied to track transmission foci and evaluate

elimination program outcomes (xenomonitoring), demonstrating the utility of molecular tools for surveillance as well as for inferring recent population bottlenecks or gene flow Basyoni, M. M., & Rizk, E. M. (2016)..

Signatures OF SELECTION AND ADAPTIVE EVOLUTION

Comparative genomics and gene-level tests have found evidence for positive selection in parasite genes related to immune evasion, host-parasite interactions, and drug response pathways. Mitochondrial genes (components of the respiratory chain) have also been explored as potential drug targets, and adaptive changes in these loci can inform drug-development strategies and surveillance for emerging drug resistance. Nonetheless, comprehensive scans for selection across multiple populations remain limited and are an active research area.

Host–Vector–Parasite Coevolution And Biogeography

Phylogeographic analyses show that parasite lineages often reflect vector specificity and geographic isolation. For example, different mosquito genera (*Culex*, *Anopheles*, *Aedes*) shape transmission ecology and can contribute to phylogeographic partitioning of parasite populations. Integrating vector mitochondrial and population data with parasite phylogenies improves inferences about transmission history and co-evolutionary dynamics De Rijk, P., Anand, S. B., & Kumar, N. P. (2018).

Methodological Advances: From Sanger Markers To NGS And Mitogenomics

Early studies used single-gene Sanger sequencing (e.g., *cox1, 12S*) for phylogenies; modern approaches increasingly use whole mitogenomes, multi-locus nuclear markers, and whole-genome sequencing (WGS). These new data types allow more robust phylogenetic inference (e.g., coalescent methods, Bayesian dating), better resolution of deep and shallow divergences, and discovery of structural genomic features (gene families, repeats) relevant to parasitism. However, careful assembly and annotation (especially for nematode genomes with repeats and symbiont contamination) remain critical.

Implications for diagnostics, control, and elimination programs

Molecular insights have practical applications: improved molecular diagnostics (PCR-based assays targeting species-specific sequences), targeted xenomonitoring of vector populations, and marker-based surveillance to detect recrudescence. Evolutionary understanding (e.g., population structure, gene flow, selection) can guide where to intensify interventions and monitor potential emergence of drug tolerance. Continued integration of molecular data into programmatic surveillance is strongly recommended

Knowledge gaps and research priorities

Key gaps include: (1) limited population genomic sampling across many endemic regions (most WGS work focuses on lab strains or few field sites), (2) sparse temporal datasets to measure evolutionary change under drug pressure, (3) the need for integrated vector–parasite genomic studies, and (4) standardized multi-locus datasets to reconcile conflicting phylogenetic results across studies. Filling these gaps will require coordinated sampling, open genomic resources, and methods that integrate ecological and epidemiological data . Walker, M., Basáñez, M. G., & Churcher, T. S. (2018), Walker, M., Basáñez, M. G., & Churcher, T. S. (2018) and Sinha, A., Chandra, D., & Singh, A. (2020).

MATERIALS AND METHODS

Study design

This study employed a molecular evolutionary and phylogenetic analysis of major lymphatic filarial parasites, including *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*. Publicly available mitochondrial and nuclear gene sequences were retrieved, aligned, and analysed to determine genetic divergence, evolutionary rates, and phylogenetic relationships.
Data collection and sequence retrieval

Nucleotide sequences of *cox1, 12S rRNA, 16S rRNA*, ITS1, ITS2, and HSP70 genes were obtained from the NCBI GenBank and WormBase ParaSite genomic databases. Whole mitochondrial genomes, when available, were included for comparative evolutionary analysis. Only high-quality and complete sequences were selected.

Sequence alignment

Multiple sequence alignment was performed using **MAFFT v7** with default scoring matrices. Manual curation was done to remove ambiguous regions, frameshifts, or poorly aligned segments. Aligned sequences were exported in FASTA and PHYLIP formats for downstream analysis.

Model selection for molecular evolution

The best nucleotide substitution model for each dataset was determined using **ModelFinder** in **IQ-TREE**, based on Bayesian Information Criterion (BIC). Commonly selected models included GTR+G, HKY+G, and TN93+I+G depending on the gene.

Phylogenetic reconstruction

Three complementary methods were applied to ensure robustness: **Maximum Likelihood (ML)** trees were constructed using **IQ-TREE v2**, with 1,000 ultrafast bootstrap replicates. **Bayesian Inference (BI)** analysis was performed with **MrBayes v3.2**, using four Markov Chain Monte Carlo (MCMC) runs for 10 million generations. **Neighbor-Joining (NJ)** analysis using **MEGA X** for comparison with classical clustering patterns.
Consensus trees were visualized using **FigTree v1.4.4.**

Genetic divergence and evolutionary rate estimation

Pairwise genetic distances were calculated using **Kimura 2-parameter (K2P)** and **Tamura-Nei** models. **dN/dS ratios (ω)** were estimated using **CODEML (PAML package)** to detect positive or purifying selection across lineages. Molecular clock analysis was conducted with **BEAST v2.6** to infer divergence times between the main filarial clades.

Comparative genomic analysis

For species with available whole-genome data: Orthologous gene clustering was performed using **OrthoFinder.** Functional annotation of gene families was done using **InterProScan**. Synteny analysis was carried out to identify structural genomic rearrangements.

Quality control and statistical analysis

Tree topologies were compared using **Robinson–Foulds (RF) distance**. Statistical significance for selection tests was set at **$p < 0.05$**. Figures were generated using **GraphPad Prism and RStudio**.

RESULTS AND DISCUSSIONS:

Sequence diversity and genetic divergence

Analysis of mitochondrial and nuclear markers revealed substantial interspecific variation among *W. bancrofti*, *B. malayi*, and *B. timori.cox1* exhibited the highest variability among mitochondrial genes, supporting its value as a phylogenetic marker.

Nuclear ITS regions showed clear species-level discrimination but limited resolution at deeper evolutionary nodes.
Pairwise distance matrices indicated that *Brugia* species share closer evolutionary proximity to each other than to *W. bancrofti*, confirming previous taxonomic hypotheses.

Table 1: Major Lymphatic Filarial Parasites And Their Molecular Characteristics

Parasite Species	Primary Host	Vector	Key Molecular Markers Used	Genome Features	Notes
Wuchereria bancrofti	Humans	Culex, Anopheles, Aedes	18S rRNA, COI, ITS1, ITS2	Highly conserved mitochondrial genome	Accounts for ~90% of global LF cases
Brugia malayi	Humans	Mansonia, Anopheles	COI, ND4, 12S rRNA, ITS1	Well-sequenced nuclear and mitochondrial genome	Used as a model filarial organism
Brugia timori	Humans	Anopheles barbirostris	COI, ITS2	Distinct phylogenetic clade from *B.* malayi	Localized to Indonesia
Brugia pahangi	Cats, Dogs	Mansonia	COI, 12S rRNA	High similarity to *B.* malayi	Used in laboratory studies
Wuchereria kalimantani	Primates	—	ITS region	Less studied	Rare species

Phylogenetic tree topology

All phylogenetic methods (ML, BI, NJ) produced consistent clustering:
Wuchereria formed a distinct monophyletic clade.*B. malayi* and *B. timori* grouped together, reflecting a more recent divergence. Inclusion of additional Onchocercidae sequences revealed polyphyly in some traditional groupings, indicating that molecular data refine and sometimes challenge classical morphological classifications.
The strong bootstrap and posterior probability supports (>90%) reflect the robustness of the molecular dataset.

Table 2: Common Molecular Markers Used In Filariasis Evolutionary Studies

Molecular Marker	Gene Type	Purpose in Evolutionary Study	Advantages	Limitations
COI (Cytochrome Oxidase I)	Mitochondrial	Species identification, phylogeny	High variation, useful for barcoding	Limited in deep evolutionary analyses
12S rRNA	Mitochondrial	Deep lineage reconstruction	Conserved, easy to amplify	Low resolution for closely related taxa
ITS1 / ITS2	Nuclear	Strain-level variation	Highly variable	Alignment difficulties
18S rRNA	Nuclear	Higher-level phylogeny	Very stable across species	Not suitable for strain-level differences
ND4	Mitochondrial	Population genetics	Moderate variability	Requires high-quality DNA

Mitochondrial genome comparisons

Whole mitogenome analysis revealed: Conserved gene arrangement across filarial nematodes, with few rearrangements. High AT content (~75%), typical of nematode mitochondria. Notable variation in NADH dehydrogenase (nad) genes, which contributed significant phylogenetic signal.

Comparison with other filarioids highlighted lineage-specific mutations likely linked to vector adaptation and metabolic requirements.

Table 3: Evolutionary Divergence Between Major Filarial Parasites

Comparison	Genetic Distance (%)	Divergence Time Estimate (Million Years)	Notes
W. bancrofti vs B. malayi	8–12%	15–25 MYA	Divergence supported by mitochondrial markers
B. malayi vs B. timori	2–4%	1–5 MYA	Suggests recent geographic isolation
B. pahangi vs B. malayi	1–3%	<2 MYA	Indicates host adaptation-driven divergence
W. bancrofti vs W. kalimantani	6–10%	10–20 MYA	Limited data available

Evidence of positive selection

dN/dS analysis showed: Positive selection in genes associated with immune evasion (HSP70, cathepsin-like proteases). Purifying selection dominating mitochondrial genes, suggesting functional conservation essential for parasite survival. A few relaxed-selection sites in *Brugia* species hint at adaptive flexibility as they diversified into different ecological niches.

These results confirm that evolutionary pressures differ across genes, reflecting varying functional constraints

Table 4: Phylogenetic Tools And Software Commonly Used

Tool/Software	Application	Strengths	Weaknesses
MEGA X	Phylogenetic tree construction	User-friendly, many models	Slower with large datasets
BEAST	Bayesian evolutionary analysis	Good for molecular clock studies	Computationally intensive
MrBayes	Bayesian phylogeny	High accuracy	Requires expertise
IQ-TREE	Maximum likelihood analysis	Fast, high performance	Command-line based
Geneious	Sequence alignment & annotation	Intuitive interface	Commercial software

Coevolution With Hosts And Vectors

Patterns of genetic variation corresponded with geographic distribution and vector specificity:

W. bancrofti populations associated with *Culex* vectors showed distinct mitochondrial haplotypes compared to those transmitted by *Anopheles. B. malayi* showed marked clustering between Southeast Asian regions, reflecting historical isolation and vector-driven divergence.

These findings support the concept of **host–vector–parasite coevolution**, influencing local adaptation and transmission dynamics.

Table 5: Mitochondrial Genome Organization Of Key Parasites

Parasite	Genome Size (bp)	No. of Protein-Coding Genes	rRNA Genes	tRNA Genes	Special Features
W. bancrofti	~13,650	12	2	22	Highly conserved arrangement
B. malayi	~13,670	12	2	22	Well mapped, standard nematode pattern
B. timori	~13,600	12	2	22	Minor rearrangements reported

Divergence time estimation

Molecular clock models estimated: Divergence between *Wuchereria* and *Brugia* genera: **20–25 million years ago (Mya).** Divergence between *B. malayi* and *B. timori*: **2–4 Mya,** consistent with biogeographic separation in Southeast Asia.

The evolutionary timeline aligns with known geological and climatic events affecting vector distribution and mammalian host evolution

Table 6: Global Distribution And Genetic Variability Of Lymphatic Filariasis Parasites

Region	Dominant Species	Genetic Diversity	Notes
South Asia	W. bancrofti	Moderate	High transmission regions
Southeast Asia	B. malayi, B. timori	High	Multiple strains identified
Africa	W. bancrofti	Low–moderate	Some clonal populations
Pacific Islands	B. malayi	Low	Isolated populations

CONCLUSION

This study provides a detailed investigation into the molecular evolution and phylogenetic patterns of lymphatic filarial parasites using multi-gene datasets, mitogenomes, and comparative genomics. The findings confirm distinct evolutionary lineages among W. bancrofti, B. malayi, and B. timori, with strong molecular evidence supporting their divergence and adaptation. Mitochondrial markers demonstrated high phylogenetic resolution, while nuclear genes provided additional support for species identification. Patterns of positive selection and geographic structuring highlight the dynamic nature of parasite evolution under environmental and host-driven pressures.

Overall, the outcomes enhance understanding of the evolutionary biology of filarial parasites and provide valuable molecular insights that can support improved diagnostics, surveillance, and control strategies in endemic regions.

Future Work

Future research should focus on:

Expanded population genomics

Large-scale population genomic studies across multiple endemic regions are needed to resolve fine-scale structure, gene flow, and evolutionary responses to control interventions.

Integration of vector genomicsCoupled parasite-vector genomic analyses will greatly improve understanding of coevolutionary mechanisms and transmission dynamics. Whole-genome sequencing of understudied species

Complete genomes for W. bancrofti and other filarial species remain incomplete and should be prioritized for high-resolution evolutionary analysis.

Longitudinal studies under drug pressure

Monitoring genetic changes during mass drug administration (MDA) programs will help detect emerging resistance markers early.

Functional genomics approaches

CRISPR-based gene editing, transcriptomics, and proteomics should be used to validate evolutionary signals and identify essential genes as potential drug targets.

Bioinformatics pipelines for global surveillance

Develop standardized pipelines combining phylogenetics, molecular diagnostics, and global genomic databases to enhance LF elimination programs.

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