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# Mitochondrial Genome of *Brugia malayi* Microfilariae Isolated From a Clinical Sample

*Xiong Qing*[1†], *Kasem Kulkeaw*[2†], *Sirichit Wongkamchai*[2*] *and Stephen Kwok-Wing Tsui*[1,3,4*]

[1] School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong, Hong Kong, [2] Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand, [3] Hong Kong Bioinformatics Center, The Chinese University of Hong Kong, Hong Kong, Hong Kong, [4] Center for Microbial Genomics and Proteomics, The Chinese University of Hong Kong, Hong Kong, Hong Kong

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***Correspondence:**
Stephen Kwok-Wing Tsui
kwtsui@cuhk.edu.hk
Sirichit Wongkamchai
sirichit.won@mahidol.ac.th

[†]These authors have contributed equally to this work and share first authorship

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Lymphatic filariasis is a neglected parasitic disease that is a leading cause of long-term disability. Information obtained from genome sequencing of filarial worm can help us identify systems in the worm that are likely to be useful for novel drug design. *Brugia* (*B.*) *malayi* is still the only lymphatic-dwelling filarial parasite with a nearly complete, fully annotated, and published genome. However, most previous studies were based on the FR3 strain of *B. malayi*, which originally was isolated from a human patient, and was adapted to the rodent model, then maintained in laboratories for more than 60 years. It is uncertain whether genetic variation exists, thus, sequencing of clinical isolates of lymphatic dwelling filarial parasites is a high priority. Here, we report for the first time the complete mitochondrial genome of *B. malayi* microfilariae from clinical isolate. Complete mitochondrial (mt) genome of the microfilariae isolated from a blood sample taken from a Thai subject living in Narathiwat Province, which is an endemic area of brugian filariasis, was assembled with sequencing reads obtained by Illumina sequencing. Gene annotation, phylogenetic analysis and single nucleotide polymorphism (SNP) were deployed. A complete 13,658-bp mt genome of *B. malayi* microfilaria was obtained, and it shows 68x coverage. Based on gene annotation, the mt genome consists of 12 protein-coding, two rRNA, and 23 tRNA genes. Phylogenetic analysis using all protein sequences of DNA sequences of mt genome or cytochrome c oxidase subunit I (*COX1*) revealed a close relationship among three lymphatic filariae (i.e., *B. timori*, zoonotic *B. pahangi*, and *Wuchereria* spp.). The SNPs in the *COX1* gene can differentiate microfilariae of *B. malayi* in human from those found in canine. Furthermore, the number, order and transcription, and direction of *B. malayi* microfilariae mitochondrial genes were the same as those found in the FR3 strain of *B. malayi*. The comparison on mitochondrial genome of *B. malayi* could have important implications on the development of a new intervention or vaccine to treat or prevent this disease in endemic areas/regions around the world.

**Keywords: *Brugia malayi*, microfilaria, mitochondrial genome (mtDNA), phylogeny, filariasis**

## INTRODUCTION

Lymphatic filariasis is an often-neglected tropical disease that adversely affects society and economics due to associated high morbidity, social stigmatization, and inability to work. In 2000, the World Health Organization launched the Global Programme to Eliminate Lymphatic Filariasis with the aim to eliminate lymphatic filariasis by 2020 (Turner et al., 2016). The primary pillar of this program is mass drug administration of diethylcarbamazine, albendazole, and ivermectin. However, antifilarial drug resistance has emerged (Schwab et al., 2005; Cobo, 2016), which presents a threat to the success of this treatment and elimination program. Genetic mutation of the parasite due to mass drug administration for several years is hypothesized to be an underlying cause of drug resistance (Schwab et al., 2005). To cope with drug resistance and to pave the way for the development of more effective drugs, data specific to the genetic variability of this parasite among different geographic areas are needed.

Mitochondrion, which is an intracellular organelle, functions in adenosine 5′-triphosphate (ATP) production for cellular respiration *via* oxidative phosphorylation and the citric acid cycle. In metazoans, including the *Onchocercidae* family, the mitochondria have their own genome, which is a small, circular, double-stranded DNA sequence with an average length of 15–20 kb (Boore, 1999). Metazoan mitochondrial DNA (mtDNA) typically encodes 37 genes, including 13 protein subunits of electron transport in oxidative phosphorylation, two rRNAs, and 22 tRNAs, which are for translation of proteins encoded by mtDNA. Recent complete mitochondrial genome sequencing of the filarial nematode *Wuchereria bancrofti* from isolates from three different geographic regions yielded evidence of a complex demographic history. Complete mt genome of *W. bancrofti* was performed *via* analysis of isolates from Papua New Guinea, India, and West Africa (Ramesh et al., 2012). A mitochondrial gene of the heme-dependent respiratory chain was reported to be a potential target for the development of a new anti-filarial drug (Strubing et al., 2010). For these reasons, mtDNA is useful for identifying drug targets, and for studying genetic variability in the same parasite from different regions.

*Brugia malayi* is still the only lymphatic-dwelling filarial parasite with a nearly complete, fully annotated, and published genome. Whole genome and mitochondrial genome of *B. malayi* are available; however, the database is derived from a laboratory strain of adult *B. malayi* FR3 (Ghedin et al., 2007). Since the FR3 strain has been maintained in Mongolian gerbil for many years, it is uncertain whether genetic variation exists. The sequencing of clinical isolates of lymphatic-dwelling filarial parasites is a high priority (McNulty et al., 2013). The present study reports for the first time the complete mitochondrial genome of microfilarial stage of *B. malayi* directly isolated from clinical blood sample of human subject living in an endemic area of nocturnally subperiodic *B. malayi* in Southern Thailand.

## MATERIALS AND METHODS

### Microfilaria Isolation and DNA Extraction

Microfilariae of *B. malayi* were isolated from blood samples from three Thai subjects (named BM2, BM3, and BM4) living in the Sugnai-Padee District of Narathiwat Province in Thailand, which is an endemic area of nocturnal subperiodic *B. malayi*, using a semi-automated microfluidic device developed by Phuakrod et al. (2019). DNA was extracted from microfilariae using a High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH, Penzberg, Germany) according to the manufacturer's instructions. DNA concentration was determined using a NanoDrop Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), after which the DNA was used as the template for sequencing.

### DNA Sequencing, *de novo* Assembly, and Genome Annotation

Genomic DNA sample was sequenced to totally obtain 44.1 Gb data of Illumina short reads in three samples using Novaseq PE150 technology in 2 × 150 paired-end library with 500-bp insertion (**Supplementary Table 2**). After mapping to the host *Homo sapiens* genome (GRCh38.85) and removed by Bowtie2 v2.3.4.3 and Samtools v1.9 (**Supplementary Table 2**), 1.06-Gb remaining data from the BM2 sample were *de novo* assembled by SPAdes v3.11.0. The mitochondrial genome was *de novo* assembled into a single contig (named BM2 mitochondrion) and annotated by the online tool GeSeq, which combines Blat, Hmmer, Aragorn v1.2.38, Arwen v1.2.3, and tRNAscan-SE v2.0.5, and which is visualized by OGDRAW v1.3.1. Secondary structure of tRNA was predicted using ARWEN v1.2 (Laslett and Canback, 2008). Pairwise dot plot was created with the mitochondrial genome of *B. malayi* FR3 strain (accession no. AF538716) by Gepard 1.40. According to the dot plot, originally assembled BM2 mitochondrial genome sequence was adjusted into the final version as the mitochondrial genome of *B. malayi* FR3 strain.

### Multiple Sequence Alignment and Phylogenetic Analysis

All mitochondrial protein sequences and cytochrome c oxidase subunit I (*COX1*) gene sequences were downloaded from NCBI database. The mitochondrial protein sequences of *B. malayi*, *B. timori*, *B. pahangi*, *W. bancrofti*, *Loa loa*, *Onchocera volvulus*, *Dirofilaria repens*, *D. immitis*, *O. ochengi*, *O. flexuosa*, and *Acanthocheilonema viteae* were obtained. For the whole mitochondrial phylogeny (**Figure 2**), protein sequences of all 12 annotated mitochondrial genes were collected and aligned independently, and then multiple alignment results of 12 annotated mitochondrial proteins were merged for phylogenetic analysis. For the *COX1*-based phylogeny (**Figure 3**), DNA sequences of *COX1* genes of *B. malayi*, *B. timori*, *B. pahangi*, and *Gongylonema pulchrum* were collected and aligned. MEGA v7.0.26 was used for multiple sequence alignment with Clustal W and phylogenetic analysis in Maximum Likelihood method

with 90% site coverage and 100 bootstrap replicates, as well as for identification of single nucleotide polymorphism.

## Data Collection and Access

The complete mitochondrial sequence of *B. malayi* is available upon request to the corresponding author and was deposited in GenBank (accession no. MT149211). All sequencing data (removed human reads) are available in NCBI (BioProject ID: PRJNA613552).

## Ethical Consideration

The study's research protocol for the use of blood samples from human subjects was approved by the Human Research Protection Unit, Faculty of Medicine Siriraj Hospital, Mahidol University (COA no. Si459/2019).

# RESULTS

## *De novo* Assembly and Genome Annotation

*De novo* assembled circular mitochondrial genome of *B. malayi* has a 13,658-bp sequence and shows 68x coverage with no heterozygosity observed in the sample BM2 (**Figure 1A**) (hereafter referred to as BM2 mt). Regarding type of nucleotide composition, the BM2 mt genome has 24.48% GC content. BLASTn of *de novo* assembled BM2 mt showed 99.72% identity to that of *B. malayi* FR3 strain (accession no. AF538716). Genome annotation revealed 12 protein-coding, two rRNA, and 23 tRNA genes (**Supplementary Table 1**), and they are summarized in **Figure 1B**. Arrangement of the mitochondrial genes of BM2 mt was similar to that of *B. malayi* FR3 strain (**Figures 1C,E**). The extra and duplicated lysine (K) tRNA was annotated in BM2 mt (**Figure 1F**), while the DNA sequence of this lysine (K) tRNA also identically exists in the mitochondrial sequence of *B. malayi* FR3 strain, but did not annotated. Cyclization of the assembled mitochondrial sequences was confirmed in dot plot with the mitochondrion of FR3 strain (**Figure 1D**) and the final mitochondrial sequences were adjusted with the complementary strand and the same start site as the mitochondrion of FR3 strain. Among the annotated 23 tRNA genes, two leucine-tRNA (trnL) and two lysine-tRNA (trnK) genes were identified. There were two sets of trnL, including trnL-UAG and trnL-UAA. However, in contrast to the FR3 isolate, two sets of tRNA-lysine (trnK-CUU and trnK-UUU) were also observed in the BM2 mt. The trnK-UUU was predicted at position 4,561–4,615 of the BM2 mt genome which is a region that overlaps with cytochrome b (*COB*) (4,578–5,664), and the sequence of this trnK-UUU also identically existed in the mitochondrial genome of FR3 but not annotated. The 5′-end of *COB* gene overlapped with the 3′-end of trnK (**Figure 1E**; **Supplementary Table 1**). According to ARWEN, a tool for detection of tRNA in metazoan mitochondrial DNA, a D-loop structure of trnK-UUU was predicted (**Figure 1F**). Moreover, mitochondria-specific ATP synthase 6 was identified. According to the arrangement of genes in the mitochondrial genome, all annotated genes were predictably transcribed in the same direction of plus strand (**Figure 1C**). The region between COX3 and trnA-UGC is the AT-rich control region for replication initiation.

## Phylogeny of the Mitochondrial Genome of Family Onchocercidae

Lymphatic filariasis that results from *B. malayi*, *B. timori*, and *W. bancrofti* causes high morbidity, followed by loasis and onchocerciasis (Zoure et al., 2011; Hotez et al., 2014). Therefore, we decided to compare the protein sequences of all 12 annotated mitochondrial genome of *B. malayi* with those of *B. timori*, *B. pahangi*, *W. bancrofti*, *Loa loa*, and *Onchocera volvulus*, as well as the zoonotic filarial nematodes *B. pahangi*, *Dirofilaria repens*, and *D. immitis*. To identify genetic variability within the *Onchocercidae* family, protein sequences of all 12 annotated mtDNA of *O. ochengi* (causative agent of cutaneous onchocerciasis in cattle), *O. flexuosa* (causative agent of onchocerciasis in deer), and *Acanthocheilonema viteae* (causative agent of rodent filariasis) were phylogenetically analyzed (**Figure 2**). The mtDNA of *Gongylonema pulchrum*, a parasitic nematode, was used as outgroup control.

Mitochondrial genome-based phylogenetic analysis categorized the aforementioned parasite species in a biological niche-specific manner. Depending on their habitat of adult worm within the host, there were three major groups, as follows: (1) lymphatic system, (2) skin and subcutaneous, and (3) heart and lung. Phylogenetic tree of the BM2 mt genome was in a clade of lymphatic-dwelling parasites, and was found to be most closely related to the *B. malayi* FR3 strain (accession no. AF538716). Moreover, next to *B. timori*, *B. pahangi*, which is a filarial nematode that is naturally found in cats and it causes human lymphatic filariasis, is proximate to *B. malayi* of human isolate in this study (**Figure 2**).

## Phylogeny of the COX1 Genes of *B. malayi*

Using phylogenetic analysis to identify a potential source of human Brugian filariasis, we set forth to compare the mt genome sequences of *Brugia* isolates in Thailand. However, given the unavailability of the mt genome sequence of Thai isolates, we used the *COX1* gene for phylogenetic analysis. As shown in **Figure 3**, *COX1*-based phylogenetic analysis showed a similar result, in which BM2 isolate is closely related to the FR3 laboratory strain. Interestingly, several isolates of *B. malayi* that were collected from domestic dogs in Bangkok, Thailand were found to be close to the human isolate (BM2) identified in the blood of a man living in Narathiwat Province, Thailand, which suggests a potential reservoir host.

## Single Nucleotide Polymorphism (SNP) of COX1 Gene

To examine type and codon alteration of the SNPs identified in the assembled *COX1* gene, we compared the DNA sequence of the *COX1* gene in this study with that of the FR3. There were six SNPs altered in at least two isolates and identified, as follow: C2511T, C2514T, T2637C, T2799C, G3000A, and C3130T (**Figure 4A**). None of SNPs had altered codons of isoleucine, glycine, phenylalanine, arginine, or leucine. Since *COX1* gene sequences of canine *B. malayi* microfilaria were available, and all were isolated from domestic dogs in Thailand, we aligned the DNA sequences of the COX1 genes of human isolates in

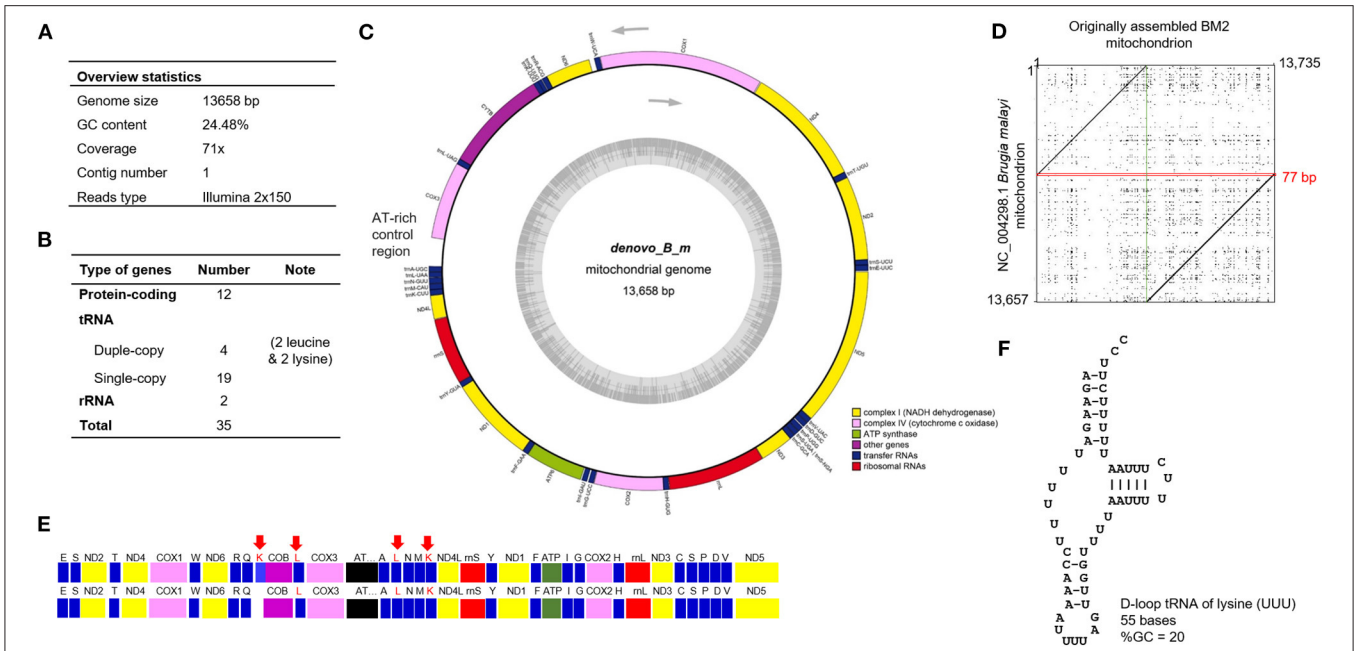


**FIGURE 1 |** Whole mitochondrial genome of *B. malayi* isolated from clinical blood samples. **(A)** Overview of mitochondrial genome of *B. malayi*. **(B)** Summary of gene types annotated from mitochondrial genome of *B. malayi*. **(C)** A circular layout of mt genome visualized using GeSeq. **(D)** Dot plot with the sequences of originally assembled BM2 mitochondrion and the mitochondrion of FR3 strain (accession no. AF538716). Overlapped 77-bp sequences on the originally assembled BM2 mitochondrion were marked in the red square, while the junction site without any overlap of two sequences was marked with the vertical green line. **(E)** Illustration of genes arranged in mt genome of *de novo* assembled BM2 (upper) and AF538716 (lower). Each type of mt gene is illustrated as a colored box: tRNA (blue), cytochrome (violet), NADH dehydrogenase (yellow), cytochrome c oxidase (pink), ribosomal subunit RNA (red), and ATP synthase (green). Arrows indicate double copy of annotated leucine- and lysine-tRNA genes. **(F)** Secondary structure of predicted trnK-UUU using ARWEN.

this study with those of the FR3 strain and canine *B. malayi* microfilaria using Clustal Omega. As shown in **Figure 4B**, we observed with following three SNPs, which are specific to human isolates: C2511T, C2514T, and C3130T.

## Comparison of Mitochondrial Proteins of the *de novo* Assembled BM2 and the FR3 Strain

Totally, 38 nucleotides are not identical between mitochondrial genomes of *de novo* assembled BM2 mt and the FR3 strain and cause amino acid changes in nine proteins. The amino acid sequences of *COX1* of BM2 mt and AAN17806 derived from the FR3 strain (AF538716) were subjected to Phyre2 for modeling protein structure. The C1M56G, a cytochrome c oxidase of the gram-negative facultative photosynthetic bacterium *Rhodobacter sphaeroides*, was matched, and it ranked number 1 with both the BM2 mt and AAN17806 sequences. The matches were ranked based on the number of aligned residues and the quality of alignment (similarity of residue probability distributions for each position), secondary structure similarity, and the presence or absence of insertions and deletions. Overall, the 513 residues (93% of the sequence) of COX1 of BM2 mt were modeled with 100.0% confidence by the single highest scoring template. Thus, the secondary structure of C1M56G was used as a template for protein structure prediction. Despite a serial of amino acid changes (**Supplementary File 1a**, boxed), secondary structure of the protein remains unchanged (**Supplementary File 1b**). Given amino acid changes detected in other mitochondrial proteins (NDL4, COB, ND1, ND5, ND4, COX3, ND2, and ATP6), none of them predictively altered the secondary structure of protein (**Supplementary File 2**). Although there were amino acid changes in ND6 of BM2 mt; however, there were only fragments of the secondary structures of the deposited database matched with ND6, prohibiting prediction of alteration of ND6 protein structure (**Supplementary File 3**).

## DISCUSSION

To our knowledge, this study reports the first complete mitochondrial genome of microfilarial stage of *B. malayi* isolated from a clinical isolate obtained from a Thai subject that lives in Southern Thailand. Major hurdle in genome sequencing of human-isolated *B. malayi* is an insufficient number of microfilariae circulating in blood of human host. Moreover, in an endemic area microfilaria nocturnally circulate in blood in a subperiodic manner, making the isolation of microfilaria-containing blood a difficult task. With many efforts and the recent advance in next generation sequencing, the complete mitochondrial genome of a *B. malayi* strain in the human host were successfully obtained at a high accuracy.

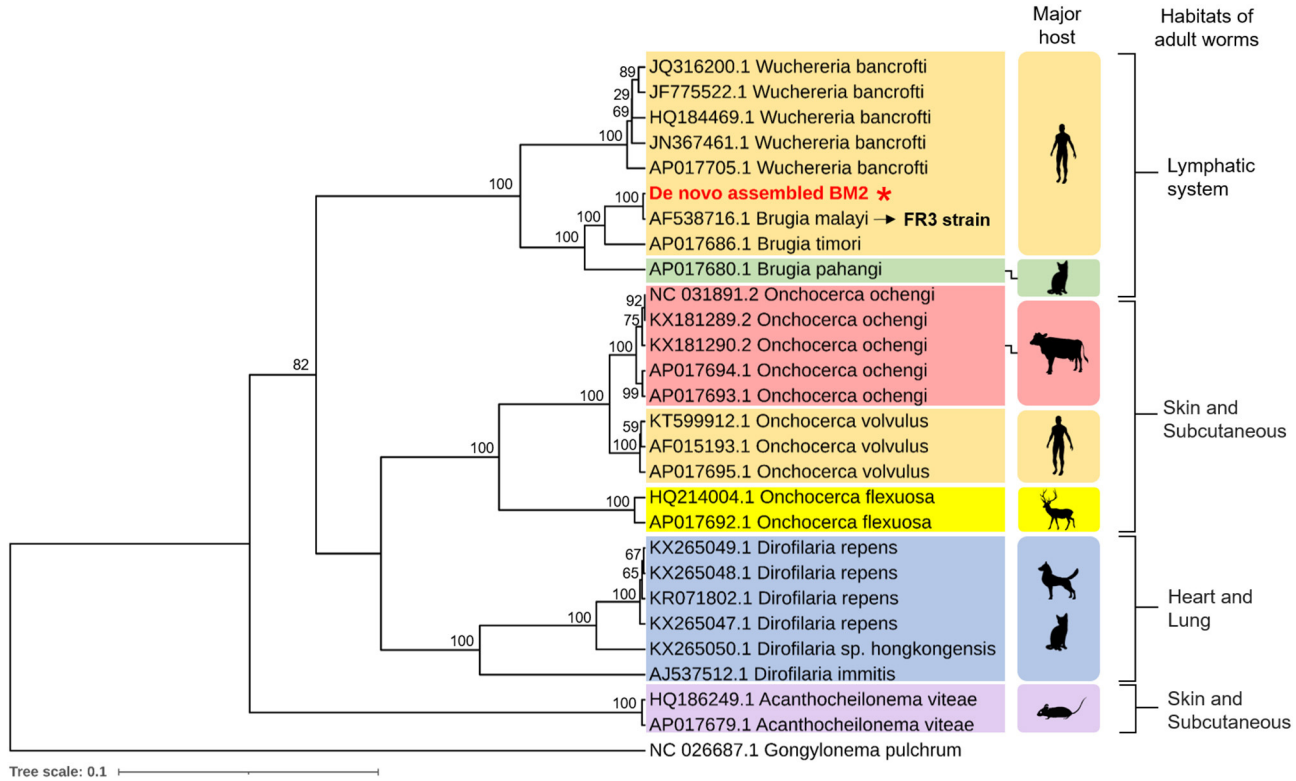


**FIGURE 2 |** Phylogenetic analysis of mt genome. The protein sequences of all 12 annotated mitochondrial genome of causative agents of human filariasis (*B. malayi*, *B. timori*, *B. pahangi*, *W. bancrofti*, *Loa loa*, and *Onchocera volvulus*), zoonotic filariasis (*B. pahangi*, *Dirofilaria repens*, and *D. immitis*), and cutaneous onchocerciasis in cattle (*O. ochengi*), onchocerciasis in deer (*O. flexuosa*), and rodent filariasis (*Acanthocheilonema viteae*). Major hosts and their habitats of adult worms within the host are indicated on right hand side. The protein sequences of all 12 annotated mt genome of *de novo* assembled BM2 was compared to that of *B. malayi* FR3 strain (accession no. AF538716). The mt genome of *Gongylonema pulchrum*, a free-living nematode, was used as outgroup control.

## Importance of Mitochondrial Genome of Microfilarial *B. malayi*

Mitochondrial genome sequences are widely used as molecular markers for phylogenetic studies. Mitochondrial genome sequencing is often preferred to whole genome sequencing. Given the rapid evolution of mitochondrial genome in animals compared to nuclear genome, the mitochondrial genome is useful for study of genetic variability and phylogenetic relation (Joy et al., 2006; Hassanin et al., 2013; Tyagi and Das, 2015; Yilmaz et al., 2016, 2019). Second, the shorter length of the mitochondrial genome makes the sequencing process easier. Third, since the adult worm habitat is in the lymphatic system of the human host, collection of adult worms is difficult and requires an invasive biopsy of tissue. Fourth, phylogenetic analysis in this study showed that *B. timori*, a filarial nematode causing human lymphatic filariasis, is proximate to *B. malayi* from human isolate. Thus, the use of mt genome for phylogenetic analysis is comparable to that of whole genome. Moreover, a mitochondrial gene of heme-dependent respiratory chain was reported to be a potential target for the development of new drugs (Strubing et al., 2010). Thus, anti-microfilaricidal drug targeting mitochondrion remains an effective therapy.

## Success and Failure of DNA Sequencing and *de novo* Assembly

Among three DNA samples, we could obtain most reads from one sample (BM2). The main reasons for failure include: (1) the number of microfilariae in the blood vary depending on host and time of blood collection; and, (2) the integrity and purity of DNA samples. Although the success rate was low, the accuracy of the mtDNA sequence was higher than that of *W. bancrofti* microfilaria (Ramesh et al., 2012), as indicated by 68x coverage. The high accuracy of the mtDNA allowed us to construct a map of the mitochondrial genome and identify gene duplication and a unique arrangement of mitochondrial genes, which is information that was lacking in the study of *W. bancrofti* microfilariae.

## Comparing Mitochondrial Genome Sequences of Microfilaria

Like nuclear genome-based phylogenetic analysis, mt genome of BM2 *B. malayi* is closely related to that of *B. timori* and zoonotic *B. pahangi* (Lau et al., 2015). Given that the genome of *O. volvulus* is available, phylogenetic tree of mt genome of *B. malayi* resembled that of *O. volvulus* (Cotton et al., 2016). In agreement with the previous analysis of Lefoulon et al., our mt

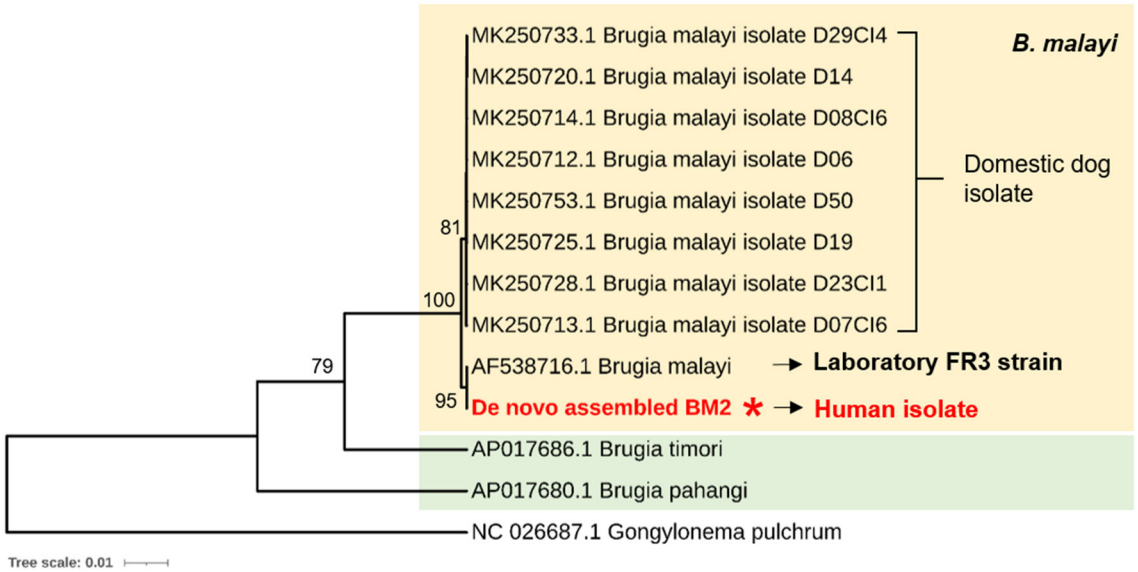


**FIGURE 3 |** Cytochrome c oxidase subunit I (*COX1*)-based phylogenetic analysis to identify potential sources of human brugian filariasis. *COX1* genes were obtained from two human isolates; one from Thailand (*de novo* assembled BM2 mt) and another from FR3 strain, and eight isolates of *B. malayi* collected from domestic dogs in Bangkok, Thailand. The *COX1* gene of *Gongylonema pulchrum*, a free-living nematode, was used as outgroup control.

**A**

| Location on BM2 mt | SNP situation | SNP | Codon alteration | Amino acid alteration | Amino acid | Symbol |
|---|---|---|---|---|---|---|
| 2511 | C(2)/T(8) | C2511T | AT C/T | I/I | Isoleucine | Ile |
| 2514 | C(2)/T(8) | C2514T | GG C/T | G/G | Glycine | Gly |
| 2637 | T(4)/C(6) | T2637C | TT T/C | F/F | Phenylalanine | Phe |
| 2799 | T(8)/C(2) | T2799C | CG T/C | R/R | Arginine | Arg |
| 3000 | G(8)/A(2) | G3000A | GG G/A | G/G | Glycine | Gly |
| 3130 | C(2)/T(8) | C3130T | C/T TA | L/L | Leucine | Leu |

**B**

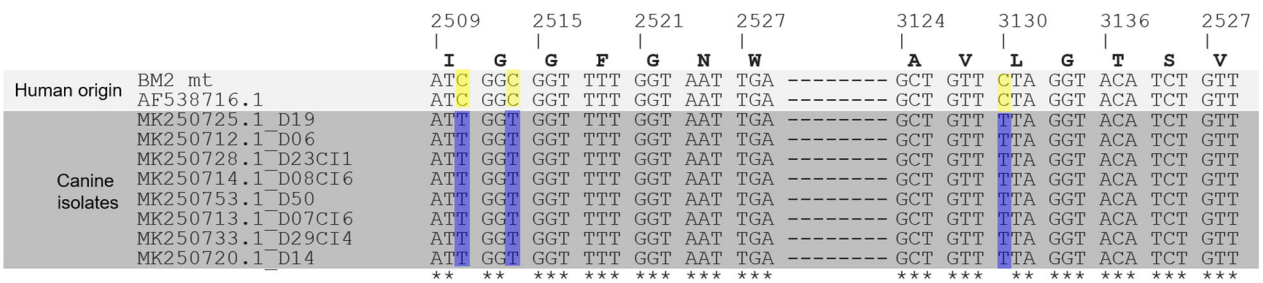


**FIGURE 4 |** Single nucleotide polymorphism of *COX1*. *COX1* genes of *B. malayi* were obtained from FR3 strain and from those isolated in dogs (D19, D06, D23CI1, D08CI6, D50, D07CI6, D50, D07CI6, D29CI4, and D14). **(A)** Types and locations of SNPs in the assembled mt DNA sequence. Six SNP sites were presented in which each nucleotide type was supported by at least two sequences, while other SNP sites caused by only one sequences were listed in the **Supplementary Table 3**. No SNP alterations caused a change in the type of amino acid. **(B)** *COX1* genes of BM2 mt and other nine isolates were subjected to multiple sequence alignment using CLUSTAL OMEGA. The locations of the C/T SNPs located at 2,511, 2,514, and 3,130 were able to separate 10 *B. malayi* isolates into two types: human or canine origin.

genome-based phylogenetic tree was supported for the clades of: (1) *Wucheriria* spp. and *Brugia* spp., (2) *Onchocerca* spp., and (3) *Dirofilaria* spp. (Lefoulon et al., 2015). Moreover, similar to other animal mitochondrial genome, mitochondria-specific ATP synthase 6 gene was identified in this study. Collectively, our analyses of the mt genome are valid and they yielded a similar result to those of the mt genomes of *B. timori* and zoonotic *B. pahangi*.

The nucleotide sequences of the mitochondrial DNA (mtDNA) molecules of two nematodes, *Caenorhabditis elegans* (13,794 bp) and *Ascaris suum* (14,284 bp), were previously reported. However, ATPase subunit 8, which is common to other metazoan mtDNA, has not been identified in the mtDNA of either of these nematodes (Okimoto et al., 1992). By using Translation Table 5 (The invertebrate mitochondrial code), *B. malayi* mt-genetic codes are similar to *C. elegans* and *A. suum* mt-genetic codes. The *O. volvulus* mitochondrial genome lacks an open reading frame encoding ATPase subunit 8 (Keddie et al., 1998), a similar finding to our study. Moreover, overlap of *trnK* gene with adjacent *COB* genes and non-overlapped *NAD1* and *trnF* gene were in agreement with annotation of W. bancrofti mt genome. By contrast, the *NAD1* and *trnF* gene were predicted to overlap in the mt genomes of *D. immitis*, *O. volvulus*, and *S. digitata* (Ramesh et al., 2012).

## Source of Microfilaria: From Animal Model or Humans

The first complete mitochondrial genome sequence of *B. malayi* is available in 2007 (Ghedin et al., 2007) and the Brugia nematode was a human-derived FR3 strain that maintained in a Mongolian gerbil (*Meriones unguiculatus*). To our knowledge, this is the first report of complete mitochondrial genome of microfilarial *B. malayi* isolated from peripheral blood of human subject in endemic area. Phylogenetic tree of the BM2 mt genome sequence was closely related to the *B. malayi* FR3 strain (accession no. AF538716), implying accuracy of sequencing. Although, the FR3 strain has been maintained in animals since first collection from human in Kuala Lumpur, Malaysia in 1950s/early 1960s (Michalski et al., 2011), mt genome seem to be conserved and has no significant genetic variation.

## Reservoir Host Potential of Domestic Dogs

Apart from vector control strategies, the infection control of reservoir hosts is necessary for more effective filariasis control. Thus, identification of the parasite reservoir host is importance. Ambily et al. firstly reported the *B. malayi* infection in dogs using Giemsa and histochemical staining, PCR and DNA sequencing. The authors emphasized the possible role of dogs in transmission of human filariasis (Ambily et al., 2011). In contrary, Ravindra et al., used 5.8S-ITS2-28S of rDNA gene to confirm suspected *B. malayi*-like parasite in the dog blood, and found that the parasite was genetically closer to *B. pahangi* (dogs) than to *B. malayi* (human). Thus, they did not find the potential of dogs as reservoirs of *B. malayi* (Ravindran et al., 2014). Recently, Satjawongvanit et al., using cytochrome c oxidase subunit I (COI) and internal transcribed spacer 1 (ITSI) gene-based PCR to detect filarial nematode DNA in blood collected from domestic dogs from the Bangkok Metropolitan Region in Thailand. Of the 57 blood samples, the COI and ITSI genes of 50 (87.72%) samples were amplified. Based on the partial nucleotide sequences of the COI gene (∼690 bp), they classified filarial nematodes detected in the domestic dogs into three species: *D. immitis* (57.89%), *B. pahangi* (22.81%), and *B. malayi* (7.02%). The study also suggests that COI gene is a useful marker for distinguishing between *D. immitis*, *B. pahangi*, and *B. malayi*, whereas use of the ITSI gene was able to detect only *D. immitis* and *B. pahangi*. Therefore, the COI-based PCR is suitable tool for the detection of filarial nematode infections in dog (Satjawongvanit et al., 2019).

Given the unavailability of the mt genome sequence of *Brugia* isolates in Thailand, we set forth to compare cytochrome c oxidase subunit I or *COX1* gene using phylogenetic analysis in order to identify a potential source of human brugian filariasis (**Figure 3**). Interestingly, several isolates of *B. malayi* collected from domestic dogs in Bangkok, Thailand (Satjawongvanit et al., 2019) were found to be close to the human isolate (BM2) identified in the blood of a man living in Narathiwat Province, Thailand, implying a potential reservoir host of domestic dogs. For lymphatic filariasis, the presence of *B. malayi* in dogs has been suspected for a long time; however, rare reports of the *B. malayi* infection in dogs were available. Our data is an evidence of the potential of a dog as *B. malayi* reservoir host. Nevertheless, further field study in human and domestic dogs is required prior to conclude the reservoir ability of dogs in endemic area.

In conclusion, the complete mitochondrial genome of microfilariae from clinical isolates was obtained in this study. To our knowledge, this report is the first to show complete mitochondrial genome of microfilariae isolated from clinical isolates. The number, order and transcription, and direction of *B. malayi* microfilariae mitochondrial genes were the same as those in the FR3 strain, which is *B. malayi* that has been adapted to the rodent model and maintaining laboratories for a long period of time.

# DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://www.ncbi.nlm.nih.gov/genbank/, MT149211; https://www.ncbi.nlm.nih.gov/, PRJNA613552.

# ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Human Research Protection Unit, Faculty of Medicine Siriraj Hospital, Mahidol University (COA no. Si459/2019). The patients/participants provided their written informed consent to participate in this study.

# AUTHOR CONTRIBUTIONS

XQ and KK preformed the experiments, analyzed and interpreted the data, and wrote the manuscript. SW and ST designed the study, analyzed and interpreted the data, and wrote the manuscript. All authors read and approved the final manuscript.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.637805/full#supplementary-material

**Supplementary File 1 |** Comparison of amino acid sequences of COX1 of the *de novo* assembled BM2 and the FR3 strain. **(a)** Alignment of the predicted amino acid sequences of COX1 obtained from FR3 strain (AAN17806) and the *de novo* assembled BM2 mt. Amino acid changes are in the blue boxes. **(b)** Prediction of secondary structure was performed using Phyre2. The models constructed for COX1 of two isolates were based on the C1M56G template. The position of the amino acids and the predicted secondary structure are indicated on the first and second lines, respectively. Amino acid residues are colored according to the biochemical property of the side chain of the amino acid, as follows: A,S,T,G,P - small/polar (orange); M,I,L,V - hydrophobic (green); K,R,E,N,D,H,Q - charged (red); and, W,Y,F,C - aromatic + cysteine (blue). The predicted α-helix is in green color. Changes of amino acids are indicated in the red boxes.

**Supplementary File 2 |** Multiple sequence alignment **(a)** and predicted protein structure **(b)** of NDL4, COB, ND1, ND5, ND4, COX3, ND2, and ATP6 using CLUSTAL 2.1 and Phyre2, respectively. In Phyre2, the predicted amino acid sequences of the *de novo* BM2 mt are subjected to matching with the others amino acid sequences, which their protein structures are known. The matches were ranked according to the number of aligned residues and the quality of alignment (similarity of residue probability distributions for each position), secondary structure similarity, and the presence or absence of insertions and deletions. Those at the top rank are selected for examining any altered secondary structure of protein.

**Supplementary File 3 |** Predicted protein structure of ND6 using Phyre2. Only fragments of the secondary structures of the deposited database are matched with ND6; thus, prediction of alteration of ND6 protein structure is not possible.

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.