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Research Note

Inferring the phylogenetic position of *Brugia pahangi* using 18S ribosomal RNA (18S rRNA) gene sequence

Fong, M., Asha Thanabalan, Azdayanti Muslim, Yee-Ling Lau, Sinnadurai Sivanandam[1] and Rohela Mahmud
Department of Parasitology, Faculty of Medicine, University of Malaya
50603 Kuala Lumpur, Malaysia
[1] Parasitology Unit, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur, Malaysia
E-mail: fongmy@um.edu.my
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Abstract. This paper presents the first reported use of 18S rRNA gene sequence to determine the phylogeny of *Brugia pahangi*. The 18S rRNA nucleotide sequence of a Malaysian *B. pahangi* isolate was obtained by PCR cloning and sequencing. The sequence was compared with 18S rRNA sequences of other nematodes, including those of some filarial nematodes. Multiple alignment and homology analysis suggest that *B. pahangi* is closely related to *B. malayi* and *Wuchereria bancrofti*. Phylogenetic trees constructed using Neighbour Joining, Minimum Evolution and Maximum Parsimony methods correctly grouped *B. pahangi* with other filarial nematodes, with closest relationship with *B. malayi* and *W. bancrofti*. The phylogeny of *B. pahangi* obtained in this study is in concordance with those previously reported, in which the 5S rRNA gene spacer region and cytochrome oxidase subunit I (COI) sequences were used.

INTRODUCTION

Brugia pahangi is a filarial nematode known to parasitize the lymphatic system of dogs and cats (Buckley & Edeson, 1956). The life cycle of this nematode involves an intermediate vector (mosquito) and the primary hosts (dogs and cats). Experimental infection studies have demonstrated that *B. pahangi* could develop to adult stage in humans (Edeson *et al*., 1960). Recently, *B. pahangi* microfilariae have been recovered in cats in some suburban areas in Kuala Lumpur (Rohela Mahmud, data not published). Due to the close relationship between cats and the human population, the risk of zoonotic infection of this parasite is possible.

Brugia pahangi shares many morphological and biological characteristics with *B. malayi*, one of the aetiologic agents for human filariasis and elephantiasis. However, not many studies have been carried out to investigate the genetic relationship between these brugian species (McReynolds *et al*., 1986; Xie *et al*., 1994; Casiraghi *et al*., 2001). In fact, hitherto there is a dearth of genetic information on *B. pahangi*. Understanding the genetic relationship of *B. pahangi* with other filarial nematode species, especially *B. malayi*, is important as it may provide clues as to why *B. pahangi* does not naturally infect man, despite sharing many common biological and morphological characteristics with *B. malayi*. The genetic information can also be used as basis for developing specific molecular methods (e.g. PCR-based assays) to detect and distinguish *B. pahangi* from *B. malayi* infections.

The 18S rRNA (also known as small subunit ribosomal RNA, SSU rRNA) gene is

widely used as marker for determining phylogenetic relationships among species. Our study presented here is the first to report the 18S rRNA sequence of *B. pahangi* and the inferred phylogeny of this nematode by comparing its sequence with those of other nematode species.

The adult *B. pahangi* worm used in this study was harvested from an infected male gerbil. Prior to harvest, this gerbil was intraperitoneally injected with L3 larvae which were recovered from female *Armigeres subalbatus* mosquitoes caught in Kampung Kerinchi, Kuala Lumpur, Malaysia. The DNA of the *B. pahangi* was extracted and purified using the QIAGEN Tissue DNA Extraction kit (Hilden, Germany). Primers for the PCR of 18S rRNA gene were designed according to the conserved regions flanking this gene of several filarial worm species (forward: 5'-GCTTGTCTCAAAGATTAAGCC-3'; reverse: 5'-TCCTTCCGCAGGTTCAC-3') (Bhandari *et al.*, 2005). The typical parameters of PCR were used to amplify the gene sequence. PCR was carried out in a 25 ml reaction mixture containing 10 mM Tris–HCl (pH 8.3), 2 mM MgCl2, 50 mM KCl, 0.01% gelatin, 200 mM of each deoxynucleoside triphosphate, 20 pmol of each primer, 1 U of *Taq* polymerase (Fermentas Life Sciences, Canada). The PCR mixture was pre-heated at 95°C for 10 min for initial denaturation before 30 cycles of amplification, which consisted of denaturation at 94°C for 1 min, annealing at 54°C for 1 min, and elongation at 72°C for 2 min. Final extension of the reaction was carried out at 72°C for 10 min. PCR fragment was cloned into plasmid pGEM-T, as according to the manufacturer (Promega Corporation, Madison, USA). Recombinant pGEM-T plasmids harbouring the cloned fragment were sent to a commercial laboratory for DNA sequencing.

The 18S rRNA

Onchocerca cervicalis, DQ094174; *Toxocara canis*, AF036608; *Toxocara cati*, EF180059; and *Wuchereria bancrofti*, AF227234.

The nucleotide sequences were aligned using the CLUSTAL-W programme which is available on-line (http://www.ebi.ac.uk/Tools/clustalw2). Homology among the sequences was computed using CLUSTAL-W. Phylogenetic trees were constructed using the Neighbour Joining (NJ), Minimum Evolution (ME), and Maximum Parsimony (MP) methods described in MEGA version 3.1 (Kumar *et al.*, 2004). In each method, bootstrap replicates of 1000 were used to test the robustness of the trees (Felsenstein, 1985).

The alignment the *B. pahangi* 18S rRNA sequence (GenBank Accession Number EU496884) with those of other nematodes is shown in Figure 1. A close inspection of the alignment reveals length heterogeneity of the 18S rRNA sequences, including among the filarial nematodes. The sequences of *B. pahangi* and *B. malayi* are of equal length (641 nucleotides), whereas *W. bancrofti*, *L. loa* and *O. cervicalis* have shorter sequences (637, 634 and 634 nucleotides, respectively). The *D. immitis* sequence, however, is 646 nucleotides in length. The similarity of the *B. pahangi* 18S rRNA sequence with other nematodes is summarized in Table 1. It can be observed that the *B. pahangi* sequence shares high similarity with other filarial nematodes. Very high similarity is shared with *B. malayi* (99%) and *W. bancrofti* (98%), followed by *L. loa* (97%), *O. cervicalis* (97%) and *D. immitis* (93%). When compared with non-filarial nematodes, a relatively lower similarity (89%–93%) was observed. These findings are reflected in the phylogenetic trees generated using the NJ, ME and MP methods (Figure 2). We used these methods, which are based on different algorithms, in

Figure 1. Comparison of *Brugia pahangi* 18S rRNA nucleotide sequence with those of other nematodes in a multiple sequence alignment. Dot (.) indicates identical nucleotide with that of *B. pahangi*. Hyphen (-) indicates a gap.

Figure 2. Phylogenetic trees based on 18S rRNA sequences, constructed using Neighbour Joining Minimum, Evolution, and Maximum Parsimony methods. Numbers on the nodes are bootstrap values. The topology of the filarial nematode group (Filarioidea) is consistent in all trees.

genetically quite distant from the *Brugia-Wuchereria* group.

The phylogeny obtained in this study is in concordance with previous reports. Xie *et al.* (1994) based their pylogenetic trees on the 5S rRNA gene spacer region and observed the placement of *B.* pahangi in the *Brugia-Wuchereria* group. A similar phylogeny was obtained by Casiraghi *et al.* (2001), who used the cytochrome oxidase subunit I (COI) to construct the trees.

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