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Complete mitochondrial genome of *Angiostrongylus malaysiensis* lungworm and molecular phylogeny of Metastrongyloid nematodes

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ABSTRACT

Angiostrongylus malaysiensis is a nematode parasite of various rat species. When first documented in Malaysia, it was referred to as *A. cantonensis*. Unlike *A. cantonensis*, the complete mitochondrial genome of *A. malaysiensis* has not been documented. We report here its complete mitogenome, its differentiation from *A. cantonensis*, and the phylogenetic relationships with its congeners and other Metastrongyloid taxa. The whole mitogenome of *A. malaysiensis* had a total length of 13,516 bp, comprising 36 genes (12 PCGs, 2 rRNA and 22 tRNA genes) and a control region. It is longer than that of *A. cantonensis* (13,509 bp). Its control region had a long poly T-stretch of 12 bp which was not present in *A. cantonensis*. *A. malaysiensis* and *A. cantonensis* had identical start codon for the 12 PCGs, but four PCGs (*atp6*, *cob*, *nad2*, *nad6*) had different stop codon. The cloverleaf structure for the 22 tRNAs was similar in *A. malaysiensis* and *A. cantonensis* except the TΨC-arm was absent in *trnV* for *A. malaysiensis* but present in *A. cantonensis*. The *Angiostrongylus* genus was monophyletic, with *A. malaysiensis* and *A. cantonensis* forming a distinct lineage from that of *A. costaricensis* and *A. vasorum*. The genetic distance between *A. malaysiensis* and *A. cantonensis* was p = 11.9% based on 12 PCGs, p = 9.5% based on 2 rRNA genes, and p = 11.6% based on 14 mt-genes. The mitogenome will prove useful for studies on phylogenetics and systematics of *Angiostrongylus* lungworms and other Metastrongyloid nematodes.

1. Introduction

The rat lungworm *Angiostrongylus malaysiensis* (Bhaibulaya and Cross, 1971) is a Metastrongyloid nematode of the family Angiostrongylidae (Eamsobhana, 2014; Spratt, 2015). It has a relatively simple life cycle, involving rodent definitive hosts and mollusk intermediate hosts (Lim and Ramachandran, 1979). The adult worms live in the pulmonary arteries of rats. When first documented in Malaysia it was referred to as *A. cantonensis* (Lim et al., 1965). It was subsequently recognized as a valid species and named as *A. malaysiensis* (Bhaibulaya and Cross, 1971). Likewise, another rat lungworm when first reported in Australia was named as *A. cantonensis* (Mackerras and Sandars, 1955) but later elevated as a distinct species, *A. mackerrasae* (Bhaibulaya, 1968).

Unlike *A. cantonensis* which causes eosinophilic encephalitis in human, *A. malaysiensis* has not been unequivocally incriminated as a zoonotic parasite (Spratt, 2015). The potential needs to be investigated as it occurs widely in Thailand (an endemic country of human angiostrongyliasis) (Yong et al., 2015a) and mixed infection has been reported in rats (Bhaibulaya and Techasophonmani, 1972).

Both mitochondrial and nuclear genes have been used to differentiate *A. cantonensis* and *A. malaysiensis*. The nuclear genes, 66 kDa protein gene (Eamsobhana et al., 2010a) and small subunit ribosomal RNA (18S rRNA) gene (Eamsobhana et al., 2015) clearly separated *A. cantonensis* and *A. malaysiensis*. Likewise, the mitochondrial genes cytochrome *c* oxidase subunit I (*cox1*) (Eamsobhana et al., 2010b) and cytochrome *b* (*cob*) (Yong et al., 2015a) unequivocally separated *A. cantonensis* and *A. malaysiensis*.

To date, of the 13 species of *Angiostrongylus* genus (Spratt, 2015) the complete mitochondrial genomes (mitogenomes) of *A. cantonensis* (Lv et al., 2012), *A. costaricensis* Brazil taxon (Lv et al., 2012), *A. costaricensis* Costa Rica taxon (Yong et al., 2015b), *A. mackerrasae* (Aghazadeh et al., 2015) and *A. vasorum* (Gasser et al., 2012) have been documented. We report here the mitogenomes of *A. malaysiensis* and *A. cantonensis* (Thailand taxon) determined by next-generation sequencing and the phylogenetic relationships with their congeners and other Metastrongyloid taxa.

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