



Molecular phylogeography and genetic diversity of *Angiostrongylus cantonensis* and *A. malaysiensis* (Nematoda: Angiostrongylidae) based on 66-kDa protein gene



Praphathip Eamsobhana^{a,*}, Hoi-Sen Yong^b, Sze-Looi Song^c, Xiao-Xian Gan^d, Anchana Prasartvit^e, Anchalee Tungtrongchitr^{a,*}

^a Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

^b Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia

^c Institute of Ocean and Earth Sciences, University of Malaya, Kuala Lumpur, Malaysia

^d Institute of Parasitic Diseases, Zhejiang Academy of Medical Sciences, Hangzhou, Zhejiang, PR China

^e Department of Disease Control, Ministry of Public Health, Nonthaburi, Thailand

ARTICLE INFO

Keywords:

Angiostrongylus nematodes
Molecular phylogeography
Haplotype diversity
Genetic divergence
Thailand

ABSTRACT

Angiostrongylus cantonensis is the main causative agent of human angiostrongyliasis. A sibling species, *A. malaysiensis* has not been unequivocally incriminated to be involved in human infections. To date, there is only a single report on the application of the partial 66-kDa protein gene sequence for molecular differentiation and phylogeny of *Angiostrongylus* species. Nucleotide sequences of the 66-kDa protein gene of *A. cantonensis* and *A. malaysiensis* from Thailand, as well as those of the laboratory strains of *A. cantonensis* from Thailand and Hawaii, *A. cantonensis* from Japan and China, *A. malaysiensis* from Malaysia, and *A. costaricensis* from Costa Rica, were used for the reconstruction of phylogenetic tree by the maximum likelihood (ML) method and the haplotypes by the median joining (MJ) network. The ML phylogenetic tree contained two major clades with a full support bootstrap value – (1) *A. cantonensis* and *A. malaysiensis*, and (2) *A. costaricensis*. *A. costaricensis* was basal to *A. cantonensis* and *A. malaysiensis*. The genetic distance between *A. cantonensis* and *A. malaysiensis* ranged from $p = .82\%$ to $p = 3.27\%$, that between *A. cantonensis* and *A. costaricensis* from $p = 4.90\%$ to $p = 5.31\%$, and that between *A. malaysiensis* and *A. costaricensis* was $p = 4.49\%$ to $p = 5.71\%$. Both *A. cantonensis* and *A. malaysiensis* possess high 66-kDa haplotype diversity. There was no clear separation of the conspecific taxa of *A. cantonensis* and *A. malaysiensis* from different geographical regions. A more intensive and extensive sampling with larger sample size may reveal greater haplotype diversity and a better resolved phylogeographical structure of *A. cantonensis* and *A. malaysiensis*.

1. Introduction

The rat lungworm *Angiostrongylus cantonensis* (Chen, 1935) is the main causative agent of human angiostrongyliasis, a disease characterized by eosinophilic meningitis or eosinophilic meningoencephalitis [1,2]. To date, more than 2800 cases of human angiostrongyliasis have been recorded worldwide [3]. A sibling species, *A. malaysiensis* Bhaibulaya and Cross, 1971, reported in Malaysia, Thailand, Indonesia, Japan, Laos and Myanmar [4–6], has a similar life cycle but has not been unequivocally incriminated to be involved in human infections [2,4,7]. The distributions of these two *Angiostrongylus* species broadly overlap in Laos, Cambodia, Myanmar, and Thailand [5,6]. *A.*

cantonensis does not occur in Malaysia.

Rodents are normally the definitive hosts of *A. cantonensis* [8–11], while snails and slugs are the intermediate hosts [2,12]. Humans, as an accidental host, are infected by consumption of raw or poorly cooked snail meat and a variety of paratenic hosts which harbor the third-stage infective larvae [4,13]. This zoonotic parasite appears to have spread from its native areas in recent years, with cases of *A. cantonensis* infection reported throughout the Southeast Asia, Pacific islands, Africa, Australia, North, Central and South America and the Caribbean islands [1–3]. It is of increasing public health importance as globalization contributes to the geographical spread and more international travelers encounter the disease. The rapid global spread of the disease has posed

* Corresponding authors at: Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand.

E-mail addresses: praphathip.eam@mahidol.ac.th (P. Eamsobhana), yong@um.edu.my (H.-S. Yong), szelooi@um.edu.my (S.-L. Song), anchalee.tun@mahidol.ac.th (A. Tungtrongchitr).

<https://doi.org/10.1016/j.parint.2018.09.006>

Received 7 August 2018; Received in revised form 30 August 2018; Accepted 25 September 2018

Available online 26 September 2018

1383-5769/ © 2018 Elsevier B.V. All rights reserved.