



## SHORT COMMUNICATION

# Molecular survey of head lice (*Pediculus humanus capitis*) infestation among disadvantaged children in Klang Valley, Malaysia

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### ABSTRACT

Ectoparasitic infestations including pediculosis capitis are common in people of disadvantaged communities as they live in overcrowded institutions, a condition that often favourable for disease transmission. In this study, we evaluated the prevalence of head lice infestation among disadvantaged children aged between five to 14 years-old living in five poor conditions located across the Klang Valley, Malaysia. Of total 335 children examined, 14% were positively infected with head lice. Molecular analysis using the universal cytochrome c oxidase subunit I (*COI*) barcoding gene on total of 167 head lice collected in this study indicated they are belonging to the A and C clades; confirming the global distribution of clade A and expansion of clade C in Southeast Asia, which is reported for the first time in Malaysia.

**Keywords:** *COI*; DNA barcoding; disadvantaged children; head lice; Malaysia.

### INTRODUCTION

Infestation by head lice is termed pediculosis capitis and it commonly affects younger age groups worldwide, particularly among those living in densely-packed conditions (Falagas *et al.*, 2008; Nutanson *et al.*, 2008). Epidemiological studies have been conducted to access the prevalence of pediculosis capitis in Malaysian children since 1981 (Sinniah *et al.*, 1981; Sinniah *et al.*, 1983; Bachok *et al.*, 2006; Muhammad Zayyid *et al.*, 2010; Yap *et al.*, 2010) however, information of genetic diversity of head lice remains scanty. In Malaysia, children welfare institutions are mostly non-profitable and they often rely on public donations to fund and sustain their operations. Insufficient funding hinders adequate care for the occupants and lack of awareness further contributes to unhygienic practices in the premises. Therefore, this study aims to screen the prevalence of pediculosis capitis and their genetic diversity amongst disadvantaged children inhabiting Klang Valley's areas, where pediculosis infestation is likely to occur.

### MATERIALS AND METHODS

The protocol involving human subjects was approved by the University of Malaya Medical Centre Ethics Committee (MEC Reference Number: 20201210-9589) and all participants provided written consent for this study.

Head lice sampling was conducted in Klang Valley, Malaysia from July 2019 to February 2020. Five welfare homes sheltering orphans, neglected children and refugees were randomly selected to represent the appropriate host population. Occupants were examined for the presence of head lice and pediculosis capitis is confirmed when living adults, nymphs or viable nits is detected. Head lice were collected from the host and directly transferred into microcentrifuge tube containing 70% alcohol; as preservative. All specimens were brought back to the laboratory for further processing.

DNA barcoding (Ratnasingham & Hebert, 2013) was employed to determine the clade of the head lice specimens. Head lice DNA was extracted from the whole specimen using the GeneJET Genomic DNA purification kit (Thermo Fisher Scientific, Lithuania). The *COI* partial sequences were amplified according to Sunantaraporn *et al.* (2015). Cycle sequencing were performed by Apical Scientific Sdn. Bhd. (Selangor, Malaysia) using the forward primer. The *COI* DNA barcodes obtained were aligned with representative Barcode Index Numbers (BINs) of five clades; AAA1556 (Clade A), AAA1557 (Clade B), AAA1558 (Clade C), AAW5034 (Clade D) and ACR6059 (Clade E) using BioEdit Sequence Alignment Editor Software, Version 7.2.6 (Hall, 1999). The pairwise genetic distances for *COI* were computed using Kimura 2-parameter

method and Maximum Likelihood (ML) phylogenetic tree was constructed in MEGA X version 10.2.6 (Kumar *et al.*, 2018).

## RESULTS

A total of 167 head lice were collected from 47 (14%) out of overall 335 children participants aged five to 14 years old examined in this study.

ML analysis of the 168 *COI* barcodes has revealed the genetic diversity of head lice in this study belonging to *Pediculus humanus capitis* corresponding with two clades: clade A (BOLD: AAA1556) and clade C (BOLD: AAA1558), as shown in Figure 1. K2P model calculation results using MEGA X showed that intra- and inter-specific genetic distance exists obvious difference. The intra-specific K2P divergence averaged 0.005 % for clade A and 0.003% for clade C whereas the inter-specific K2P divergence between the two clades averaged 0.079 %. The inter-specific divergence value was greater than intra-specific values, more than 15 times.

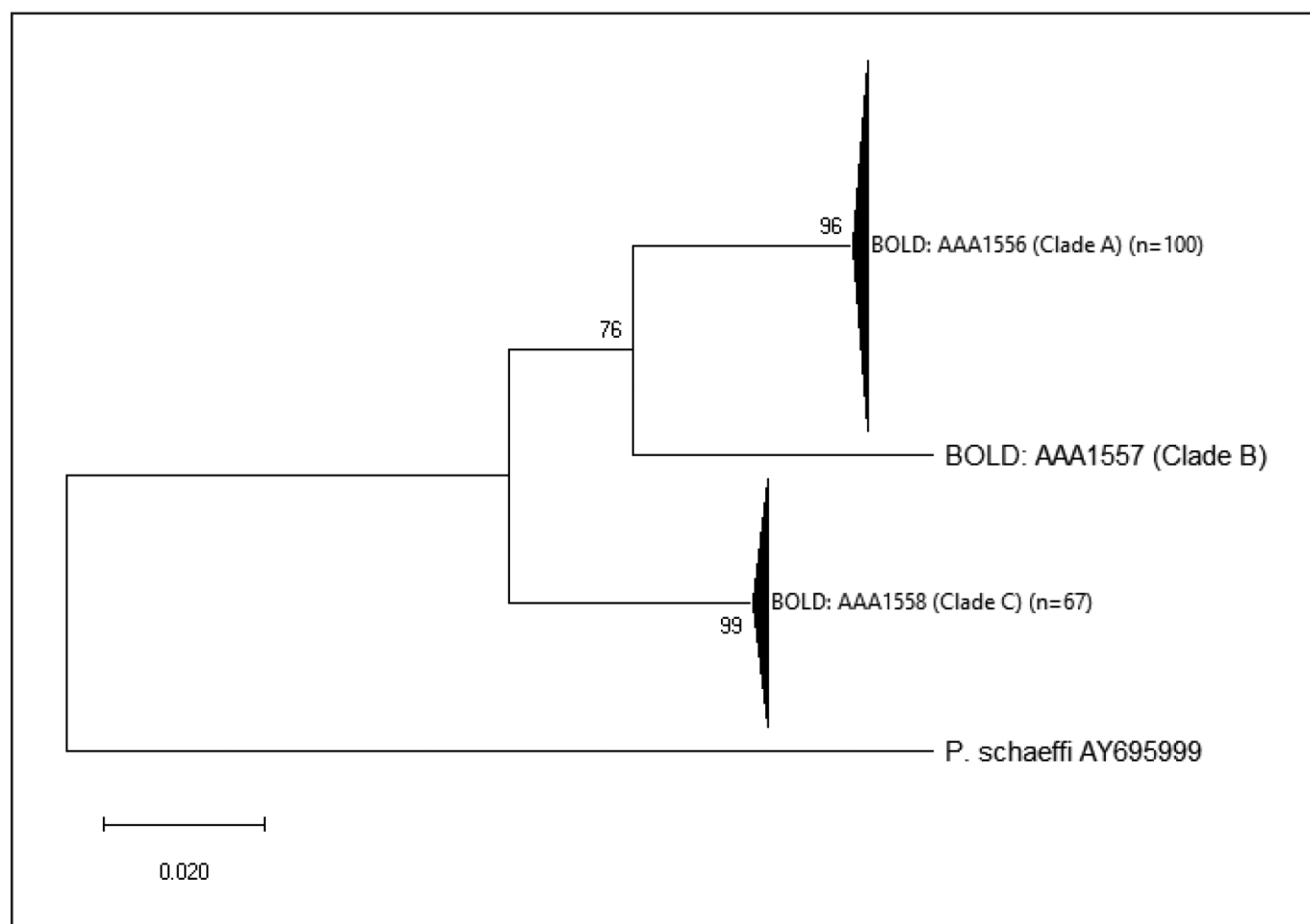
Representative *COI* barcodes discovered in this study were assigned GenBank accession numbers: OK175617 and OK175618 for clade A; and OK175619 for clade C.

Four shelters presented head lice belonging to clades A and C and one shelter (Ahmadiyah Refugee Committee) had the occurrence of only clade C head lice. In addition, the occurrence of two clades within the same individual were detected in occupants from three shelters.

The prevalence of pediculosis capitis among occupants and the occurrence of clades A and C in the five welfare homes are summarized in Table 1.

## DISCUSSION

Pediculosis capitis remains a health issue throughout the world and Malaysia is no exception to this problem. High prevalence of pediculosis capitis especially amongst children is attributable to various factors including crowded living condition (Nutanson *et al.*, 2008) and poor hygiene practices (Mokhtar *et al.*, 2019). Despite the vector status of head lice in the transmission of pathogens to humans is not fully understood, there are increasing reports on the detection of pathogenic bacteria in head lice. *Acinetobacter baumannii* (Bouvresse *et al.*, 2011; Kempf *et al.*, 2012; Sunantaraporn *et al.*, 2015; Mokhtar *et al.*, 2019; Abd Majid *et al.*, 2020), *Bartonella recurrentis* (Boutellis *et al.*, 2013; Amanzougaghene *et al.*, 2016), *Bartonella quintana* (Sasaki *et al.*, 2006; Bonilla *et al.*, 2009; Angelakis *et al.*, 2011) and *Serratia marcescens* (Mokhtar *et al.*, 2019) are amongst human pathogens that have been reported in the literature, suggesting head lice as a potential environmental reservoir posing a health threat to the infested children.



**Figure 1.** Maximum Likelihood (ML) phylogram of the *COI* barcodes showing the placement of the 167 head lice into two BINs of Clade A and C. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Selected subtrees were compressed for visual reasons. *Pediculus schaeffi* (Accession Number: AY695999) was used as an outgroup.

**Table 1.** Prevalence of pediculosis capitis and occurrence of clades A and C head lice in welfare homes in Klang Valley

City	Welfare homes	No. of occupants examined	No. of occupants infested by head lice	No. of collected head lice	Prevalence (%)	Clades	
						A	C
1 Selayang	Madrasah Hashimiah	99	16	83	16.2	65	18
2 Puchong	United Arakan Institute Malaysia	63	6	31	9.5	4	27
3 Cheras	Ahmadiyah Refugee Committee	56	1	2	1.8	0	2
4 Kajang	Al-Islamiah Centre	39	39	17	23.1	5	12
5 Sungai Buloh	Tahfiz Sri Al-Ain	78	78	34	19.2	26	8

Current data on the genetic variation of *COI* of head lice in Malaysia are scarce and to our knowledge, only one such study was conducted by Mokhtar *et al.* (2019). Our finding is the first to demonstrate the occurrence of clade C head lice in Malaysia, amongst the clade A collected from the shelter homes in Klang Valley. The NJ cluster analysis based on *COI* barcodes has successfully showed the phylogenetic placement of the 167 head lice into two BINs of clades A and C. The detection of clade A lice in this study further confirmed its global distribution. Clade C was previously detected in head lice from Nepal and Africa (Light *et al.*, 2008a). In 2015, Sunantaraporn *et al.* (2015) first reported the occurrence of clade C in Southeast Asia. Data in this study shows that both clade A and C occur in sympatry. Concurrent occurrence of clades A and C was also reported in Thailand (Sunantaraporn *et al.*, 2015), Ethiopia (Angelakis *et al.*, 2011; Veracx *et al.*, 2013) and Senegal (Boutellis *et al.*, 2012). Clade A comprises both head and body lice whereas clade C comprises only head lice (Light *et al.*, 2008b; Drali *et al.*, 2016). Both clades have been shown to carry pathogens, therefore, addressing the clade diversity is fundamental to provide insight into the transmission dynamics of their potential associated pathogens in Malaysia setting. Despite the low prevalence of pediculosis capitis encountered in the current study, the data obtained provide baseline data useful for the relevant authorities including health and welfare departments to work on necessary planning, funding and control strategies to prevent the occurrence of pediculosis capitis especially in children welfare institutions. It is anticipated that *COI* DNA barcoding has revealed the genetic diversity of head lice in Malaysia where sympatry of clades A and C offers tremendous scope for extensive studies, especially on the potential associated pathogens these two clades might carry.

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#### Conflict of interest statement

The authors declare that they have no competing interest.

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