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Comparison of genetic variation in the blow fly, *Chrysomya megacephala* (Diptera: Calliphoridae), thirteen countries

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Chrysomya megacephala (Fabricius, 1794), also called the oriental latrine fly, is a member of the family Calliphoridae and is a medically important blow fly species. *C. megacephala* has a wide geographical distribution, including Asia, Australasia, and the Palaearctic, Afrotropical, Nearctic, and Neotropical realms. The study of genetic variation in organisms to understand organismal biodiversity is very important to know the genetic structure of populations, one of the basic data required for disease control. In this research, we conducted a study of worldwide genetic variation in the blow fly *C. megacephala* by comparing mitochondrial DNA sequences (cytochrome c oxidase subunit I [COI]) obtained from the National Center for Biotechnology Information (NCBI) GenBank and the Barcode of Life Database (BOLD). Fifty COI sequences of *C. megacephala* from 13 countries comprising Australia, Brazil, China, Costa Rica, Ecuador, Egypt, India, Malaysia, Peru, Philippines, Saudi Arabia, Thailand, and the USA were obtained. The results of this analysis revealed that 62.65% of the variation occurred within the populations with statistically significant difference ($p< 0.05$). Only eight haplotypes of *C. megacephala* populations comprising four singular and four shared species were detected in this study, indicating that *C. megacephala* has low genetic diversity. A Pairwise FST analysis showed significant genetic differences between the populations from Malaysia and Philippines, Malaysia and Thailand, the USA and Philippines, and the USA and Thailand.

Keywords: ***Chrysomya megacephala*, Genetic, Variation, Mitochondrial, Sequences.**

INTRODUCTION

Blow flies (Calliphoridae) are non-biting flies that can be mechanical vectors of several human pathogens including bacteria, viruses, protozoan cysts, helminth eggs, and fungi (Killick-Kendrick, 1996). *Chrysomya megacephala* (Fabricius, 1794), also known as the oriental latrine fly, is a member of the family Calliphoridae and is a medically important blow fly species (Chaiwong et al., 2014). The adults of *C. megacephala* serve as mechanical vectors mainly of bacteria. Intestinal pathogens can adhere to external surfaces such as the body, legs, or other organs, and these microorganisms can subsequently be transmitted to humans (Killick-Kendrick, 1996). Non-fermentative Gram-negative bacilli and coagulase-negative staphylococci are common in *C. megacephala* (Sukontason et al., 2007). Previous research has investigated the bacterial enteric pathogens for which *C. megacephala* is a vector, and has found three species of bacterial enteric pathogens which cause diarrheal disease including *Aeromonas hydrophila*, *Edwardsiella tarda*, and *Vibrio cholerae* non-01, and five species of possible enteric pathogens including *A. sobria*, *Citrobacter freundii*, *Escherichia coli*, *Providencia alcalifaciens*, and

Pseudomonas aeruginosa (Sukontason et al., 2000). The larval stage of *C. megacephala* can also cause myiasis in humans, when maggots feed on the tissue of humans (Sukontason et al., 2005). *C. megacephala* usually live close to human dwellings and are one of the leading causes of outbreaks of diarrhea.

C. megacephala has a wide geographical distribution, including Asia, Australasia, and the Palaearctic, Afrotropical, Nearctic, and Neotropical realms (Sontigun et al., 2018). The original distribution of *C. megacephala* was in the Oriental and Australasian regions, but there have subsequently been many reports of sightings in the Indian region and the Middle East (Badenhorst and Villet, 2018). *C. megacephala* has spread rapidly through Africa and the New World (Badenhorst and Villet, 2018). The species was identified in South Africa in 1971; in Brazil in 1977; in North America, including Baja California and Mexico, in 1987; in Florida in 1992; and in Indiana, USA, in 2012 (Wells, 1991; Badenhorst and Villet, 2018). The widespread distribution of this fly species may affect the genetic variation of populations in each area arising from geographical differences (Chong et al., 2014).

Studying genetic variation in order to understand the organismal biodiversity and the genetic structure of populations is important and can provide data required for the implementation of disease control strategies (Zhou et al., 2013). Recently, the genetic variation of *C. megacephala* populations was assessed using the cytochrome oxidase gene, and ISSR-PCR has been studied in five sites in Malaysia: Penang, Selangor, Pahang, Johor, and Sabah. This research found low genetic variation within populations (Chong et al., 2014). However, genetic data from *C. megacephala* obtained from different geographical areas should be compared in order to understand its genetic variation and the effect of geographic barriers in this species.

Until recently, little was known about the genetic variation and population structure of *C. megacephala*, even though this fly species has spread to countries across the globe. We conducted a worldwide study of the genetic variation of *C. megacephala* using mitochondrial cytochrome c oxidase subunit I (COI) DNA sequences obtained from the National Center for Biotechnology Information (NCBI) GenBank and the Barcode of Life Database (BOLD). The results of this study add to our understanding of the genetic variations of *C. megacephala* worldwide, the data of which are important for controlling this disease vector.

MATERIALS AND METHODS

Selection of *C. megacephala*

In this study, samples of *C. megacephala* in each country were selected based on the COI sequences from the NCBI GenBank and BOLD. The COI sequences were confirmed using Basic Local Alignment Search Tool (BLAST) against GenBank, and the data which did not match those in the database were discarded. Fifty COI sequences of *C. megacephala* from 13 countries comprising Australia, Brazil, China, Costa Rica, Ecuador, Egypt, India, Malaysia, Peru, Philippines, Saudi Arabia, Thailand, and the USA were used (Table 1).

Table 1:COI sequences of *C. megacephala* analyzed in this study.

Countries	Database	No	Accession no.
Brazil	GenBank	3	MF695694.1, MF695701.1, MF695706.1
China	GenBank	7	KJ129132.1, KJ129137.1, KJ129139.1, KJ129142.1, KJ129145.1, KJ145953.1,KF037970.1
USA	GenBank	3	KC617812.1, KC617813.1, KC617814.1
Australia	GenBank	4	DQ647350.1, DQ647351.1, DQ647352.1,DQ647353.1
Philippines	GenBank	4	KP310063.1, KP310068.1, KP310069.1,KP310070.1
Peru	GenBank	4	KP310057.1, KP310065.1, KP310066.1,KP310067.1
India	GenBank	2	KT353002.1, MG780140.1
Saudi Arabia	GenBank	2	MF322595.1, MF322594.1
Thailand	GenBank	3	KT894991.1, KT894992.1, KT894993.1
Malaysia	GenBank	5	KF562106.1, KC855270.1, KC855271.1,KC855272.1, KC855286.1
Costa Rica	BOLD	4	ASIND3378-12, ASIND3379-12, ASIND3380 12,ASIND3382-12
Ecuador	BOLD	5	FFECU250-16, FFECU065-14, FFECU066-14,FFECU067-14, FFECU068-14
Egypt	GenBank	4	KM434363.1, KM434364.1, KM434365.1,KM434366.1

Statistical analysis of the genetic data

All COI sequences were aligned using Clustal X (Larkin et al., 2007) and manually edited. Genetic variation in the different populations was assessed by analyzing the numbers of haplotypes (N), polymorphic sites, nucleotide diversity (π), and haplotype diversity (h) using DnaSP6 (Rozas et al., 2017).

The population structure of *C. megacephala* was calculated using an analysis of molecular variance (AMOVA) based on 10,000 permutations using the Arlequin 3.5.2.2 software (Excoffier and Lischer, 2010). Populations were grouped for analysis according to their continent. Genetic differentiation among populations in this study was assessed using Pairwise FST based on 10,000 permutations using the Arlequin 3.5.2.2 software (Excoffier and Lischer, 2010).

Phylogenetic analysis

Phylogenetic analysis using the maximum likelihood method with 1000 bootstraps was carried out using Mega7 (Kumar et al., 2016). A median-joining haplotype network (Bandelt et al., 1999) was constructed to show the genetic diversity and geographic relationships using Network 5.0.1.1 (freely available at http://www.fluxus-engineering.com).

RESULTS

Genetic diversity

Nucleotides from fifty COI sequences, 658 bp in length, of *C. megacephala* were analyzed, and 12 polymorphic sites were identified (Table 2). The total haplotype diversity and nucleotide diversity were 0.391 ± 0.087 and 0.002 ± 0.001, respectively (Table 2). Eight haplotypes were found: four shared haplotypes, including H1 shared between Australia, Brazil, China, Costa Rica, Ecuador, Egypt, India, Malaysia, Peru, Philippines, Saudi Arabia, Thailand and the USA; H4 shared between China, Egypt, and India; H7, two shared haplotypes in Saudi Arabia; and H8, two shared haplotypes in Egypt, and four singular haplotypes H2, H3, H5, and H6, specific to China (Table 3).

Population structure

Population structure analysis of *C. megacephala* using an AMOVA found 62.65% genetic variation within populations (ΦST = 0.376, $p < 0.05$) (Table 4). The results of Pairwise FST analysis showed significant genetic differences between the populations in Malaysia and Philippines, Malaysia and Thailand, the USA and Philippines, and the USA and Thailand ($p < 0.05$) (Table 5).

Table 2: **Haplotype diversity and nucleotide diversity of *C. megacephala* from 13 countries.**

Countries	No.	No. haplotypes	No. polymorphic sites	Haplotype diversity (h) (mean ± SD)	Nucleotide diversity (π) (mean ± SD)
Australia	4	1	0	0.000 ± 0.000	0.000 ± 0.000
Brazil	3	1	0	0.000 ± 0.000	0.000 ± 0.000
China	7	6	7	0.952 ± 0.096	0.003 ± 0.002
Costa Rica	4	1	0	0.000 ± 0.000	0.000 ± 0.000
Ecuador	5	1	0	0.000 ± 0.000	0.000 ± 0.000
Egypt	4	3	4	0.833 ± 0.222	0.004 ± 0.002
India	2	2	2	1.000 ± 0.050	0.003 ± 0.002
Malaysia	5	1	0	0.000 ± 0.000	0.000 ± 0.000
Peru	4	1	0	0.000 ± 0.000	0.000 ± 0.000
Philippines	4	1	0	0.000 ± 0.000	0.000 ± 0.000
Saudi Arabia	2	1	0	0.000 ± 0.000	0.000 ± 0.000
Thailand	3	1	0	0.000 ± 0.000	0.000 ± 0.000
USA	3	1	0	0.000 ± 0.000	0.000 ± 0.000
Total	50	8	12	0.391 ± 0.087	0.002 ± 0.001

Table 3: Haplotype distributions of *C. megacephala* from 13 countries.

Countries	Haplotypes								Total
	H1	H2	H3	H4	H5	H6	H7	H8	
AUS	4	-	-	-	-	-	-	-	4
BRA	3	-	-	-	-	-	-	-	3
CHI	2	1	1	1	1	1	-	-	7
COS	4	-	-	-	-	-	-	-	4
ECU	3	-	-	-	-	-	-	2	5
EGY	3	-	-	1	-	-	-	-	4
IND	1	-	-	1	-	-	-	-	2
MAL	5	-	-	-	-	-	-	-	5
PER	4	-	-	-	-	-	-	-	4
PHI	4	-	-	-	-	-	-	-	4
SAU	-	-	-	-	-	-	2	-	2
THA	3	-	-	-	-	-	-	-	3
USA	3	-	-	-	-	-	-	-	3
Total	39	1	1	3	1	1	2	2	50

Table 4. Analysis of molecular variance (AMOVA) of *C. megacephala* from 13 countries.

Source of variation	df	Sum of squares	Variance components	Percentage of variation	*p*-value
Among groups	3	1.107	−0.00745 Va	−3.75	Φ_{CT} = −0.037
Among populations with groups	9	3.866	0.08167 Vb	41.10	Φ_{SC} = 0.396*
Within populations	37	4.607	0.12452 Vc	62.65	Φ_{ST} = 0.373*
Total	49	9.580	0.199		

Table 5. Pairwise comparisons of *C. megacephala* among 13 countries.

Countries	AUS	BRA	CHI	COS	ECU	EGY	IND	MAL	PER	PHI	SAU	THA	USA
AUS	-												
BRA	-0.128 (0.915)	-											
CHI	0.000 (1.000)	-0.128 (0.909)	-										
COS	0.000 (1.000)	-0.060 (0.838)	0.000 (1.000)	-									
ECU	0.000 (1.000)	-0.060 (0.828)	0.000 (1.000)	0.000 (1.000)	-								
EGY	0.000 (1.000)	-0.060 (0.837)	0.000 (1.000)	0.000 (1.000)	0.000 (1.000)	-							
IND	0.250 (0.386)	-0.240 (0.906)	0.000 (0.391)	0.250 (0.327)	0.000 (0.323)	0.385 (0.341)	-						
MAL	1.000 (0.095)	0.799 (0.027)	0.000 (0.103)	1.000 (0.064)	0.385 (0.065)	1.000 (0.066)	0.875 (0.338)	-					
PER	0.000 (1.000)	-0.128 (0.913)	0.000 (1.000)	0.000 (1.000)	1.000 (1.000)	0.000 (1.000)	0.250 (0.404)	1.000 (0.095)	-				
PHI	0.000 (1.000)	-0.016 (0.597)	0.000 (1.000)	0.000 (1.000)	0.000 (1.000)	0.000 (1.000)	0.474 (0.281)	1.000 (0.045)*	0.000 (1.000)	-			
SAU	0.000 (1.000)	-0.060 (0.834)	0.000 (1.000)	0.000 (1.000)	0.000 (1.000)	0.000 (1.000)	0.385 (0.324)	1.000 (0.063)	0.000 (1.000)	0.000 (1.000)	-		
THA	0.000 (1.000)	-0.016 (0.591)	0.000 (1.000)	0.000 (1.000)	0.000 (1.000)	0.000 (1.000)	0.474 (0.285)	1.000 (0.049)*	0.000 (1.000)	0.000 (1.000)	0.000 (1.000)	-	
USA	0.465 (0.147)	0.231 (0.094)	0.000 (0.142)	0.465 (0.143)	0.533 (0.139)	0.533 (0.145)	-0.108 (0.731)	0.739 (0.066)	0.465 (0.138)	0.583 (0.048)*	0.5333 (0.138)	0.583 (0.047)*	-

*= significant difference ($p < 0.05$)

Phylogenetic tree and median-joining haplotype network

A phylogenetic analysis based on maximum likelihood with 1,000 bootstrap replicates is shown in Figure 1. The maximum likelihood tree shows a genetic relationship between samples. However, this information shows that the phylogenetic tree cannot separate the groups by country except for Saudi Arabia and Egypt (Figure 1). The median-joining haplotype network tree is a star-like network with H1 as the dominant haplotype (Figure 2).

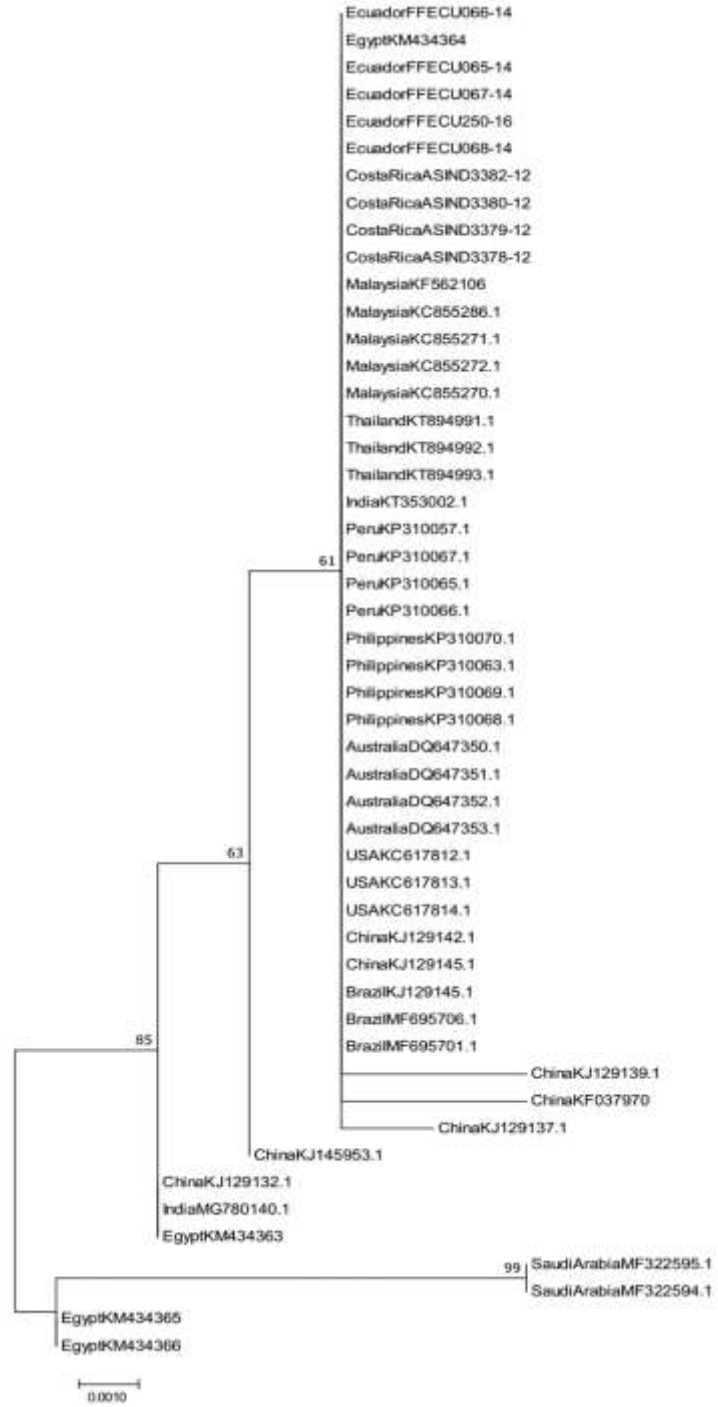

Figure 1: A phylogenetic tree based on maximum likelihood.

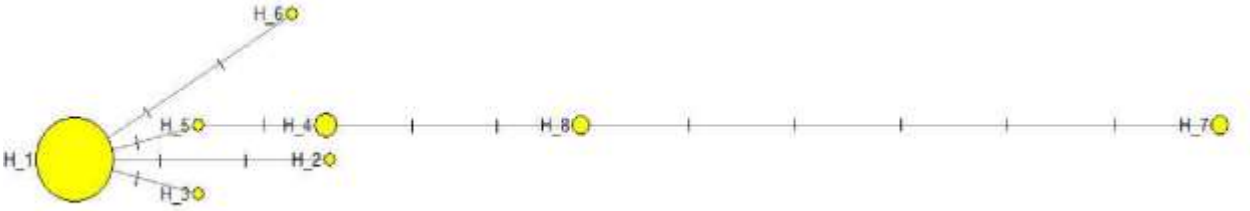

Figure 2: **A minimum spanning network tree. Each haplotype is represented by a circle, and the size of the circles is proportional to the number of individuals with each haplotype.**

DISCUSSION

This study analyzed 50 COI sequences of *C. megacephala* from 13 countries comprising Australia, Brazil, China, Costa Rica, Ecuador, Egypt, India, Malaysia, Peru, Philippines, Saudi Arabia, Thailand, and the USA. The sequences were retrieved from public databases including NCBI GenBank and BOLD. Over the last decade, DNA barcoding has been one of the methods used for the identification of animal species and the investigation of genetic variation. DNA barcoding involves the analysis of a short DNA sequence in a defined region of the genome, and this approach is precise and has high efficiency (Yang et al., 2018). Currently, a 650-base-pair region on the 5' end of the mitochondrial COI gene has been very popular as a candidate sequence for DNA barcoding. BLAST is a similarity-based algorithm that compares sequences of sample with reference databases (Lim et al., 2009). After 2004, a comprehensive barcode library has been developed and has been applied to the genetics of living organisms (Ratnasingham and Hebert, 2007).

The results of our analysis of genetic diversity of *C. megacephala* populations in 13 countries reveal that 62.65% of the variation occurred within populations with statistically significant difference ($p< 0.05$). Only eight haplotypes of *C. megacephala* populations comprising four singular haplotypes and four shared haplotypes were detected indicating that *C. megacephala* has low genetic diversity. All four singular haplotypes (H2, H3, H5, and H6) were specific to China and can be used as an index to identify the genetic groups from China. The results of this study are unexpected because geographically restricted species often have lower genetic diversity than more widespread species (Wysocka et al., 2011). However, the results of this study were consistent with a previous research that found that the worldwide COI barcode diversity of *C. megacephala* is low (Qiu et al., 2016).

Normally, the population genetic structure of *C. megacephala* populations is influenced by evolutionary factors including natural selection, gene flow, the mating system, and the mode of reproduction (Chong et al., 2014). In this study, population structure analysis based on AMOVA revealed differences in genetic structure within *C. megacephala* populations, and when comparing differences between pairs of populations using Pairwise FST analysis, we found genetic differences between Malaysia and Philippines, Malaysia and Thailand, the USA and Philippines, and the USA and Thailand ($p < 0.05$).

The haplotype network did not show clear separation of *C. megacephala* groups. The examination of genetic relationships based on maximum likelihood with 1000 bootstraps revealed that the phylogenetic tree cannot separate the population in each country except for Saudi Arabia and Egypt. This result may be because of the two shared haplotypes; H7 has two shared haplotypes that were specific to Saudi Arabia and H8 has two shared haplotypes that were specific to Egypt. Genetic variation in insects is affected by many environmental factors (Huang et al., 2016). Previous research in Malaysia found that the *C. megacephala* populations in Penang and Selangor were different from the *C. megacephala* populations in Johor and Pahang (Chong et al., 2014). The environment also has a strong influence on the characteristics of the blow fly such as behavior and population density. Recently, medically important flies in the central region of Thailand were divided into five groups according to landscape: the coastal area, urban area, upper alluvial area, lower alluvial area, and mountainous area. The environment was observed to affect the density of *C. megacephala* (Chaiphongpachara et

al., 2018). However, genetic variation in *C. megacephala* in some countries is not yet clear due to low sample sizes and therefore waits upon the addition of more COI sequences from *C. megacephala* to the public databases.

CONCLUSION

Fifty COI sequences of the blow fly *C. megacephala* from 13 countries were shown to have low genetic diversity. We generated a median-joining haplotype network, which is a star-like network indicating the possibility of a recent bottleneck event followed by most probably postglacial population expansion of *C. megacephala*.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

TC designed and performleed the experiments and also wrote the manuscript.

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