Short communication

COI Gene Based Molecular Characterization and Phylogenetic Assessment of Head Louse of Bangladesh

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Abstract

The head louse is the primary vector of pediculosis and transmits some pathogenic bacterial agents in human. The *COI* gene of the head louse *Pediculus humanus capitis*was sequenced and submitted to NCBI Gen Bank. The accession number is MH293113.This research is considered as the first molecular identification of head louse species from Bangladesh.Nucleotide base composition of louse revealed that AT base content was higher (63.6%) than GC base content (36.4%). The intraspecific genetic divergence range of louse was found 0.005-0.080. Multiple sequence alignment analysis revealed 7.36% intraspecific nucleotide variation among the head louse species. The phylogenetic analysis confirmed that studied louse sequence along with other available sequences of the head louse was in the same major clade.

Keywords: Head louse, mitochondrial *COI* gene, phylogenetic analysis

1. Introduction

In Bangladesh, both ecto and endo parasitic diseases are very common [1]. Lice (Anoplura: Pediculidae) are highly species-specific parasites [2]. This order comprises of many species of which, only two have succeeded to feed on humans. These are the common head lice, *Pediculus humanus capitis* and the body lice *Pediculus humanushumanus* [3]. Human head lice (*P. humanus capitis*) is obligate parasites and rely solely on human blood for growth and survival [4-5]. *P.humanus capitis* or head louse infestation have been a worldwide public health problem especially among school aged children [5-7]. Pediculosis, caused by the human head louse, is the most prevalent parasitic infestation of the humans. Head louse feeds on blood which may lead to anemia and the scratch sites can lead to secondary infection [8]. It causes irritation, annoyance and disturbance in sleep, might cause loss of appetite and disturbed mental condition apart from psychological and social distress [9-10]. Sucking blood may cause pruritis (itching) to host due to sensitization to louse saliva, and sub sequence skin excoriation which also may lead to secondary bacterial infections. The lice are known to transmit bacteria mechanically [11]. Several human diseases such as epidemic typhus, relapsing fever and trench fever are caused by lice transmitted gram-negative bacteria [12-13].

Accurate identification is obligatory factor for any type of research with insects [14]. In addition to morphological, molecular markers specifically DNA based molecular markers are beingwidely used in animal systematic [15]. Several researchers have suggested the use of DNA barcoding in taxonomy as a method to achieve rapid species description [16-17] and the commonly adopted standard 658 bp of mitochondrial *COI* gene segment has proven to be highly informative and useful for species level identification [18-20].

So far, there is no report on the DNA barcoding of head louse in Bangladesh. Therefore, the present study aims to conduct the molecular identification and characterization of head louse based on mitochondrial *COI* gene.

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2. Materials and Methods

2.1 Collection of head lice

Adult and young human population was selected from various schools and colleges of Dhaka, Khulna, Madaripur and Mymensingh districts of Bangladesh. After carefully examining, these lice were collected by means of hands using sterile hand gloves or when it is necessary, with a small hair brush dipped in 70% alcohol. Asheet of white art paper was placed just underneath the hair because this precaution was undertaken to avoid the chances of losing them during brushing.

2.2 Morphological identification of lice

Presumptive identifications were made while preserved in ethyl alcohol under stereozoom microscope (Leica-EZ4E, Germany). Lice were identified following the methods suggested by Soulsby [21] and Herms et al. [22]. Morphometric and meristic measurements are required to identify at species and sub species level. For further confirmation CO1 gene was sequenced.

2.3 Extraction of genomic DNA and quantification

For genomic DNA extraction, unfed adult lice were considered. The genomic DNA was extracted from collected lice using Wizard® Genomic DNA Purification Kit, USA, following the manufacturer's protocol with slight modification as discussed by Aslam et al. [23]. Extracted genomic DNA was stored at 4^0C or -20^0C.The quantity and purity of DNA was measured by using Nano drop™ 2000 spectrophotometer (Thermo Fisher Scientific, USA).

2.4 Amplification of DNAand gel electrophoresis

The DNA extract was subjected to PCR amplification of a 658 bp region near the 5′ terminus of the mitochondrial *COI* gene in a thermal cyclerof 96 well plates (Veriti, Applied biosystems by Thermofisher Scientific, USA) with 20 µl reaction volume contains 10 µl Master mix (GoTaq® Green

Master Mix, Promega), 1 µL (10 pmol) forward primer, 1 µL (10 pmol) reverse primer, template DNA 50 ng and adjustable nuclease free water. Universal Forward primer LCO1490(F)-5'GGTCAACAAATCA TAAAGA TATTGG-3' and reverse Primer HCO2198(R)5'-TAAACTTCAGGGTGACCAAAAAATCA-3' [24] were used for amplification. Mt-*COI* gene was amplified using PCR protocol, as follows: Initial step: 94 °C for 3 minutes, 32 cycles of the following profile: Denaturing step: 94 °C for 30 seconds, Annealing step: 49 °C for 30 seconds, Extending step: 72 °C for 45 seconds.The amplified product was analyzed on a 1% agarose gel electrophoresis. The DNA was then visualized under gel documentation system (BioDoc Analyzer of Biometra, Germany).

2.5 PCR product purification and gene sequencing

The PCR products were purified using Promega Wizard® SV Gel & PCR clean up system manufactured by Promega Corporation, USA. The quantity and purity of PCR purified products was checked again by spectrophotometer. DNA sequencing was performed to determine the nucleotide sequence in cytochrome oxidase I region. The sequencing was carried out using ABI PRISM 3730xl Genetic Analyzer (Applied Biosystems, USA) with manufacturers protocol of Apical sequencing, Malaysia. Each species was bi-directionally sequenced to get sequence of both (5' and 3') the DNA strands.

2.6 Submission of gene to gen bank

Sequenced data was checked for quality by Bio Edit v.7.0.5 software. After homology was checked using NCBI BLAST search, the sequence was uploaded to Gen Bank database.

2.7 Bioinformatic analysis

The chromatogram was converted to FASTA format using Finch TV chromatogram viewer software. The DNA sequence was edited using BioEdit v.7.0.5. Results of sequence editing were analyzed using BLAST (Basic Local Alignment Search Tool) NCBI to determine the homology from closest species.Available *COI* gene sequences of *P.humanus capitis* of different countries (USA, Australia, China) from NCBI database were considered for comparison. Multiple sequence alignment analysis was carried out using Jalview, version 2.9 [25]. Nucleotide composition and genetic distance analysis was performed in MEGA, X software [26]. Phylogenetic tree was constructed using maximum likelihood method, calculation using Bootstrap with 1000 times of repetition in MEGA (Molecular Evolutionary Genetic Analysis) software program X [26].

3. Results and Discussion

Twenty-three lice specimens were collected from different regions of Bangladesh. CO1 gene sequencing determined the collected species was *Pediculus humanus capitis.*

3.1 Morphological description of *Pediculus humanus capitis*

*Pediculus humanus capitis*was small wingless insects 4 mm in length. It had narrow heads and oval, flattened bodies, no ocelli, and it's compound eyes were reduced in size or absent. It's antennae was short with three to five segments, and it's mouth parts, which were retractable into head, were adapted for piercing and sucking. The thoracic segments were fused, the abdominal segments are separate, and there was a single large claw at the tip of each of the six legs (Fig. 1).

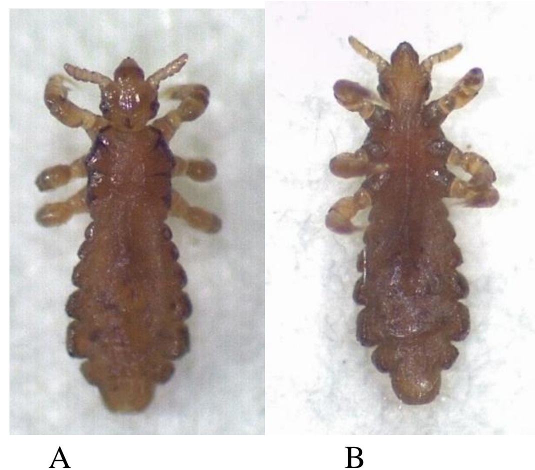

Fig.1: Head louse *Pediculus humanus capitis* (A) Dorsal view and (B) Ventral view

Partial region of cytochrome c Oxidase subunit I gene of the louse was sequenced and submitted to the NCBI Gen Bank for accession number. The Gen Bank accession number of *Pediculus humanus capitis*was (MH293113) and the GPS locationwas (23.7594226 N, 90.4367056 E). Chromatogram of sequenced specimen are as follows:

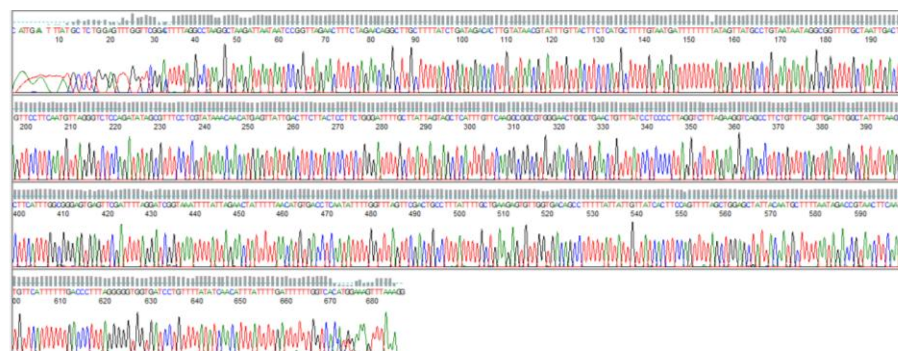

Fig. 2 Chromatogram (derived from forward primer) of sequenced specimen

FASTA sequence of sequenced specimen are as follows:

>ATTTATGCTCTGGAGTTTGGTTCGGACTTTTAGGC
CTAAGGCTAAGATTAATAATCCGGTTAGAACTTTC
TAGAACAGGCTTGCTTTTATCTGATAGACACTTGT
ATAACGTATTTGTTACTTCTCATGCTTTTGTAATGA
TTTTTTTTATAGTTATGCCTGTAATAATAGGCGGTT
TTGCTAATTGACTAGTTCCTTCAATGTTAGGGTCTC
CAGATATAGCGTTTCCTCGTATAAACAACATGAGT
TATTGACTTCTTACTCCTTCTGGGATTTTGCTTATT
AGTAGCTCATTTGTTCAAGGCGGCGTGGGAACTGG
CTGAACTGTTTATCCTCCCCTTAGGTCTTTAGAAGG
TCAGCCTTCTGTTTCAGTTGATTTGGCTATTTTAAG
CCTTCATTTGGCGGGAGTGAGTTCGATTTTAGGAT
CGGTAAATTTTATTAGAACTATTTTTAACATGTGAC
CTCAATATTTTGGTTTAGTTCGACTGCCTTTATTTT
GCTGAAGAGTGTTGGTGACAGCCTTTTTATTATTGT
TATCACTTCCAGTTTTAGCTGGAGCTATTACAATGC
TTTTAATAGACCGTAACTTCAATTGTTCATTTTTTG
ACCCTTTAGGGGGTGGTGATCCTGTTTTATATCAA
CATTTATTT

BLAST search was done to check homology between the retrieved sequences and database of sequences. The analysis revealed that the obtained sequence showed 99% similarity with the sequences of Gen Bank which means it has been derived from *Pediculus humanuscapitis* (Table 1).

Table 1: BLAST analysis of *COI* gene of head louse, *Pediculus humanus capitis*

Species name	Country	Total Score	Query cover	Identity	E value	Gen Bank Acc. no.
P. humanus capitis	Australia	1085	97%	99%	0.0	KC685851
P. humanus capitis	China	1125	95%	99%	0.0	KC685833
P. humanus capitis	USA	985	94%	99%	0.0	KC685843
P. humanus capitis	Australia	950	93%	99%	0.0	KC685835
P. humanus capitis	China	1095	92%	99%	0.0	KC685849

3.2 Nucleotide base composition

Nucleotide base composition was analyzed using MEGA, X software. A-T base content was found higher (63.6%) than G-C base content (36.4%) in mt-DNA of the studied louse species (Table 2).

3.3 Genetic distance

To estimate the intraspecific genetic distance, analysis was conducted using the Kimura 2-parameter model [27]. The analysis involved 6 nucleotide sequences. All positions containing gaps and missing data were eliminated. Intraspecific genetic divergence range of medically important lice was 0.005 - 0.080 and shown in Table 3. Highest distance (0.080) was found between *P. humanuscapitis* (KC685851) and *P. humanuscapitis* (KC685849). Lowest genetic distance (0.005) was found between *P. humanuscapitis* (KC685851) and *P. humanuscapitis* (KC685833), *P. humanuscapitis* (KC685843) and *P. humanuscapitis* (KC685835).

Table 2: Nucleotide base contents of louse species of the COI barcode region

Species	T (%)	C (%)	A (%)	G (%)	AT (%)	GC (%)
MH293113_*Pediculus_humanus_capitis	41.4	16.4	22.2	20.1	63.6	36.4
KC685851_*Pediculus_humanus_capitis*	41.2	16.3	23.1	19.4	64.3	35.7
KC685833_*Pediculus_humanus_capitis*	41.5	16.0	23.2	19.2	64.8	35.2
KC685843_*Pediculus_humanus_capitis*	41.2	16.5	21.1	21.2	62.3	37.7
KC685835_*Pediculus_humanus_capitis*	41.4	16.3	21.4	20.9	62.8	37.2
KC685849_*Pediculus_humanus_capitis*	41.5	16.2	21.1	21.2	62.6	37.4

MH293113_*Pediculus_humanus_capitis- present studied louse

Table 3: K2P sequence divergence of the COI barcode region among the *Pediculus humanus capitis* from different countries

Name of countries	1	2	3	4	5	6
1. *MH293113_*P._humanus_capitis* (BD*)						
2. KC685851_*P._humanus_capitis* (Aust*)	0.073					
3. KC685833_*P._humanus_capitis* (China)	0.071	0.005				
4. KC685843_*P._humanus_capitis* (USA)	0.032	0.069	0.071			
5. KC685835_*P._humanus_capitis* (Aust*)	0.030	0.067	0.066	0.005		
6. KC685849_*P._humanus_capitis* (China)	0.025	0.080	0.078	0.013	0.011	

*MH293113_*Pediculus_humanus_capitis*isour studied louse, BD*-Bangladesh, Aust*-Australia

3.4 Multiple sequence alignment

Available *COI*gene sequences of *P.humanus capitis* of different countries (USA, Australia, China) from NCBI database were compared with our studied sequence for multiple sequence alignment. Multiple sequence alignment was presented by showing the non-conserved region in letter and 70% identical or conserved region by dots. Total 14 polymorphic sites were observed in the present study. *P.humanus capitis* (Accession no: MH293113) was compared with the other *P.humanus capitis* species from other counties to know the conserve region among them. About 7.36% intraspecific variation was observed among the louse species (Fig. 3).

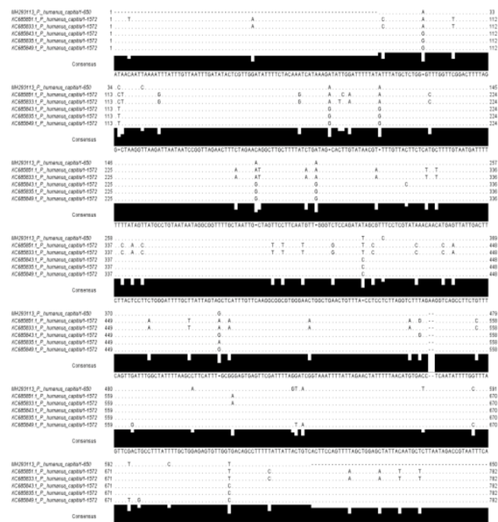

Fig. 3: Multiple sequence alignment of *Pediculus humanus capitis* based on the partial region of *COI* gene sequences constructed by Jalview. Dot denotes the conserved region. *MH293113_ *Pediculus_humanus_capitis*isthe sequence obtained in the present study

3.5 Phylogenetic analysis

Phylogenetic analysis through the construction of phylogenetic tree was performed by MEGA, Version X [26]. *Rhipicephalus microplus*was considered as out group in Maximum-Likelihood tree. *COI* gene sequences of *P.humanus capitis* of available subcontinents (USA, Australia, China) from NCBI database were considered for phylogenetic analysis. In tree construction, sixsequences of *P.humanus capitis* were grouped into two minor clades. Sequencesofour studied louse was observed in the same major clade along with other sequences taken from Gen Bank (Fig. 4).

The aim of the research was to identify the louse species of Bangladesh by DNA barcoding method. *COI* gene was used for DNA barcoding because this is the most popular gene used worldwide and very efficient for species identification [28].

Karim et al. [29] recorded one head louse *P.humanus capitis* responsible for pediculosis in human. Akhteret al. [30] found only one louse species named *P. humanus capitis* which cause pediculosis in primary school going students

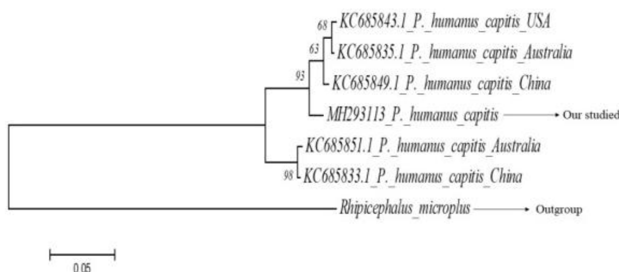

Fig. 4: Phylogenetic relationship of lice performed by MEGA X. The bar at the bottom '0.05' denotes the genetic change

Nucleotide base content composition analysis revealed that A-T base content was higher than G-C base content in mtDNA of our studied louse species (Table 2).Other head louse sequences from Gen Bank data also showed similar result.G-C bond is more stable since it has three hydrogen bonds, compared to A-T bond that only has two hydrogen bonds.Various studies have revealed that the mitochondrial *COI* gene was AT biased [30-31].

The intraspecific genetic distance range of louse was found 0.005-0.080 (Table 3). The intraspecific divergences are rarely greater than 0.02 and most are less than 0.01, and higher genetic divergencesgenerally, involve taxonomic uncertainty and imply recognition of new species [32]. Ashfaq et al. [33] studied high diversity and rapid diversification in the head louse, *Pediculus humanus* (Pediculidae: Phthiraptera). Analysis indicates a maximum K2P interspecific distance of 0.103 among lice *COI* gene sequences. Phylogenetic analysis revealed that the sequencesofour studied louse was observed in the same major clade along with other head louse sequences of Gen Bank with high bootstrap value (98) when *R. microplus* was used as out group (Fig. 4). Most of the phylogenetic information has been derived from mitochondrial DNA variations [32] and *COI* gene sequences have turn into a vital and increasingly used tool as part of an integrative taxonomy in recent species descriptions [34-35].

4. Conclusion

Pediculus humanus capitis infestation have been a worldwide public health problem. In present study, 23 lice specimens were collected from different areas of Bangladesh and were identified morphologically under a single species i.e. *Pediculus humanus capitis*. From the present research, a COI barcoding database was developed and the molecular identification of louse was supported by nucleotide base content composition, multiple sequence alignment and phylogenetic analyses. To confirm molecular identification further study is required based on 16s rRNA, 12s rRNA, Cyt-b gene and other nuclear genes. The present result may be of an important contribution for aiming control measure against lice species.

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