

Prevalence and Molecular Characterization of *cytb* Gene of *Pediculus humanus capitis* in the Northern Regions of Basrah Province, Iraq

Sarmad Awad Mozan AL-Asadi¹, Asmaa Abdul-Zahra Sabaa Al-Edany², Luay AL-Kanan³

¹ Department of Biology, College of Education for Pure Sciences, University of Basrah, Basrah, Iraq.

² Department of Biology, College of Education for Pure Sciences, University of Basrah, Basrah, Iraq.

³ Department of Biology, College of Education for Pure Sciences, University of Basrah, Basrah, Iraq.

*Corresponding Author.

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Abstract

Human head lice are widely dispersed ectoparasites in the world and have been divided into 6 haplogroups (A to F). There are no studies about the louse haplogroup type in Iraq. Thus, the study aimed to determine the species of head lice and their prevalence rate in primary school students in north Basrah and also aimed to determine the genotype and haplogroup of head lice in these regions. All louse samples belong to *Pediculus humanus capitis* and their prevalence rate was significantly higher in female students (34.9%) than in male students (23.2%). Molecular genotyping of the *cytb* gene using PCR together with the Sanger sequencing method was employed to determine the louse haplogroups and subgroups. Based on species-specific primers, the *cytb* amplified size was 348 bp for all samples. The *cytb* sequence results for randomly selected samples showed that samples shared 99.43 to 100% identities and shared 100% identities with GenBank samples (*Pediculus humanus capitis*). A phylogeny showed all samples belonged to haplogroup A, and the subgroup network separated these sequences into two unique subgroups (S1 and S2). These subgroups shared 99.43% and 100% identities based on nucleotide and amino acid sequences, respectively. The current study reported that the head lice in primary school students in north Basrah belonged to *Pediculus humanus capitis* and their prevalence rate ranged from 23.2 to 34.9%. It was also informed for the first time in Iraq that these lice were ancestry from two unique subgroups of haplogroup A.

Keywords: Cytochrome b, Haplogroup A, Head lice, Human, Iraq.

Introduction

Human lice are ectoparasites that invade the head, body, and pubic area and feed on the host's blood¹. There are only two species that infest humans; these are *Pediculus humanus* and *Phthirus pubis*. The *P. humanus* species is divided into two sub-species, *P. humanus corporis* and *P. humanus capitis*¹. Human body lice belong to *P. humanus corporis* whereas, human head lice belong to *P. humanus capitis*. However, human pubic lice belong to *Phthirus pubis*. Head lice infest humans of all socioeconomic statuses and belong to Psocodea: Pediculidae^{1, 2}. This species exists on the host surface and transmits via head-to-head contact. It infests more

than 100 million people around the world and causes a general health issue affecting humans, especially children³⁻⁵. The human louse genotype diversity has been broadly investigated by applying mitochondrial cytochrome oxidase 1 (*CO1*), cytochrome b (*cytb*), and 12S *rRNA* genes⁶⁻⁸. At the beginning of molecular investigations on the human louse mDNA from different countries, the results revealed that human lice had three main distinct haplogroups (clades): A represents body and head lice, B and C represent head lice^{9, 10}. In addition to these three haplogroups, a study reported other two new haplogroups (D and E), based on the sequence analysis of *CO1* and *cytb* genes in South Africa, Egypt, and Pakistan¹¹. Haplogroup D contains the body and head lice, while haplogroup E contains head lice only. Finally, another study introduced the sixth haplogroup F, representing Amazonian head lice, according to 12S *rRNA*, *CO1*, and *cytb* gene sequence analyses⁸. Although human louse clades are distinct from each other, every single clade has many distinct haplotypes^{6-8, 12}. Geographically, haplogroup A is widely spread across all countries, while the rest of the other haplogroups (B, C, D, E, and F) are limited to some countries^{7, 8, 12}. Haplogroup B exists in Algeria, America, Saudi Arabia, Europe, South Africa, Palestine, and Australia, and haplogroup C is presented in certain Asian and African nations. Haplogroup D seems to be found in Malaysia and some African nations (DR Congo, Zimbabwe, Ethiopia, and Congo Braz) and haplogroup E appears to be found in Niger, Mali, and Senegal. Lastly, the haplogroup F is found in Mexico, French Guyana, America (Amazon), and Argentina. As can be seen, the haplogroup of Iraqi lice is unknown. The current study focused on the community of north Basrah, which is well known to be a rural community, and based on search, no studies on pediculosis capitis have been conducted there. Thus, the study aimed to investigate the species of head lice and the prevalence rate in primary school students in north Basrah and aimed to determine the genotype and haplogroup of these human head lice in students in these regions using molecular and bioinformatics approaches.

Materials and Methods

Study area

Basrah province, located in the south of Iraq, which is close to Iranian borders, is the third biggest and hottest city in Iraq. The northern regions of Basrah, where the Tigris and Euphrates rivers are linked to form the Shatt AL-Arab river and drain into the Arab Gulf, are well famous in animal husbandry. The north Basrah community is rural, and like other Iraqi regions, primary and secondary schools are present in the north of Basrah regions. Based on the search, studies on the prevalence rate of head pediculosis have not been carried out in these regions.

Sample collection

Louse samples were collected from October 2021 to May 2022 from 1,456 straight-haired primary school students (840 females and 616 males) during autumn, winter and spring in the northern regions of Basrah province, Iraq. The students' ages ranged from 6 to 12 years old. The lice were collected from moderate-girl hairs and short-boy hairs. The collected lice from the head student were placed in a 1.5 ml tube and washed three times with normal saline. The washed specimens were pooled in a plastic container labeled with an age group and then preserved in 70% (v/v) ethanol until employed in the molecular study.

DNA extraction

Hundred-fifty specimens were randomly selected (25 lice per age group) to enrich the molecular side. The total DNA was obtained from each louse sample by applying the insect protocol of commercial DNA extraction tools (Geneaid) according to the instructions of the manufacturer. Eluted DNA's concentration and purity were determined using Nanophotometer™ N50 (Implant), and then DNA was stocked at -20°C till it was required.

Polymerase chain reaction (PCR)

PCR was done using species-specific primers for targeting the cytochrome b (*cytb*) of head lice¹³. These primers were the antisense primer 5'- GAGCGACTGTAATTACTAATC -3' and the sense primer 5'- CAACAAAATTATCCGGGTCC -3' manufactured by MacroGen¹³. A reaction mixture contained Promega GoTaq® G2 Green Master Mix (25 µl), the antisense primer (2 µl of 10 pmole/µl), and the sense primer (2 µl of 10 pmole/µl) and DNA (5 µl) in a 50 µl-entire volume^{14,15}. The PCR amplification reactions were carried out using a thermal cycler (MiniAmp™ Plus) utilized to amplify a 348bp piece. The cycling conditions were a pre-denaturation cycle (5 min at 95°C), then denaturation 1 min at 94°C, annealing 30 sec at 56°C, and expansion 1 min at 72°C for thirty-five cycles¹³. The final cycle was an extension 5 min at 72°C. 1.5% agarose gel with 3µl of ethidium bromide dye was employed to separate the amplified PCR products at 85 volt for 45 minutes. In agarose gel, 5µl of either PCR products or 100bp DNA marker (Bionner) was added into each well. The gel was then run at 85 voltage for 45 minutes. After electrophoresing, DNA bands were visualized using a UV transilluminator and captured using a digital camera.

Sequencing and bioinformatics analyses

The amplified PCR products were cleaned up using a Promega kit to remove the residual of the mixture reaction according to the instructions of the manufacturer. The sequencing was performed for purified products in both directions using *cytb* forward and reverse primers¹³ by the MacroGen company (South Korea) as previously described^{14, 15}. After editing the sequences of each sample, the forward and reverse data were assembled using Sequencher 5.4.6 software. After that, a BLAST search was performed for each sample, and the sequences were compared and aligned both within GenBank sequences and with each other. The phylogeny was built via MEGA X employing the NJ test, and the K2P test was used to compute evolutionary distances. In addition to this, the bootstrap of phylogeny was 1000 replicates. The subgroup network⁸ was generated based on the *cytb* gene using Popart 1.7 software.

Statistical analysis

The pediculosis data was statistically examined via SPSS version 22 (Chi-squared test). When $p < 0.05$, the data was considered significant.

Results and Discussion

Pediculosis capitis prevalence

The current study results showed that all louse samples collected from female and male students at primary schools in the northern regions of Basrah province belonged to head lice *P. humanus capitis* (Fig 1). The study results showed the highest prevalence of pediculosis capitis was seen in female students (34.9%) whereas in male students, the percentage of pediculosis was 23.2% (Fig 2). Like the current study, the previous study conducted in the Sulaimani province of Iraq found that the infested female students with pediculosis capitis were 16.7% in the primary schools compared with 1.82% for the infested male students¹⁶. In the Al-Kut province of Iraq, the prevalence of pediculosis capitis was 13.3 and 21.8% in males and females, respectively¹⁷. However, in both female and male students, the prevalence of pediculosis capitis in north Basrah was higher than that for both previous studies. The current study focused on the community of north Basrah, which is well known to be a rural (countryside) community. The pediculosis in a countryside community was 33.6%, whereas it was 11.5% in an urban community¹⁷. Thus, these differences in the prevalence of pediculosis capitis from previous studies could be due to the lack of health awareness in the rural community compared with the urban community.

Pediculosis prevalence in both male and female students was significantly associated with the age group, in which the increase in the pediculosis prevalence notes when the student age groups were close to 6 > 7-year-olds (data not shown) . Similar to the present study, Laabusi and Rhadi¹⁷ reported that pediculosis capitis prevalence is relatively associated with a decrease in the age group. This situation was not consistent with

other studies that found the increase in pediculosis prevalence associated with the increase in the age group^{16, 18}.



Figure 1. The head louse *P. humanus capitis* was collected from male and female students.

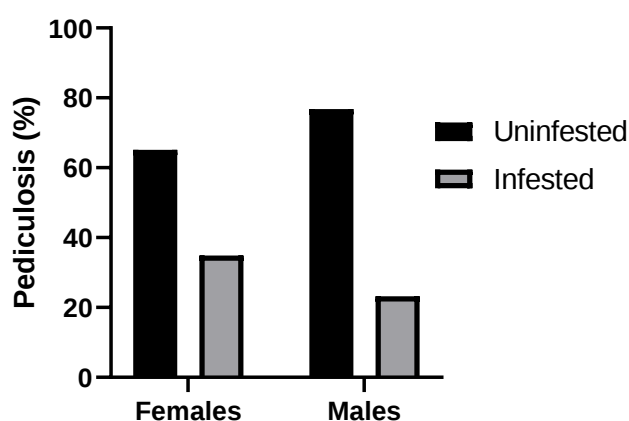


Figure 2. Pediculosis capitis prevalence between female and male students. The data were statistically significant ($X^2 = 23.058$, $p = 0.00$).

Detection of the partial *cytb* gene

The amplification of the partial *cytb* gene of louse samples with the expected size of 348 bp is shown in Fig 3. A selection of 24 samples (4 heads for each age group) was sequenced, and most of these samples had the same sequences. Thus, twelve of these sequenced samples were placed in the GenBank with the following accession numbers: OK257846.1, OQ580989.1, OQ580990.1, OQ580991.1, OQ580992.1, OQ580993.1, OK257845.1, OQ580994.1, OQ580995.1, OQ580996.1, OQ580997.1 and OQ580998.1 (Table 1). In addition to this, the deduced amino acid sequences were generated for each sample (Table 1). The BLAST search results are also shown in Table 1. The results displayed that the sequenced samples (1 - 6) shared 100% identity with the *P. humanus capitis* GenBank sample (FJ267431.1). Similarly, the samples (7 - 12) revealed 100% identity with the *P. humanus capitis* GenBank sample (MK248879.1). Furthermore, the samples (1 - 6) revealed 99.43% identity with the samples (7 - 12). PCR analysis of the *cytb* gene followed by Sanger sequencing and BLAST search showed the study head louse samples belonged to *P. humanus capitis*. This corresponded with previous studies that mention human head lice belonging to *P. humanus capitis* and responsible for worldwide head pediculosis infections in humans at different age levels¹. These sequences were divided into two groups. This suggests that the low difference 0.57% between the sequences of these two groups could put them in separate clades.

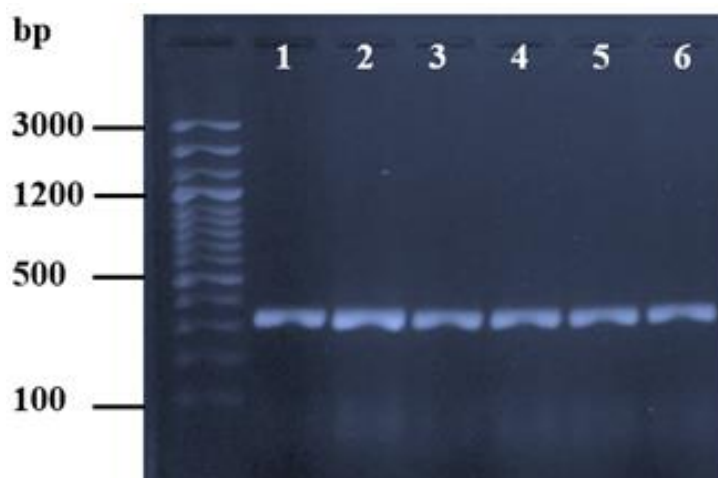


Figure 3. Agarose gel electrophoresis of the *P. humanus capitis cytb* gene. Lanes 1 – 6 represent 6 ≥ 7, 7 ≥ 8, 8 ≥ 9, 9 ≥ 10, 10 ≥ 11 and 11 ≥ 12 group ages, respectively.

Table 1. Accession numbers of the current study samples and their identities based on BLAST search.

Sample	Species	Accession number		Host	BLAST result	
		Gene	Protein		Accession No.	Identity (%)
1	<i>Pediculus humanus capitis</i>	OK257846.1	UBK11767.1	<i>Homo sapiens</i>	FJ267431.1	100
2		OQ580989.1	WKR38613.1	<i>H. sapiens</i>	FJ267431.1	100
3		OQ580990.1	WKR38614.1	<i>H. sapiens</i>	FJ267431.1	100
4		OQ580991.1	WKR38615.1	<i>H. sapiens</i>	FJ267431.1	100
5		OQ580992.1	WKR38616.1	<i>H. sapiens</i>	FJ267431.1	100
6		OQ580993.1	WKR38617.1	<i>H. sapiens</i>	FJ267431.1	100
7		OK257845.1	UBK11766.1	<i>H. sapiens</i>	MK248879.1	100
8		OQ580994.1	WKR38618.1	<i>H. sapiens</i>	MK248879.1	100
9		OQ580995.1	WKR38619.1	<i>H. sapiens</i>	MK248879.1	100
10		OQ580996.1	WKR38620.1	<i>H. sapiens</i>	MK248879.1	100
11		OQ580997.1	WKR38621.1	<i>H. sapiens</i>	MK248879.1	100
12		OQ580998.1	WKR38622.1	<i>H. sapiens</i>	MK248879.1	100

Phylogenetic analysis of the head louse clades

The phylogenetic relationships among the *cytb* nucleotide sequences of the head louse A, B, C, D, E, and F clades obtained from GenBank databases and the nucleotide sequences of current study samples are shown in Fig 4. To root the tree, the *P. schaeffi cytb* sequence was an out-group. Phylogram was divided into two main branches. The first branch contained the two head louse sister clades (C and E). The C clade was restricted to four countries (DR Congo, Ethiopia, France and Nepal). The E clade was limited to five countries, which were France, Mali, Niger, Senegal and DR Congo (4.35%). Both C and E clades only existed in France and DR Congo. The second branch contained the four head louse clades (A, B, D, and F). The A and D were head louse sister clades while the F and B were head louse sister clades. The A clade was widespread and had sequences from DR Congo, Taiwan, Australia, The Philippines, France, Palestine, Amazonia and Iraq (from Erbil) clustered under it. In addition to this, all current study sequences originally from the northern regions of Basrah province were clustered together with those sequences in the A clade, and they were far away from sequences of other clades. The D clade was limited to France, Congo Braz, DR Congo and Ethiopia. Both A and D clades only existed in France and DR Congo. The B clade was restricted to France, USA and Honduras

while the F clade was completely restricted to Amazonia. To determine whether louse samples belonged to one clade or more. The phylogenetic analysis was performed using specific sequences from different countries for each clade ^{7,8}. The phylogram showed that all head louse selected samples for sequencing belonged to the common haplogroup A, and they were away from the other haplogroups (B, C, D, E, or F). Previous studies stated that haplogroup A is the largest clade and is widely spread across all countries ^{7,8,12}.

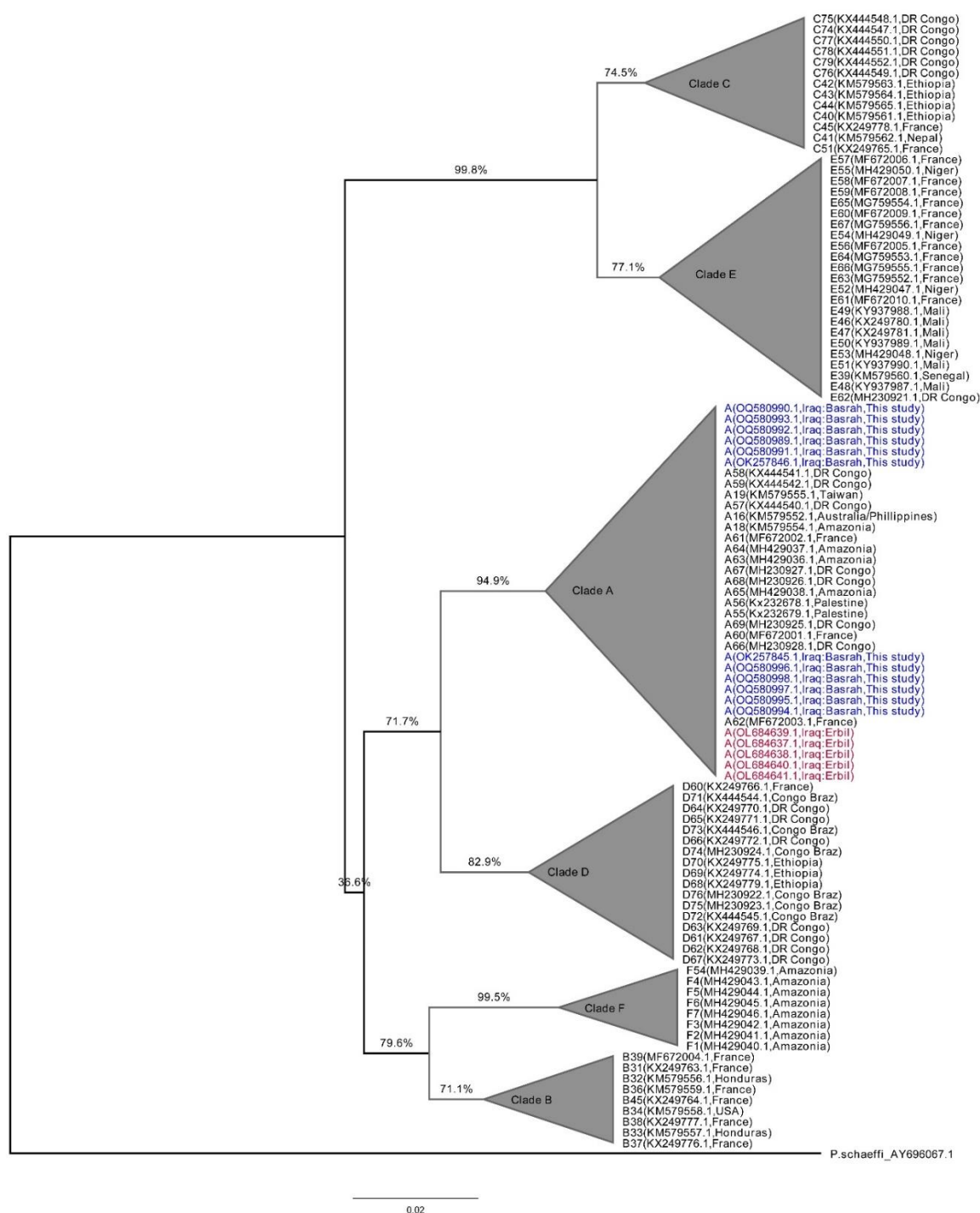


Figure 4. The phylogram of *P. humanus capitis* showed the sequences of the *cytb* gene of current study samples and sequences from different clades and countries obtained from the GenBank database. The accession numbers obtained from NCBI are mentioned in the tree.

The current study results reported for the first time that the human head lice from south Iraq (Basrah province) belonged to clade A. In addition to this, bioinformatics investigations also reported that human head louse samples from north Iraq (Erbil province) belonged to the haplogroup A. It seems to be that the common head louse haplogroup in Iraq was A. Based on the sequence analyses of *COX1* and *cytb* and *12S rRNA* genes, it was observed that all continents, including Asia, contained haplogroup A as well as other haplogroups⁸. For instance, Africa and Europe had five haplogroups (A, B, C, D, and E), while Asia had three haplogroups (A, B, and C) and America had three haplogroups (A, B, and F). Furthermore, Oceania possessed only two haplogroups, A and B. Like Iraq, it was well known that haplogroup A was present in all nations and this haplogroup included both body and head lice^{1, 6, 7}. Previous studies also mentioned that more than one haplogroup could exist in the same country^{7, 18}. In Saudi Arabia, there were only two haplogroups A and B reported¹⁸. The percentage of haplogroup A (68%) was higher than the percentage of haplogroup B (32%). Similarly, in Thailand, there were also two haplogroups, A and C, found with up to 65 and 35%, respectively⁷. While in Malaysia, there were three haplogroups (A, B, and D) found⁶. From current results and bioinformatics analyses, haplogroup A was only observed in the southern and northern regions of Iraq. There was no evidence about the existence of the other human head louse clades in Iraq, in particular the northern regions of Basrah. Thus, the current study suggests that further studies on Iraqi human head lice need to be performed in different cities to determine whether Iraq has another human louse clade or not.

Clade A subgroups

The relationship of current study sequences with their counterparts in clade A from Iraq (Kurdistan, Erbil), Australia, The Philippines, Amazonia, Taiwan, Palestine, Dr-Congo, and France based on the sequences of the *cytb* gene is shown in Fig 5. The network showed there were 21 subgroups in clade A and the current study samples were separated into two subgroups while each known subgroup (4 - 21) obtained from different countries stayed in its subgroup. The first Iraq subgroup 1 (S1) contained six sequences from Basrah province (OK257845.1, OQ580994.1, OQ580995.1, OQ580996.1, OQ580997.1 and OQ580998) whereas the second one (S2) contained other six sequences from Basrah province (OK257846.1, OQ580989.1, OQ580990.1, OQ580991.1, OQ580992.1 and OQ580993). In addition to this, analyses showed that Iraq had another subgroup (S3), containing the Iraqi samples from Kurdistan, Erbil (OL684637.1, OL684638.1, OL684639.1, OL684640.1 and OL684641.1).

As noted in Iraqi samples, there were three subgroups belonging to haplogroup A, and these were S1, S2, and S3. The two subgroups (S1 and S2) were found in Basrah province and had only one nucleotide difference between them. However, the third subgroup (S3) was only found in Kurdistan-Iraq and possessed six differences with S1 and seven differences with S2 from Basrah province. Previous studies reported that more than one subgroup could be noted in the same country or province^{4, 12, 19}. This completely corresponds with the present study results.

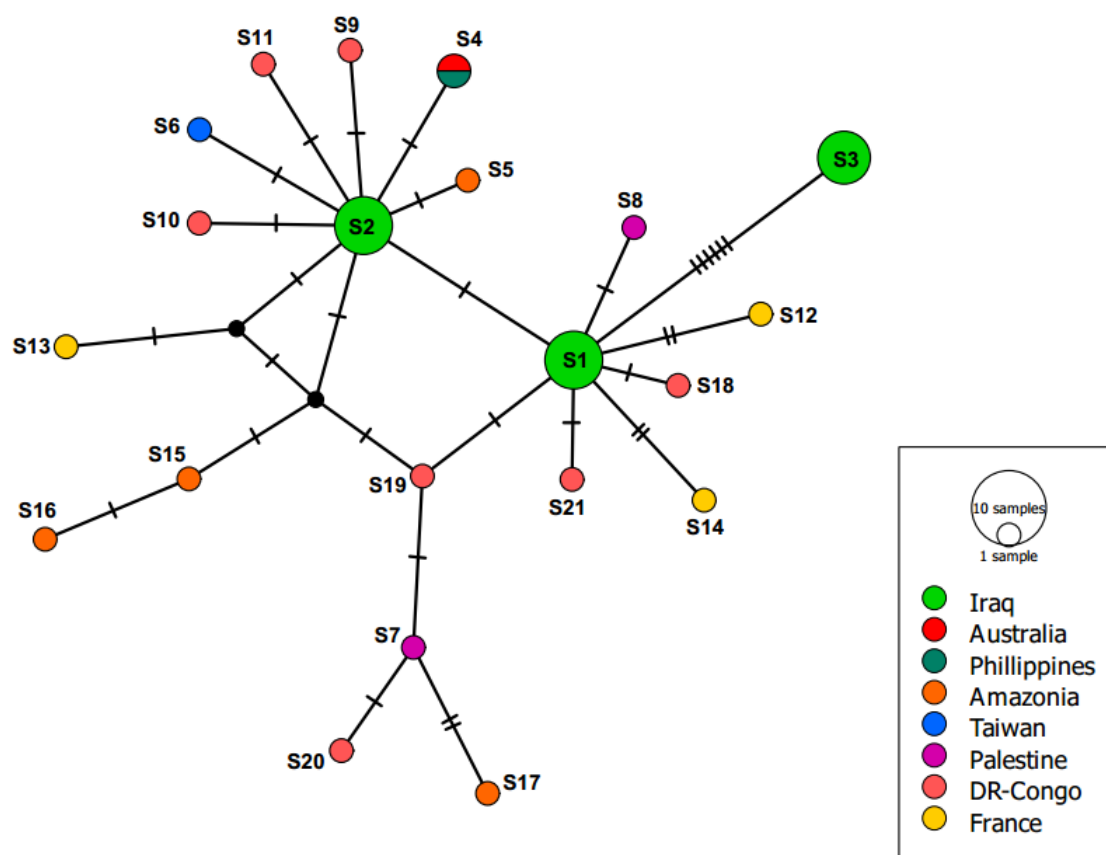


Figure 5. The subgroups of clade A from different countries were generated based on the *cytb* gene. S1 and S2 are the present data while the rest of the subgroup data is obtained from NCBI (see Fig.4 for the accession numbers). A dash between subgroups represents the mutated positions and a small dark circle represents the node of the median vector.

Alignment of *P. humanus capitis* *cytb* proteins

The deduced amino acid sequences of the selected present study samples, 1-12, were lined up with those of known subgroups of haplogroup A obtained from GenBank (Fig 6). The results showed that all the present study samples shared 100% identity within each other and with those seven subgroup sequences (A16, A55, A56, A60, A63, A65 and A67) of haplogroup A and there were no differences among their amino acid residues. Contrarily, the results revealed that the present amino acid sequences differed in one or two amino acid residues (Table 2) from 10 (58.82%) known subgroups (A19, A57-A59, A61, A62, A64, A66, A68 and A69). To gain more understanding, of whether the amino acids of current subgroups were identical/similar to the other known GenBank subgroups of the A clade or not. The alignment of amino acids of the current study subgroups with the GenBank amino acid sequences showed that haplogroup A could be divided into three sub-haplogroups (Aa, Ab, and Ac). The sub-haplogroup Aa consisted of up to 41.2% subgroups that shared 100% identity within each other in CYBT amino acid sequences and the sub-haplogroup Ab consisted of up to 47% subgroups different in only one amino acid residue at diverse locations, whereas the sub-haplogroup Ac consisted of up to 11.8% subgroups different in only two amino acid residues at also diverse locations. As can be seen from the results, all present study samples belonged to the sub-haplogroup Aa and were different from other sub-haplogroups Ab and Ac in either one or two amino acid residues, respectively.

The differences in one amino acid could be either in serine³⁰ (S³⁰), S⁶⁴, glycine⁶⁹ (G⁶⁹), aspartate⁷¹ (D⁷¹), S⁷⁴, leucine⁹⁵ (L⁹⁵) or I¹⁰¹ residues conserved in the present study sequences and sub-haplogroup Aa. These amino acids were replaced with tryptophan³⁰ (W³⁰) in the subgroup A66, phenylalanine⁶⁴ (F⁶⁴) in the subgroup A59,

	1	10	20	30	40	50	60	
Sample 1_UBK11767.1	:	GATVITNLLSA	IP	IVGSD	LV	IVW	GGF	SVSHPTLERLFTLHFLLPFVLLGFVMAHIILLH : 60
Sample 2_WKR38613.1	:	GATVITNLLSA	IP	IVGSD	LV	IVW	GGF	SVSHPTLERLFTLHFLLPFVLLGFVMAHIILLH : 60
Sample 3_WKR38614.1	:	GATVITNLLSA	IP	IVGSD	LV	IVW	GGF	SVSHPTLERLFTLHFLLPFVLLGFVMAHIILLH : 60
Sample 4_WKR38615.1	:	GATVITNLLSA	IP	IVGSD	LV	IVW	GGF	SVSHPTLERLFTLHFLLPFVLLGFVMAHIILLH : 60
Sample 5_WKR38616.1	:	GATVITNLLSA	IP	IVGSD	LV	IVW	GGF	SVSHPTLERLFTLHFLLPFVLLGFVMAHIILLH : 60
Sample 6_WKR38617.1	:	GATVITNLLSA	IP	IVGSD	LV	IVW	GGF	SVSHPTLERLFTLHFLLPFVLLGFVMAHIILLH : 60
Sample 7_UBK11766.1	:	-ATVITNLLSA	IP	IVGSD	LV	IVW	GGF	SVSHPTLERLFTLHFLLPFVLLGFVMAHIILLH : 59
Sample 8_WKR38618.1	:	-ATVITNLLSA	IP	IVGSD	LV	IVW	GGF	SVSHPTLERLFTLHFLLPFVLLGFVMAHIILLH : 59
Sample 9_WKR38619.1	:	-ATVITNLLSA	IP	IVGSD	LV	IVW	GGF	SVSHPTLERLFTLHFLLPFVLLGFVMAHIILLH : 59
Sample 10_WKR38620.1	:	-ATVITNLLSA	IP	IVGSD	LV	IVW	GGF	SVSHPTLERLFTLHFLLPFVLLGFVMAHIILLH : 59
Sample 11_WKR38621.1	:	-ATVITNLLSA	IP	IVGSD	LV	IVW	GGF	SVSHPTLERLFTLHFLLPFVLLGFVMAHIILLH : 59
Sample 12_WKR38622.1	:	-ATVITNLLSA	IP	IVGSD	LV	IVW	GGF	SVSHPTLERLFTLHFLLPFVLLGFVMAHIILLH : 59
A16_AKD27978.1	:	-----	IP	IVGSD	LV	IVW	GGF	SVSHPTLERLFTLHFLLPFVLLGFVMAHIILLH : 49
A19_AKD27981.1	:	-----	IP	IVGSD	LV	IVW	GGF	SVSHPTLERLFTLHFLLPFVLLGFVMAHIILLH : 49
A55_ANP94324.1	:	-----	IP	IVGSD	LV	IVW	GGF	SVSHPTLERLFTLHFLLPFVLLGFVMAHIILLH : 49
A56_ANP94323.1	:	-----	IP	IVGSD	LV	IVW	GGF	SVSHPTLERLFTLHFLLPFVLLGFVMAHIILLH : 49
A57_APL96989.1	:	-----	IP	IVGSD	LV	IVW	GGF	SVSHPTLERLFTLHFLLPFVLLGFVMAHIILLH : 49
A58_APL96990.1	:	-----	IP	IVGSD	LV	IVW	GGF	SVSHPTLERLFTLHFLLPFVLLGFVMAHIILLH : 49
A59_APL96991.1	:	-----	IP	IVGSD	LV	IVW	GGF	SVSHPTLERLFTLHFLLPFVLLGFVMAHIILLH : 49
A60_AWF94071.1	:	-----	IP	IVGSD	LV	IVW	GGF	SVSHPTLERLFTLHFLLPFVLLGFVMAHIILLH : 49
A61_AWF94072.1	:	-----	IP	IVGSD	LV	IVW	GGF	SVSHPTLERLFTLHFLLPFVLLGFVMAHIILLH : 49
A62_AWF94073.1	:	-----	IP	IVGSD	LV	IVW	GGF	SVSHPTLERLFTLHFLLPFVLLGFVMAHIILLH : 49
A63_QBC17249.1	:	-----	IP	IVGSD	LV	IVW	GGF	SVSHPTLERLFTLHFLLPFVLLGFVMAHIILLH : 49
A64_QBC17250.1	:	-----	IP	IVGSD	LV	IVW	GGF	SVSHPTLERLFTLHFLLPFVLLGFVMAHIILLH : 49
A65_QBC17251.1	:	-----	IP	IVGSD	LV	IVW	GGF	SVSHPTLERLFTLHFLLPFVLLGFVMAHIILLH : 49
A66_AYA29410.1	:	-----	IP	IVGSD	LV	IVW	GGF	SVSHPTLERLFTLHFLLPFVLLGFVMAHIILLH : 49
A67_AYA29409.1	:	-----	IP	IVGSD	LV	IVW	GGF	SVSHPTLERLFTLHFLLPFVLLGFVMAHIILLH : 49
A68_AYA29408.1	:	-----	IP	IVGSD	LV	IVW	GGF	SVSHPTLERLFTLHFLLPFVLLGFVMAHIILLH : 49
A69_AYA29407.1	:	-----	IP	IVGSD	LV	IVW	GGF	SVSHPTLERLFTLHFLLPFVLLGFVMAHIILLH : 49

	70	80	90	100	110	
Sample 1_UBK11767.1	:	QHGSSNPLGLDLS	DKVYFYPYFY	LKDILGGFVCLFLFVLA	CIYSPDFFMDPDNFV	: 116
Sample 2_WKR38613.1	:	QHGSSNPLGLDLS	DKVYFYPYFY	LKDILGGFVCLFLFVLA	CIYSPDFFMDPDNFV	: 116
Sample 3_WKR38614.1	:	QHGSSNPLGLDLS	DKVYFYPYFY	LKDILGGFVCLFLFVLA	CIYSPDFFMDPDNFV	: 116
Sample 4_WKR38615.1	:	QHGSSNPLGLDLS	DKVYFYPYFY	LKDILGGFVCLFLFVLA	CIYSPDFFMDPDNFV	: 116
Sample 5_WKR38616.1	:	QHGSSNPLGLDLS	DKVYFYPYFY	LKDILGGFVCLFLFVLA	CIYSPDFFMDPDNFV	: 116
Sample 6_WKR38617.1	:	QHGSSNPLGLDLS	DKVYFYPYFY	LKDILGGFVCLFLFVLA	CIYSPDFFMDPDNFV	: 116
Sample 7_UBK11766.1	:	QHGSSNPLGLDLS	DKVYFYPYFY	LKDILGGFVCLFLFVLA	CIYSPDFFMDPDNFV	: 115
Sample 8_WKR38618.1	:	QHGSSNPLGLDLS	DKVYFYPYFY	LKDILGGFVCLFLFVLA	CIYSPDFFMDPDNFV	: 115
Sample 9_WKR38619.1	:	QHGSSNPLGLDLS	DKVYFYPYFY	LKDILGGFVCLFLFVLA	CIYSPDFFMDPDNFV	: 115
Sample 10_WKR38620.1	:	QHGSSNPLGLDLS	DKVYFYPYFY	LKDILGGFVCLFLFVLA	CIYSPDFFMDPDNFV	: 115
Sample 11_WKR38621.1	:	QHGSSNPLGLDLS	DKVYFYPYFY	LKDILGGFVCLFLFVLA	CIYSPDFFMDPDNFV	: 115
Sample 12_WKR38622.1	:	QHGSSNPLGLDLS	DKVYFYPYFY	LKDILGGFVCLFLFVLA	CIYSPDFFMDPDNFV	: 115
A16_AKD27978.1	:	QHGSSNPLGLDLS	DKVYFYPYFY	LKDILGGFVCLFLFVLA	-----	: 90
A19_AKD27981.1	:	QHGSSNPLGLDLS	DKVYFYPYFY	LKDILGGFVCLFLFVLA	-----	: 90
A55_ANP94324.1	:	QHGSSNPLGLDLS	DKVYFYPYFY	LKDILGGFVCLFLFVLA	-----	: 90
A56_ANP94323.1	:	QHGSSNPLGLDLS	DKVYFYPYFY	LKDILGGFVCLFLFVLA	-----	: 90
A57_APL96989.1	:	QHGSSNPLGLDLS	DKVYFYPYFY	LKDILGGFVCLFLFVLA	-----	: 90
A58_APL96990.1	:	QHGSSNPLGLDLS	DKVYFYPYFY	LKDILGGFVCLFLFVLA	-----	: 90
A59_APL96991.1	:	QHGSSNPLGLDLS	DKVYFYPYFY	LKDILGGFVCLFLFVLA	-----	: 90
A60_AWF94071.1	:	QHGSSNPLGLDLS	DKVYFYPYFY	LKDILGGFVCLFLFVLA	-----	: 90
A61_AWF94072.1	:	QHGSSNPLGLDLS	DKVYFYPYFY	LKDILGGFVCLFLFVLA	-----	: 90
A62_AWF94073.1	:	QHGSSNPLGLDLS	DKVYFYPYFY	LKDILGGFVCLFLFVLA	-----	: 90
A63_QBC17249.1	:	QHGSSNPLGLDLS	DKVYFYPYFY	LKDILGGFVCLFLFVLA	-----	: 90
A64_QBC17250.1	:	QHGSSNPLGLDLS	DKVYFYPYFY	LKDILGGFVCLFLFVLA	-----	: 90
A65_QBC17251.1	:	QHGSSNPLGLDLS	DKVYFYPYFY	LKDILGGFVCLFLFVLA	-----	: 90
A66_AYA29410.1	:	QHGSSNPLGLDLS	DKVYFYPYFY	LKDILGGFVCLFLFVLA	-----	: 90
A67_AYA29409.1	:	QHGSSNPLGLDLS	DKVYFYPYFY	LKDILGGFVCLFLFVLA	-----	: 90
A68_AYA29408.1	:	QHGSSNPLGLDLS	DKVYFYPYFY	LKDILGGFVCLFLFVLA	-----	: 90
A69_AYA29407.1	:	QHGSSNPLGLDLS	DKVYFYPYFY	LKDILGGFVCLFLFVLA	-----	: 90

Figure 6. An alignment of *P. humanus capitis* cytb amino acid sequences of the present study and with those belonging to the A clade from GenBank.

S⁶⁹ in the subgroup A57, histidine⁷¹ (H⁷¹) in the subgroup A58, asparagine⁷⁴ (N⁷⁴) in the subgroup A19, F⁹⁵ in the subgroup A69, N¹⁰¹ in the subgroup A64 or methionine¹⁰¹ (M¹⁰¹) in the subgroup A68. The S³⁰ and S⁶⁴ residues were from the polar uncharged R group, while the W³⁰ and F⁶⁴ residues were from the nonpolar aromatic R group^{14, 15}. The polar uncharged R group was represented by the S⁶⁹ residue, whereas the nonpolar aliphatic R group was represented by the G⁶⁹ residue^{14, 15, 20}. The positively charged R group contained the H⁷¹ residue, whereas the negatively charged R group contained the D⁷¹ residue^{14, 15}. The residues S⁷⁴ and N⁷⁴ belonged to the same polar uncharged group^{14, 20}. While the F⁹⁵ residue belonged to the nonpolar aromatic R group, the L⁹⁵ residue belonged to the nonpolar aliphatic R group^{14, 15}. The I¹⁰¹ and M¹⁰¹ residues were from

the same nonpolar aliphatic R group^{14, 15, 20}. The differences in the amino acid group between subgroups at a certain location could lead to variation in the protein function of a specific subgroup.

The differences in two amino acids, I¹⁴ and I¹⁰¹ residues conserved in the present study sequences and the sub-haplogroup Aa, were replaced with the V¹⁴ and M¹⁰¹ residues in the haplotype A61. The conserved and replaced residues were all from the same nonpolar aliphatic R group, and thus, the protein function could be unaffected. However, in the present study sequences and the sub-haplogroup Aa, the S¹⁷ residue was from the polar uncharged R group, while in the subgroup A62, the G¹⁷ residue was from the nonpolar aliphatic R group^{14, 15, 20}. The difference in these residues could affect the protein function. Unlike this, V⁴⁷ conserved in the present study sequences and the sub-haplogroup Aa and I⁴⁷ replaced in the subgroup A62 were both from the nonpolar aliphatic R group^{14, 20}. Therefore, the replacement of these two amino acids could keep the protein functioning as it is.

Table 2. Amino acid differences among the current study sample and the 17 subgroups of clade A (From GenBank).

Sequence	Position										Difference
	14	17	30	47	64	69	71	74	95	101	
Sample 1	I	S	S	V	S	G	D	S	L	I	-
Sample 2	I	S	S	V	S	G	D	S	L	I	-
Sample 3	I	S	S	V	S	G	D	S	L	I	-
Sample 4	I	S	S	V	S	G	D	S	L	I	-
Sample 5	I	S	S	V	S	G	D	S	L	I	-
Sample 6	I	S	S	V	S	G	D	S	L	I	-
Sample 7	I	S	S	V	S	G	D	S	L	I	-
Sample 8	I	S	S	V	S	G	D	S	L	I	-
Sample 9	I	S	S	V	S	G	D	S	L	I	-
Sample 10	I	S	S	V	S	G	D	S	L	I	-
Sample 11	I	S	S	V	S	G	D	S	L	I	-
Sample 12	I	S	S	V	S	G	D	S	L	I	-
A16_ AKD27978.1	I	S	S	V	S	G	D	S	L	I	-
A19_ AKD27981.1	I	S	S	V	S	G	D	N	L	I	1
A55_ ANP94324.1	I	S	S	V	S	G	D	S	L	I	-
A56_ ANP94323.1	I	S	S	V	S	G	D	S	L	I	-
A57_ APL96989.1	I	S	S	V	S	S	D	S	L	I	1
A58_ APL96990.1	I	S	S	V	S	G	H	S	L	I	1
A59_ APL96991.1	I	S	S	V	F	G	D	S	L	I	1
A60_ AWF94071.1	I	S	S	V	S	G	D	S	L	I	-
A61_ AWF94072.1	V	S	S	V	S	G	D	S	L	M	2
A62_ AWF94073.1	I	G	S	I	S	G	D	S	L	I	2
A63_ QBC17249.1	I	S	S	V	S	G	D	S	L	I	-
A64_ QBC17250.1	I	S	S	V	S	G	D	S	L	N	1
A65_ QBC17251.1	I	S	S	V	S	G	D	S	L	I	-
A66_ AYA29410.1	I	S	W	V	S	G	D	S	L	I	1
A67_ AYA29409.1	I	S	S	V	S	G	D	S	L	I	-
A68_ AYA29408.1	I	S	S	V	S	G	D	S	L	M	1
A69_ AYA29407.1	I	S	S	V	S	G	D	S	F	I	1

Conclusion

In conclusion, all head louse samples collected from primary school students in north Basrah belong to *P. humanus capitis*. The present study showed that the percentage of pediculosis is significantly higher in female students than in male students. The prevalence rate significantly decreases when the student age increases in both male and female students. The molecular analysis of the *cytb* gene revealed that the louse samples in north Basrah belong to haplogroup A. There is no evidence about the existence of the other human head louse haplogroups in Basrah. For the first time in Iraq, these lice are divided into two unique subgroups (S1 and S2)

of haplogroup A which share 99.43 and 100% identities according to the analysis of nucleotide and amino acid sequences. Further studies on Iraqi human head lice need to be performed in different cities to determine whether Iraq has another human louse clade or not.

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Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours.
- We collected louse samples from primary school students. Thus, we signed on ethical consideration's approval.
- Ethical Clearance: The project was approved by the local ethical committee in the Department of Biology, College of Education for Pure Sciences.

Authors' Contribution Statement

SAM AL-Asadi wrote the paper as well as designed and analyzed experiments. AAS Al-Edany collected and kept the samples. L. AL-Kanan commented on the paper.

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الانتشار والتوصيف الجزيئي لجين *cytb* في قمل الرأس في المناطق الشمالية في محافظة البصرة، العراق

سرمد عواد موزان الاسدي¹، اسماء عبد الزهرة سبع²، لؤي الكنعان³

¹ علوم الحياة، كلية التربية للعلوم الصرفة، جامعة البصرة، بصرة، العراق.

² علوم الحياة، كلية التربية للعلوم الصرفة، جامعة البصرة، بصرة، العراق.

³ علوم الحياة، كلية التربية للعلوم الصرفة، جامعة البصرة، بصرة، العراق.

الخلاصة

يعد قمل الرأس البشري من الطفيليات الخارجية المنتشرة على نطاق واسع في جميع انحاء العالم، وينقسم الى ستة مجاميع احادية (A-F). لم تتناول الدراسات السابقة تلك المجاميع الاحادية في العراق. لذا هدفت الدراسة الحالية الى تحديد نوع قمل الرأس ومعدل انتشاره لدى طلبة المدارس الابتدائية في شمال محافظة البصرة كما هدفت الى تحديد النمط الجيني ونوع المجموعة الاحادية لقمل الرأس في تلك المناطق. أظهرت الدراسة ان جميع عينات قمل الرأس تعود الى النوع *Pediculus humanus capitis* وأن معدل انتشاره كان أعلى في الطالبات (34.9%) وبفارق معنوي كبير منه في الطلاب (23.2%). وظفت تقانة البلمرة المتسلسلة جنباً الى جنباً مع طريقة تتابع DNA لسانكر في تحديد المجاميع الاحادية بالاعتماد على بادئات متخصصة لاستهداف الجين *cytb* في قمل الرأس، وبينت النتائج تضخيم 348 زوج قاعدي من جين *cytb* في جميع عينات الدراسة. فضلاً عن ذلك اظهر دراسة تتابع الجين *cytb* لعينات تم اختيارها عشوائياً تطابق عينات الدراسة الحالية وبنسبة تراوحت بين 99.43 - 100% مع بعضها البعض و 100% مع عينات بنك الجينات (*Pediculus humanus capitis*). أوضحت نتائج الشجرة التطورية انتماء تلك التسلسلات الى المجموعة الاحادية A كما فصلت شبكة المجاميع الفرعية عينات الدراسة الحالية الى مجموعتين فرعيتين (S1 و S2). فضلاً عن ذلك أظهرت المجموعتين الفرعيتين تطابق بنسبة 99.43 و 100% اعتماداً على تسلسل القواعد النيتروجينية وتسلسل الاحماض الامينية على التوالي. اشارت الدراسة الحالية أن قمل الرأس لدى طلاب المدارس الابتدائية في شمال البصرة ينتمي إلى نوع *Pediculus humanus capitis* وأن معدل انتشاره يتراوح بين 23.2 إلى 34.9%. كما بينت لأول مرة في العراق أن قمل الرأس انحدر من مجموعتين فرعيتين فرديتين من المجموعة الاحادية A.

الكلمات المفتاحية: سايتوكروم b، المجموعة الاحادية A، قمل الرأس، الانسان، العراق.