Genetic diversity of the human head lice, Pediculus humanus capitis, *among primary school girls in Saudi Arabia, with reference to their prevalence*

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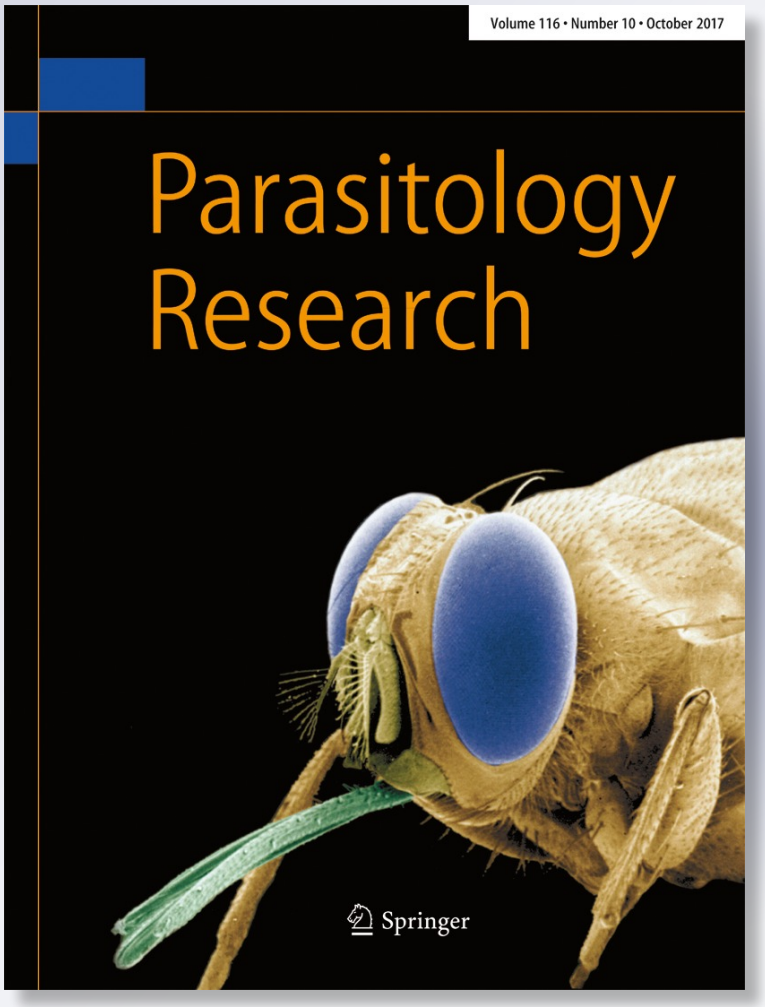

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ORIGINAL PAPER

Genetic diversity of the human head lice, *Pediculus humanus capitis*, among primary school girls in Saudi Arabia, with reference to their prevalence

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Abstract The present work aimed at investigating the genetic diversity of the head louse *Pediculus humanus capitis* (*P. humanus capitis*) among infested primary school girls at Bisha governorate, Saudi Arabia, based on the sequence of mitochondrial cytochrome b (mt cyt b) gene of 121 *P. humanus capitis* adults. Additionally, the prevalence of pediculosis capitis was surveyed. The results of sequencing were compared with the sequence of human head lice that are genotyped previously. Phylogenetic tree analysis showed the presence of 100% identity (n = 26) of louse specimens with clade A (prevalent worldwide) of the GenBank data base. Louse individuals (n = 50) showed 99.8% similarity with the same clade A reference having a single base pair difference. Also, a number of 22 louse individuals revealed 99.8% identity with clade B reference (prevalent in North and Central Americas, Europe, and Australia) with individual diversity in two base pairs. Moreover, 14 louse individual sequences revealed 99.4% identity with three base pair differences. It was concluded that moderate pediculosis (~13%) prevailed among the female students of the primary schools. It was age-and hair texture (straight or curly)-dependent. *P. humanus capitis* prevalence diversity is of clades A and B genotyping.

Keywords *Pediculus humanus capitis* · Prevalence · Mitochondrial cytochrome b gene · Genetic diversity

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Introduction

Although it is not clear if head lice (*Pediculus humanus capitis*) can act as vectors of human pathogens, they can carry pathogens. DNA from *Bartonella quintana* (Sasaki et al. 2006; Bonilla et al. 2009; Angelakis et al. 2011; Boutellis et al. 2013a), *Borrelia recurrentis* (Boutellis et al. 2013a), and *Acinetobacter baumannii* (Bouvresse et al. 2011) was detected in head lice.

The head louse, *P. humanus capitis*, is an obligate ectoparasite (Burgess 2004), coexisting with human for at least 9000 years (Mumcuoglu and Zias 1988). Pediculosis capitis caused by *P. humanus capitis* occurs worldwide (Ko and Elston 2004; Mahmud et al. 2011) and is considered as a major economic and social concern throughout developing nations where it is often associated with school age children (Falagas et al. 2008; Toloza et al. 2009; Li et al. 2010).

Genetic studies of human lice using several nuclear and mitochondrial DNA sequences have been recorded (Kittler et al. 2003; Reed et al. 2004; Light et al. 2008; Raoult et al. 2008; Angelakis et al. 2011; Boutellis et al. 2012; Veracx et al. 2013; Drali et al. 2013; Boutellis et al. 2014). Using the nuclear DNA sequences, EF-1a and 18S rDNA genes have been investigated to distinguish human louse into two subgroups; the African Sub-Saharan and worldwide lice (Yong et al. 2003). Additionally, based on the mitochondrial DNA markers, cytochrome oxidase subunit one (COI) and

Table 1 Percentage of pediculosis with *Pediculus humanus capitis* in girls of the primary schools at Bisha governorate, Saudi Arabia in relation to age (year) and hair texture (straight and curly)

Age (year)	No. of examined girls	No. of infested girls	% pediculosis
6–7	492	43	8.74
7–8	450	51	11.33
8–9	410	55	13.41
9–10	416	71	17.07
10–11	463	67	14.47
11–12	402	52	12.94
Total	2633	339	12.88
$\chi^2 = 12.489$, $p < 0.05$, Phi coefficient = 0.65			
Straight hairs	1453	291	20.03
Curly hairs	1180	48	4.07
$\chi^2 = 116.413$, $p < 0.05$, Phi coefficient = 0.198			

cytochrome b (cyt b) genes have been also used in the classification of human lice into three deeply divergent clades (A, B, C); each clade has a specific geographical distribution (Reed et al. 2004; Light et al. 2008; Raoult et al. 2008; Boutellis et al. 2012). Clade A includes both head and body lice and is worldwide in distribution (Reed et al. 2004; Raoult et al. 2008). On the other hand, clade B represents only head lice that are found in North and Central Americas (USA and Honduras, respectively), in Europe, and in Australia (Light et al. 2008). Clade C is the only representative head lice from Nepal, Ethiopia, and Senegal (Reed et al. 2004; Angelakis et al. 2011; Boutellis et al. 2012).

In this work, we aimed to classify the human head lice population clades prevalent at Bisha governorate, Saudi Arabia on the basis of genetic diversity using nucleotide sequence of mt cyt b gene. Additionally, the prevalence of pediculosis capitis relative to age and hair texture (straight or curly) was also surveyed.

Materials and methods

Study area

Bisha governorate was chosen for the present study as to the best of our knowledge there are no studies to date about the prevalence of the head lice in this governorate. It lies North Asir, Saudi Arabia, between latitude 19–21° North of the equator and between longitude 42–43° East Greenwich, rising above the sea level by about 3600 ft with annual temperature ranging from 25 to 42 °C.

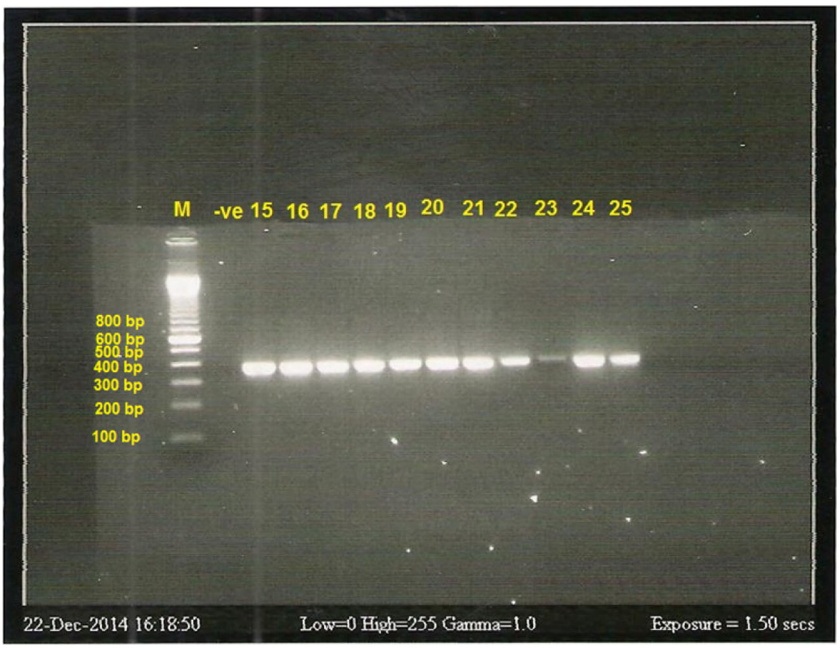

Fig. 1 Agarose gel electrophoresis profile of *Pediculus humanus capitis*. *M* DNA marker 100 bp, *−Ve* negative control, *numbers from 15 to 25* represent individual samples randomly selected that showed single base pair difference

Ethics statement

A cross-sectional school-based study was performed from February to October 2014 in 19 randomly selected public primary girl schools (village and city schools) according to the ethics principles and research protocol through informed consent of the Ministry of Education, Bisha governorate, Saudi Arabia. Girl students were screened for lice infestation, after a written consent of their parents, for the presence of pediculosis capitis. A student was defined as being infested by the presence of live or dead lice or eggs/nits. The students of the primary schools were selected as they were more liable to pediculosis capitis than any other age group (Rassami and Soonwera 2012; Gulgun et al. 2013). Pediculosis capitis relative to hair texture (straight or curly) was taken also into consideration.

Head lice collection

Adult head lice specimens were collected from the hairs of 2633 female students, representing the 19 primary schools described above, and their parents already gave a written consent, using a metal pin lice comb according to authors per se. Lice samples were randomly pooled and preserved in 95% ethanol and stored at − 80 °C till use (Kittler et al. 2003; Light et al. 2008; Ascunce et al. 2013a; Toloza et al. 2014).

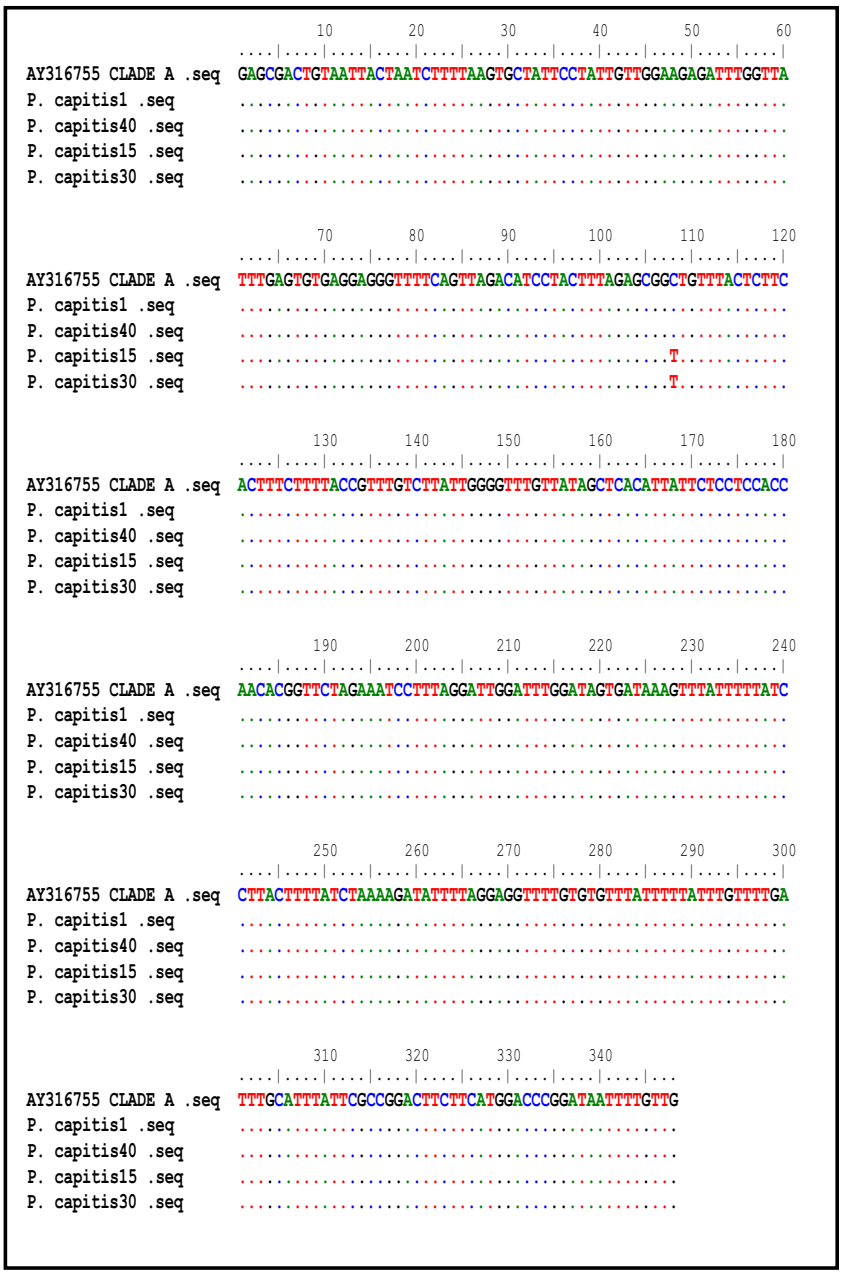

Fig. 2 Complete alignment of partial nucleotide sequence of (mt cyt b gene) for two samples randomly selected from each group with the standard reference of clade A (GenBank, accession # AY316755). *Dots* indicate identical nucleotides

DNA preparation

Of the head lice collected from 2633 female students, DNA was extracted from random 121 louse specimens using the QIAamp DNA Micro Kit (Qiagen, Valencia, USA) as described by the manufacturer. Assessment of the quantity and quality of the extracted DNA, using a NanoDrop instrument (Thermo Fisher Scientific Inc., USA), was conducted. DNA samples were preserved at − 80 °C for further use (Durand et al. 2007).

Polymerase chain reaction (PCR)

The PCR reactions were performed using a specific forward primer (5`-GAGCGACTGTAATTACTAATC-3′) and reverse primer (5`-CAACAAAATTATCCGGGTCC-3`) for mt cyt b gene (Raoult et al. 2008). PCR reaction containing 12.5 μl of GoTaq® Green Master Mix (Promega, Madison, USA), 1 μL of both forward and reverse primers, 5 μL of DNA template, and sterile nuclease-free water and completed to 25 μL was assessed. Nuclease-free water was used instead of template DNA as a negative control. The PCR was performed using automated thermal cycler (Thermal cycler, Applied Biosystems) under the following conditions: an initial denaturing step at 95 °C for 5 min, followed by 35 cycles of 40 s denaturing step at 95 °C, 40 s of primers annealing step at 55 °C, and 40 s of extending step at 72 °C, with a final extension at 72 °C for 5 min. PCR amplicons were visualized on a 2% agarose gel and stained with 10 μl ethidium bromide under UV light transilluminator.

DNA sequencing and bioinformatics analysis

DNA sequence data of each *P. humanus capitis* individual were compiled, edited, and aligned with the sequence data that are available in GenBank using the Laser gene sequence analysis software package (DNASTAR, Inc.) (Burland 1999). Data were then saved as FASTA format. Phylogenetic tree

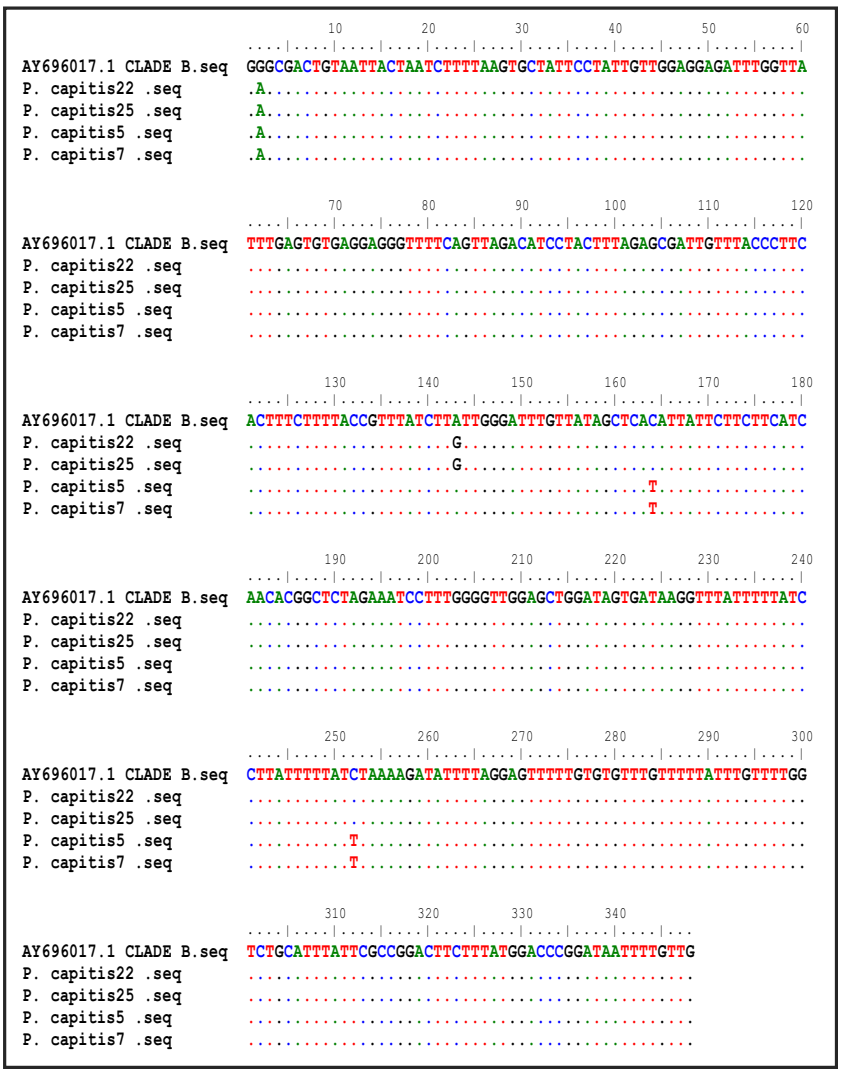

Fig. 3 Complete alignment of nucleotide sequence of partial (mt cyt b gene) for two samples randomly selected from each group with the standard reference of clade B (GenBank, accession # AY696017). *Dots* indicate identical nucleotides

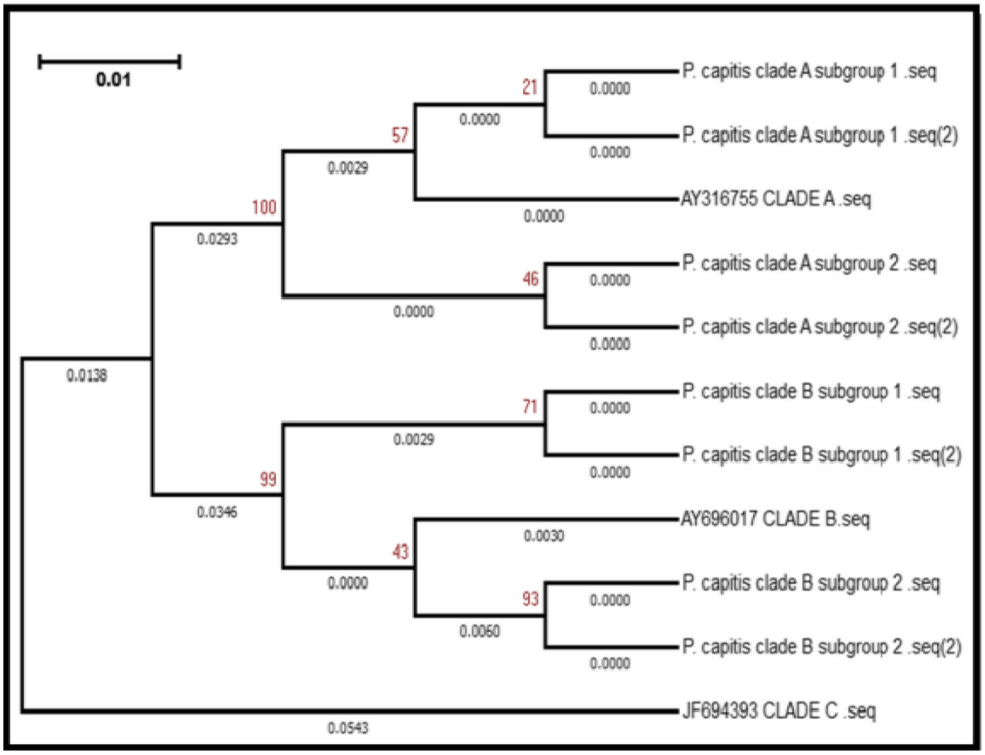

Fig. 4 Cladogram of *Pediculus humanus capitis* constructed from partial mt cyt b gene sequences. Maximum likelihood method (ML) with Tamura and Nei model, the bootstrap values of 100 replicates were used

analysis was constructed to determine percentage of similarity for sequence data by using the maximum likelihood method (ML) and Tamura and Nei model with the bootstrap value of 100 replications and the MEGA 5 software package (Molecular Evolution Genetic Analysis, Biodesign Institute, AZ, USA) (Tamura et al. 2013).

Statistical analysis

The results of percentage of pediculosis were analyzed using chi-squared test (χ^2) and Phi coefficient using IBM SPSS Statistics, Version 22, based on Dancy and Reidy (2004).

Results

Prevalence of pediculosis capitis

Table 1 shows that 6–7-year-old girls were the least age group infested with *P. humanus capitis*, where percentage of pediculosis was 8.74% ($p < 0.05$). On the contrary, 9–10-year-old girls were the highest infested girls (17.07% pediculosis) ($p < 0.05$). The total prevalence of *P. humanus capitis* among the female students of the primary schools (6–12-year-old) was about 13% ($p < 0.05$). Girls with straight hairs were about five times more infested with *P. humanus capitis* (20.03%) than those with curly hairs (4.07%) ($p < 0.05$) (Table 1). Nevertheless, the degree of association between two binary variables for each parameter was weak based on Phi coefficient.

Molecular study

The concentration of the total extracted DNA of adult lice samples ($n = 121$) was ranged from 6.2 to 82 ng/μl. About 390-bp DNA fragment was successfully amplified from the mt cyt b gene in all samples (Fig. 1). PCR amplicons of the direct sequence of *P. humanus capitis* and the phylogenetic tree analysis revealed that most of the investigated specimens ($n = 76$) were representations of clade A reference (accession # AY316755) of the GenBank data base, where individual specimens ($n = 26$) were 100% identical to this clade A, while the other individuals ($n = 50$) showed 99.8% similarity to the same reference of clade A, with a single base pair difference (Fig. 2). However, of the remaining specimens, 36 specimens belonged to clade B: 22 specimens showed 99.8% identity with this clade reference (accession # AY696017) of GenBank with diversity in two base pairs only, and 14 specimens showed 99.4% similarity to clade B reference where the diversity was detected through three base pairs (Fig. 3). The phylogenetic tree of the head lice of Bisha governorate was clearly classified into clades A and B (Fig. 4).

Discussion

To the best of our knowledge, the present study represents the first report on pediculosis capitis at Bisha governorate, Saudi Arabia. The total percentage of pediculosis (12.88%) obtained herein was similar to the results obtained by Boyle (1987) who

recorded 12% pediculosis with the head lice at Jeddah, Saudi Arabia. In comparison, other studies carried out in Saudi Arabia recorded 5.2% infestation at Al-Khobar (Al-Saeed et al. 2006) and 9.6% infestation at Abha (Bahamdan et al. 1996). Moreover, our results showed that 9–10-year-old girls were the most infested ones with the head lice. This result is in agreement with the result of Gulgun et al. (2013) for pediculosis capitis at Kayseri, Turkey. Boyle (1987) reported that 6–7-year-old girls at Jeddah, Saudi Arabia were highly infested to pediculosis capitis. This result disagrees with the results obtained in the current study.

Our results that girls with curly hairs were about five times less infested with the human head lice than those with straight hairs extend to our observation that no pediculosis capitis was recorded for girls with severe curly hairs (African hairs); although, such girls were closely associated with infested girls.

The molecular diversifications obtained in the present investigation categorized head lice into clade A, with identities ranged between 98.8 and 100%. These results are similar to other studies which indicated that this clade is globally distributed ecotype (Kittler et al. 2003; Reed et al. 2004; Light et al. 2008; Raoult et al. 2008; Boutellis et al. 2012; Veracx et al. 2013). On the other hand, the results obtained for the remaining samples that showed identities ranged between 99.8 and 99.4% revealing diversity through two and three base pairs, respectively, are in agreement with the results of Reed et al. (2004) for clade B lice from Honduras. These results successfully demonstrated the existence of clade B at Bisha as a representative of the Arabian Peninsula. However, previous reports indicated that clade B origin was unknown and its distribution was limited to the Americas (Light et al. 2008; Raoult et al. 2008; Ascunce et al. 2013b; Veracx et al. 2013). It has been suggested by Ascunce et al. (2013b) that lice from Central America may be descended from lice imported by the first people who emigrated from Asia, and this clade of lice seems likely to be imported through the international travels between America and the Old World during early globalization.

Although the present study showed that *P. humanus capitis* are phylogenetically related to clade A which is distributed worldwide, a surprising result was obtained that certain individuals were related to clade B, which is known in North and Central Americas (USA and Honduras, respectively), in Europe, and in Australia (Light et al. 2008). This phenomenon may be explained by the fact that Saudi Arabia is one of the most attractive countries of foreign labors from different continents. However, lice of clade C distributed in Nepal, Ethiopia, and Senegal (Boutellis et al. 2012; Veracx et al. 2013) are not verified in the present molecular identified ecotypes. Comparable to our results, Sunantaraporn et al. (2015) demonstrated genetic variations in head lice collected from different geographical regions in Thailand.

The high mobility of lice is rarely recognized, but lice do not exist as single groups but as actively intermingling specimens (Maunder 1983). This intermingling allows them to mate more frequently, which increase gene exchange and recombination (Veracx and Raoult 2012). Recombination of clades A and B suggests that interbreeding occurs in sympatric environments (Boutellis et al. 2013b, c). Potential recombination events between clades A and B show that their evolution is not dichotomic and that their behavior is closer to that of a rhizome (Raoult 2010; Georgiades and Raoult 2011). The latter authors also assumed that given the wide genomic plasticity of louse mitochondria, which is split on 20 mini-circular chromosomes, the exchange of a single plasmid was to be expected. Shao et al. (2012) reported that inter-minichromosome recombination occurs in head lice.

Conclusion

Moderate pediculosis prevalence among the primary school girls at Bisha governorate was recorded. These results may reflect the socio-economic status of students living in such rural environment who are more liable to pediculosis capitis than those living at different Saudi Urban governorates. Moreover, it appears that louse classification based on mt cyt b gene does not identify species but rather could be used as ecotypes marker and that globalization allows for a sympatric lifestyle in head lice.

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References

Al-Saeed WY, Al-Dawood KM, Bukhari A, Bahnasy AA (2006) Prevalence and pattern of skin disorders among female schoolchildern in eastern Saudi Arabia. Saudi Med J 22:227–234

Angelakis E, Diatta G, Abdissa A, Trape J-F, Mediannikov O, Richet H, Raoult D (2011) Altitude-dependent *Bartonella quintana* genotype C in head lice, Ethiopia. Emerg Infect Dis 17:2357–2359

Ascunce MS, Fane J, Kassu G, Toloza AC, Picollo MI, González-Oliver A, Reed DL (2013a) Mitochondrial diversity in human head louse populations across the Americas. Am J Phys Anthropol 152:118–129

Ascunce MS, Toups MA, Kassu G, Fane J, Scholl K, Reed DL (2013b) Nuclear genetic diversity in human lice (*Pediculus humanus*) reveals continental differences and high inbreeding among worldwide populations. PLoS One 8:e57619

Bahamdan K, Mhfouz AA, Tallab IA, Al-Amori OM (1996) Skin diseases among adolescent boys in Abha, Saudi Arabia. Int J Dermatol 35:405–407

Bonilla D, Kabeya H, Henn J, Kramer V, Kosoy M (2009) *Bartonella quintana* in body and head lice collected from homeless persons, San Francisco, California, USA. Emerg Infect Dis 5:912–915

Boutellis A, Veracx A, Angelakis E, Diatta G, Mediannikov O, Trape J-F, Raoult D (2012) *Bartonella quintana* in head lice from Senegal. Vector Borne Zoonotic Dis 12:564–567

Boutellis A, Mediannikov O, Bilcha KD, Ali J, Campelo D, Barker SC, Raoult D (2013a) *Borrelia recurrentis* in head lice, Ethiopia. Emerg Infect Dis 19:796–798

Boutellis A, Drali R, Rivera MA, Mumcuoglu K, Raoult D (2013b) Evidence of a co-infestation by 1 clade A and clade B head lice in pre-Columbian Chilean mummy from Cameroon. PLoS One 8: e76818

Boutellis A, Veracx A, Jonatas A, Raoult D (2013c) Amazonian head lice-specific genotypes are putatively pre-Columbian. Am J Trop Med Hyg 88:1180–1184

Boutellis A, Bitam I, Fekir K, Mana N, Raoult D (2014) Evidence that clade A and clade B head lice live in sympatry and recombine in Algeria. Med Vet Entomol 29:94–98

Bouvresse S, Socolovschi C, Berdjane Z, Durand R, Izri A, Raoult D, Chosidow O, Brouqui P (2011) No evidence of *Bartonella quintana* but detection of *Acinetobacter baumannii* in head lice from elementary schoolchildren in Paris. Comp Immunol Microbiol Infect Dis 34:475–477

Boyle P (1987) Pilot study of the prevalence of head lice infestation in a population of Saudi Arabian children. Fam Pract 4:138–142

Burgess IF (2004) Human lice and their control. Annu Rev Entomol 49: 457–481

Burland TG (1999) DNASTAR's lasergene sequence analysis software. In: Misener S, Krawetz SA (eds) Bioinformatics methods and protocols, vol 132. Humana Press, New York, pp 71–91

Dancy CP, Reidy J (2004) Statistics without Maths for Psychology, 5th edn. Prentice Hall, New Jersey

Drali R, Boutellis A, Raoult D, Rolain JM, Brouqui P (2013) Distinguishing body lice from head lice by multiplex real-time PCR analysis of the Phum_PHUM540560 gene. PLoS One 8: e58088

Durand R, Millard B, Bouges-Michel C, Bruel C, Bouvresse S, Izri A (2007) Detection of pyrethroid resistance gene in head lice in schoolchildren from Bobigny, France. J Med Entomol 44:796–798

Falagas ME, Matthaiou DK, Rafailidis PI, Panos G, Pappas G (2008) Worldwide prevalence of head lice. Emerg Infect Diseases 14: 1493–1494

Georgiades K, Raoult D (2011) The rhizome of *Reclinomonas americana*, *Homo sapiens*, *Pediculus humanus* and *Saccharomyces cerevisiae* mitochondria. Biol Direct 6:55

Gulgun M, Balci E, Karaoğlu A, Babacan O, Türker T (2013) *Pediculosis capitis*: prevalence and its associated factors in primary school children living in rural and urban areas in Kayseri, Turke. Cent Eur J Public Health 21:104–108

Kittler R, Kayser M, Stoneking M (2003) Molecular evolution of *Pediculus humanus* and the origin of clothing. Curr Biol 13:1414–1417

Ko CJ, Elston DM (2004) Pediculosis. J Am Acad Dermatol 50:1–12

Li W, Ortiz G, Fournier P-E, Gimenez G, Reed DL, Pittendrigh B, Raoult D (2010) Genotyping of human lice suggests multiple emergences of body lice from local head louse populations. PLoS Negl Trop Dis 4:e641

Light JE, Allen JM, Long LM, Carter TE, Barrow L, Suren G, Raoult D, Reed DL (2008) Geographic distributions and origins of human head lice (*Pediculus humanus capitis*) based on mitochondrial data J Prasitol 94(6):1275–1281

Mahmud S, Pappas G, Hadden WC (2011) Prevalence of head lice and hygiene practices among women over twelve years of age in Sindh, Balochistan, and North West Frontier Province: national health survey of Pakistan, 1990-1994. Parasit Vectors 4:11

Maunder JW (1983) The appreciation of lice. Proc R Inst G B 55:1–31

Mumcuoglu YK, Zias J (1988) Head lice, *Pediculus humanus capitis* (Anoplura: Pediculidae) from hair combs excavated in Israel and dated from the first century BC to the eighth century AD. J Med Entomol 25:545–547

Raoult D (2010) The post-Darwinist rhizome of life. Lancet 375:104–105

Raoult D, Reed DL, Dittmar K, Kirchman J, Rolain J-M, Guillen S, Light JE (2008) Molecular identification of lice from pre-Columbian mummies. J Infect Dis 197:535–543

Rassami W, Soonwera M (2012) Epidemiology of pediculosis capitis among schoolchildern in eastern area of Bangkok, Thailand. Asian Pac J Trop Biomed 2:901–904

Reed DL, Smith VS, Hammond SL, Rogers AR, Clayton DH (2004) Genetic analysis of lice supports direct contact between modern and archaic humans. PLoS Biol 2(11):e343

Sasaki T, Poudel SK, Isawa H, Hayashi T, Seki N, Tomita T, Sawabe K, Kobayashi M (2006) First molecular evidence of *Bartonella quintana* in *Pediculus humanus capitis* (Phthiraptera: Pediculidae), collected from Nepalese children. J Med Entomol 43:110–112

Shao R, Zhu XQ, Barker SC, Herd K (2012) Evolution of extensively fragmented mitochondrial genomes in the lice of humans. Genome Biol Evol 4:1088–1101

Sunantaraporn S, Sanprasert V, Pengsakul T, Phumee A, Boonserm R, Tawatsin A, Thavara U, Siriyasatien P (2015) Molecular survey of the head louse *Pediculus humanus capitis* in Thailand and its potential role for transmitting *Acinetobacter* spp. Parasit Vectors 8:1–7

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729

Toloza AC, Vassena C, Gallardo A, González-Audino P, Picollo MI (2009) Epidemiology of *Pediculosis capitis* in elementary schools of Buenos Aires, Argentina. Parasitol Res 104:1295–1298

Toloza AC, Ascunce MS, Reed D, Picollo MI (2014) Geographical distribution of pyrethroid resistance allele frequency in head lice (Phthiraptera: Pediculidae) from Argentina. J Med Entomol 51: 139–144

Veracx A, Raoult D (2012) Biology and genetics of human head and body lice. Trends Parasitol 28:563–571

Veracx A, Boutellis A, Raoult D (2013) Genetic recombination events between sympatric clade A and clade C lice in Africa. J Med Entomol 50:1165–1168

Yong Z, Fournier P-É, Rydkina E, Raoult D (2003) The geographical segregation of human lice preceded that of *Pediculus humanus capitis* and *Pediculus humanus humanus*. C R Biol 326:565–574