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The epidemic typhus and trench fever are risk for public health due to increased migration in southeast of Turkey

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ABSTRACT

Pediculus humanus capitis is a small ectoparasitic insect that has lived and feds on human beings for thousands of years. Molecular techniques have been used for *Pediculus* species identification and evolutionary, phylogenic, and ecological studies. A total of 23 adults of *P. h. capitis* were collected in Gaziantep, located in southeast Turkey, and DNA was isolated from all *P. h. capitis* using DNA extraction kit. All DNA samples were screened for investigate of *Ricettsia prowazekii*, *Bartonella quintana* and *Borrelia recurrentis* with real-time polymerase chain reaction. In addition, we investigated genetic variation in DNA samples of *Pediculus humanus capitis* using the cytochrome oxidase I genetic DNA sequence. We found 4 (17.4%) *Ricettsia prowazekii* and 3 (13.1%) *Bartonella quintana* in DNA samples of *Pediculus humanus capitis,* while we did not find any *Bartonella recurrentis* in any of the DNA samples. We demonstrated 1.8% genetic variations in DNA samples of *Pediculus humanus capitis* with *Bartonella quintana*. The phylogenetic tree based on the cytochrome oxidase I gene revealed that *P. h. capitis* in southeast Turkey are classified into two clades (clade A, clade B) and *Bartonella quintana* was found in only clade B. However, we did not find any genetic variations in other DNA samples in this region. The genetic variations may be related to *P. h.capitis* vector of *Bartonella quintana* has found in this study. In addition, this study was shown that *P. h. capitis* do transmit *Rickettsia prowazekii* and *Bartonella quintana* to people, epidemic typhus and trench fever may emergence in Gaziantep southeast of Turkey in the future.

1. Introduction

Pediculus is obligatory human hematophagous ectoparasites belonging to the *Pediculidae* family, and *Pediculus* is caused to *Pediculosis* infestation (Do-Pham et al., 2014; Sunantaraporn et al., 2015). Three species of lice usually infest humans: *Pediculus humanus capitis* (head lice); *Pediculus humanus corporis* (body lice); and *Phthirus pubis* (pubic lice) (Do-Pham et al., 2014).

P. h. capitis is the most common parasitic infestation in children with an annual rate of 15 million infestations (Gallardo et al., 2013; Smith and Goldman, 2012). *P. h. capitis* causes scalp itching, irritability, and occasional secondary bacterial infection as a result of scratching (Toloza et al., 2014). In addition, it may transmit epidemic typhus, which is caused by *Rickettsia prowazekii* (*R. prowazekii*), or trench fever, which is caused by *Bartonella quintana* (*B. quintana*) and the louse-borne relapsing fever caused by *Borrelia recurrentis* (*B. recurrentis*) (Coulaud et al., 2015).

The migrants have also come from areas with poor sanitary conditions and many health hazards, so they affect the distribution of *P. h. capitis* and other infectious disease in all world (Robinson et al., 2003; Seki et al., 2007; Eroglu et al., 2016). In the after math of World War I an outbreak of epidemic typhus and trench fever occurred which threatened to cause significant loss of lice (Robinson et al., 2003; Seki et al., 2007). The prevalence of *P. h. capitis* was increased 5.4% in Gaziantep after Syria civil war (mentioned the older prevalence rate before Syrian sivil war). *P. h. capitis* is a neglected infestation and it has re-emerged in Gaziantep, located in the southeast of Turkey (Eroglu et al., 2016). However, the prevalence of epidemic typhus and trench fever was not investigated. Therefore, we investigated to assess the presence of *R. prowazekii*, *B. quintana,* and *B. recurrentis* in *P. h. capitis* in Gaziantep, located in southeast Turkey. In addition, we analyzed the DNA sequence of the COI gene in *P. h. capitis,* and we investigated

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relationships between genetic variation and the vector of pathogenic bacteria.

2. Materials and methods

2.1. Pediculus humanus capitis collections and DNA extraction

Pediculus humanus capitis adult forms were collected from the heads of 23 infested primary school children using a fine-toothed louse plastic comb. *Pediculus humanus capitis* were transported in 95% ethanol and stored at −20 °C until DNA extraction was performed. DNA extraction was completed following manufacturer protocol with a QIAamp DNA mini kit (Qiagen, Courtaboeuf, France).

2.2. PCR assay and DNA sequence analysis

The primers (C1-j-1718: 5′-GGAGGATTTGGAAATTGATTAGT TCC-3′; C1-N-2191: 5′-CCAGGAAGAATAAGAATATAAACTTC-3′) were designed based on the cytochrome oxidase I (COI) gene of the *P. h. capitis* (Yong et al., 2003). The PCR reaction was set up in a final volume of 50 µl reaction mixture containing 10 µM of each primer, 10X Taq buffer DNA (Fermantas, Pittsburgh, PA), 2.5 µM dNTPs, 2.5 mM of $MgCl_2$, and 1 unit of Taq DNA polymerase (Fermantas, Pittsburgh, PA). The PCR amplification conditions were as follows; initial denaturation at 95 °C for 3 min; 35 cycles at 95 °C for 1 min, 60 °C for 1 min, and 72 °C for 1 min; and the final extension at 72 °C for 5 min. The PCR amplicons were determined via 1.5% agarose gel electrophoresis stained with ethidium bromide and visualized with ultraviolet.

2.3. Real-time PCR assay

We used previously described primers and probes for detection of *R. prowazekii*, *B. quintana* and *B. recurrentis,* with real-time PCR assay. Real-time PCR target genomic region, primers, and amplicon size are shown in Table 1.

A total of 23 *P. h. capitis* were analyzed by real-time PCR. The different real-time PCR reaction mixture was used for all bacteria (*R. prowazekii*, *B. quintana, B. recurrentis*) and primers were selected to amplification. The real-time PCR concentration and thermal condition for the PCR target is shown in Table 2. A search for inhibitors was performed in samples that showed real-time PCR negative results to assess possible real-time PCR failures to detect bacteria.

2.4. DNA sequence analysis and phylogenetic tree

The obtained nucleotide sequences were analyzed by comparison with the nucleotide sequence in the GenBank database using BLAST (http://blast.ncbi.nlm.nih.gov/blast/Blast.cgi), and all the nucleotide sequences from this study were submitted to the GenBank database. The nucleotide sequences of each region were aligned, and the percentage of intra-specific variation was calculated using BioEdit Sequence Alignment Editor, Version 7.1.9. A phylogenetic tree was constructed via the neighbor-joining method using Kimura's 2-parameter model implemented in MEGA6.06, and the tree was tested using 1000 bootstrap replicates and compared with the reference sequence.

Table 2
Real-time PCR concentration and thermal-cycler condition for detection of Ricekttsia prowazekii, Bartonella quintana and Borrelia recurrentis from P. h. capitis.

Bacteria	Real-time PCR concentration	Thermal-cycler condition
Ricekttsia prowazekii	10 µl probe master PCR kit (1X, Qiagen), 0.5 µl F-primer, 0.5 µl R-primer, 0.5 µl probe, 4 µl DNA, 4.5 µl distilled water	95 °C 1 min, 35 cycles (95 °C 15 s, 60 °C 30 s) 72 °C 10 min
Bartonella quintana	10 µl probe master PCR kit (1X, Qiagen), 0.5 µl F primer, 0.5 µl R primer,0.5 µl probe 4 µl DNA, 4.5 µl distilled water	95 °C 1 min, 35 cycles (95 °C 15 s, 60 °C 30 s), 72 °C 10 min
Borrelia recurrentis,	10 µl QuantiFastSYBRGreen PCR kit (1X) 0.5 µl F-primer, 0.5 µl R-primer, 4 µl DNA, 5 µl distilled water	95 °C 1 min, 35 cycles (95 °C 15 s, 60 °C 30 s, 72 °C 30 s), 72 °C 10 min

3. Results

The size of PCR products was approximately 524 bp for all adults of *P. h. capitis*. They were accepted as *P. h. capitis* (Fig. 1). The 4 (17.4%) *R. prowazekii* and 3 (13.1%) *B. quintana* were found in 23 *Pediculus humanus capitis*. However, *B. recurrentis* was not found in any of the *P. h. capitis* (Table 3).

The DNA of any bacteria (*R. prowazekii*, *B. quintana*, *B. recurrentis*) was not found in 16 (69.7%) children infested with *P. h. capitis*. There were no inhibitors in all real-time PCR assay. Most of the DNA sequences contained approximately 500 bp of the nucleotide sequences and all these sequences in the GenBank database. The comparisons of all DNA sequences with the GenBank database were similar to the comparisons of *P. h. capitis*. There was no nucleotide difference between *P. h. capitis* and the reference strain in GenBank (AY239286). We found that genetic variation in between 250 bp and 360 bp in *P. h. capitis*, which detected *B. quintana* in this study (Fig. 2). A 1.8% genetic variation was found in all DNA of *P. h. capitis* with *B. quintana*. Genetic distance is a measure of the genetic divergence between species or between populations within species. The genetic distance value was found 0.018 (9/500) in all *P. h. capitis* with *B. quintana*. As compared to previous records in GenBank, the similarity between sequences of *P. h. capitis* in GenBank was 98.2%. There was a 1.8% nucleotide difference between *P. h. capitis* and AY239286 in 250–360 bp. The phylogenetic tree based on the COI gene revealed that *P. h. capitis* in southeast Turkey are classified into two clades (A and B) (Fig. 3). The relationship of *B. quintana* and the *P. h. capitis* clades showed that *B. quintana* was found in only clade B.

Table 1
The gene region, primer from genomic region and amplicaon size for detection of Ricekttsia prowazekii, Bartonella quintana and Borrelia recurrentis from P. h. capitis.

Bacteria	Gene region	Primer	Reference
Ricekttsia prowazekii	*gltA*	5′-TCGGTAAAGATGTAATCGATAAG-3′ 5′-CATATCCTCGATACCATAATATGC-3′ FAM-ACTTTACTTATGATCCGGTTTTATG-TAMRA	Svraka et al.
Bartonella quintana	*fabF3*	5′-GCTGGCCTTGCTCTTGATGA-3′ 5′-GCTACTCTGCGTGCCTTGGA-3′ FAM-TGCAGCAGGTGGAGGAGAACGTG-TAMRA	Angelakis et al.
Borrelia recurrentis	*16SrRNA*	5′-TTCGCCACTGAATGTATTGC-3′ 5′-TGCCAATGTTCTTGTTGGTC-3′	Boutellis et al.

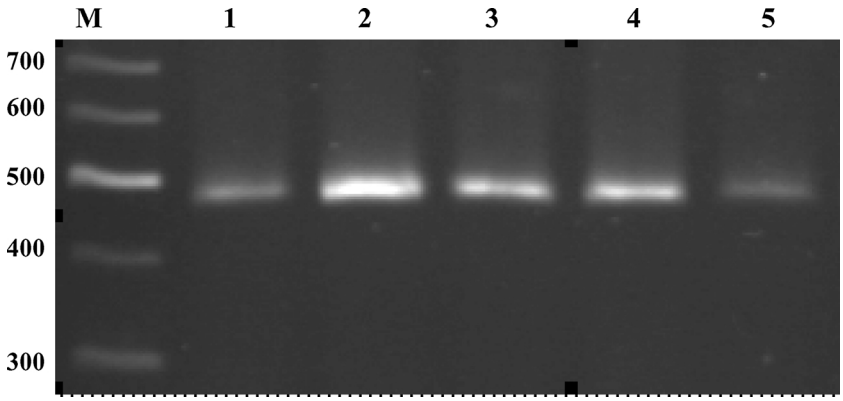

Fig. 1. The results of PCR assay from DNA samples of *P. h. capitis*. M; 100bp–1000 bp DNA Marker, Lanes 1–5; DNA of *P. h. capitis*.

Table 3
The results of real-time PCR assay for research pathogen bacteria (*Ricettsia prowazekii, Bartonella quintana, Borrelia recurrentis*) in this study.

P. h. capitis (n = 23)	Positive (%)	Negative (%)
Ricekttsia prowazekii	17.4	82.6
Bartonella quintana	13.1	86.9
Borrelia recurrentis	0	100

4. Discussion

Analysis of data on the global incidence of *P. h. capitis* has shown that this remains a major health problem in many countries (Grecchi et al., 2016). *P. h. capitis* is known to invest millions of primary school children worldwide with symptoms like itching, social stigma, and loss of sleep; however, no human pathogen transmission has been reported (Bressa et al., 2015). Some studies using molecular methods reported that *R. prowazekii*, *B. quintana* and *B. recurrentis* could be found in *P. h. capitis* (Robinson et al., 2003; Sangare et al., 2014). We found that *R. prowazekii* (4/23, 17.4%) and *B. quintana* (3/23, 13.1%) in *P. h. capitis* in this study. The role of the *P. h. capitis* in this study was shown that transmission of *Ricettsia prowazekii* and *Bartonella quintana*. In addition, the genetic variations may be related to *P. h. capitis* vector of *Bartonella quintana*.

Epidemic typhus results from infection by *R. prowazekii*, a gram-negative, obligate, intracellular bacterium, and it has been found worldwide (Portillo et al., 2015). Epidemic typhus has caused more deaths than all of the wars in history; its transmission by the *P. h. corporis* was demonstrated by Charles Nicolle (Boutellis et al., 2014). Most medical workers and scientists consider the body louse to be the only vector of *R. prowazekii*, yet there is strong experimental evidence that *P. h. capitis* can be competent vectors of *R. prowazekii* as well (Robinson et al., 2003). Fournier et al. have identified *R. prowazekii* in *P. h. corporis,* but they have not identified it in *P. h. capitis* (Fournier et al., 2002). In contrast, Robinson et al. have identified *R. prowazekii* in *P.h. capitis,* and their study has been suggested that *P. h. capitis* as vector of *R. prowazekii* (Robinson et al., 2003). Our findings supported Robinson et al.'s study. We found that *R. prowazekii* in *P. h. capitis* in southeast Turkey, and our results supported that the *P. h. capitis* is a potential vector of *R. prowazekii*.

B. quintana is a facultative intracellular bacterium that causes diseases including trench fever, chronic bacteremia, endocarditis, bacillary angiomatosis, and chronic lymphadenopathy (Sunantaraporn et al., 2015). *P. h. capitis* and *P. h. corporis* have the potential to be vectors of *B. quintana* worldwide. The first evidence that *B. quintana* might infect *P. h. capitis* was shown by Sasaki et al. (Sasaki-Fuatsu et al., 2006). The bacterial DNA of *B. quintana* was detected in *P. h. capitis* collected from homeless individuals from the USA and Nepalese slum children (Bonilla et al., 2009; Sasaki-Fuatsu et al., 2006). We identified B. quintana from *P. h. capitis,* and our result is similar to that of a previous report conducted on *P. h. capitis* of primary school children.

B. recurrentis is the etiologic agent of louse-borne relapsing fever (Houhamdi and Raoult 2005). The mortality rate of louse-borne relapsing fever varies widely and may reach 80% in untreated cases (Houhamdi and Raoult (2005). The transmission of relapsing fever to humans occurs by the rupturing of *P. h. capitis* and *P. h. corporis*

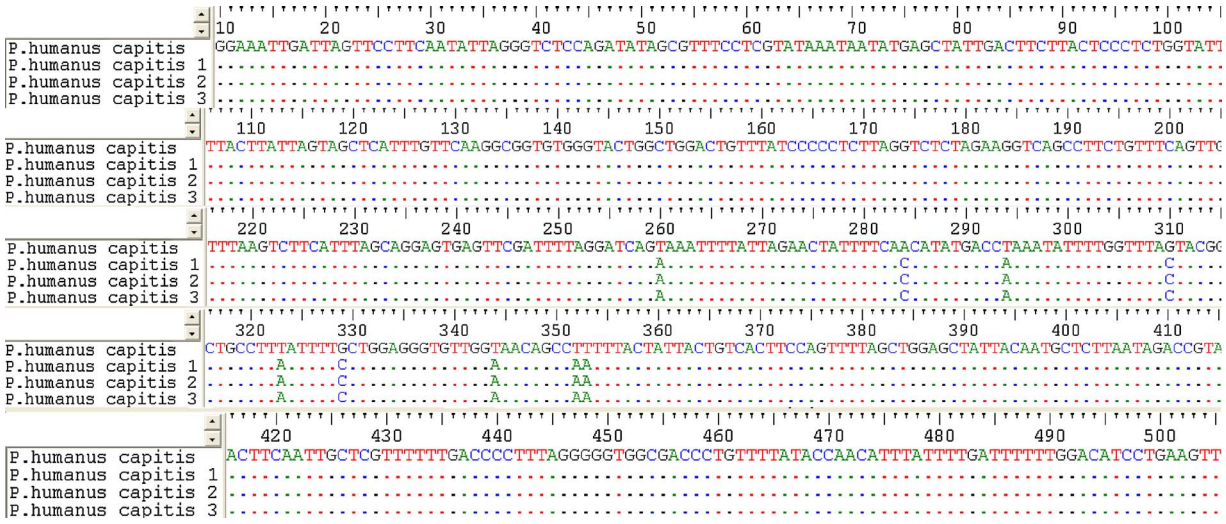

Fig. 2. The results of COI genomic DNA sequence in *P. h. capitis* with *Ricettsia prowazekii* and *Bartonella quintana* in this study. There are 1.8% genetic variation between 250 and 360 bp in *P. h. capitis* with *Bartonella quintana* while there are no variation in other *P. h. capitis* (*P.h. capitis* 1–3 are with *Bartonella quintana*).

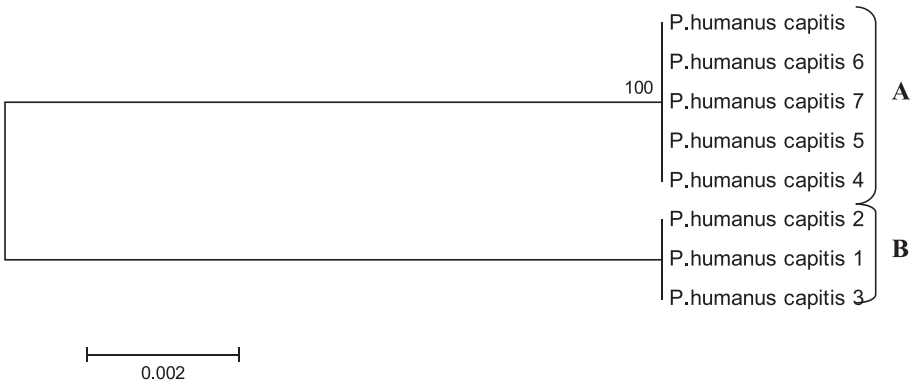

Fig. 3. The phylogenetic tree relationship of *Ricettsia prowazekii* and *Bartonella quintana* detected in *P. h. capitis* was compared with *P. h. capitis* without pathogen bacteria using the Kimura 2 parameter model with the neighbor-joining method by testing with 1000 bootstrap values. Clade A is including *P. h. capitis* with *Ricettsia prowazekii* and Clade B is including *P. h. capitis* with *Bartonella quintana*.

(Boutellis et al., 2014). The epidemiologic studies of *P. h. capitis* collected from more patients with louse-borne relapsing fever should be conducted for to determine whether *B. recurrentis* in *P. h. capitis* (Boutellis et al., 2014). Although some research identified *B. recurrentis* in *P. h. capitis*, we did not find *B. recurrentis* in *P. h. capitis*. This event might have a relationship to the low number of samples and geographic region where the study was conducted.

There are several studies about genetic variations of *Pediculus* species worldwide. Kittler et al. (2003) and Reed et al. (2004) revealed that the phylogenetic tree analyses of their studies examined the COI gene sequence data, which showed 3 clades of human lice collected from Europe, Africa, and Asia. Sunantaraporn et al. (2015) investigated the genetic variations of *P. h. capitis* collected from several geographic regions of Thailand (Sunantaraporn et al., 2015). Using COI genomic DNA sequences, they demonstrated genetic variation in *P. h. capitis* collected from different geographical regions of Thailand (Sunantaraporn et al., 2015). Their phylogenetic tree analyses of the *P. h. capitis* sequences classified the lice into two clades. This study demonstrated that *P. h. capitis* in Gaziantep, located in southeast Turkey, belong to the two different clades, A and B. We did not find *B. recurrentis* in this study, but we found *R. prowazekii* and *B. quintana* in this study. In addition, we found genetic variation in *P. h. capitis* with *B. quintana,* and this digested different clade by phylogenetic tree analysis.

The results of this study suggest that pathogen bacteria such as *R. prowazekii*, and *B. quintana* might host microbes and contribute to making *P. h. capitis* a vector for human infection diseases. Therefore, detection of pathogenic bacteria in *P. h. capitis* is crucial for monitoring the *Pediculosis capitis*-borne pathogens transmitted to humans. The relationship of *B. quintana* and *P. h. capitis* clades showed that *B. quintana* was found in a different clade. The results of this study might be used to develop effective planning for *Pediculosis capitis* control. However, future studies that include epidemiological data and a larger sample size of *P. h. capitis* should be carried out to further explore this issues.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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References

Bonilla, D.L., Kabeya, H., Henn, J., Kramer, V.L., Kosoy, M.Y., 2009. Bartonella quintana in body lice and head lice from homeless persons, San Francisco, CA, USA. Emerg. Infect. Dis. 15, 912–915.

Boutellis, A., Abi-Rached, L., Raoult, D., 2014. The origin and distribution of human lice in the world. Infect. Genet. Evol. 23, 209–217.

Bressa, M.J., Papeschi, A.G., Toloza, A.C., 2015. Cytogenetic features of human head and body lice (Phthiraptera: pediculidae). J. Med. Entomol. 52, 918–924.

Coulaud, P.J., Lepolard, C., Bechah, Y., Berenger, J.M., Raoult, D., Ghigo, E., 2015. Hemocytes from Pediculus humanus are hosts for human bacterial pathogens. Frant. Cell. Infect. Microbiol. 30, 183.

Do-Pham, G., Monsel, G., Chosidow, O., 2014. Lice. Semin. Cutan. Med. Surg. 33, 116–118.

Eroglu, F., Basaran, U., Kurklu, C.G., Yuceer, M., Yalcıntürk, R.G., Tanrıverdi, M., Daglı Icbay, E., Koltas, I.S., 2016. Pediculosis capitis is a growing neglected infestation due to migration in southeastern Turkey. Parasitol. Res. 115, 2397–2401.

Fournier, P.E., Ndihokubwayo, J.B., Guidran, J., Kelly, P.J., Raoult, D., 2002. Human pathogens in body and head lice. Emerg. Infect. Dis. 8 (12), 1515–1518.

Gallardo, A., Toloza, A., Vassena, C., Picollo, M.I., Mougabure-Cueto, G., 2013. Comparative efficacy of commercial combs in removing head lice (Pediculus humanus capitis) (Phthiraptera: pediculidae). Parasitol. Res. 112, 1363–1366.

Grecchi, C., Zanotti, P., Pontarelli, A., Chiari, E., Tomasoni, L.R., Gulletta, M., Barbui, A., Caligaris, S., Matteelli, A., Castelli, F., 2016. Louse-borne relapsing fever in a refugee from Mali. Infection. http://dx.doi.org/10.1007/s 15010-017- 0987-2.

Houhamdi, L., Raoult, D., 2005. Excretion of living Borrelia recurrentis in feces of infected human body lice. J. Infect. Dis. 191, 1898–1906.

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

Portillo, A., Santibonez, S., Garcia-Alvarez, L., Palomar, A.M., Oteo, J.A., 2015. Rickettsioses in Europe. Microbes Infect. 17, 834–838.

Reed, D.L., Smith, V.S., Hammound, S.L., Rogers, A., Clayton, D.H., 2004. Genetic analysis of lice supports direct contact between modern and archaic humans. Plos Biol. 2, e340.

Robinson, D., Leo, N., Prociv, P., Barker, S.C., 2003. Potential role of head lice, Pediculus humanus capitis, as vectors of Rickettsia prowazekii. Parasitol. Res. 90, 209–211.

Sangare, A.K., Boutellis, A., Drali, R., Socolovschi, C., Barker, S.C., Diatta, G., Rogier, C., Olive, M.M., Doumbo, O.K., Raoult, D., 2014. Detection of Bartonella quintana in African body and head lice. Am. J. Trop. Med. Hyg. 9, 294–301.

Sasaki-Fuatsu, K., Koga, R., Nikoh, N., Yoshizawa, K., Kasai, S., Mihara, M., Kobayashi, M., Tomita, T., Fukatsu, T., 2006. Symbiotic bacteria associated with stomach discs of human lice. Appl. Environ. Microbiol. 72, 7349–7352.

Seki, N., Kasai, S., Saito, N., Komagata, O., Mihara, M., Sasaki, T., Tomita, T., Sasaki, T., Kobayashi, M., 2007. Quantitative analysis of proliferation and excretion of Bartonella quintana in body lice, pediculus humanus L. Am J. Trop. Med. Hyg. 77 (3), 562–566.

Smith, C.H., Goldman, R.D., 2012. An incurable itch: head lice. Cam. Fam. Phys. 58, 839–841.

Sunantaraporn, S.V., Sanprasert, T., Pengsakul, A., Phumee, A., Boonserm, R., Tawatsin, A., Tharavara, 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, 127.

Toloza, A.C., Ascunce, M.S., Reed, D., Picollo, M.I., 2014. Geographical distribution of pyrethroid resistance allele frequency in head lice (Phthiraptera: pediculidae) from Argentina. J. Med. Entomol. 51, 139–144.

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