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< Short Communication >

Molecular epidemiological characterization of poultry red mite (*Dermanyssus gallinae*) collected from Korea

Sang-Ik Oh, Guntai Noh, Seung Won Yi, Yoon Jung Do, Eunju Kim, Jae Gyu Yoo*

Division of Animal Disease & Health, National Institute of Animal Science, Rural Development Administration, Wanju 55365, Korea

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Abstract

The poultry red mite (PRM), *Dermanyssus gallinae*, causes great economic losses to poultry industries in Korea. The molecular epidemiological characterization of PRM has been investigated in some countries, but those analysis has been not conducted yet in Korea. The aim of this study is to determine the genetic diversity of PRMs in Korea compared with those from other countries. Here, 13 PRM samples collected from Korea were analyzed with a part of the mitochondrial cytochrome oxidase subunit I (COI) gene and nuclear internal transcribed spacers (ITS) region. All the samples showed an identical COI sequence, which has also been reported in European countries and Japan. Phylogenetic diversity analysis showed that the mites from Korea were genetically related to those in other countries. The nuclear ITS region sequences were classified into three sequence types. Additionally, one of the ITS sequences was an intermediate type, implying that a hybridization event occurred among the mite populations in Korea. These findings suggested PRMs from Korea showed low genetic diversity with respect to mitochondrial COI gene, but three different populations inhabited in Korea with respect to nuclear ITS region sequences.

Key words : Korea, Mitochondrial COI gene, Nuclear ITS region, Phylogenetic diversity, Poultry red mite

INTRODUCTION

The poultry red mite (PRM), *Dermanyssus gallinae*, is a blood-feeding ectoparasite of poultry, which is characterized by five developmental stages, namely, egg, larva, protonymph, deutonymph, and adult (Sparagano et al, 2009). PRM is a small parasite (approximately 1.5 mm in length) which spend most of their life cycle away from the hens, hiding in cracks and crevices within the poultry house (Sparagano et al, 2009). The PRM should feed on blood meal to develop from a protonymph to an adult, and to reproduce (Axtell and Arends, 1990). During these developmental stages, the mites cause irritation, anemia, and weight loss; increase water intake, and reduce egg production and quality in hens, leading to economic loss in the poultry industry (Chauve, 1998; Brännström et al, 2008; Sparagano et al, 2009). Recently, the issue of pesticide residues in eggs, which have been misused to eradicate PRMs in Korean layer farms, have resulted in significant concerns for poultry industry and public health (Nam et al, 2018). Although several studies have been conducted to develop PRM management methods since the emergence of pesticide egg contamination issue in Korea, but studies were limited in the development of novel chemicals against PRM (Lee et al, 2017; Kim et al, 2018; Lim et al, 2018; Lee et al, 2019).

The initial infection of PRMs in layer poultry farms is known to be caused by the arrival of new hens in the farms, introduction of contaminated equipment, transmission via the shoes and clothes of farm workers, or movement of wild birds (Øines and Brännström, 2011). Genetic diversity and phylogenetic studies on PRMs are

*Corresponding author: Jae Gyu Yoo, Tel. +82-63-238-7220, Fax. +82-63-238-7235, E-mail. vetjack@korea.kr

important to understand the source of mites—whether they are transmitted by infected wild birds or infected hosts when hens are transferred between breeding facilities and layer farms (Marangi et al, 2014). Moreover, this information is also essential for the development of biological pest control strategies by identifying the dissemination of resistant PRM population between layer poultry farms (Cruickshank, 2002; Øines and Brännström, 2011). To date, their genetic variability patterns based on nucleotide sequences have been investigated in Europe, the United States, Australia, and Brazil (Brännström et al, 2008; Marangi et al, 2009; Roy et al, 2009; Øines and Brännström, 2011; Marangi et al, 2014; Huong et al, 2015). In these studies, the DNA of PRMs has been sequenced with mitochondrial cytochrome oxidase subunit I (COI) gene and nuclear internal transcribed spacers (ITS) region. Among Asian countries, there is just one study conducted in Japan, which described the genetic relationship of PRMs with those in their country (Houng et al, 2015).

In the present study, we investigated both mitochondrial and nuclear sequences data from PRMs distributed in Korea, which is useful for understanding the genetic diversity and their intricate histories of populations. The findings could also provide valuable basic information for increasing awareness about the molecular epidemiological characterization of PRMs from layer farms in Korea.

MATERIALS AND METHODS

Thirteen samples of PRM pools were obtained from 10 different layer poultry farms between January 2018 and July 2019 in Korea. A total of 40 adult mites were contained to each samples of PRM pools. Among them, six samples were collected from two different hen house buildings on three layer farms (K2.1 and K2.2, K3.1 and K3.2, and K4.1 and K4.2 derived from K2, K3, and K4 farms, respectively). All samples were stored at −72℃ until further use. DNA was extracted using the Qiamp DNA Mini Kit (Qiagen Inc., Valencia, CA, USA) according to the manufacturer's protocol, with some modifications. The mites were homogenized with zirconia beads using TissueLyser II (Qiagen Inc.) in 25 μL of PBS, and the volume was adjusted to 100 μL with AL buffer. The volume of other reagents was also modified (10 μL of proteinase K, 100 μL of AL buffer, 100 μL of ethanol, and 260 μL of AW1 and AW2 buffer). The step with proteinase K was prolonged overnight at 56℃. The genetic material was finally eluted with 50 μL of AE buffer and stored at −24℃ until further use.

A part of the mitochondrial COI gene and nuclear ITS region was amplified using the primers which designed in a previous study (Houng et al, 2015). The amplified fragment corresponded to nucleotide positions 61～742 of the reference COI gene sequences (accession no. AM921853) and 97～657 of the reference ITS region sequences (accession no. GQ129212). Polymerase chain reaction (PCR) was performed according to a previous study and amplification was carried out using the BioRad T100 Thermocycler (Bio-Rad Laboratories Ltd., Hercules, CA, USA) (Houng et al, 2015). Sequencing was conducted using the Bigdye^{TM} Terminator Cycle Sequencing Ready Reaction kit V.3.1 in combination with the 3730XL Capillary DNA sequencer machine (Applied Biosystems, Foster city, CA, USA) by SolGent Co., Ltd. (Daejeon, Korea).

The COI gene sequences obtained in this study were aligned using Clustal Omega Alignment Tools (http://www.ebi.ac.uk/tools/msa/clustalo/). A total of 476 bp that covered the position from 121 to 596 of the reference COI gene sequences was used to reveal the phylogenetic relationship with 1027 COI gene sequences reported worldwide, including the European countries, the United States, Australia, Brazil, and Japan (Øines and Brännström, 2011; Houng et al, 2015). The COI gene sequences of PRMs from other countries were clustered into four haplogroups A, B, C, and D by Øines and Brännström (2011) and the mites from Japan were grouped into four sub-haplogroups AJ1, AJ2, BJ1, and BJ2 by Houng et al. (2015). The mites with identical sequences were designated as one haplotype. In the ITS region sequence, 522 bp corresponding to the reference sequences of the ITS region at the position from 116 to 637 was used for the phylogenetic analysis to reveal the relationship with sequences of PRMs from Korea. To determine the closest ITS region sequences obtained,

identification was performed in public data libraries using the basic local alignment search tool (BLAST) on the National Center for Biotechnology Information (NCBI, USA). For the phylogenetic analysis, we also used the ITS region sequences from other countries which had an identical COI gene sequences. The phylogenetic trees were constructed using the neighbor-joining method. The sequence data were sampled at 1000 replicates for bootstrap analysis using Mega-X software with Kimura two-parameter option.

RESULTS

The 13 mitochondrial COI gene sequences from mite samples in Korea have been deposited in the GenBank database (accession numbers MN249072-MN249084). The phylogenetic analysis results of this study are shown in Fig. 1. All samples in this study showed 100% identical 476-bp COI gene sequences, which were aligned to analyze the phylogenetic relationship of PRMs. The mites in this study were classified into only one haplotype (the Korean haplotype). The phylogenetic analysis revealed that the COI gene sequences of PRMs were included in haplogroup B, reported in a previous study (Øines and Brännström, 2011). The Korean haplotype was also included in sub-haplogroup BJ1 and showed identical sequences with those of BJ1.2 haplotype previously reported in Japan (Houng et al, 2015). In addition, this haplotype was identical to the eight COI gene sequences of PRMs from other countries, including Japan (*n*=4), Poland (*n*=2), the UK (*n*=1), and Sweden (*n*=1).

The 13 nuclear ITS region sequences of PRMs in this study were 522 bp, and they have been deposited in the GenBank database (accession numbers MN251171-MN251183). The results revealed that there are three sequence types that had different alleles in two positions corresponding to 199 and 516 of the reference ITS region sequences. Positions 199 and 516 are included in the ITS1 and ITS2 regions, respectively. Among the 13 analyzed samples from 10 farms in this study, seven samples showed C at positions both 199 and 516 (Type I), whereas five samples showed T and A at positions 199 and 516 (Type II), respectively. One of our collected sample showed intermediate ITS region sequences (Type III) between Type I and II, which had T and C at positions 199 and 516, respectively. The phylogenetic analysis results of the ITS region sequences are shown in Fig. 2. Of the eight samples from other countries that had the Korean haplotype of COI gene sequences, two ITS region sequences of PRMs in Japan were obtained in the database, namely Type I sequence collected in Hokkaido and Type II in Iwate, respectively (Houng et al, 2015). Geographical distribution of the ITS region sequence types in Korea is shown in Fig. 3. The PRMs with Type I sequence were restricted to Gyeonggi and Gyeongnam Provinces in Korea, while Type II sequence was distributed in Chungbuk, Chungnam, and Jeonnam Provinces in Korea. The intermediate ITS region sequence type (Type III) was found in Jeonbuk Province in Korea. The six mite samples from three farms of two different hen house buildings showed also identical sequences within the same farm.

DISCUSSION

Our findings revealed the genetic relatedness of PRMs within Korea and with other countries, with respect to the mitochondrial and nuclear sequences of the mites. Through the knowledge of the phylogeny of PRMs from layer farms in Korea, we could understand about the molecular epidemiological characterization and assume the main infection routes of PRMs in layer farms.

The mitochondrial COI gene sequences in this study indicated that PRMs collected in Korea consists of one homogeneous population, implying that the mites that had a low genetic diversity background were recycled within Korea by intraregional migration. The result is inconsistent with that of previous studies in Europe and Japan, which showed phylogenetic diversities in PRMs in the respective countries (Øines and Brännström, 2011; Houng et al, 2015). In this study, the PRMs were included in haplogroup B, which is composed of the COI gene sequences from Norway, Sweden, the UK, the Netherlands, Poland, Denmark, France, and Japan. Although we could not reveal the origin of this COI haplotype,

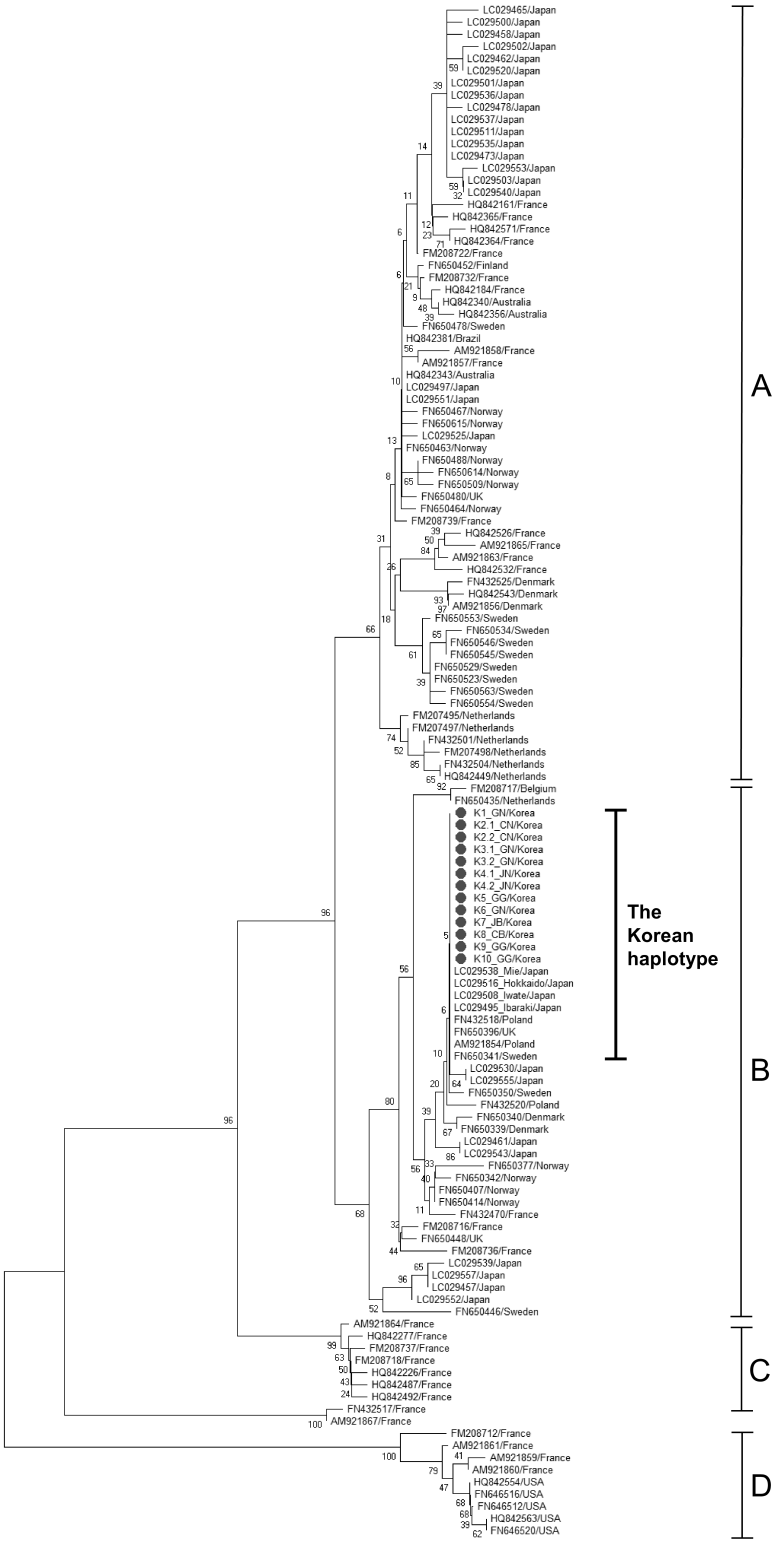

Fig. 1. Phylogenetic tree constructed with the nucleotide sequences of the mitochondrial COI gene using the neighbor-joining method. The tree was constructed with 476 bp of the COI gene sequences of poultry red mite in this study and that previously reported in other countries (Roy et al, 2009; Øines and Brännström, 2011; Houng et al, 2015). The COI gene sequence of 13 mite samples collected from Korea is labeled with a red circle. All sequences obtained from previous studies are shown with the accession number and the country where the mites were detected. Tree reliability was tested by running 1000 bootstrap replicates. The haplotypes, A, B, C, and D from a previous study are indicated with thin vertical lines (Øines and Brännström, 2011). The haplotype found in this study (the Korean haplotype) is indicated with thick vertical lines.

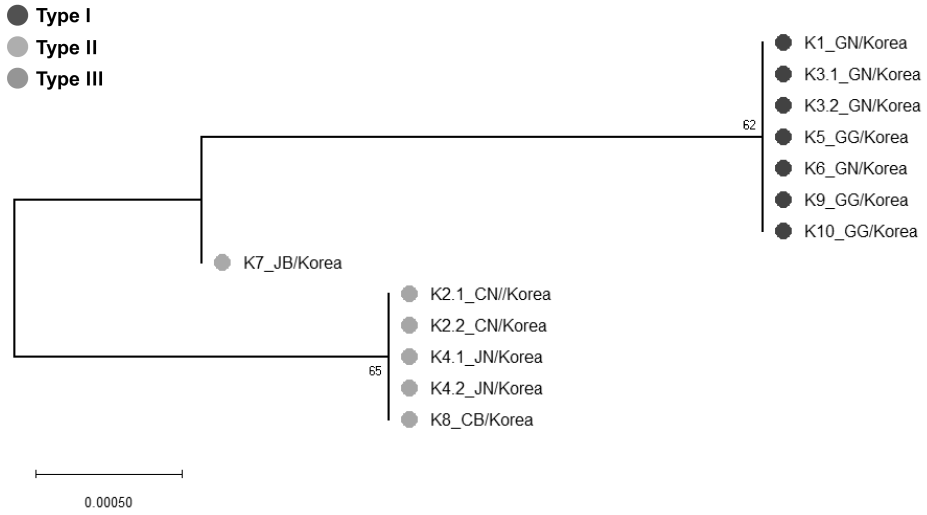

Fig. 2. Phylogenetic tree constructed with nucleotide sequences of the nuclear internal transcribed spacers (ITS) region using the neighbor-joining method. The tree was constructed with 522 bp of the ITS region sequences from poultry red mites in this study. Type I, II, and III sequences of the ITS region are labeled with blue, green, and orange circles, respectively. Tree reliability was tested by running 1000 bootstrap replicates.

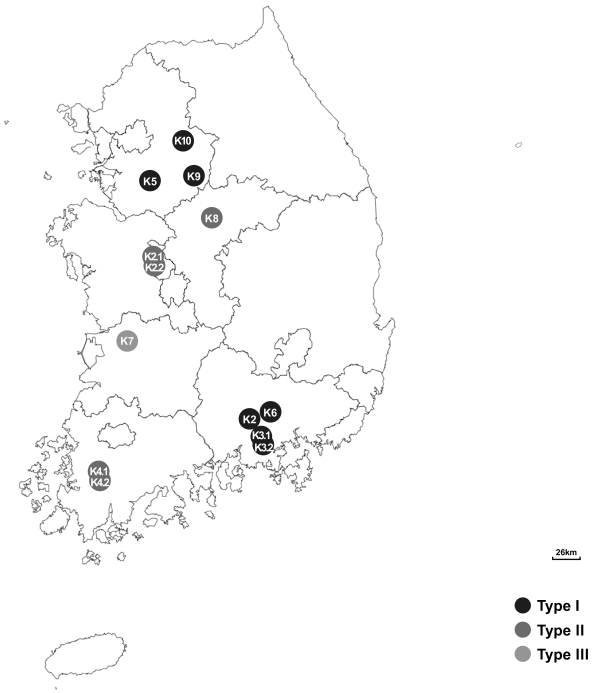

Fig. 3. Geographical distribution of the sequence types in poultry red mites collected in this study based on the ITS region sequence. Type I, II, and III of ITS region sequences are labeled with blue, green, and orange circles, respectively. The farms from which mite samples were collected in this study are indicated with a circle.

the results imply that PRMs collected from Korea are genetically related to the mites in the European countries and Japan. It is worthy to note that the Korean haplotype of the COI gene sequences was coincidence with haplotype BJ1.2 which included in sub-haplogroup BJ1, reported by Huong et al. (2015). Given that PRMs clustered into BJ1 sub-haplogroup were distributed throughout Japan, the results supported the hypothesis that PRMs collected from Korea are strongly related to those from Japan (Houng et al, 2015). Overall, PRMs distributed in Korea was identified to have the same mitochondrial COI gene sequence, but this Korean haplotype was not an indigenous type. As PRMs have the possibility of international migration, continuous monitoring is essential to detect emerging a novel COI haplotype of PRM from other countries and obtain updated genetic information.

In the present study, of the 13 mite samples, 12 ITS region sequences (7 and 5 samples of Types I and II, respectively) from Korea were 100% identical to 32 identified ITS region sequences of mite samples (25 and 8 of Types I and II, respectively) from Japan (Houng et al, 2015). The results implied PRMs in Korea were genetically close to those from Japan, although the COI gene sequence was different. Notably, one mite sample from layer farms in Jeonbuk Province of Korea showed an intermediate ITS region sequence type (Type III), which had a different allele with both Type I at position 199 of the ITS1 region and Type II at position 516 of the ITS2 region. This intermediate ITS region sequence type (Type III) could not be found in any other country. Furthermore, three ITS region sequence types in this study were divided according to the geographical distribution in Korea. However, the sample size of the present study was relatively small to establish a defini-

tive conclusion. For a comprehensive understanding of the geographical distribution of types of ITS region sequences from PRM, further sequence analyses of the mites collected from all the layer farms in Korea are needed.

Although all the PRM samples collected in this study contained the same COI gene sequences, the ITS region sequences were divided into three different types. The results were dissimilar with those of previous reports on PRM samples derived from the same COI haplogroups showed no differences in the ITS region sequences, except two sample of haplotype BJ1.2 collected from Hokkaido and Iwate (Øines and Brännström, 2011; Houng et al, 2015). The mitochondrial and nuclear sequence data have been used as markers to study intricate histories of mite populations, including hybridization and discrimination between mites which had different genetic background (Roy and Buronfosse, 2011). A previous study has suggested discordance between the haplotypes based on the sequence of mitochondrial gene and that of nuclear gene implies a hybridization event that occurred between different haplotypes of the mites, because the COI gene and ITS region are mitochondrial and nuclear genes, respectively (Roy and Buronfosse, 2011; Houng et al, 2015). Therefore, the results suggested that a hybridization event occurred among the PRM populations in Korea. Furthermore, the emergence of intermediate ITS region sequences (Type III) in this study indicate hybridization between PRM populations of Type I and II ITS region sequences.

This is the first phylogenetic study of PRMs collected from poultry layer farms in Korea. The results revealed genetic homogeneity of PRMs distributed in Korea with respect to the mitochondrial COI gene. Regarding the nuclear ITS region, the PRMs collected in Korea were classified into three types, which were distributed according to the geographic locations. Notably, the intermediate ITS region sequences type (Type III) in this study is a strong evidence for hybridization of PRM populations with an identical mitochondrial COI haplotype in Korea. The findings would help understand the phylogenetic relationships among PRMs distributed in Korea and other countries and provide scientific background to develop control strategies for the mites.

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REFERENCES

Axtell R, Arends J. 1990. Ecology and management of arthropod pests of poultry. Annu Rev Entomol 35: 101-126.

Brännström S, Morrison, D, Mattsson J, Chirico J. 2008. Genetic differences in internal transcribed spacer 1 between *Dermanyssus gallinae* from wild birds and domestic chickens. Med Vet Entomol 22: 152-155.

Chauve C. 1998. The poultry red mite *Dermanyssus gallinae*: current situation and future prospects. Vet Parasitol 8: 364-376.

Cruickshank RH. 2002. Molecular markers for the phylogenetics of mites and ticks. Syst Appl Acarol 7: 3-15.

Huong, CTT, Murano T, Uno Y, Usui T, Yamaguchi T. 2015. Molecular epidemiological characterization of poultry red mite, *Dermanyssus gallinae*, in Japan. J Vet Med Sci 77: 1397-1403.

Kim HK, Lee SJ, Hwang BY, Yoon JU, Kim GH. 2018. Acaricidal and repellent effects of Cnidium officinale-derived material against *Dermanyssus gallinae* (Acari: Dermanyssidae). Exp Appl Acarol 74: 403-414.

Lee SJ, Kim HK, Kim GH. 2019. Toxicity and effects of essential oils and their components on *Dermanyssus gallinae* (Acari: Dermanyssidae). Exp Appl Acarol 78: 65-78.

Lee SJ, Yoon JU, Park GH, Kim HK, Kim GH. 2017. Evaluation of susceptibility of red poultry mite, *Dermanyssus gallinae* (Acari: Dermanyssidae) in Five regions to 11 acaricides. Korean J Appl Entomol 56: 427-434.

Lim CI, Park SG, Choe HS, Ryu KS. 2018. Effect of spraying chamaecyparis obtusa essential oil on the elimination of red mite (*Dermanyssus gallinae*) in laying hens. Kor J Poult Sci 45: 193-200.

Marangi M, Cantacessi C, Sparagano O, Camarda A, Giangaspero A. 2014. Molecular characterization and phylogenetic inferences of *Dermanyssus gallinae* isolates in I taly within an E uropean framework. Med Vet Entomol 28: 447-452.

Marangi M, De Luna C, Cafiero MA, Camarda A, Le Bouquin S, Huonnic D, Giangaspero A, Sparagano O. 2009. Phylogenetic relationship between *Dermanyssus gallinae* populations in European countries based on mitochondrial COI gene sequences. Exp Appl Acarol 48: 143-155.

Nam SH, Kwak JI, Kim D, An YJ. 2018. A comparative study of management system of unregulated agricultural pesticides in Korea, the European Union, and the United States of America: a review. J Appl Biol Chem 61: 195-204.

Roy L, Buronfosse T, 2011. Using mitochondrial and nuclear sequence data for disentangling population structure in complex pest species: a case study with *Dermanyssus gallinae*. PLoS One 6: e22305.

Roy L, Dowling A, Chauve C, Lesna I, Sabelis M, Buronfosse T. 2009. Molecular phylogenetic assessment of host range in five *Dermanyssus* species. Exp Appl Acarol 48: 115-142.

Sparagano O, Pavlićević A, Murano T, Camarda A, Sahibi, H, Kilpinen O, Mul M, Van Emous R, Le Bouquin S, Hoel K, Cafiero MA. 2009. Prevalence and key figures for the poultry red mite *Dermanyssus gallinae* infections in poultry farm systems. Exp Appl Acarol 48: 3-10.

Øines Ø, Brännström S. 2011. Molecular investigations of cytochrome c oxidase subunit I (COI) and the internal transcribed spacer (ITS) in the poultry red mite, *Dermanyssus gallinae*, in northern Europe and implications for its transmission between laying poultry farms. Med Vet Entomol 25: 402-412.