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Molecular Characterization of *Dermanyssus gallinae* in Türkiye Based on 16S and 18S rDNA

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ABSTRACT

The poultry red mite, *Dermanyssus gallinae* (De Geer, 1778), is widely regarded as the significant ectoparasite of egg-laying hens worldwide. Since many molecular studies on poultry red mites have focused on analyzing COI and ITS1-2 genes, the present study aimed to identify 16S rDNA and the relatively understudied nuclear 18S rDNA genes of Turkish *D. gallinae* populations. Twenty-eight different *D. gallinae* populations were collected from henhouses throughout Türkiye, and the target genes were amplified using conventional PCR after morphological analysis. Haplotype analyses of the 16S rDNA sequences revealed 14 different haplotypes, with Turkish *D. gallinae* grouped into two of these haplotypes. The intra-species genetic variation of the 18S rDNA and 16S rDNA sequences examined in the present study and the available sequences in public GeneBank were determined as 0.17% and 0.53%, respectively. The obtained sequences belonging to *D. gallinae* from Türkiye were submitted to GenBank for the first time. Given the importance of identifying genetic diversity within and between species across different geographical regions, the obtained data may contribute substantially to the genetic knowledge of the PRMs.

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Introduction

The poultry red mite, PRM, *Dermanyssus gallinae* (De Geer, 1778) is a vital ectoparasite threatening to the poultry industry worldwide (Sparagano et al., 2014). The PRM is an obligate blood-feeding parasite and leads to a considerable reduction in the production of eggs (Sleeckx et al., 2019). The PRM can also transmit disease-causing pathogens to hens (De Luna et al., 2008). As a result of these direct and indirect adverse effects, welfare problems and significant economic losses (estimated to be 231 million euros in Europe) often occur in the egg-laying hen industry (Sigognault Flochlay et al., 2017). According to a recent review, 83% of European poultry houses are infested by *D. gallinae* (George et al., 2015). This prevalence is even higher, reaching 100% in Türkiye's farms (Koç and Nalbantoglu, 2021; Konyalı and Savaş, 2021).

Over the last several decades, various molecular markers have been employed in several Acari to explore a variety of purposes, including the definition of taxonomy, the understanding of population structure, the detection of the geographical origin, and even their ecological adaptation as well as food preferences (Hebert et al., 2003; Dong et al., 2021). Several nuclear- and mitochondrial-encoded genes have preferably been amplified as molecular markers so far (Dabert, 2006). The mitochondrial DNA (mtDNA) genes, on the other hand, have been frequently employed in phylogenetic studies due to a large number of copies of mtDNA, their maternal inheritance, haploid state, and rapid mutation rates, as well as the simplicity of designing primers to amplify conserved mitochondrial genes (Avise, 1987; Behura, 2006). In the case of PRM, the genetic diversity studies have targeted several genes, including the *COI* (Karp-Tatham et al., 2020; Roy et al., 2021), 16S rDNA (Roy et al., 2009; Roy et al., 2010), nuclear internal transcribed spacer regions (Chu et al. 2015; Brännström et al., 2008; Oines and Brännström, 2011; Roy and Buronfosse, 2011) Tropomyosin, and elongation factor 1-alpha (EF-1α) (Roy et al., 2010; Roy et al., 2021).

The present study aims to determine and compare the genetic variation of molecular markers, including a nuclear (18S rDNA) and a mitochondrial (16S rDNA) gene in 28 *D. gallinae* populations collected from different locations in Türkiye. In addition, the comparisons were further expanded using available sequences in the public GenBank. The results will contribute to mite phylogeny and population-genetic studies on *D. gallinae*. The sequences of 18S rDNA and 16S rDNA genes of Turkish *D. gallinae* were submitted to GenBank for the first time.

Materials and Methods

Mite samples

Twenty-eight populations of *D. gallinae* were sampled using a fine brush from different integrated laying hen farms in Türkiye during 2022-2023 (Table 1). The collected mites were transported to the laboratory in 70% and 90% ethanol for further morphological and molecular processing, respectively. The sampled mites were identified at the species level based on their morphology using a stereomicroscope (Stemi 2000-C, Zeiss, Germany) (Naegele, 1963; Di Palma et al., 2012).

Genomic DNA extraction

All mites were grouped, including ten mites according to the poultry houses to extract the genomic DNA (gDNA). Pooled mites were rinsed with sterilized water and dried on a filter paper then extracted using the "Qiagen DNeasy Blood and Tissue Kit" following the manufacturer's instructions. Last, the quality of gDNA was evaluated using a spectrophotometer (NanoDrop™ 2000, Thermo Scientific). The extracted gDNAs were kept at -20°C until PCR was performed.

Amplification of target genes and phylogenetic analysis

The primers, Rh16S-1 GCTCAATGATTTTTTAAAT TGCTG and Rh16S-2 CCGGTCTGAACTCAGATCATG were employed to amplify the 442 bp of 16S ribosomal DNA at an annealing temperature of 55°C (De Rojas et al., 2001). The primers, 18S_F ATATTGGAGG GCAAGTCTGG and 18S_R1 TGGCATCGTTTATG GTTAG were used to amplify the 500 bp of 18S rDNA at an annealing temperature of 50°C (Otto and Wilson, 2001).

Each PCR reaction was conducted in a final volume of 30 µL, consisting of 2 µL of mite gDNA (61-74 ng/µL), one µL of each primer (forward and reverse), 11 µL of PCR-grade water, and 15 µL of EmeraldAmp Max PCR Master Mix (which includes buffer, MgCl2, and dNTPs) (Takara, Japan). The PCR products were purified using the "HighPrep PCR clean-up system" (MagBio Genomics Inc.) and followed by sequencing at Macrogen Inc. (Amsterdam, Netherlands).

Sequencing chromatographs were initially checked using 'BioEdit v7.0.9.0' (Hall, 1999). The obtained sequences were then examined in the GenBank database using the BLAST analysis. Multiple sequence alignment was carried out using 'MAFFT v.7' with the 'Auto' strategy (Katoh et al., 2019) and further refined with 'Bioedit v.7.0.5' software (Hall, 1999). Intra- and interspecific genetic distances were assessed using 'MEGA X' (Kumar et al., 2018) with the 'Kimura-2 parameter' (K2P) model with 1000 bootstrap supports. Due to the lack of available sequences in NCBI, the genetic distance assessments of 18S rDNA were performed using the obtained sequences from Türkiye and the sequences of the published draft genome of *D. gallinae* from Scotland (Burgess et al., 2018; accession number: QVRM00000000) and a sequence submitted from the USA (Dowling and Oconnor, 2010; accession number: FJ911836.1). In addition, haplotype determinations of PRMs were utilized with 'DnaSP v.6' (Rozas et al., 2017). A Maximum likelihood (ML) phylogenetic tree based on 16S rDNA sequences belonging to *Dermanyssus* spp. was constructed with 'MEGA X' using the 'HKY+G model' (identified to be the best-fit model in 'MEGA') (Tamura, 1992) with 1000 bootstraps.

Results

According to morphological analysis, the females of *D. gallinae* were typically measured 0.8-1.5 mm in length and around 0.4 mm in width, male mites were significantly smaller at about 0.6 mm in length and 0.3 mm in width. Additionally, the second cheliceral segment in females was notably elongated, extending well beyond the basal segment. *D. gallinae* possessed a genito-ventral shield that is narrowly rounded at the posterior, with the anus located at the posterior aspect. Furthermore, there were two setae on both the anterodorsal and posterodorsal sides of Tibia 1, and one seta each on the anterolateral sides of Tibia 2, 3, and 4.

The fifty-six sequences were subsequently obtained from *D. gallinae* populations belonging to partial fragments of 18S and 16S rDNA genes. All sequences showed the best BLAST hit (>99.1% identity) with the PRM sequences found in the NCBI database. Obtained sequences were submitted to Public GenBank, and accession numbers are presented in Table 2. Haplotype analyses of the 16S rDNA sequences of Turkish PRMs identified 14 haplotypes, presented in Table 3.

Alignments and intra- and interspecific genetic distances (Table 2) of 18S rDNA were mainly performed using the genome of *D. gallinae* and a sequence submitted from the USA (accession number: FJ911836.1) because of the absence of the sequences in the GenBank. According to this, the intra-species genetic variation among 18S rDNA sequences was calculated to be 0.11%. The 16S rDNA sequences showed an average intra- and inter-genetic distance of 0.22% (min-max, 0-1.07) and 11.84% (min-max, 7.96-16.75) among Turkish *D. gallinae* populations (Table 2). Additionally, results demonstrated that 16S rDNA sequences of Turkish PRMs were clustered in two haplotypes in the phylogenetic tree (Figure 1).

Discussion

Although there is a range of molecular data available for COI, 16S, and ITS sequences of *D. gallinae* from various countries (Çiloğlu et al., 2020; Karp-Tatham et al., 2020; Roy et al., 2021; Koç et al., 2022), no sequence data belonging to 18S and 16S rDNA of Turkish *D. gallinae*, considering the significance of the identification of genetic diversity within and between species from various geographical locations, there was a gap in the literature for the sequences of poultry red mites.

Poultry red mites collected from 28 poultry houses were initially identified based on morphological characteristics. This identification involved assessing factors such as size, setae localization, the structure of chelicerae, and the genito-ventral shield, as described in Naegele (1963) and Di Palma et al. (2012).

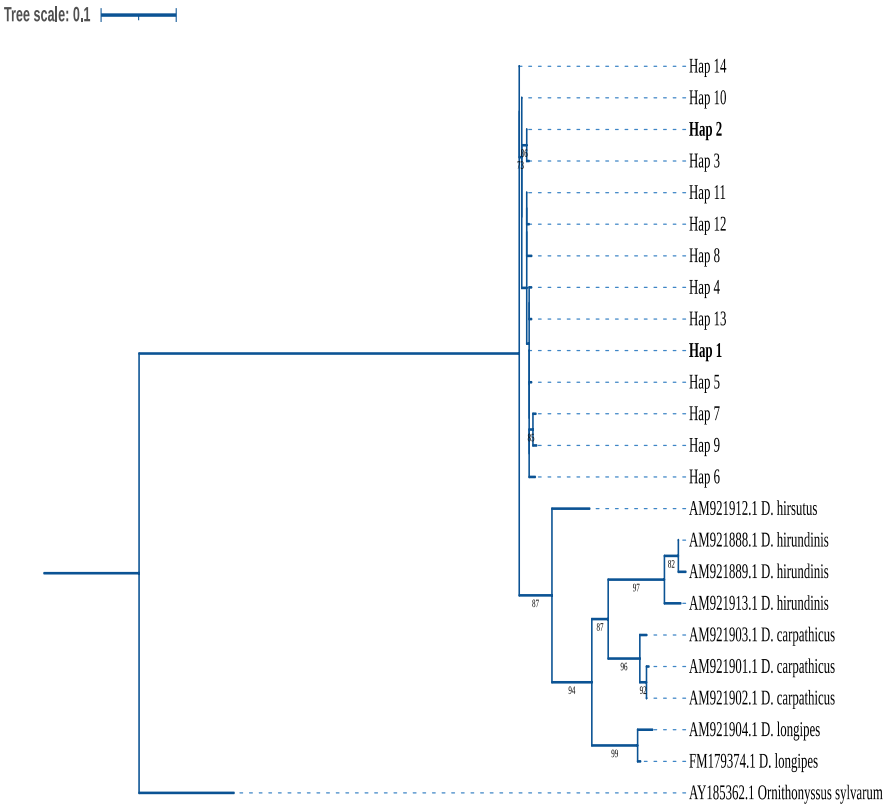

Figure 1. Phylogenetic tree of obtained *Dermanyssus gallinae* species based on 16S sequences belonging to the haplotypes available in GenBank. The sequences obtained in the present study are indicated in bold. Only the bootstrap values higher than 70% are shown. *Ornithonyssus sylviarum* sequence was used as an outgroup.

Table 1. Geographic origin, hen breeds, age of hens, and sampling dates of 28 *Dermanyssus gallinae* populations used in the present study

No	Location	Abbreviations	Hen breeds	Age of hens (weeks)	Collection date
1	Afyon	AFY1	Lohman brown	62	Sept 2022
2	Afyon	AFY2	Lohman brown	57	June 2023
3	Afyon	AFY3	Nick chick	55	June 2023
4	Ankara/Beypazarı	BYP1	Nick chick	73	August 2023
5	Ankara/Beypazarı	BYP2	Nick brown	82	August 2023
6	Ankara/Çubuk	CBK1	Nick chick	96	June 2023
7	Ankara/Çubuk	CBK2	Nick brown	89	August 2023
8	Ankara/Çubuk	CBK3	Lohman brown	64	Sept 2023
9	Ankara/Çubuk	CBK4	Nick brown	56	Sept 2023
10	Ankara/Elmadağ	ELM1	Nick brown	84	June 2023
11	Ankara/Elmadağ	ELM2	Lohman brown	98	June 2023
12	Ankara/Haymana	HYM	Nick chick	71	July 2023
13	Ankara/Gölbaşı	GLB	Lohman brown	70	July 2023
14	Ankara/Kalecik	KAL1	Lohman brown	64	Oct 2022
15	Ankara/Kalecik	KAL2	Lohman brown	65	May 2023
16	Ankara/Kalecik	KAL3	Lohman brown	68	Sept 2023
17	Ankara/Kalecik	KAL4	Nick brown	88	Sept 2023
18	Ankara/Kazan	KZN1	Nick brown	49	April 2023
19	Ankara/Kazan	KZN2	Nick brown	53	April 2023
20	Balıkesir	BAL1	Nick chick	68	August 2023
21	Balıkesir	BAL2	Nick brown	42	August 2023
22	Bayburt	BAY1	Lohman brown	56	May 2023
23	Bayburt	BAY2	Lohman brown	48	May 2023
24	Eskişehir	ESK1	Nick brown	45	Sept 2022
25	Eskişehir	ESK2	Lohman brown	56	Sept 2022
26	Konya	KNY1	Nick brown	71	June 2022
27	Konya	KNY2	Nick brown	96	June 2022
28	Uşak	USK	Lohman brown	62	May 2023

Table 2. Mean genetic distance (%) (mean, min-max) between *D. gallinae* from Türkiye and other countries, and other species in the same genus

Gene	Genetic distance				Nucleotide diversity within D. gallinae (π)	Accession numbers
	Between *D. gallinae* populations			Within the genus *Dermanyssus*		
	From Türkiye	From Türkiye and other countries	Other species within the genus *Dermanyssus*			
18S	0.11 (0-0.70)	0.17 (0-0.69)	0.87 (0.46-1.64)	0.21 (0-0.70)	0.00117	OR960601-OR960628
16S	0.22 (0-1.07)	0.53 (0-1.90)	11.84 (7.96-16.75)	1.77 (0-17.28)	0.00614	OR960571-OR960598

Table 3. Haplotypes of *Dermanyssus gallinae* populations based on 16S sequences

Haplotypes	n	Populations
Hap_1	43	AFY1, AFY2, AFY3, CBK1, CBK2, CBK3, CBK4, ELM1, ELM2, HYM, GLB, KAL1, KAL2, KAL3, KAL4, KZN1, KZN2, BAL1, BAL2, BAY1, BAY2, KNY1, KNY2, USK, FM207492, AM921890, AM921887, AM921884, LC029621, LC029622, LC029675, LC029677, LC029678, LC029679, LC029680, LC029681, LC029697, LC029698, LC029699, LC029706, LC029754, LC029755, LC029797
Hap_2	34	BYP1, BYP2, ESK1, ESK2, AM921914, AM921883, LC029566, LC029570, LC029592, LC029599, LC029625, LC029644, LC029687, LC029696, LC029711, LC029725, LC029730, LC029731, LC029732, LC029735, LC029737, LC029741, LC029749, LC029751, LC029752, LC029753, LC029759, LC029760, LC029766, LC029774, LC029776, LC029783, LC029792, LC029793
Hap_3	2	L34326, LC029798
Hap_4	1	FM207494
Hap_5	1	FM207493
Hap_6	1	AM921911
Hap_7	1	AM921910
Hap_8	1	AM921885
Hap_9	1	AM921886
Hap_10	43	LC029560, LC029565, LC029571, LC029575, LC029584, LC029585, LC029590, LC029591, LC029603, LC029606, LC029614, LC029617, LC029623, LC029626, LC029654, LC029661, LC029684, LC029688, LC029689, LC029693, LC029694, LC029707, LC029708, LC029713, LC029715, LC029716, LC029718, LC029719, LC029726, LC029728, LC029736, LC029740, LC029747, LC029748, LC029750, LC029757, LC029762, LC029768, LC029775, LC029779, LC029782, LC029789, LC029795
Hap_11	143	LC029561- LC029563, LC029567- LC029569, LC029572- LC029574, LC029576- LC029583, LC029586- LC029589, LC029593- LC029598, LC029600- LC029602, LC029604, LC029605, LC029607, LC029609- LC029613, LC029615, LC029616, LC029618- LC029620, LC029624, LC029627- LC029643, LC029645- LC029653- LC029660, LC029662- LC029667, LC029669- LC029674, LC029676, LC029682, LC029683, LC029686, LC029690- LC029692, LC029695, LC029700- LC029702, LC029704, LC029709, LC029710, LC029712, LC029714, LC029717, LC029720- LC029724, LC029727, LC029729, LC029733, LC029734, LC029738, LC029739, LC029742, LC029743, LC029745, LC029756, LC029758, LC029761, LC029763- LC029765, LC029769- LC029773, LC029777, LC029778, LC029780, LC029781, LC029784- LC029788, LC029790, LC029791, LC029794, LC029796
Hap_12	7	LC029564, LC029608, LC029668, LC029685, LC029703, LC029744, LC029746
Hap_13	1	LC029705
Hap_14	1	LC029767

Nuclear ribosomal DNA still provides one of the most complete tools for many molecular tasks. Among nuclear gene/gene regions, 18S rDNA, ITS1, and ITS2 have proved helpful in phylogenetic classification (Doolittle, 1999; Hebert et al., 2003). However, the evolutionary rates of the nuclear ribosomal genes are lower; therefore, they have used as molecular markers for phylogenies at higher taxonomic levels (Eickbush and Eickbush, 2007). In the current study, intra-species genetic variation among 18S rDNA sequences was calculated to be 0.11%. This distance indicates only a slight differentiation between populations in Türkiye from various geographical origins. The low difference is even revealed in the interspecific variance of 0.87% which is with the results of Dowling and Oconnor (2010) in superfamilies, Dermanyssoidea. Therefore, species-level identification should be avoided due to the low inter-specific distance of 18S rDNA sequences, or a combination of an additional marker should be favored.

The mitochondrial genome also contains two ribosomal RNA (rRNA) genes, including 12S and 16S rRNA. 16S rRNA is a small ribosomal RNA subunit responsible for the translation of genetic codes to functional cell components in all organisms (Woese and Fox, 1977). 16S rRNA region has been used for many years as a valuable tool to infer phylogenetic relationships for distantly related taxa (Dong et al., 2021). Due to its species-specific characteristics, it has been mainly employed in determining phylogenetic relationships between bacteria (Woese, 1987) and also popularly in ticks (Navajas and Fenton 2000). It was also included in several phylogenetic studies on PRM (Roy et al., 2010, Roy et al., 2009; Chu et al., 2015), and intraspecific variation was determined between 0-4% (Roy et al., 2010). Regarding the 16S rRNA results in the current study, the intra- and inter-genetic distances were determined consistent with Roy et al. (2010). Although low divergences were detected within species, the 16S rRNA gene could be particularly informative at the interspecific levels, as documented before. Supportingly, the phylogenetic tree shows a good clustering pattern at the species level (Figure 1).

In general, the molecular characterization of 18S rDNA and 16S rDNA of *D. gallinae* sampled from poultry houses in Türkiye were performed. The sequence data that was obtained was submitted to the NCBI database. This study may significantly contribute to the genetic data of poultry red mites regionally and globally. Still, additional sequences are required to elucidate the genetic diversity in PRMs fully.

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Ethical approval

Not applicable

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