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Original Article

Phylogenetic Diversity of *Dermanyssus gallinae* (Dermanyssidae) based on Mitochondrial Cytochrome Oxidase-1 Gene Sequence Collected from Different Bird Species in Iran

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Abstract

A wide range of hosts, especially birds, can be infested with *Dermanyssus gallinae* (*D. gallinae*), as an obligate hematophagous mite. In this study, cytochrome oxidase 1 (*CO1*) gene sequences were employed to perform molecular and phylogenetic analyses of *D. gallinae* collected from different bird species in Iran. Adult mites were collected from the body surface and cage material of ornamental and wild birds in industrial farms located in the Western and Northwestern regions of Iran. The infestation was identified in layer poultry farming by inspecting the eggs and the whole surfaces of the birds' bodies. The holding area and body surface of the ornamental and wild birds were also thoroughly examined. The *D. gallinae* samples were assigned to two subgroups of haplogroup A (i.e., A1 and A2). The phylogenetic tree suggested that the *D. gallinae* samples collected from wild birds in the A1 sub-haplogroup should be placed beside Japanese, Norwegian, Italian, and French samples isolated from wild birds in the A2 sub-haplogroup. Additionally, the highest phylogenetic similarity in the A2 sub-group was observed between mites isolated from ornamental and industrial birds in Australia. The findings of the present study suggest that crows and sparrows may play an important role in the transmission of *D. gallinae* infestation to other species of wild birds due to their high population, as well as their presence in most areas.

Keywords: CO1; Mite; Phylogenetic relationship; Reservoir host; Wild bird

1. Introduction

As an obligate hematophagous ectoparasite with a wide range of hosts, especially birds and rodents (1), the poultry red mite, *Dermanyssus gallinae* (Acari: Dermanyssidae), is globally considered the most damaging parasite of laying hens (2). Female mites have been known to feed more on blood, compared to male mites (3). The infestation of farmed birds and poultry with *D. gallinae* results in their dermatitis, weight loss, irritation, decreased egg production, anemia, and even death in severe cases, which in turn leads to huge economic losses (4). In the infested layer farms, viral and bacterial poultry diseases, including Colibacillosis, Salmonellosis, Pasteurellosis, and Newcastle (2, 5), spread by *D. gallinae* as their vector (6). These mites have also caused a growing concern in human medicine since the infestation can cause skin lesions, such as gamasoidosis in humans, especially those in close contact with poultry (7, 8).

The determination of the phylogenetic diversity of mites is critical for their tracing (i.e., specifying whether they are transmitted by the infected hosts or wild birds, which transfer between layer farms and breeding facilities (9)). *D. gallinae* and *D. hirundinis*

have been observed in eight and nine different phylogenetically-distant types of birds, respectively. The phylogenetic reconstruction of birds by Livezey and Zusi (10) showed the development of *D. gallinae* in Galliformes and Passeriformes, which are basally and distally situated in a large clade of Neognathae.

In this study, Partial cytochrome c oxidase subunit 1 (*CO1*) gene sequences were utilized to perform molecular analyses of *D. gallinae* isolated from different bird species in the West and Northwest of Iran.

2. Materials and Methods

2.1. Sampling

The adult mites were collected from the body surface and cage materials of turkeys, egg-laying poultry, and ornamental birds. In the case of wild birds, the samples were collected either from body surfaces or nests of birds, as shown in table 1 (11-14). The collected samples were placed in 70% ethanol and transferred to the parasitology laboratory of the Faculty of Veterinary Medicine in Urmia University, Urmia, Iran. Mites were identified using diagnostic entomological keys (15).

Table 1. Birds and infestation rates of *Dermanyssus gallinae* regarding to sampling regions in Iran

Birds: Types and Species			No. of Samples (Positive %)						
Type	**Common name**	**Scientific name**	**Ku**	**Hm**	**Zn**	**Wa**	**Ea**	**Ar**	**Total**
Ornamental birds	Canary	*Serinus canaria domestica*	10 (0)	22 (4.5)	8 (0)	7 (14.3)	36 (13.9)[a]	5 (0)	88 (7.9)
	Pigeon	*Columba livia domestica*	30 (10)	32 (0)	19 (5.3)	23 (4.3)	45 (6.7)	10 (0)[b]	159 (5)
	Budgerigar	*Melopsittacus undulatus*	10 (10)	22 (0)	8 (0)[c]	7 (14.3)	36 (8.3)	5 (0)	88 (5.7)
	Finch	*Taeniopygia guttata*	10 (0)	22 (0)	8 (25)	7 (14.3)[d]	36 (22.2)	5 (20)	88 (13.6)
	Parrots	*Psittacula eupatrica*	10 (10)	22 (4.5)[e]	8 (0)	7 (0)	36 (2.8)	5 (20)	88 (4.5)
	Cockatiel	*Nymphicus hollandicus*	10 (0)[f]	22 (0)	8 (0)	7 (0)	36 (5.5)	5 (20)	88 (3.4)
Wild birds	Sparrow	*Passer domesticus*	56 (1.8)	110 (2.7)	33 (0)	193 (4.1)	340 (4.7)[h]	86 (2.3)	818 (3.7)
	Owl	*Strix nebulosa*	0	2 (0)	0	10 (10)[i]	18 (11.1)	0	30 (10)
	Crow	*Corvus corone*	33 (6.1)[j]	55 (1.8)	21 (0)	110 (3.6)	189 (6.9)	56 (1.8)	464 (4.5)
	Partridge	*Alectoris chukar*	0	0	9 (0)	38 (7.9)	138 (5.8)	49 (2)[k]	234 (5.1)
	Peacock	*Pava muticus*	0	0	0	5 (0)	4 (25)[l]	0	9 (11.1)
Industrial farms	Turkey	*Meleagris gallopavo*	20 (5)	15 (0)	88 (6.8)[g]	9 (11.1)	92 (3.7)	7 (0)	231 (4.8)
		Laying hens farm	6 (16.7)[m]	10 (10)[n]	13(23.1)[o]	19 (10.5)[p]	22 (22.7)[q]	8 (12.5)[r]	78 (16.7)

Sampling sites/regions: West (Ku=Kurdistan, Hm=Hamadan), and Northwest (Zn=Zanjan, Wa=West Azerbaijan, Ea=East Azerbaijan, Ar=Ardabil) in Iran.
Accession numbers: a=MT416629, b=MT416627, c=MT416624, d=MT416625, e=MT416628, f=MT416626, g=MT416608, h=MT416607, i=MT416605, j=MT416606, k=MT416609, l=MT416604, m=MT791527, n=MT791526, o=MT79130, p=MT791529, q=MT791528, r=MT791525.

2.2. DNA Extraction and Molecular Diagnosis

The mites were first crushed in 20-µl phosphate-buffered saline. Genomic DNA was extracted using MBST tissue kits (Molecular Biological System Transfer Company, Iran), according to the instructions given in the manufacturer's manual.

Molecular detection and proliferation of 400 bp fragments were performed using a pair of primers for mitochondrial *CO1* gene (i.e., CO1F (5'-TGATTTTTTGGTCACCCAGAAG-3') and CO1R (5'-TACAGCTCCTATAGATAAAAC-3') (16). The reactions were regulated in a 25-µL volume of the polymerase chain reaction (PCR) reaction mixture. The PCR program was as follows: 10 min at 94°C, followed by 44 cycles at 94°C, 53°C, and 72°C for 1 min, and the final extension at 72°C for 4 min. Amplified products were confirmed using 1.2% agarose gel electrophoresis after staining with ethidium bromide (0.3 µg/ml) and visualization under UV light (16). Finally, the purified PCR products were sent to Takapozist Company (Tehran, Iran) for sequencing.

2.3. Sequencing and Genomic Analysis

All the obtained nucleotide sequences of each host and various mite collection regions were deposited in the GenBank with the assigned accession numbers

(Table 1). A bioinformatics analysis was ultimately performed using the Basic Local Alignment Search Tool on the National Center for Biotechnology Information site, and the phylogenetic tree was examined in DNASIS Max 3.0, BioEdit 7.2, and MEGA X. The results were interpreted as follows:

Sequence trimming and editing construction were conducted using DNASIS MAX 3.0 (MiraiBio, Hitachi Ltd.) software. Sequence alignments were performed using BLOSUM matrices and CLUSTAL W tool in BioEdit 7.2 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html) software. The sequences were compared in a table in BioEdit 7.2 based on the percentage of similarity and closeness of the sequence identity matrix and the analysis of maximum composite similarity. Phylogenetic relationship was examined and constructed by Maximum-likelihood method using the MEGA, version 6.0. The reliability of an inferred tree was tested by 1000 bootstrap.

The analysis was performed on the phylogenetic tree based on haplotypes and the classification proposed by Chu, Murano (7).

3. Results

Table 1 presents the rate of infestation with *D. gallinae* categorized by bird types and sampling locations. The highest infestation rate with the mites was observed at 16.7% in laying hen farms, followed by 13.6% in finch (i.e., ornamental birds group), and 11.1% in peacock (i.e., wild birds group). Figure 1 show the sequence alignment based on the *D. gallinae* samples isolated from ornamental, industrial, and wild birds in Urmia, Iran. A transition occurred between nucleotides T and C. This mutation was visible in all *D. gallinae* specimens isolated in this study, compared to the reference specimens. In nucleotides 617 and 731, transitions were observed both between A and G, as well as between C and T. These two Single Nucleotide Polymorphisms (SNP) were also observed in the *D. gallinae* isolated from wild birds. The alignment of amino acid sequences was performed in order to investigate the effects of SNP (Figure 2). The sequences, in this study, which were isolated from industrial, ornamental, and wild birds, showed a similarity of 100%. Compared to the isolates of other countries, the isolates in this study showed that SNPs had no effect on gene expression.

Mites in Iran and other countries were clustered into haplogroups A, B, C, and D, according to the phylogenetic tree constructed based on the 400-bp *CO1* nucleotide sequence (Figure 3) (7). Isolates of *D. gallinae* lay in A1 and A2 sub-haplogroups of haplogroup A. According to the phylogenetic tree, the *D. gallinae* samples isolated from wild birds in A1 sub-haplogroup were placed beside the isolates from Japan (LC029559, LC029476, LC029466, LC029463 and LC029553), Norway (MK599418), Italy (KC774792 and KC774766), and France (HQ842161 and FM208732), all of which were isolated from wild birds and assigned to a separate sub-haplogroup. The samples of *D. gallinae* in the A2 sub-haplogroup, which were isolated from ornamental and industrial birds, were placed beside those in a separate sub-haplogroup, collected from industrial birds in Turkey (KU866541), France (AM921858), and Australia (HQ842356). Based on phylogenetic analysis, the highest similarity (>98%) was observed in A1 subgroup among the isolates in this study (MT416604-09) and wild bird isolates from Italy (KC774766 and KC774792) and France (HQ842161 and FM208732). In addition, in the A2 subgroup, the highest similarity (>99%) was noted between the isolates from this study (MK791525-30 and MT424624-29) and those derived from ornamental and industrial birds in Australia (HQ842356). In the present study, the isolates derived from haplogroup A were classified into two sub-haplogroups (i.e., A1 and A2).

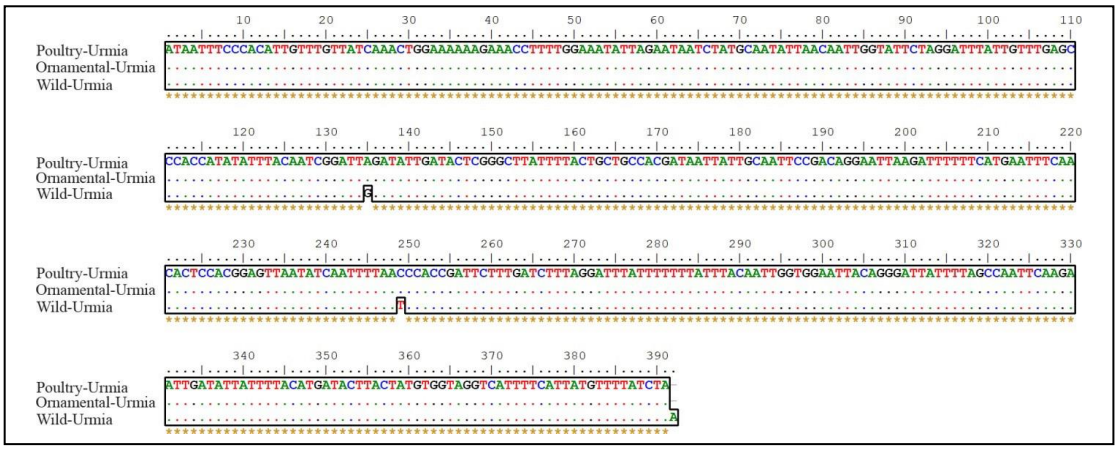

Figure 1. Nucleotide sequence alignments based on *D. gallinae* samples isolated from ornamental, industrial, and wild birds in Urmia, Iran

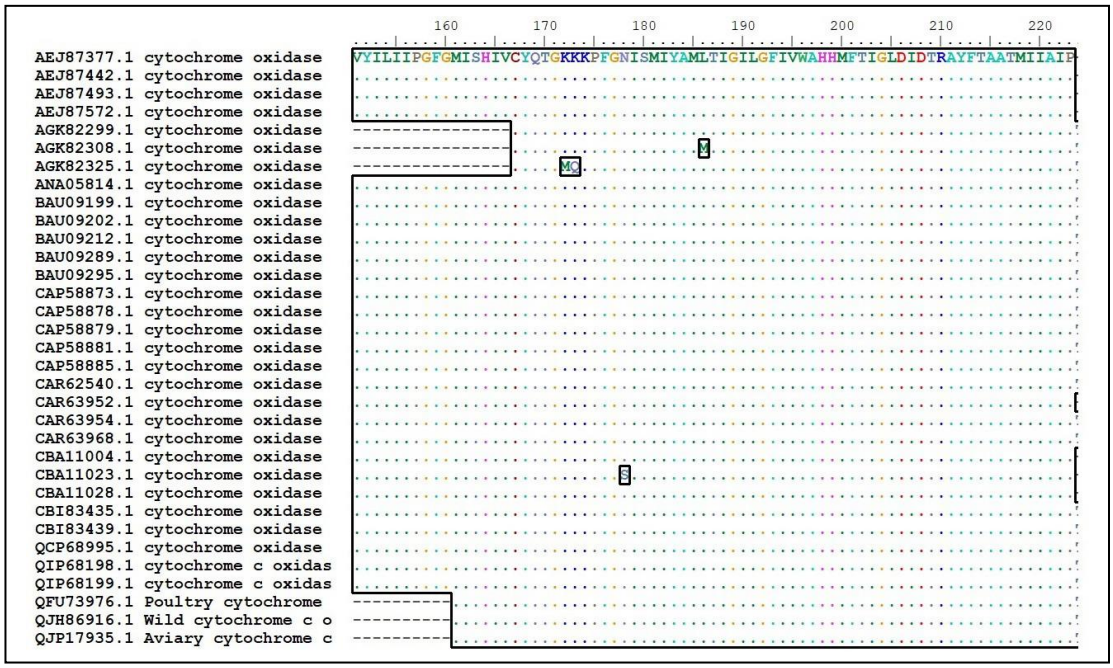

Figure 2. Effects of point mutations and the alignment of the amino acid sequences of the *D. gallinae* samples isolated from ornamental, industrial, and wild birds

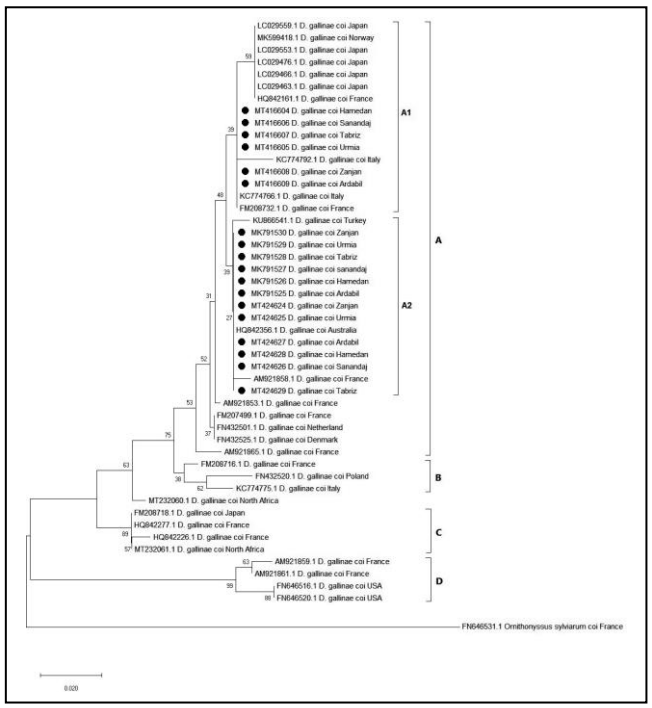

Figure 3. Phylogenetic tree constructed using neighbor-joining and *CO1* gene sequences of *D. gallinae* in Iran

4. Discussion

The phylogenic diversity of *D. gallinae* populations was investigated through sequencing mitochondrial *CO1* gene amplicons, collected from six different regions in Iran. *COI* diversity in *D. gallinae* has demonstrated multiple lineages with comparative analysis, concluding that the presence of cryptic species is essential (17-19). The isolates from different regions in the world were used as reference isolates for comparing with the isolates used in the present study and drawing the phylogenetic tree. The ineffectiveness of these SNPs in the gene expression and the characteristics of functional proteins can be explained by the functional stability of this protein in evolution. There are a limited number of wild birds in Iran due to its climatic condition; therefore, most of the birds for the present study were selected from a zoo. The sequence analysis showed significant correlations between A1 and A2 sub-haplogroups in the *CO1* tree and the sequences of haplotypes in France (AM921858), Australia (HQ842356), and Turkey (KU866541). The A2 sub-haplogroup, which is originally from Turkey, was also found in West Azerbaijan, Iran, because of a common geographical border between the two countries. The sequences of the mitochondrial *CO1* gene can be used to reveal the history of hybridization between different lineages. In line with the results of a study by Brannstrom, Morrison (12), the differences in the *CO1* sequences showed that two genotypes in the mites were associated with wild birds and domesticated poultry (both laying and ornamental). Limitations of the present study include sampling the mites from the nests of the wild birds, and the fact that although the experimental boxes used as nests were convenient in terms of mating location and ethological research, they were sometimes far from ornamental birds and poultry facilities. Regarding the identical genetic sequences of isolated mites, irrespective of geographical location and bird species, a similar sequence was detected among farm birds and different species of wild birds that nested near the farms.

The findings of the present study suggest the insignificant role of wild birds as a *D. gallinae* reservoir for commercial poultry production systems at least in Iran. Wild birds appeared to be infested with the *D. gallinae* acquired from crows and sparrows nesting in poultry farms. Since the population of crows and sparrows is high in all regions, the poultry production management system would be responsible for transmission routes by transporting live birds and eggs in rural regions and farms, as well as wildlife.

The isolates of *D. gallinae* were generally categorized as at least as two genotypes, one infesting wild birds, and the other egg-producing poultry. Moreover, wild birds were found not to play a major role as a reservoir for *D. gallinae.* Further studies are recommended to be conducted on *CO1* diversity in other countries to gain a more comprehensive understanding of the present subject.

In conclusion, this study pioneered the molecular characterization of *D. gallinae* in Iran for the first time. These findings could be useful as the basis of future studies on *D. gallinae* infestation.

Authors' Contribution

Study concept and design: H. R.
Acquisition of data: H. R.
Analysis and interpretation of data: H. R.
Drafting of the manuscript: H. R.
Critical revision of the manuscript for important intellectual content: M. T. and B. E.
Statistical analysis: H. R.
Administrative, technical, and material support: M. T. and B. E.

Ethics

Ethical approval for this study was obtained from the Ethical Review Board in Urmia University, Urmia, Iran.

Conflict of Interest

The authors declare that they have no conflict of interest.

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