Morphological and molecular genetic analysis of dermanyssus gallinae of the genus dermanyssus dugès

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Abstract. Nowadays, global climate change is leading to the widespread distribution of bird ectoparasites, specifically mites of the *Dermanyssidae* family, and an increase in their harmful effects. This has caused a sharp decline in poultry farming development and productivity. Therefore, studying the bioecology of bird ectoparasites and developing new methods to protect poultry from them is a pressing issue. During the spring, summer, and autumn of 2022–2023, acarological examinations were conducted to identify infestations of *Dermanyssus gallinae* (red poultry mites) in poultry farms and household flocks across 25 locations in the Dehqonobod, Kasbi, Kitob, Koson, and Mirishkor districts of the Kashkadarya region, Uzbekistan. In the research, 457 Gallus gallus domesticus (chickens) were examined using both route and stationary methods, and 1,700 mite specimens belonging to the Dermanyssidae family were collected. The species composition and morpho-biological characteristics of *Dermanyssus gallinae* were analyzed. Molecular genetic studies were conducted on *D. gallinae*, focusing on the mitochondrial DNA 16S rRNA region, and the nucleotide sequences were analyzed. The obtained sequences were compared with those in the NCBI database. Additionally, the phylogenetic tree of species belonging to the *Dermanyssus genus* was studied.

1 Introduction

Dermanyssus gallinae (De Geer 1778) (poultry red mite) is a cosmopolitan hematophagous ectoparasitic mite of wild, domestic and synanthropic birds [1, 2]. and which may also feed upon mammalian hosts [3]. *D. gallinae* is a significant pest of poultry worldwide and a serious economic threat mainly to the laying hen sector [4] in any farming system (cages, barns, free-range and organic farming), including the recently introduced “colony” system [5]. *D. gallinae* is responsible for stress behaviour in its poultry hosts, reduced egg production and egg grade, anaemia, and diminished disease resistance [6, 7, 8]. *D. gallinae* is also a vector of several infectious disease agents [9].

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For this reason, it is felt that those involved with the broader systematic, molecular, health or economic aspects of *D. gallinae* might benefit from a gallery of light and scanning electron micrographs illustrating the characters used by Moss [10, 11] to identify this species. Accordingly, we have photographed every *D. gallinae* feature mentioned in Moss' keys and have labelled them to pinpoint their ap pearance and location. We have also included micrographs illustrating the most important morphological differences between *D. gallinae* and *O. sylviarium* to differentiate these morphologically similar species better.

2 Materials and methods

In the spring, summer and autumn seasons of 2022-2023, a total of 457 chickens were examined from 25 poultry farms located in Dehkanabad, Kasbi, Kitab, Koson and Mirishkor districts of Kashkadarya region (Fig. 1).

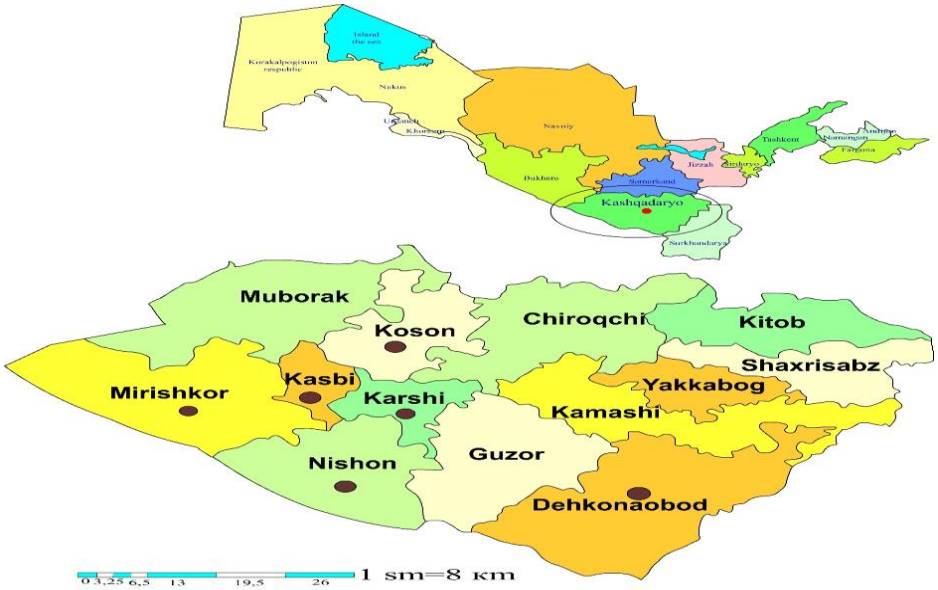

Fig. 1. Researched areas (Kashkadarya region).

1700 samples of mites belonging to the genus Dermanyssidae from Gallus gallus domesticus (chickens) based on route and stationary methods (МУК 4.2.1479-03). collected based on the method. It was carried out based on species composition and morpho-biological characteristics of mites [12,13,14,15].

DNA isolation. To carry out molecular genetic research methods, genomic DNA was extracted from the leg part of D. gallinae species belonging to the genus Dermanyssus. The reagents of Thermo Scientific GeneJET PCR Purification Kit (Germany) were used for genomic DNA extraction. Polymerase chain reaction. The 16S fragments of mitochondrial (mDNA) DNA of D. gallinae belonging to the genus Dermanyssus were isolated using the forward 16Sf 5TTAAATTGCTGTRGTATT3, reverse 16Sr 5CCGGTCTGAACTCASAWC3 primers widely used in molecular taxonomy for studying nucleotide sequences [16, 17]. When preparing Master-mix for PCR, Water (distilled) - 7.1 µl, 10x PCR buffer 1 µl, dNTP - 0.2 µl, primers - 0.5 µl, Taq-polymerase - 0.2 µl=10 µl prepared. Polymerase chain reaction from isolated DNA samples was carried out using an automatic programmable amplifier (PR-96E) in the following mode. Touchdown PCR

protocol for 16S as follows: an initial denaturation step (94ºC 5 min), followed by 5 cycles of 94 ºC for 30 s, 52 ºC for 30 s, and 68 ºC for 1 min; 5 cycles of 94ºC for 30 s, 50ºC for 30 s, and 68ºC for 1 min; 5 cycles of 94ºC for 30 s, 48ºC for 30 s, and 68ºC for 1 min; 25 cycles of 94ºC for 30 s, 46ºC for 30 s, and 68ºC for 1 min; followed by a final extension step of 68ºC for 5 min.

Constructing a phylogenetic tree. Nucleotide sequences of the sequenced species D. gallinae and DNA sequences obtained from the International Center for Biotechnology Information database (https://www.ncbi.nlm.nih.gov/) (NCBI) were used and these sequences were manually aligned using Genius prime software. edited, consensus sequences were calculated using Mega X computing software. Primer data from this program and additional sequences from the GenBank database were aligned using MAFFT v.7 online software using default settings and Clustal Omega 1.2.2 software and edited with Genius prime software (Table 1).

Table 1. Species and their accession numbers from the National Center for Biotechnology Information

№		Accession number	Country
1.	*D. hirundinus*	AM921889, AM921888, AM921913	France
2.	*D. carpathicus*	AM921903, AM921902, AM921901	France
3.	*D. longipes*	FM179374, AM921904,	France
4.	*D. hirsutus*	AM921912	USA
5.	*D. gallinae*	LC029793, LC029792	Japan
6	*Menopon gallinae*	PP345483	Uzbekistan

Nucleotide sequences belonging to the 16S domain of the obtained mRNA were determined by ultrafast bootstrapping with maximum likelihood-ML phylogenetic tree performed with 1000 iterations in IQ-TREE version 1.6.12, and analyses were performed in CIPRES Science Gateway V 3.3. The nucleotide sequence of the 16S region of the species Menopon gallinae (Linnaeus, 1758) (Accession number: PP345483) belonging to the genus Menopon Nitzsch, 1818 was included as an outgroup to facilitate the generation of consensus trees. The resulting phylogenetic tree was analyzed and edited in iTOL v6.6 software

3 Results

Morphological studies. The red chicken mite, D. gallinae, belonging to the genus Dermanyssus, is considered an obligate hematophagous mite, its body is oval, brown-red in color, and its body is covered with hairs (Fig. 2).

Fig. 2. Dorsal (a) and ventral (b) views of the red chicken mite Dermanysus gallinae (original).

The body length of the male D. gallinae mite is 0.6-0.63 mm. The back shield is slightly wider and rounded than the back shield of the female tick. The larva is oval in shape, 0.34-0.42 mm long. The cuticle is thin, shields are not formed. Oral apparatus is not developed. The body of the protonymph is oval in shape, the back is bulging, and it is transparent with a yellowish tint. Length 0.4 mm. On the back there is a large, round, cephalo-thoracic shield with a slightly curved posterior edge and a row of small pairs of bristles. The oral apparatus is beginning to sag, the chelicerae are long and spear-like.

The body of the female D. gallinae mite is oval, oblong, 0.75-0.84 mm long, 0.4 mm wide. It is covered with a shield on the back (dorsal) and belly (ventral), and this body narrows from the beginning to the end. The body is completely covered with hairs on the outside. The chelicera is very long, and spear-shaped.

Daytonymph. The body length of unfed Deutonymphma is 0.58 mm. The covering of the body is dense. At the back there is a single rear shield with the trailing edge cut off. The chest shield is significantly elongated. The anal shield is large, straight in front and broadly rounded at the back. Sexual dimorphism is manifested in the deutonymph phase. Male deutonymphs are significantly smaller than female deutonymphs in terms of body size.

Results of molecular genetic research. Based on the results of molecular genetic research (sequence chromatography) on the species D. gallinae belonging to the genus Dermanyssus, nucleotides with 309 base pairs belonging to the 16S area of rDNA were isolated (Fig.3)

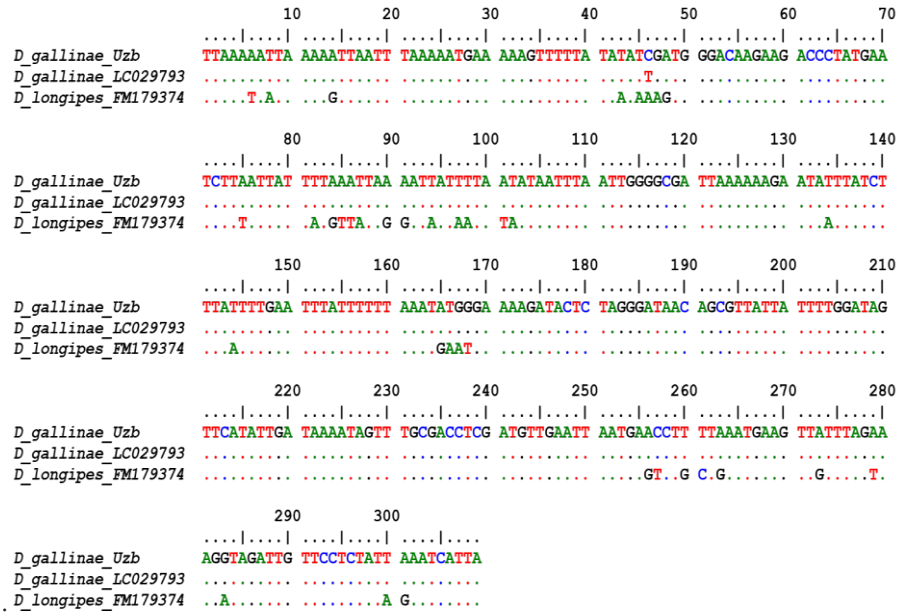

Fig. 3. Nucleotide sequence comparison of rDNA 16S region of mite species belonging to the genus Dermanyssus.

When the species belonging to the genus Dermanyssus were analyzed using bioinformatic methods, there was 1 difference with the type of D. gallinae (Accession number: LC029793) from the database of D. gallinae (Accession number: LC029793). In the sample of D. gallinae (Accession number: LC029793), it was found that T-temin nucleotides were exchanged. The difference between total nucleotides was 0.3%. This difference between these moist soils can be explained by the environmental and geographical conditions of the places where the samples were collected.

Differences in 37 nucleotides were noted between the nucleotides of D. longipes (Accession number: LC029793) and the type of D. gallinae (NCBI), and the difference between the total nucleotides was 11.9%.

The nucleotide sequence of D. gallinae, a member of the genus Dermanyssus, has been deposited in the National Center for Biotechnology Information and received accession number (Accession number: PP345482).

Phylogenetic tree. According to the analysis of the studied D. gallinae species belonging to the genus Dermanyssus and the mRNA 16S nucleotide sequences of the species belonging to this genus obtained from the GenBank database, representatives of this genus have 54-99% bootstrap support for 4 clades (groups). was found to be united (Fig. 4).

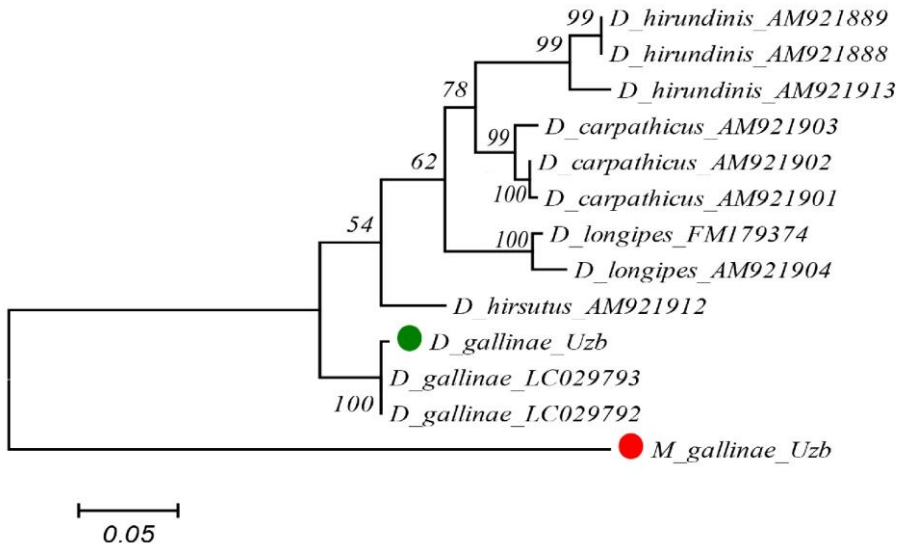

Fig 4. A phylogenetic family tree developed based on the maximum likelihood-ML method of mites of the genus Dermanyssus.

The first group includes 99% of D. hirundine specimens, the second group includes 99-100% of D. carpathicus specimens, the third group includes 100% of D. longipes specimens, and the fourth group includes D. hirsutus and D. gallinae specimens. compared to the main joint, 54%, and specimens of D. gallinae species combined to form a butistrap support of 100%.

4 Conclusion

Morphological and morphometric studies of D. gallinae mite belonging to the genus Dermanyssus revealed that the length of the male mite is 0.6-0.63 mm, and the body length of the female mite is 0.75-0.84 mm. Females are larger than males. was found to be distinguished by D. gallinae (Accession number: LC029793) obtained from the D. Gallinae species base (NCBI) studied as a result of molecular genetic studies was found to be 99.7% similar to the sample distributed in Japan.

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