Prevalence, Molecular Identification, and Phylogenetic Analysis of Ectoparasites Infesting Ornamental Birds in Tehran, Iran.

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Abstract

Introduction: Ectoparasites such as ticks and lice pose significant health threats to ornamental birds and may act as vectors of infectious agents affecting animals and humans. Despite the diversity and trade of ornamental birds in Tehran Province, Iran, limited information exists regarding the molecular characteristics and genetic diversity of their ectoparasites. This study aimed to investigate the prevalence and molecular phylogeny of ectoparasites infesting ornamental birds in Tehran province.

Materials and Methods: A total of 2,000 ornamental birds from 200 bird shops were examined by visual inspection of the wings, vent, and cage cracks; 505 birds (25.25%) were found infested. Parasites were identified morphologically and subjected to molecular characterization. Genomic DNA was extracted, and fragments of the mitochondrial COX1, 16S rRNA, and 18S rRNA genes were amplified and sequenced. Phylogenetic relationships were analyzed using the Neighbor Joining method based on Kimura's two-parameter model.

Results: Morphological and molecular analyses confirmed the presence of *Menacanthus stramineus* (chewing lice) and *Argas persicus* (soft ticks). Out of the 2,000 birds examined, 245 (12.25%) were infested with lice and 260 (13%) with ticks. Representative ectoparasite specimens from the infested birds were used for molecular characterization. Phylogenetic analysis of COX1 and 16S rRNA showed that the Iranian isolate of *A. persicus* clustered with Spanish isolates, whereas 18S rRNA analysis of *M. stramineus* exhibited genetic divergence from previously described strains.

Conclusion: These findings provide molecular evidence of ectoparasites infesting ornamental birds in Iran and highlight the need for surveillance because of the potential epidemiological importance of ectoparasites in bird markets.

Keywords: Avian ectoparasites; Molecular phylogeny; Ornamental bird; Parasite diversity; Tehran Province.

1- Introduction

Ectoparasite infestations are among the most common health problems in birds worldwide [1]. These parasites infect both wild and domestic birds and may have considerable effects on host health, welfare, and productivity. Infestations with ectoparasites can cause irritation, feather

damage, blood loss, and reduced growth performance [2], and decreased egg production. In addition, several ectoparasites act as vectors for important pathogens of poultry and other birds, including the agents of pasteurellosis, fowl pox, and Newcastle disease [3]. Free-ranging and captive birds may also contribute to the maintenance and spread of these parasites, facilitating their transmission between different environments and host species. Among avian ectoparasites, lice (order Phthiraptera) are widely distributed and commonly infest a broad range of bird species. These permanent parasites spend their entire life cycle on the host and feed mainly on feathers, skin debris, and occasionally blood. Heavy infestations can cause severe irritation, feather deterioration, anemia, and increased susceptibility to secondary infections, particularly in birds kept under crowded or poor hygienic conditions. One of the most frequently reported species is *M. stramineus* (family Menoponidae), commonly known as the chicken body louse [4]. This species parasitizes various domestic and ornamental birds and may contribute to economic losses in poultry production due to reduced growth and productivity [5]. Ticks represent another important group of ectoparasites affecting birds [6]. These obligate blood-feeding arthropods are distributed worldwide and are capable of transmitting a wide range of pathogens to both animals and humans [7]. Among them, soft ticks of the family Argasidae are particularly important in avian hosts. *A. persicus*, commonly known as the fowl tick, is widely associated with domestic and wild birds and has been reported in many poultry-producing regions. Infestations with this tick can cause anemia, decreased productivity, and increased mortality in birds, and the species is also recognized as a vector of several pathogens, including the agent of avian borreliosis [8]. Although the occurrence and biological characteristics of avian ectoparasites have been widely studied, information on their genetic diversity and molecular characterization remains limited in several regions, including Iran. Molecular markers such as mitochondrial and ribosomal genes have proven useful for accurate species identification and for investigating evolutionary relationships among parasite populations. Such analyses can provide valuable insights into parasite diversity, distribution patterns, and host–parasite interactions. Tehran Province, located at the foothills of the Alborz Mountains, supports a diverse population of ornamental and captive birds that are commonly kept in bird shops and households. Close contact among birds in such environments may facilitate the transmission of ectoparasites and increase the risk of parasite spread. However, data on the molecular characteristics of ectoparasites infesting ornamental birds in this region are scarce. Therefore, the present study aimed to investigate the molecular characteristics and phylogenetic relationships of ectoparasites collected from ornamental birds in Tehran, Iran, using mitochondrial and ribosomal genetic markers.

2- Materials and Methods

2-1- Study Area and Sample Collection

A cross-sectional survey was conducted to investigate ectoparasite infestations in ornamental birds. Between 25 August 2022 and 22 April 2023, a total of 2,000 ornamental birds from 200 bird shops were examined. The examined bird species included *Serinus canaria, Melopsittacus undulatus*, *Columba livia*, *Nymphicus hollandicus*, *Agapornis roseicollis*, *Psittacus erithacus*, and *Gallus gallus domesticus* (Cochin breed). Sampling was carried out in Tehran city (35.6892° N, 51.3890° E) and several western regions of Tehran Province, including Shahriar (35.6599° N, 51.0596° E), Robat Karim (35.4848° N, 51.0821° E), and Parand (35.4853° N, 50.8666° E). Bird shops were randomly selected, and birds were individually examined for ectoparasites. In some locations, infected birds were observed in close proximity to ornamental birds, which may facilitate parasite transmission.

2-2- Collection and Preservation of Ectoparasites

Ectoparasites were collected by direct visual examination of birds and their cages. Birds were carefully inspected, particularly around the wings, feathers, ventral region, femoral area, and cloacal region, where ectoparasites are commonly found. Cages, floors, and wall cracks were also examined to detect hidden parasites. Specimens were collected using forceps, scalpels, and a hand lens. In addition, traps were placed to capture *Dermanyssus gallinae* mites and were retrieved after 24 hours. Collected ectoparasites were preserved in 96% ethanol in 50-mL tubes and transported to the laboratory for further examination. For morphological identification, specimens were processed using a 10% potassium hydroxide (KOH) digestion method and examined under a light microscope at 40–100× magnification. Identification of ectoparasites was performed according to standard taxonomic keys described in Soulsby's reference texts and related literature [9].

2-3- DNA Extraction

After morphological identification of lice and ticks, genomic DNA was extracted individually from the specimens using MBST Tissue DNA Extraction Kits (Molecular Biological System Transfer Co., Iran) according to the manufacturer's instructions. The extracted DNA samples were stored at −20 °C until further molecular analysis.

2-4- PCR Amplification

PCR amplification was performed using a Bio-Rad T100 thermal cycler (Bio-Rad, USA). The COXIArGAs primer set was used to amplify a 606 bp fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene. The 16SArGAs primer pair amplified a 240 bp fragment of the 16S rRNA gene, while the SAIDG-MEN primers targeted a 539 bp fragment of the 18S rRNA gene (Table 1).

Table 1. Primer sequences and characteristics of the primers used in this study.

Primer name	Oligo sequences (5′ → 3′)	Molecular weight	GC content (%)	Tm (°C)	Extinction coefficient	Molarity (µM)	Size (bp)	Length (bp)
16SArGAs-F	TTTGGGACAAGAAGACCCTATGAATTT	8322.4	4.4	17.45	174.47	60.41	27	240 bp
16SArGAS-R	ACATCGAGGTCGCAATCAATTTTATC	7929.2	4.2	17.48	174.80	60.07	26	240 bp
COXIArGAs-F	GGAGGATTTGGAAATTGATTAGTTCC	8080.3	3	12.25	122.52	60.07	26	606 bp
COXIArGAs-R	ACTGTAAATATATGATGAGCTCA	7070.6	4.2	19.60	196.02	53.52	23	606 bp
SAIDG-MEN -F	TCTGGTTGATCCTGCCAGTA	6099	3	16.23	162.32	57.30	20	539 bp

SAIDG-	ATTCCGATTGCAGAGCCT	6093		18.	189.56	59.35	20	539
MEN -R	CG		3.5	96				bp

PCR reactions were carried out in a 25 µL reaction mixture containing 12.5 µL of 2× PCR Master Mix (SinaClon Co., Iran), specific primers, template DNA, and sterile nuclease-free water. Amplification was performed in a thermocycler under the conditions described in Table 2.

Table 2. The thermal program of the thermocycler for the PCR test of tick and lice samples.

Step	*M. stramineus* (Temp / Time*)*	*A. persicus* (Temp / Time)	Cycles
Initial denaturation	95 °C / 5 min	95 °C / 5 min	1
Denaturation	95 °C / 1 min	95 °C / 1 min	
Annealing	56 °C / 1 min	54 °C / 1 min	35
Extension	72 °C / 1 min	72 °C / 1 min	
Final extension	72 °C / 5 min	72 °C / 5 min	1

2-5- Agarose Gel Electrophoresis

PCR products were separated on a 1.5% agarose gel stained with a nucleic acid dye. A 100-bp DNA ladder was used as a molecular size marker. The amplified DNA fragments were visualized under UV illumination using a gel documentation system.

2-6- DNA Sequencing and Molecular Analysis

Selected PCR products from two ectoparasite samples were purified using the QIAquick Gel Extraction Kit (Qiagen, Germany) according to the manufacturer's instructions. The purified products were sent for sequencing to Macrogen Company (Seoul, South Korea). The obtained nucleotide sequences were compared with reference sequences available in the GenBank database using the BLAST algorithm. Molecular identification results were then compared with the morphological characteristics of the specimens to confirm species identification.

2-7- Statistical Analysis

The prevalence of ectoparasite infestation was calculated as the proportion of infected birds relative to the total number of examined birds. Descriptive statistics were used to summarize infestation rates among different bird species and locations. Differences in infestation rates were analyzed using the chi-square (χ^2) test, and p-values less than 0.05 were considered statistically significant. Genetic sequence alignment and evolutionary distance analyses were performed using MEGA software version 7.0.

3- Results

Out of 2,000 examined ornamental birds, 505 (25.25%) were infested with ectoparasites. Of these, 245 birds (12.25%) harbored *M. stramineus* and 260 birds (13.0%) harbored *A. persicus*. No bird was simultaneously infested with both parasites. Ectoparasite specimens were collected from infested birds for morphological and molecular analyses. The species-specific and regional infestation rates are detailed in Table 3.

Table 3. The occurrence of ectoparasite species in ornamental birds from various regions of Tehran Province.

Birds: Types and Species				No. of Samples						
Type	Common name	Scientific name	type of parasite	Tehran	Robat Karim	Shahriar	Parand	total NO. birds examined	Parasite-Positive (P)	Parasite Prevalence Percentage
	Canary	*Serinus canaria domestic*	_	150	50	50	50	300	_	_

Ornamental	*Budgerigar*	*Melopsittacus undulatus*	_	150	50	50	50	300	_	_
	Pigeon	*Columba livia domestic*	*Argas persicus tick*	200 (P:100)	50 (P:40)	100 (P:80)	50 (P:40)	400	260	13%
Birds	*Cockatiel*	*Nymphicus hollandicus*	_	100	25	50	25	200	_	_
	Rosy-faced lovebird	*Agapornis roseicollis*	_	100	25	50	25	200	_	_
	African Grey Parrot	*Psittacus Erithacus*	_	100	25	50	25	200	_	_
	cochins	*Chinese Shanghai fowl*	*Menacanthus Stramineus lice*	200 (P:80)	50 (P:50)	100 (P:70)	50 (p:45)	400	245	12.25%

Total	**1000**	**275**	**450**	**275**	**2000**	**505**	**25.25%**

PCR amplification of the target genes (16S rRNA, COI, and 18S rRNA) from DNA extracted from individual ectoparasite samples successfully produced fragments of the expected sizes. The amplified products corresponded to approximately 240 bp for 16S rRNA, 600 bp for COI, and 539 bp for 18S rRNA. The electrophoresis profiles confirming the amplification of these fragments are presented in Figure. 1.

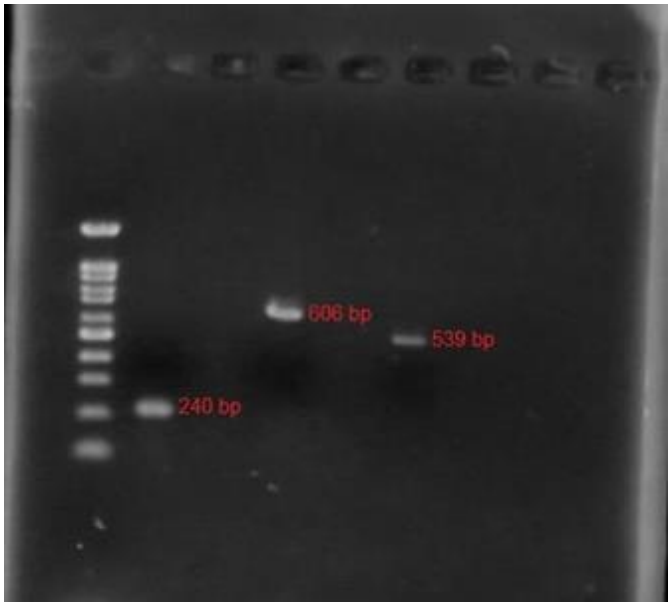

Figure. 1. Agarose gel electrophoresis of PCR products amplified from lice and tick samples. Lane M: 100 bp DNA ladder. Lane X: 240 bp fragments (16S rRNA). Lane Y: 606 bp fragments (COI). Lane Z: 539 bp fragments (18S rRNA).

Negative controls showed no amplification, confirming the specificity of the reactions. The obtained nucleotide sequences were assembled and analyzed using the Basic Local Alignment Search Tool (BLAST) to determine their similarity with reference sequences available in GenBank. Molecular analysis confirmed the presence of two ectoparasite species, *A. persicus* and *M. stramineus*. Identification of *A. persicus* was based on the mitochondrial genes COI and 16S rRNA, whereas *M. stramineus* was identified using the ribosomal 18S rRNA gene. Sequence comparison showed a high similarity (approximately 98–100%) with corresponding reference sequences available in GenBank. The sequences obtained in this study were submitted to GenBank with accession numbers OP522004 and OP532983 for *A. persicus*, and OP452933 for *M. stramineus*. Phylogenetic analyses were performed using the Neighbor-Joining method

with evolutionary distances calculated according to the Kimura two-parameter model using MEGA version 7.0 software. The phylogenetic trees constructed from the obtained sequences and reference sequences from GenBank are shown in Figures 2 and 3.

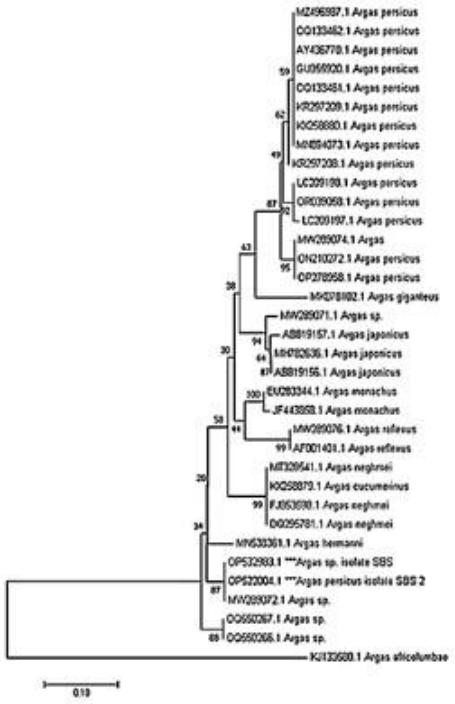

Figure. 2. Neighbor-Joining phylogenetic tree based on partial 16S rRNA gene sequences of *A. persicus* and related species. Bootstrap values (%) are indicated at the nodes. The scale bar shows the number of substitutions per site.

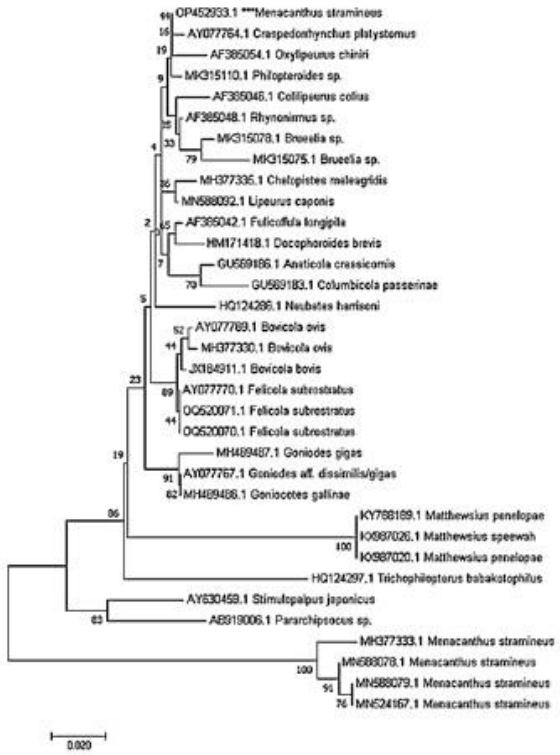

Figure. 3. Neighbor-Joining phylogenetic tree based on partial 18S rRNA gene sequences of lice isolates, including *M. stramineus*. Bootstrap values (%) are shown at the nodes. The scale bar indicates the number of substitutions per site.

Bootstrap values were calculated to assess the reliability of the phylogenetic groupings. The analyzed sequences clustered with previously reported sequences of *A. persicus* and *M. stramineus*. The closest related sequence to the obtained louse isolate originated from Spain (Almería) and was isolated from *Coracias garrulus*. Due to the limited number of available sequences for *M. stramineus* in GenBank, additional related louse species were included in the analysis.

4- Discussion

The findings of this study provide important insights into ectoparasitic infestations of ornamental birds in Tehran Province, Iran. Our results revealed the presence of two significant ectoparasites, *M. stramineus* (chicken body louse) [5] and *A. persicus* (Persian soft tick), with an overall infestation rate of 25.25% [10]. These findings highlight the potential risks posed by these parasites to both avian and human health and emphasize the need for further research and improved control measures in commercial bird markets and aviculture settings [11]. The observed infestation rates of *M. stramineus* (12.25%) and *A. persicus* (13%) indicate a

considerable presence of these ectoparasites in the examined bird populations [2]. Similar infestation patterns have been reported in poultry and wild birds in previous studies. Environmental conditions, host density, and hygiene practices play important roles in determining parasite prevalence. Overcrowded conditions in bird shops, together with inadequate sanitation measures, may facilitate the persistence and transmission of these parasites in the studied region. Our findings are consistent with studies conducted in other regions of the world, where *M. stramineus* has been reported as a common ectoparasite of domestic poultry [12]. This species can cause significant health and productivity problems, including feather damage, reduced growth rates, irritation, and increased susceptibility to secondary infections [13]. Therefore, monitoring and controlling lice infestations are essential for maintaining the health and welfare of birds in breeding and commercial environments. The molecular analysis performed in this study provided additional confirmation of parasite identification and offered insights into their phylogenetic relationships [14]. Sequencing of mitochondrial genes (COI and 16S rRNA) and the ribosomal gene (18S rRNA) confirmed the identity of *M. stramineus* and *A. persicus* [15]. These results contribute to the existing knowledge of the genetic diversity of these parasites. Phylogenetic analysis revealed a close genetic relationship between the identified *A. persicus* isolate and previously reported isolates from Spain [16], particularly those associated with *C. garrulus*. This relationship may reflect historical or ecological connections between parasite populations, potentially influenced by bird migration or international trade of birds in contrast, the genetic distance observed between our *M. stramineus* isolate and the limited sequences available in GenBank highlights the need for additional molecular data to better understand the genetic diversity and distribution of lice affecting avian hosts. The role of *A. persicus* as a vector of zoonotic and avian pathogens is another important concern. This tick species has been associated with the transmission of several pathogens, including the agents of avian borreliosis and West Nile virus [17]. Increasing interactions between wild birds, domestic poultry, and humans in urban and peri-urban areas may facilitate parasite transmission [18]. Continuous monitoring of ectoparasite populations and their associated pathogens is therefore essential for reducing potential public health risks. This study also demonstrates the importance of combining morphological identification with molecular diagnostic methods. Traditional morphological approaches remain useful for initial identification; however, molecular techniques provide greater accuracy and enable the detection of cryptic species and genetic variations [19]. Despite the strengths of this study, several limitations should be acknowledged. Sampling was restricted to bird shops and did not include wild bird populations, which may act as additional reservoirs for ectoparasites [20]. Future

studies should include wider geographic sampling and natural habitats to provide a more comprehensive understanding of parasite–host interactions. Moreover, although molecular analyses confirmed species identity, further genomic studies are required to investigate genetic variations related to insecticide resistance, host adaptation, and pathogen transmission potential.

5- Conclusion

Overall, the findings of this study achieved the research aim and demonstrate that ornamental birds in Tehran Province harbor identifiable ectoparasite species that can be accurately detected using a combination of morphological and molecular approaches. This study provides the first comprehensive molecular investigation of *M. stramineus* and *A. persicus* associated with ornamental birds in Tehran, Iran. The findings offer important baseline information on the prevalence, genetic diversity, and phylogenetic relationships of these ectoparasites. The identification and characterization of these species using molecular approaches contribute to a clearer understanding of their distribution and biological characteristics in ornamental bird populations. The relatively high infestation rates observed in this study highlight the need for improved ectoparasite management practices in bird markets and aviaries, including regular monitoring, better sanitation, and appropriate control strategies. Further research is recommended to investigate the epidemiological significance of these parasites and to clarify their potential role as vectors of infectious diseases, which may have implications for both avian health and zoonotic disease prevention.

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Author Contributions

Soroor Edrisnia (First Author) conceived and designed the study, performed the sample collection from ornamental birds, conducted the molecular experiments and phylogenetic analyses, analyzed the data, and drafted the original manuscript. Saloomeh Shirali (Corresponding Author) supervised the project, assisted in laboratory procedures and data interpretation, and critically revised the manuscript for important intellectual content. Bahar Shemshadi supervised the project and provided critical revision of the manuscript. All authors have read and approved the final version of the manuscript.

Conflict of interest

The authors declare that they have no competing interests.

Ethics approval:

Ethical approval was not required for this study as it did not involve human or animal subjects. All sampling procedures were non-invasive (visual inspection and gentle combing) and caused no harm or distress to the birds. Verbal informed consent was obtained from the owner or manager of each bird shop prior to sampling.

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Data Availability:

All data generated in this study are included in this article and its supplementary material. Artificial intelligence (AI) tools were not used in the preparation, analysis, or writing of this manuscript.

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