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ORIGINAL ARTICLE

MORPHOLOGICAL AND MOLECULAR STUDY OF CHEWING LICE INFESTING POULTRY IN BASRAH PROVINCE, IRAQ

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ABSTRACT

Birds are exposed to infection by various ectoparasites, including chewing lice (Order: Phthiraptera), which affect nearly all avian species. The present study aimed to diagnose the species of lice parasitizing poultry using morphological traits, and molecular analysis (PCR). Additionally, the study included the construction of a phylogenetic tree of the among the species sequenced on the basis of genetic sequencing by using 18S rRNA. The survey was conducted during the period from November 2023 to October 2024. A total of 300 chickens and pigeons were examined from different locations in Basrah Province. In the study, four species were recorded, and registered in GenBank with the following accession numbers: PQ636457 to *Columbicola columbae* (Linnaeus,1758), PQ636458 to *Campanulotes bidentatus* (Scopoli,1763), PQ636459 to *Menacanthus stramineus* (Nitzsch,1818), and PQ636460 to *Menopon gallinae* (Linnaeus,1758).

Keywords**:** Birds, Chewing lice, Chicken, Molecular Characterization, Pigeon.

INTRODUCTION

Birds were among the first animals to be domesticated by humans. They have been used for thousands of years for various purposes, including livestock industry (Alemu *et al*.,2015). Birds, like other animals, are exposed to infestations by external parasites, such as lice, ticks, and mites (Oliveira *et al*., 2011). These infections affect bird behavior and physiology due to the activity of the parasites within the tissues or through the secretion of some substances that may be harmful (Wall and Shearer, 1997). The physiological activities of bird bodies are affected by hosts, leading to reduced their nutritional performance (Alemu *et al*., 2015). Parasitic infections can result in stunted growth and decreased egg production (Saikia *et al*., 2017). Ectoparasites are also dangerous to birds because can they transmit many pathogens, including bacteria, viruses and fungi, and therefore, transmitting infection to healthy birds (Saif *et al*., 2003). External parasites cause significant health issues in birds and can even lead to death. Additionally, they may act as addition to being intermediate hosts for endoparasites (Derakhshanar *et al*., 2006). Poultry are infected with many ectoparasites, the most important

of which is chewing lice, which attacks chickens, especially prevalent in open farming systems , such as rural farming and poultry farms (Wall, 2007). Lice are the most widespread parasites in birds and are found on different parts of the body, such as the head, chest, wings, and abdomen (Mccrea *et al*., 2005). They feed by biting feathers and skin, and it completes its life cycle on the host (Wall and Shearer, 1997). Lice are wingless insects, with a body divided into a head, thorax and abdomen, and have legs modified for clinging to feathers (Pickworth and Morishita, 2007). They are equipped with claws and have antennae that are visible or hidden in a groove on the head. The eyes are vestigial or absent (Lehane, 2005). Adult lice typically range in length from 2-3 mm. Among their distinguishing characteristics is that they have a broad, triangular head that is wider than the thorax, and their mouthparts are of the chewing type (Pape and Roza, 2005). Recently, all lice species are classified classified under the order Phthiraptera, which includes four suborders: Ischnocera, Amblycera, Rhyncophthirinae and Anoplura. The old classification included the first three orders (also known as Biting lice or Chewing lice) were grouped under the order Mallophaga (Mullen and Durden, 2019). This study is important because it fills there is a gap in the molecular identification of lice species in Iraq.

MATERIALS AND METHODS

Study area: Lice samples were collected from 300 birds - 142 domestic chickens *Gallus gallus* (Linnaeus, 1758), and 158 domestic pigeons *Columba livia* J. F. Gmelin, 1789 randomly selected from different rural and urban areas of Basrah Province. The study was conducted from November 2023 to October 2024. There are differences in environmental and geographical characteristics among these areas of collection. Some of which are agricultural, and others are residential.

Sampling, preservation and Photography of specimens: Specimens were examined visually throughout the year, focusing specific regions regions of the bird's body such as the head, neck, wings, and perianal area. Lice were collected and preserved in 70% ethyl alcohol. Morphological identification was performed using standard identification keys (Barriga, 1995; Kareem, 2006). Identification was confirmed by the Iraq Natural History Research Center and Museum at the University of Baghdad. Lice were placed in a 10% sodium hydroxide solution. Then washed with distilled water. The specimens were passed through some concentrations of ethyl alcohol, then transferred to xylol. Each specimen was placed on a glass slide, stained with Canada Balsam, and covered with a cover glass. The specimens were examined under a dissecting microscope (Leica Wild M3) and photographed using an advanced imaging system.

Molecular study of chewing lice using PCR technology: Molecular identification of lice species was conducted through DNA analysis using the PCR (Polymerase chain reaction). This procedure was carried out at the Iraqi association for medical researches and studies in Baghdad.

DNA extraction: Lice specimens were redried by leaving them exposed to room temperature for 24 hours, then ground using a ceramic mortar to obtain a homogeneous powder. After that 18S rRNA was used for DNA amplification; type of primers (forward and reverse) are

showed in Table (1). The Thermocycler software included steps shown in Table (2), and performed an electrical relay of the products of PCR.

Electrophoresis: Electrophoresis of the PCR products was performed after completing the PCR process using a 100bp DNA Ladder and DNA was extracted from 18 samples. The reaction components are as listed in Table (3).

Sequence analysis: Samples of PCR products for sequencing the 18S rRNA gene of the selected samples of the lice were sent to Macrogene Company in Korea. The studied segments were aligned and filtered using the Blast program. The sequence segments of each species were registered in the gene bank.

RESULTS

Morphological study

In this study, four species of chewing lice were collected from chickens and pigeons. Two species were found infesting pigeons: *Columbicola columbae* Linnaeus,1758, and *Campanulotes bidentatus* (Scopoli**,**1763). While, the other two species were recorded on chickens: *Menacanthus stramineus* (Nitzsch,1818) and, *Menopon gallinae* Linnaeus (1758). Below is a brief morphological description of the above species:

1- *Columbicola Columbae* Linnaeus,1758: An elongated body, dark gray in colour, found on the wings. The head is rounded anteriorly, and the antennae have five segments. The head has six pairs of hairs laterally, and a pair of hairs ventrally. The mesothorax is partially separated from the back, and has a tuft of hairs laterally, with lower hair density on the metathorax. Abdominal rings are elongated and narrow, and at the end of the abdomen, there are a few hairs of various lengths. Abdominal segments have three long hairs laterally (Pl.1A).

2- *Campanulotes bidentatus* (Scopoli,1763): A small bodied species, found in various parts of the host's body. The head is triangular in shape, and rounded anteriorly; the temporal region appears wide. Head with long hairs laterally, and no ventral spine in the front of the head. The maxillary palps and jaws are small and dark in color. The antennae are filiform and composed of five segments, with the basal segment being square. The thorax is separated in the middle, while the middle unites with the back. The abdomen is short and oval, with few hairs, and the segments are separate. The end of the abdomen is irregular in shape (Pl.1 B).

3- *Menacanthus stramineus* (Nitzsch,1818): A large-bodied species, dark yellow in color, found all over the host's body. The head is triangular in shape and rounded ventrally. The antennae are club-shaped and hidden inside a cavity. The mandibles consist of four segments. The prothorax is triangular, and the Mesothorax and Metathorax are fused and bear dense hairs. The abdomen is oval with long hairs, but there are short hairs laterally. Abdominal segments have two rows of short hairs and three rows of transverse hairs dorsally (Pl.1C).

4- *Menopon gallinae* (Linnaeus,1758): A medium sized body, light yellow in color, found on feather blades. The head is triangular dorsally with lateral temporal areas. A small set of hairs

is present on the top of the head and three hairs laterally. The antennae are capitate, with four segments partially drawn inward. The mandibles are large and dark. The prothorax is separated from the Mesothorax and Metathorax. Thoracic segments have a set of medium-length hairs. The abdomen consists of seven segments; the fourth is the largest, and has tuft of hairs of varying length dorsally. A pair of long hairs is present laterally, and three rows of fine hairs are at the end (Pl.1 D).

Identification key for chewing lice species of pigeons in the current study:

1. Antennae visible and composed of 5 segments; body slender, head longer than wide ……..……. *Columbicola columbae*

- Antennae not clearly visible; body with oval shape, head equal in length and width ……..…….... *Campanulotes bidentatus*

Identification key of chewing lice species of chickens in the current study:

1. Head with a pair of spines-like ventrally, abdomen densely covered with setae; 12 setae on the dorsal plates of the metathorax and mesothorax……. …*M. stramineus*

- Head without spine-like structures, abdomen not densely covered with setae ……..…*Menopon gallinae*

Molecular study

DNA extraction and amplification: Extracted DNA concentrations ranged from 25to35 µg/µL, with A260/A280 purity ratios between 1.6–1.8. The results of electrophoresis on agarose gel showed successful amplification of the 18S rRNA gene using specialized primers (Pl. 2). Nucleotide sequences of PCR-amplified 18S rRNA fragments were aligned and compared with GenBank reference sequences using BLAST, revealing identity levels between 95% and 100%. The size of the PCR fragments ranged from 300 to 350 base pairs. All lice species were subjected to similarity analysis using the BLAST program, and the 18S rRNA gene sequences of the selected species were documented in GenBank, with an independent accession number assigned to each fragment (Tab. 4).

Phylogenetic tree of the species: The results are shown of the phylogenetic tree of the 18S rRNA gene among the lice species in the current study, along with their corresponding species worldwide are presented. *Columbicola columbae* showed the highest similarity to the Dohuk specimens (MN588094.1), indicating regional genetic homogeneity. The species *Campanulotes bidentatus* was closely related to the isolate GU569170.1 from Japan. The species *Menacanthus stramineus* was similar to the isolate MN588078.1 from Duhok, Iraq. The species *Menopon gallinae* showed similarity to the isolate GU569169.1 from Japan (Diag. 1).

DISCUSSION

The results of the present study witnessed the recorded four species of chewing lice parasitizing local chickens and pigeons in Basrah Province. The species *Columbicola Columbae* was recorded for the first time in Iraq by Abu Al-Hab (1975). It is characterized by its elongated body and the length of the head. It is also characterized by the presence of transverse bands on the fifth to seventh ventral segments. The antennae are black-gray or brown in colour, consisting of five pieces (Crespo *et al.*, 2012). While *Campanulotes bidentatus* was recorded for the first time in Iraq by Khalaf (1959). It is characterized by the absence of spine-like appendages at the front of the head, and by filiform antennae consisted of five pieces in both sexes (Kareem, 2006).

The species *Menacanthus straminus* was first recorded in Iraq by Al-Hubaity (1976). It is characterized by containing thorn-like appendages on the ventral side of the head and by the presence of two rows of transverse hairs on the third to seventh ventral rings and the presence of numerous short hairs on the dorsal side of the mesothorax and metathorax (Mani, 1974). The species *Menopon gallinae* was also recorded for the first time in Iraq by Al-Hubaity (1976); it is characterized by the absence of spine-like appendages at the front of the head and on the base of the jaw texture (Emerson, 1956). Other studies in Iraq related to chewing lice parasitizing birds include those by Al-Mawla and Al-Saffar (2008), Al-Iraqi and Hamad-Ameen (2012), Hatem *et al.* (2021), and Al-Neema and Al-Hayali (2024).

The molecular identification of lice remains inconsistent due to a lack of sufficient information about molecular traits. Morphological similarity does not represent genetic similarity, as it sometimes places the phenotypic characteristics among different species close to each other due to external similarities, making them appear as one species (Brown *et al*, 2000). DNA extraction techniques and their polymerase reaction method have significantly advanced the accurate identification of species (Wells *et al*., 2001). In the current study, specialized primers commensurate with the studied species in order to obtain 18S rRNA gene sequences. This contributed to finding congruence in the diagnosis of the studied species. The high matching rates of the genetic sequences of the studied species can indicate their close correlation, as it was shown through the results of alignment of the sequences with the sequences preserved in the gene bank (Xu *et al*., 2024).

A phylogenetic tree was constructed for the studied species. The phylogenetic tree of chewing lice is often constructed via a combination of molecular data, such as DNA sequences and chromosomal variation, and morphological data features, like body shape, hairs, and mouthparts (Xu *et al*., 2024). Smith (2001) was the first to use a phylogenetic approach to investigate lice relationships, as he used EFl and 18S rRNA nuclear genes to take a molecular approach.

This study is consistent with the result of Al-Badrani and Al-Muftti (2023) in the Kurdistan Region, who also used 18S rRNA primers in what was the first molecular study of chewing lice in Iraq. Other Iraqi research on the molecular characterization of biting lice on birds, were the study of Al-Neema and Al-Hayali (2024). Globally, in Vietnam, Najir *et al.* (2014)

conducted molecular identification of seven lice species in ten species of wild birds using the COI gen. In Saudi Arabia, Alajmi *et al.* (2021) recorded six species of chicken lice using COI gene analysis.

Table (1): Type of primers (forward and reverse).

Design primers	Seq.	TM	Size bp
18s rRNA	F:TGAAACCGCGAAAGGCTCAT R: TACCCGTTACCACCACGGTA	60C	20
18s rRNA	F:TGTCTCAGTGCAAGCCGAAT R:TCCGGGAGTGGGTAATTTGC	60C	20

Table (2): PCR reaction components.

Components		Volume (µl)
Master Mix		12.5
Primers	Forward	1
	Reverse	1
DNA sample		6
Nuclease-Free water		4.5
The total volume of the reaction		25

Table (3): The program for selected gene amplifications.

Steps	Temperature (°C)	Time	Cycles
Initial Denaturation	95	5 min	1
Denaturation	95	30 sec	30
Annealing	57±5	30 sec	
Extension	72	30 sec	
Final extension	72	7 min	1

Table (4): Percentage of Match of the Target 18s rRNA Gene. Sequence with the species preserved in the Genebank and Genbank Accession Numbers.

Species	Accession number of Genbank	Number of Species Matching in Genbank	Gene name	Matching ratio
Columbicola columbae	PQ636457	MN588094.1	18S rRNA	98.43%
Campanulotes bidentatus	PQ636458	GU569170.1	18S rRNA	100%
Menacanthus stramineus	PQ636459	MN588078.1	18S rRNA	99%
Menopon gallinae	PQ636460	PQ636460	18S rRNA	95%

Plate (1): The species of chewing lice that recoded in the study: (A) *Columbicola columbae*, (B) *Campanulotes bidentatus*, (C) *Mencanthus stramineus*, (D) *Menopon gallinae*.

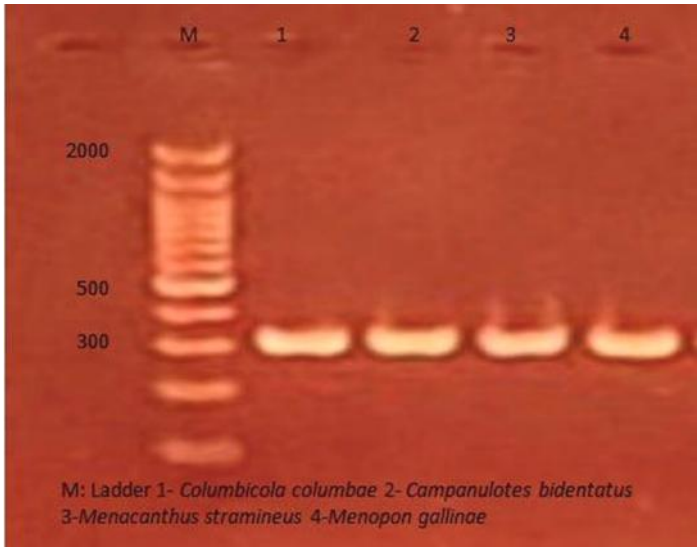

Plate (2): Electrophoresis of PCR amplification products for the 18S rRNA gene in the studied species.

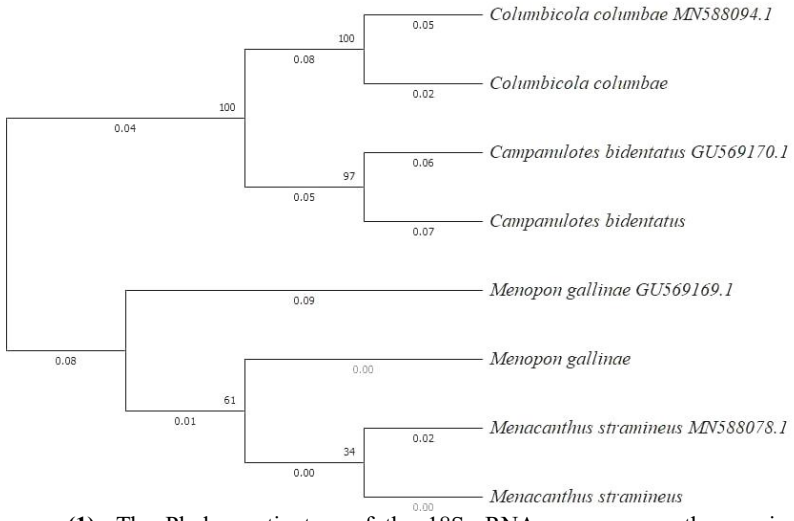

Diagram (1): The Phylogenetic tree of the 18S rRNA gene among the species of chewing lice in Basra - southern Iraq.

CONCLUSIONS

The species of chewing lice found on local chickens differ in morphological characteristics from those found on domestic pigeons. Molecular identification of species is a modern and highly reliable method. Some lice species showed very close genetic relationships based on the sequence of the gene 18S rRNA.

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CONFLICT OF INTEREST STATMENT

"The authors have no conflicts of interest to declare".

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دراسة مظهرية وجزيئية للقمل القارض المتطفل على الدواجن في محافظة البصرة، العراق

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الخلاصة

تصاب الطيور بعدد من الطفيليات الخارجية المختلفة ومنها القمل القارض (رتبة القمل Phthiraptera)، والذي يتطفل على جميع أنواع الطيور تقريبًا. لذا هدفت هذه الدراسة إلى تشخيص أنواع القمل التي تتطفل على الدجاج والحمام المنزلي عن طريق الصفات المظهرية والتحليل الجزيئي باستخدام تقانة PCR. كما تضمن البحث إنشاء شجرة تطورية بين الأنواع التي تم تسلسلها وراثيًا باستخدام 18S rRNA. وتمت مقارنة بيانات التسلسل بالبيانات المتوفرة في بنك الجينات.

أجريت الدراسة خلال الفترة من تشرين الثاني 2023إلى تشرين الأول 2024. بلغ العدد الكلي للدجاج والحمام 300 طائر من مواقع مختلفة في محافظة البصرة. سجلت أربعة أنواع من القمل القارض في الدراسة. شخصت هذه الأنواع مظهريا، وتم تسليط الضوء على أهم الصفات المظهرية التشخيصية. وكما تم تشخيص هذه الأنواع جزيئيا بواسطة تقانة PCR. وتم تسجيل الأنواع في GenBank بأرقام انضمام محددة وكما يأتي: PQ636457 للنوع *Columbicola columbae* (Linnaeus,1758)، و PQ636458 للنوع *Capanulotes bidentatus* (Scopoli,1763)، وPQ636459 للنوع *Menacanthus stramineus* (Nitzsch,1818)، وPQ636460 للنوع *Menapon gallinae* (Linnaeus,1758).