Annals of Parasitology 2021, 67(2), 161–168
doi: 10.17420/ap6702.325

Original paper

Phylogenetic analysis of lice infested chicken (*Gallus gallus domisticus*) with new records in Kurdistan of Iraq

Mohammed Adnan AL-BADRANI, Shamal Abdullah AL-MUFFTI

Department of Biology, College of Sciences, University of Duhok, Kurdistan Region-Iraq

Corresponding Author: Shamal Abdullah Al-Muffti; e-mail: shamal.al-muffti@uod.ac

ABSTRACT. The domestic chickens are the most important protein sources of human populations in every part of the world; many parasites' species may affect birds. Ectoparasites can be found practically in all birds. They feed on their body components like blood, feathers. The effects of louse parasitism on birds are often severe, including retarded growth, low egg production and susceptibility to other infections and due to lice possess chewing mouthpart, it feeds on dry skin scales, scab tissues, and feather parts and it causes skin irritation and sucks blood (anemia), discomfort, loss of plumage, and decrease in productivity of the host. The aim of this study is to investigate lice genera and create the phylogenetic tree among the sequenced lice, using both mitochondrial DNA (COI gene) and nuclear gene (18S rRNA gene). Sequencing data were aligned with the sequences available in the NCBI GenBank. From October 2017 until July 2018, two hundred outdoor local chickens from three governorates (Duhok, Erbil and Sulaymaniyah), were examined for lice collection. After the morphological identification of the lice, the total genomic DNA of each louse was extracted individually and universal primers L6625, H7005 were used to amplify the DNA of mitochondrial gene COI (Cytochrome Oxidase Subunit I) and three designed specific primers (18SrRSHM1, 18SrRSHM2 and 18SrRSHM3) were applied to amplify the DNA of the nuclear gene 18S rRNA**.** Sequencing DNA results were submitted to the GenBank, For the first time in Iraq, twelve species of chicken lice have been submitted in a GenBank with accession numbers of MN531684 from *Menopon gallinae*; MN524167, MN588078, MN588079 belonged to the species of *Menacanthus stramineus*; MN524168, MN524182, MN588080 belonged to the species of *Goniocotes gallinae*; MN524180, MN588092 belonged to the species of *Lipeurus caponis*; MN524181, MN588091 belonged to the species of *Goniocotes gigas* and MN588089 belonged to the species of *Goniodes dissimilis*.

Keywords: chicken, *Menopon gallinae*, *Menacanthus stramineus*, *Goniocotes gallinae*, *Lipeurus caponis*, *Goniocotes gigas*, *Goniodes dissimilis*

Introduction

Poultry production is one of the important economic fields. Domestic chickens are the most important protein sources of human populations in every part of the world [1]. Many parasites species may affect birds when they were breeding outdoor. Ectoparasites can be found practically in all birds. The effects of louse parasitism on birds are often severe, including retarded growth, low egg production and susceptibility to other infections and due to lice possess chewing mouthpart, it feeds on dry skin scales, scab tissues, and feather parts and it causes skin irritation and sucks blood (anemia), discomfort, loss of plumage, and decrease in productivity of the host**.** Approximately 4.000 species of chewing lice (Order Phthiraptera) have been recorded on birds worldwide [2]**.** Lice are obligate ectoparasitic insects that infest birds and mammals [3] They have no free-living stage; they are the most completely committed to parasitism [4]. Most birds and mammals can be infested by specific lice [5]. The order Phthiraptera is currently divided into four suborders: Amblycera, Ischnocera, Rhyncophthirina, and Anoplura [6]. The taxonomic division put the lice into two groups sucking lice (Anoplura) and chewing lice (Mallophaga) [7].

The goal of the present study is to investigate the lice species and create the phylogenetic tree among sequenced lice in local chickens of Kurdistan of

Table 1. Types of primers (forward and reverse)

Universal Prime	Sequences (5' to 3')	Tm	Size bp
F: L6625	CCGGATCCTTYTGRTTYTTYGGNCAYCC	46°C	28
R: H7005	CCGGATCCACNACRTARTANGTRTCRTG	46°C	28
Design Primers*	Sequences (5' to 3')	Tm	Size bp
IHU	F: TGAAACCGCGAAAGGCTCAT R: TACCCGTTACCACCACGGTA	60°C	20
IHU-Gg	F: TGTCTCAGTGCAAGCCGAAT R: TCCGGGAGTGGGTAATTTGC	60°C	20
IHU-Gg	F: ATGTCTCAGTGCAAGCCGAA R: TCCGGGAGTGGGTAATTTGC	60°C	20

*Designed primer for the amplification of the 18S rRNA gene

Iraq, using both mitochondrial DNA and nuclear sequence data and to compare them with similar data available in the NCBI (National Center for Biotechnology Information) GenBank.

Materials and Methods

Study area

Two hundred local chickens were randomly examined for the collection of lice from their bodies. All samples were collected from the outdoor area between October 2017 and July 2018. The study included urban area in the three governorates: Duhok, lies 416 km northwest of Baghdad (included Zakho, lies 524 km northwest and Akre, lies 498 km north of Baghdad); Erbil, lies 388 km north of Baghdad (included Shaqlawa, lies 408 km north and Koya lies, 356 km north of Baghdad) and Sulaymaniyah, lies 375 km northeast of Baghdad (included Rania, lies 401 km northeast and Dokan lies, 406 km northeast of Baghdad).

Collection and preservation of samples

Chicken specimens were collected from random homes and sewage areas. The examination of chicken's bodies included wings, feathers, ventral and femoral areas and anus, using naked eyes and hand lens for collecting lice. Total, 386 lice (male 241, female 145) from chicken were taken to a laboratory in the Department of Biology at College of Sciences, University of Duhok. Lice were collected by handpicking forceps and then transferred to a Petri dish containing water. After that, they were stored in 50 ml tubes containing 96% ethanol to preserve the DNA samples.

The morphological characteristics is according to the identification keys [18,19], using a dissecting microscope (type Lambomed made in U.S.A).

DNA extraction

After the identification of lice, the total genomic DNA was extracted from individual lice head carefully. The head of 20 samples was stored separately in the buffer provided by the Qiagen tissue extraction kit. Digestion proceeded overnight at 55°C. DNA extraction was done according to the manufacturer's protocols (Qiagen) Germany Company. For DNA amplification, species-specific primer (forward primer L6625 and reverse primer H7005) was used to amplify mitochondrial gene COI (Cytochrome Oxidase Subunit I) gave 406 bp, whereas the designed species-specific primers forward and reverse (18SrRSHM1 primer gave 291 bp; 18SrRSHM2 primer gave 405 bp and 406 bp; and 18SrRSHM3 primer gave 394 bp and 407 bp) were used to amplify the nuclear gene 18S rRNA (Table 1). The thermocycler (BioRad, USA) program included an initial denaturation step of 2 min at 94°C, followed by 35 cycles of a 30 s denaturation step at 94°C, and 30 s annealing temperature at 46°C with the primer L6625 and H7005. The annealing temperature of 60°C was used for other designed primers, following 30 s extension at 72°C, with a final extension step of 7 min at 72°C (Table 2). DNA purification was done by the Qiagen PCR purification kit (Cat No./ID: 28506) (20).

Table 2. The thermocycler program for the COI and 18S-rRNA gene

Steps	Temperature (°C)			
	COI gen	18S-rRNA gen	m:s	Cycle (s)
Initial denaturation	95	95	05:00	
Denaturation	95	9	00:30	1
Annealing	46	46	00:30	
Extension	72	72	00:30	30
Final extension	72	72	07:00	
Hold	4	4	100:00	1

Table 3. The chemical reactions for PCR amplification

Master mix components	Volume used for one sample/µl
Master Mix	12.5
Forward primer	1 µl (10 pg mol 1 µM)
Reverse primer	1 µl (10 pg mol 1 µM)
Nuclease Free Water	7.5
DNA Template	3 ng/µl
Total volume	25 µl

Table 4. The prevalence of lice species on local chicken of some areas in the Kurdistan of Iraq

Study location	Latitude Y	Longitude X	No. chicken examined	No. lice	Relative prevalence (%)
Duhok city	36.8632°	42.9885°	22	143	6.5
Zakho	37.1505°	42.6727°	22	41	1.8
Akra	36.7411°	43.8808°	21	51	2.4
Erbil city	36.1901°	43.9930°	24	38	1.5
Shaqlawa	36.4098°	44.3202°	24	20	0.8
Koya	36.0751°	44.6199°	22	70	3.1
Sulaymaniyah	35.5558°	45.4351°	22°	14	0.6
Rania	36.2391°	44.8855°	22	7	0.3
Dokan	35.9496°	44.9621°	21	2	0.09
Total			200	386	1.93

Agarose gel electrophoresis

Gel electrophoresis used DNA Marker (1000 bp), has been performed on 1.5 % agarose gel for DNA extracted from thirteen samples. The reaction mixture (Tag polymeras, Nuclease Free Water, $MgCl_2$ and buffers) used for each sample (Table 3).

Sequence analysis

The PCR products were purified, using the QIAquick gel extraction kit (Qiagen) [21]. Thirteen different samples of PCR products were sent for sequencing in Macrogen Company in Korea. The nucleotide sequences were aligned with the gene

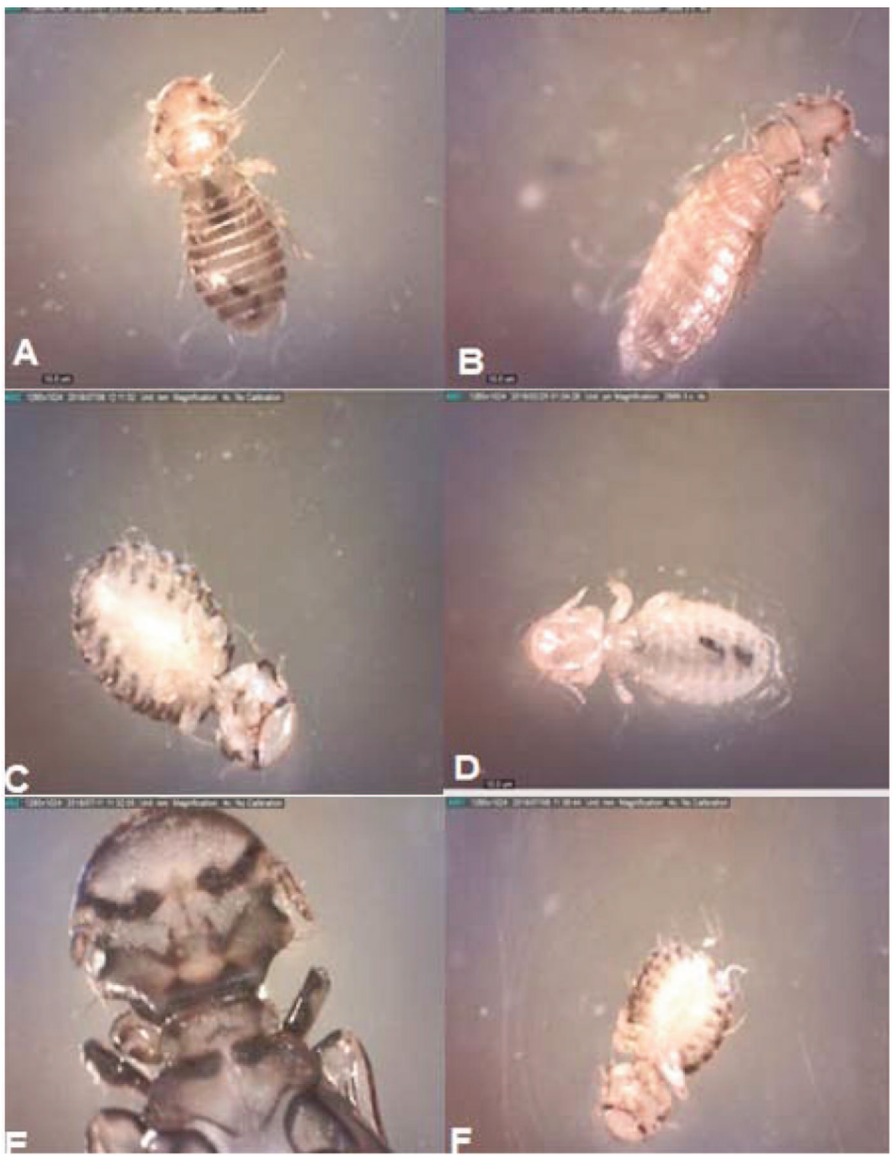

Figure 1. Lice of chicken under a dissecting microscope (40×)
(A) *Menopon gallinae,* (B) *Menacanthus stramineus,* (C) *Goniocotes gallinae*, (D) *Lipeurus caponis* (E) *Goniocotes gigas*, (F) *Goniodes dissimilis*

sequences of mitochondrial (COI) or nuclear 18S rRNA in the GenBank, using the BLAST program. The results were confirmed with the above-mentioned morphological data [22].

Results

Three hundred eighty-six lice were collected from two hundred local chickens from some areas in the Kurdistan of Iraq (Table 4). The results detected twelve species (*Menopon gallinae* one species, *Menacanthus stramineus* three species, *Goniocotes gallinae* three species, *Lipeurus caponis* two species, *Goniocotes gigas* two species and *Goniodes dissimilis* one species). The infected percentage ration of parasitic lice were 42% (n=84) (Table 5). The pictures of six genera of lice are in the Figure 1. The universal primers (F: L6625 and R: H7005) had detected one genus *Menopon gallinae* and their PCR product gave 406 bp as demonstrated in Figure 2. While the species-specific designed forward and reverse primers 18SrRSHM1, 18SrRSHM2 and 18SrRSHM3 had detected five lice genera with different PCR products as the following: *Menacanthus stramineus* (291 bp); *Goniocotes gallinae* (405 bp); *Lipeurus caponis* (394 bp); *Goniocotes gigas* (407 bp); and *Goniodes dissimilis* (406 bp), as depicted in Figure 3. Nucleotides bases for both genes, mitochondria (COI) and 18S rRNA gene were submitted to

Table 5. Prevalence of lice species in local chickens in Kurdistan Region/Iraq

Host	Governorates	No. examined chickens	No. infested chickens	Prevalence (%)
Chickens	Duhok	65	41	20.5
	Erbil	70	28	14
	Sulaymaniyah	65	15	7.5
		200	84	42

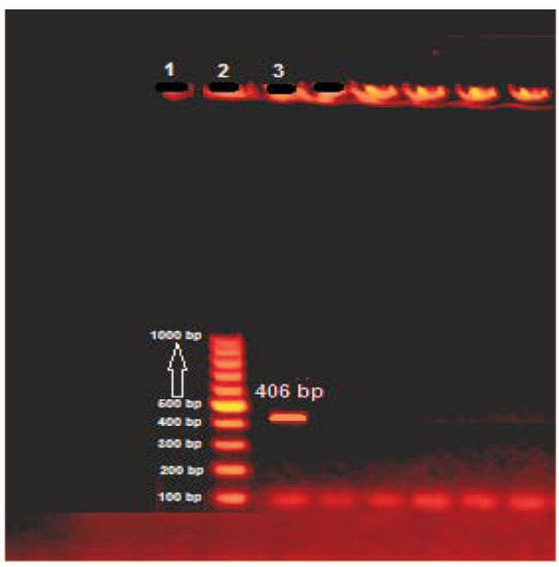

Figure 2. Agarose gel electrophoresis of species-specific PCR amplification of *Menopon gallinae* genomic DNA**.** Electrophoresis was performed on 1.5% agarose gel and run with 3 volt/cm. The lane 1=Negative control (without genomic DNA), lane 2=Marker (Molecular weight marker is a 1000 bp ladder; it means that each band with the next one has 100 bp difference), lane 3=*Menopon gallinae*. The lengths of polymerase chain reaction products are approximately 406 bp.

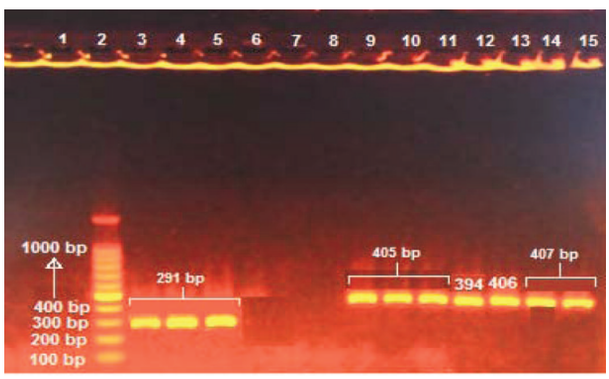

Figure 3. Agarose gel electrophoresis of species-specific PCR amplification of *Menacanthus stramineus*, *Goniocotes gallinae*, *Lipeurus caponis*, *Goniodes dissimilis* and *Goniodes gigas* genomic DNA. Electrophoresis was performed on 1% agarose gel and run with 3 volt/cm. The lane 1=Negative control (without genomic DNA), lane 2=Marker (Molecular weight marker is a 1000 bp ladder; it means that each band with the next one has 100 bp difference), lane 3, 4 and 5=*Menacanthus stramineus*; lane 9, 10 and 11= *Goniocotes gallinae*; lane 12=*Lipeurus caponis*; lane 13= *Goniodes dissimilis*; lane 14 and 15=*Goniodes gigas*. The lengths of polymerase chain reaction products are 291 bp for three samples of *Menacanthus stramineus*; 405 bp three samples of *Goniocotes gallinae*; 394 bp for *Lipeurus caponis*; 406 bp for *Goniodes dissimilis* and 407 bp for two samples of *Goniodes gigas*.

GenBank. For the first time in Iraq, twelve genera of chicken lice have been submitted to GenBank. One genus had recorded by the mitochondria (COI) gene, as *Menopon gallinae* with the accession number MN531684 while the other eleven species had recorded by the ribosomal gene 18S rRNA. Three records for the species *Menacanthus stramineus* with the accession number MN524167, MN588078 and MN588079. This species was higher than the other types of lice in all governorates of the Kurdistan region, and the highest prevalence was in the city of Dohuk. Three records for the species *Goniocotes gallinae* with the accession number MN524168, MN524182 and MN588080. Two records for the species *Lipeurus caponis* with the accession number MN524180 and MN588092. Two records for the species *Goniocotes gigas* with the accession number MN524181 and MN588091. One record for the species *Goniodes dissimilis* with the accession number MN588089. The twelve recorded species of lice were aligned in the phylogenetic tree in Figure 4.

Discussion

The rate of infection in Duhok was bigger than other governorates may be due to the lack of hygiene in these villages. This study agreed with the study of Nigeria showing that 41% of chickens were

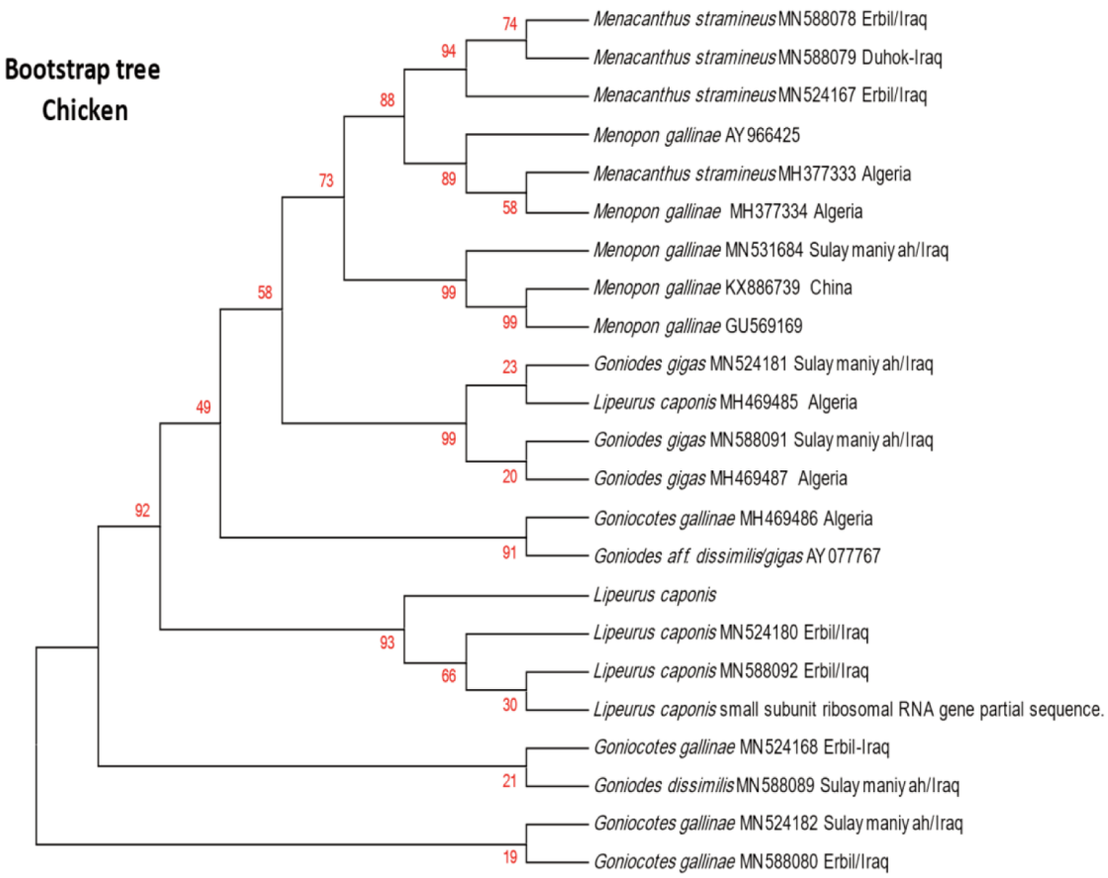

Figure 4. The phylogenetic tree of twelve lice species recorded in the GenBank with their evolutionary taxa. The evolutionary history of molecular phylogenetic analysis by using the Maximum Likelihood method based on the Jukes-Cantor model [1].

The evolutionary history was inferred by using the Maximum Likelihood method based on the Jukes-Cantor model [1]. The bootstrap consensus tree inferred from 1000 replicates [3] is taken to represent the evolutionary history of the taxa analyzed [3]. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches [3]. Initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. The analysis involved 23 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There was a total of 242 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 [2].

infested with ectoparasites lice [23], while the present study is in disagreement with Thi-Qar (22.36%) of lice infested aquatic birds [24]**.** Alemu et al. [25] recorded the prevalence of three lice species *Menacanthus stramineus*, *Menopon gallinae*, and *Cuclotogaster heterographa* at 87.68% in Bishoftu Town of Ethiopia. Further, 81.36% (48/59) were recorded with lice (*Menacanthus stramineus*, *Goniocotes gallinae*, *Menopon gallinae*, *Lipeurus caponis*, *Goniocotes gigas*, and *Goniodes dissimilis*) in Sulaymaniyah of Iraq [9], the reason for the differences between this study and the study of Sulaymaniyah was attributed to many factors like environmental differences, increasing awareness of poultry farmers and repeated attention to hygiene in the Kurdistan of Iraq. The phylogenetic relationships and classification of the four main groups of lice have been matters of contention for some time. These new loci should be potentially valuable data for a broad range of future molecular systematic studies of parasitic lice [26]. The familial relationships among most major groups of lice are difficultly understood, and for resolution of these relationships, it requires more available morphological data, as well as gathering of molecular information including phylogenetic trees. The relationships among lice will

not easy to uncover their groups due to their long and complex independent history [27].

Based on the sequences of both gene COI and 18SrRNA, a neighbor-joining tree had been done for 23 taxa of different lice belong to two suborders Ischnocera and Amblycera of the order Phthiraptera. Numbers branches indicated support from 1000 bootstrap replicates. The focusing in the phylogenetic tree indicated the following:

1. The three lice species *Menacanthus stramineus* MN588078; *Menacanthus stramineus* MN588079; and *Menacanthus stramineus* MN524167 had to make the Iraqi subgroup in one cluster, while species *Menacanthus stramineus* MH37733A Algeria, is in another cluster maybe it has various qualities.

2. The *Menopon gallinae* MN531684 seems to be a new species and needs more further study.

3. Another cluster contains *Goniocotes gigas* MN524181 that had made variant A, while *Goniocotes gigas* MN588091 had to make variant B in the same cluster.

4. Another cluster contains *Lipeurus caponis* MN524180 and had to make variant A, while *Lipeurus caponis* MN588092 had to make variant B in the same cluster.

5. The *Goniocotes gallinae* MN524168 and *Goniodes dissimilis* MN588089 had to make variant A in new cluster; while *Goniocotes gallinae* MN52182 and *Goniocotes gallinae* MN588080 had to make variant B for the same cluster.

References

[1] Al-Saed A.T.M., Al-Badrani M.A.I. 2014. Study of the parasites of the local chickens (*Gallus gallus domisticus*) in Duhok province, Kurdistan Region-Iraq. *Journal of the University of Zakho* 2: 18-23. https://sjuoz.uoz.edu.krd/index.php/sjuoz/article/view/85

[2] Dik B., Uslu U. 2012. Studies on chewing lice (Phthiraptera) species found on some duck (Anseriformes: Anatidae) species at Lake Akşehir, Turkey. *Kafkas Universitesi Veteriner Fakultesi Dergisi* 18: 1055-1060. doi:10.9775/kvfd.2012.7020

[3] Soto-Patiño J., Londoño G.A., Johnson K.P., Weckstein J.D., Avendaño J.E., Catanach T.A., Sweet A.D., Cook A.T., Jankowski J.E., Allen J. 2018. Composition and distribution of lice (Insecta: Phthiraptera) on Colombian and Peruvian birds: new data on louse-host association in the Neotropics. *Biodiversity Data Journal* 6: e21635 doi:10.3897/BDJ.6. e 21635

[4] Johnson K.P., Yoshizawa K., Smith V.S. 2004. Multiple origins of parasitism in lice. *Proceedings of the Royal Society of London* B 271: 1771-1776. doi:10.1098/rspb.2004.2798

[5] Mirzaei M., Ghashghaei O., Yakhchali M. 2016. Prevalence of ectoparasites of indigenous chickens from Dalahu Region, Kermanshah Province, Iran. *Turkiye Parazitoloji Dernegi* 40: 13-16. doi:10.5152/tpd.2016.4185

[6] Palma R.L., Jensen J. 2005. Lice (Insecta: Phthiraptera) and their host associations in the Faroe Islands. *Steenstrupia* 29: 49-73.

[7] Sweet A.D., Allen J.M., Johnson K.P. 2014. Novel primers from informative nuclear loci for louse molecular phylogenetics (Insecta: Phthiraptera). *Journal of Medical Entomology* 51: 1122-1126. doi:10.1603/ME13218

[8] Al-Dulimy E.J.F., Abd-Alamery A.M. 2015. Isolation and diagnosis of the ectoparasites of house chickens in the province of Baghdad and study their effects on the blood. *Al-Qadisiyah Journal of Veterinary Medicine Sciences* 14: 1-6.

[9] Abdullah S.H., Mohammed A.A. 2013. Ecto- and endoparasites prevalence in domestic chickens in Sulaimani Region. *The Iraqi Journal of Veterinary Medicine* 37: 149-155. doi:10.30539/iraqijvm.v37i2.275

[10] Al-Zubaidei H.H. 2015. Isolation and identification of some ecto- and endoparasites on native chickens in Diyala province. *Al-Qadisiyah Journal of Veterinary Medicine* 14: 1-5.

[11] Mansur M.K., Mahmoud N.M., Allamoushi S.M., El Aziz M.M.A. 2019. Biodiversity and prevalence of chewing lice on local poultry. *Journal of Dairy, Veterinary and Animal Research* 8: 26-31.

[12] Naseem S., Tahir H.M. 2016. Use of mitochondrial COI gene for the identification of family Salticidae and Lycosidae of spiders. *Mitochondrial DNA Part A* 29: 96-101. doi:10.1080/24701394.2016.1248428

[13] Banos S., Lentendu G., Kopf A., Wubet T., Glöckner F.O., Reich M. 2018. A comprehensive fungi-specific 18S rRNA gene sequence primer toolkit suited for diverse research issues and sequencing platforms. *BMC Microbiology* 18: 190. https://doi.org/10.1186/s12866-018-1331-4

[14] Adang K.L., Oniye S.J., Ezealor A.U., Abdu P. A., Ajanusi O.J. 2008. Ectoparasites of domestic pigeon (*Columba livia domestica*, Linnaeus) in Zaria, Nigeria. *Research Journal of Parasitology* 3: 79-84.

[15] Thamer N.K., Almeelman M.D., Abdulaz Norei D.A. 2016. Taxonomical *s*tudy of lice from some birds at southern Iraq. *Journal of International Academic Research for Multidisciplinary* 3: 25-34.

[16] Al-Mayali H., Abdul Kadhim H. 2015. Ectoparasites of domestic turkey (*Meleagris gallopavo*) in Al-Diwaniya City/Iraq. *International Journal of Current Microbiology and Applied Sciences* 4: 669-677. https://www.researchgate. net/publication/309159585

[17] Smith V.S., Page R.D.M., Johanson K.P. 2003. Data incongruence and the problem of avian louse phylogeny. *The Norwegian Academy of Science and Letters Zoologica Scripta* 33: 239-259. doi:10.1111/j.0300-3256.2004.00149x

[18] Soulsby E.J.L. 1982. Helminths Arthropods and Protozoa of domesticated animals. Bailliere Tindal London U.K.

[19] Price M.A., Graham O.H. 1997. Chewing and sucking lice as parasites of and mammals and birds. U.S. Department of Agriculture, Technical Bulletin No. 1849.

[20] Simon C., Frati F., Beckenbach A., Crespi B., Liu H., Flook P. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America* 87: 651-701. https://doi.org/10.1093/aesa/87.6.651

[21] Mutinelli F., Corro M., Catania S., Melchiotti E. 2008. Multiple feather follicle cysts in a Moroseta hen (*Gallus gallus*). *Avian Diseases* 52: 345-347. doi:10.1637/8153-101907-Case.1

[22] Al-Muffti S.A. 2008. Genetic diversity analysis of *Anopheles* species in Kurdistan Region-Iraq, Using Molecular Biology Techniques. Ph.D. Thesis, University of Baghdad.

[23] Nnadi P.A., George S.O.A. 2010. Cross-sectional survey on parasites of chickens in selected villages in the subhumid zones of South-Eastern Nigeria. *Journal of Parasitology Research* 2010: 141824. doi:10.1155/2010/141824

[24] Awad A.H., Mohammad Z.A. 2015. Chewing lice found on aquatic birds collected from some marshes of Thi-Qar Governorate-South of Iraq. *Journal of Babylon University/Pure and Applied Sciences* 23: 694-702.

[25] Alemu N., Muktar Y., Kassaye D., Hiko A. 2015. Prevalence of lice and fleas in backyard chickens of Bishoftu Town, Ethiopia. *American-Eurasian Journal of Agricultural and Environmental Sciences* 11: 2136-2142. doi:10.5829/idosi.aejaes.2015.15.11.10181

[26] Smith V.S. 2001. Avian louse phylogeny (Phthiraptera: Ischnocera): a cladistic study based on morphology. *Zoological Journal of the Linnean Society* 132: 81-144. https://doi.org/10.1111/j.1096-3642.2001.tb02272.x

[27] Kansal G., Singh H.S. 2014. Incidence of ectoparasites in broiler chicken in Meerut. *Journal of Agriculture and Veterinary Science* 7: 55-58. doi:10.9790/2380-07115558

Received 08 January 2021
Accepted 19 March 2021