Host habitat and position on host affecting the evolution of chewing lice (Phthiraptera): Phylogenetic analysis of Ischnocera in Saudi Arabia

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ABSTRACT. There are very few reports discussing the higher level phylogeny of the chewing lice (Phthiraptera) and their roles in the evaluation of these insects. So, the aim of this work is to study the phylogeny of genera of the suborder Ischnocera in Saudi Arabia using morphological, ecological and molecular criteria trying to answer a very important question about ecological factors that affecting their evolution. The results show high degree of similarity between phylograms produced by morphological and ecological criteria and that produced by genes sequencing data of Cytochrome Oxidase Subunit I (COI) and elongation factor-1 alpha (EF-1). The rooted trees showed several clusters for head chewing lice and this indicated that the phenomenon of parasitizing birds’ heads evolved several times in the clade of chewing louse. This is also indicating that the position on host played a very important role in shaping the diversity of these insects. The unrooted tree which produced by analysis of COI and (EF-1) also supports the previous conclusion but added to it the impact of host habitat on such diversity. The evolutionary distance between marine birds’ chewing lice was very small and all of them appear in single line or group which far from that of terrestrial taxa of the chewing lice. Both the host habitat and the feeding position of chewing lice on their hosts played a very important role in the way by which this group of insects evolved.

Key words: Chewing lice, Ischnocera, Evolution, Saudi Arabia, Host habitat, Phylogeny

Introduction

The chewing lice are a group of exceptional interest due to the way by which these organisms were evolved. All of them are completely permanent ectoparasites of mammals and birds and their evolution has lagged behind their hosts (Price & Graham, 1997). Therefore, the phylogeny of this group when properly understood will be constantly...
reflected on our knowledge of mammals and birds systematics (Hopkins, 1941). From this point of view, the study of chewing lice phylogeny becomes more and more critical need for any modern chewing lice investigations.

The importance of studying chewing lice phylogeny started early on the beginning of last century by the article published by Harrison (1914). He found that the chewing lice of genus Philopterus Nitzsch, 1818 from the common Australian cuckoo (Cacomantis flabelliformis Latham, 1801) usually hardly separated from the chewing lice species of the same genus that parasitized European cuckoos. In addition, all the Philopterus species that parasitized different species of cuckoo around the globe have common characters and structures that separated them from any other species groups of Philopterus that infesting any other birds. So, he concluded that the chewing lice will be a possible clue to birds’ evolutionary events.

After that, the study of chewing lice phylogeny had been continued for more than 100 years through all taxonomic levels. Several studies have discussed the phylogeny and systematics of the order Phthiraptera and the relationships within its suborders and families (Lyal, 1985; Johnson et al., 2004). The evolutionary interaction between the chewing lice and their hosts mark the importance of studying host/lice coevolution (Hafner & Nadler, 1988). Several recent works discussed this point for certain species of chewing lice and their specific hosts (Paterson et al., 2000; Clayton & Johnson, 2003; Banks & Paterson, 2004). The host/parasite coevolution usually described as species arms race because when parasites develop a mechanism to help them in adaptation to their hosts, the hosts also develop a defense against these parasites and vice versa (Majerus et al., 1996). This fact is not completely true with chewing lice/birds coevolution because sometimes the bird is infected with very few lice individuals and in this case, the host gain nothing to remove these parasites (Clayton, 1991).

The other factor that affects the chewing lice phylogeny and evolution is host isolation and speciation. From this point of view, the lice/birds evolution takes one of three phylogenetic scenarios (Gullan & Cranston, 2010). The first scenario is subject to the rule of “Fahrenholz”. In the late 1800’s, Fahrenholz referred for the first time to parallel evolution between host and parasite and as the host becomes isolated and speciation process took place and new species is evolved. The same process also affects the parasite and new species of this parasite is also evolved (Timm, 1983). Eichler (1948) noticed about the work of Fahrenholz as “Fahrenholz Rule” and defined this rule as: “In groups of permanent parasites the classification of the parasites usually corresponds directly with the natural relationships of the hosts”. This rule is applicable for some chewing lice species especially those showing monoxenous habit such as species of genus Dennyus Neumann, 1906 on swifts (Clayton et al., 2004).

The other two scenarios of lice/birds evolution occur when either the birds or lice have an independent phylogenetic way. In this case, when bird species becomes isolated and the speciation process took place. The same thing didn’t happen to its ectoparasite and vice versa. In this case, the lice phylogeny doesn’t reveal host systematics (Johnson et al., 2002). The Lice/birds evolution passed through several stations during the long history of our planet. Sometimes there was a great congruence between them, other time there was a conflict, and in few cases, there was no relation at all. Various factors governed their race for survival including cospeciation, isolation, host switching, geographical distribution of
host birds, abundance and many other ecological and behavioral factors (Clay, 1964). Some species like *Colpocephalum turbinatum* Denny, 1842 found to infest hosts through three different avian orders (Accipitriformes, Columbiformes, and Strigiformes) while other species are monoxenic restricted only to one host like *Quadraceps brunneus* (Nitzsch 1866) on crab plover (Nasser, 2015a).

Finally, the complete picture of chewing lice/ birds’ phylogeny and coevolution is not entirely understood. There is a great lack of information for many lice species either on ecological or molecular levels (Shobrak et al., 2015; Adly et al., 2019). In addition, the previous phylogenetic analysis neglected some important taxonomic categories as there are very few works discussed the phylogeny of lice at generic level (Smith, 2000). The factors that affect the evolutionary process of such interesting creatures are also neglected. So, the aim of this study is to answer one important question that arose during the study of Saudi Arabia chewing lice fauna: Is the evolution of chewing lice genera of suborder Ischnocera depend on the position of these parasites on the host or it depends on host habitat either aquatic or terrestrial.

**Material and methods**

**Morphological and ecological data**

A group of eleven genera of previously recorded Saudi Arabians Ischnocera chewing lice (Nasser, 2015b) were used through this study. Set of 29 morphological and qualitative ecological parameters were used to study the attribution of relations among these genera (Table 1; Table 2). The selection of the characters was done according to two factors: the importance of the parameter itself in separating of the genera through the taxonomic key and the use of these parameters in some previous studies which concerning lice evolution (Smith, 2000). The Neighbor-Joining classification test based on Euclidean distance was used for calculating the relation between the twelve genera (Moftah et al., 2011). The PAST 3.06 (Hammer et al., 2001) software was used to induce the final Dendrogram using the previous tests.

The database of the National Center for Biotechnology Information (NCBI) was used as a source of DNA sequences that helped to analyze the phylogenetic relationships among all recorded Saudi Arabia genera of Ischnocera. The sequences were aligned using MEGA 6 (Tamura et al., 2013). This involves establishing which regions of the sequences are homologous and can be compared. Two conservative genes Cytochrome Oxidase Subunit I gene (COI) and elongation factor-1 alpha gene (EF-1) were used to achieve the aim of this part of the study. Sequences of two genes from 11 species of Ischnocera chewing lice representing all Saudi Arabia recorded genera were extracted in FASTA format from the GeneBank database (Table 3).

MEGA 6 (Tamura et al., 2004) was used for alignment and phylogenetic analysis. The evolutionary history was inferred using the Neighbor-Joining method (Saitou & Nei, 1987). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004) and are in the units of the number of base substitutions per site. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There was a total of 724 positions for the two genes in the final dataset.
Table 1. List of the 29 morphological and ecological characters of Ischnocera chewing lice genera used to produce the final data matrix in Table 2.

| No. | Character                                      | state                                                                 |
|-----|-----------------------------------------------|-----------------------------------------------------------------------|
| 1   | Body shape                                    | Broad and Stout (1), Oblong and slender (0)                           |
| 2   | Head front shape                              | Broadly convex (1), Narrow pointed (0)                               |
| 3   | Head Index                                    | More than or equal 1 (1), Less than 1 (0)                            |
| 4   | Hyaline margin                                | Present (1), Absent (0)                                             |
| 5   | Hyaline margin                                | Normal (1), Bilobed (0)                                             |
| 6   | Marginal Carina                               | Thick (1), Faint (0)                                                |
| 7   | Carina                                        | Continues at anterior margin (1), Interrupted at anterior margin (0) |
| 8   | Clypeal plate                                 | Transparent (1), Not transparent (0)                                |
| 9   | Clypeal suture                                | With Clypeal suture (1), Without Clypeal suture (0)                 |
| 10  | Antenna Sexual dimorphism                     | Present (1), Absent (0)                                             |
| 11  | Conus                                         | Large (1), Very small or reduced (0)                                 |
| 12  | Temple margins                                | Rounded (1), Pointed (0)                                            |
| 13  | Postocular setae                              | Thorn like (1), Not thorn like (0)                                  |
| 14  | Post temporal setae                           | Present (1), Absent (0)                                             |
| 15  | Prothorax shape                               | Pantagonal or trapezoidal (1), Longitudinally or transversally rectangular (0) |
| 16  | Pairs of three to five setae bundles on lateral margin of metathorax | Present (1), Absent (0)                                          |
| 17  | Legs length                                   | Equal or almost equal (1), Unequal (0)                              |
| 18  | Abdominal lateral margin                      | Normal chitinized margin (1), Vertebra shape chitinized margin (0)  |
| 19  | Column of intermediate sternal abdominal setae | Present (1), Absent (0)                                           |
| 20  | Polygonal marks over the cuticular surface of the abdomen especially on female | Present (1), Absent (0)                                          |
| 21  | Male abdominal segment IX                     | Extended prominently beyond the abdominal nature margin (1), Not extended prominently beyond the abdominal nature margin (0) |
| 22  | Male genitalia                                | Symmetrical (1), Asymmetrical (0)                                   |
| 23  | Male genitalia                                | Highly chitinized (1), Weakly chitinized (0)                        |
| 24  | Female genital plate chitinization            | Present (1), Absent (0)                                             |
| 25  | Female hairy genital chamber                  | Present (1), Absent (0)                                             |
| 26  | Chitinized Ring spermatheca                   | Present (1), Absent (0)                                             |
| 27  | Body transparency                             | Body not transparent (1), Body transparent (0)                      |
| 28  | Position on host                              | Head (1), Body (0)                                                  |
| 29  | Found on host wing                            | Yes (1), No (0)                                                     |
Table 2. The final data matrix of 29 characters of the Ischnocera chewing lice genera.

| TAXA             | CHARACTERS |
|------------------|------------|
|                  | 1-10       | 11-20      | 21-29      |
| Goniocotes       | 1100011000 | 0101101110 | 110000110  |
| Goniodes         | 1100011001 | 0001101110 | 110000110  |
| Brueelia         | 0010000100 | 0101001110 | 010101101  |
| Quadraceps       | 0011101110 | 0101000110 | 011101111  |
| Meropoeus        | 1001000110 | 1100101100 | 011101111  |
| Saemundssonia    | 1011100110 | 1100101111 | 011101111  |
| Lipeurus         | 0010111001 | 0100000110 | 011100100  |
| Columbicola      | 0010111001 | 0100000110 | 011001000  |
| Pectinopygus     | 0011100011 | 0100110111 | 001110101  |
| Carduiceps       | 0011100011 | 1101101110 | 011101101  |
| Campanulotes     | 1100111000 | 0001100001 | 110000110  |

Table 3. Sequences of Two Genes (COI & EF-1) were extracted in FASTA format from 11 species of Ischnoceran chewing lice representing all recorded genera in Saudi Arabia.

| Louse genera of suborder: Ischnocera | GenBank No. of Cytochrom Oxidase | GenBank No. of Elongation Factor-1 |
|--------------------------------------|----------------------------------|-----------------------------------|
| Brueelia sp.                         | DQ887246.1                       | DQ887211.1                        |
| Campanulotes sp.                     | AF278653.1                       | AF278671.1                        |
| Carduiceps sp.                       | JN900135.1                       |                                   |
| Columbicola sp.                      | EF678986.1                       | AF278636.1                        |
| Goniocotes sp.                       | AF348852.1                       | AF320403.1                        |
| Goniodes sp.                         | AF348851.1                       | AF320404.1                        |
| Lipeurus sp.                         | AY314818.1                       | AY314837.1                        |
| Meropoeus sp.                        | JX121675.1                       | JX121689.1                        |
| Pectinopygus sp.                     | DQ482971.1                       | AF320444.1                        |
| Quadraceps sp.                       | JX121678.1                       | AF447209.1                        |
| Saemundssonia sp.                    | JN900113.1                       | AF320464.1                        |

Results

Several evolutionary trees were produced as a result of statistical analysis of morphological, ecological and molecular data matrices to get the answers to the main question of this work which concerning Ischnocera genera evolution. Two data sets were used to reconstruct the phylogenetic relations between the genera of Ischnocera.

Morphological and Ecological Data

The tree was produced using Neighbor-Joining classification based on Euclidean distance for a set of 29 morphological and ecological parameters for the eleven Saudi Arabia Ischnocera genera using PAST 3.06 platform for the analysis (Fig. 1). The resulting tree showed that the genera are divided into two clusters. The first cluster included only three head chewing lice genera (Campanulotes Kéler 1939, Goniodes Clay, 1940a, and Goniocotes
Burm., 1838). Within this cluster, the *Goniocotes* and *Goniodes* form a monophyletic group while acted as paraphyletic toward the third genus *Campanulotes*.

The second cluster which contains the other eight genera is polyphyletic with two main groups. The first group contains the wing chewing lice genera (*Lipeurus* Nitzsch, 1818, *Columbicola*, Ewing, 1929 and *Pectinopygus* Mjöberg, 1910b). As the *Lipeurus* and *Columbicola* infesting terrestrial birds, they are monophyletic while the marine parasite *Pectinopygus* is paraphyletic to them. The other group is polyphyletic contains two head chewing lice (*Meropoecus* Eichler, 1940b and *Saemundssonia* Timmer, 1936) which act as a monophyletic cluster and paraphyletic to marine bird body lice *Carduiceps* Clay & Meinertzhagen, 1939c. The remaining two genera (*Quadraceps* Clay & Meinertzhagen, 1939c and *Brueelia* Kéler, 1936a) form a monophyletic group as body lice genera.

The whole dendrogram produced by PAST showed the delay of the first cluster of head chewing lice genera (*Campanulotes*, *Goniodes*, and *Goniocotes*) which means late evolve of this group. Also, the diagram showed the concurrent appearance of other two head chewing lice genera (*Meropoecus* and *Saemundssonia*) with the first cluster at the same evolutionary point.

**Figure 1.** Phylogenetic tree based on Neighbor-Joining classification using Euclidean distance of morphological and ecological Data matrix for all recorded genera of Ischnocera.
**Bioinformatics Data**

Two conservative common genes sequences (Cytochrome Oxidase Subunit I (COI) and elongation factor-1 alpha gene (EF-1)) were used to study the evolutionary relations between the same eleven Saudi Arabia Ischnocera genera. The evolutionary distances were calculated using the Neighbor-Joining statistical method.

The tree (Fig. 2) was divided into two main clusters the first cluster contains 6 genera with the polyphyletic relation. This part of the tree has one large cluster which has paraphyletic relation with marine head chewing lice *Saemundssonia*. The main cluster contains the same monophyletic group of head chewing lice that appear in morphological and ecological analysis of the genera. The *Goniocotes* and *Goniodes* are very closely related while act as paraphyletic toward the third genus *Campanulotes*. The genus *Lipeurus* and *Columbicola* are paraphyletic to each other and to the head chewing lice group. Through the unrooted tree (Fig. 3), these genera form one evolutionary lineage with very closely related tip contains the group of head chewing lice followed by the *Lipeurus* and *Columbicola* that also are very closely related and finally *Saemundssonia* which are very far from this group in evolutionary distance term.

**Figure 2.** Phylogeny tree based on Neighbor-Joining analysis of COI gene and (EF-1) gene alignments for all recorded genera of Ischnocera.
The second cluster contains 5 genera with polyphyletic manner and its main clade with one monophyletic genera *Brueelia* and *Meropoecus*. The three other genera *Pectinopygus*, *Carduiceps* and *Quadraceps* are paraphyletic to each other and to the monophyletic group (*Brueelia* and *Meropoecus*). The unrooted tree (Fig. 3) of this cluster show that it takes a different evolutionary direction from the first cluster and its tip contains *Brueelia* and *Meropoecus* with very small evolutionary distance. The other tree marine chewing lice are more related to each other, but with somewhat long evolutionary distance where *Quadraceps* occupy about the mid-way between *Pectinopygus* and *Carduiceps*. By analyzing the entire unrooted tree (Fig. 3), the results indicated that the head chewing lice are scattered through the tree. *Campanulotes*, *Goniodes*, and *Goniocotes* are found on one tip and *Meropoecus* on the other tip while *Saemundssonia* was found on the center of the tree. Also, the four marine genera were found to be somewhat closely related and they separated by almost equal evolutionary distance.

**Figure 3.** Unrooted tree based on Neighbor-Joining analysis of COI gene and elongation factor-1 alpha gene alignments for all recorded Saudi Arabia genera of Ischnocera (Red flash indicates head chewing lice; Blue flash indicates aquatic chewing lice).

**Discussion**

Understanding of the ecological processes that governed the chewing lice evolution is very important to evaluate their relations with their hosts and some time to reveal the evolution of these hosts themselves (Nasser et al., 2015, 2019). There are a lot of obstacles face scientists to achieve a full awareness of birds/chewing lice phylogeny. One of these obstacles is the lack of clear phylogenetic analysis of many host birds especially on lower taxonomic levels (Smith, 2000). Also, an ambiguity that surrounds many genera and species of chewing lice including cases of host switching and struggling which in many cases become misleading for
understanding host/parasite interaction (Whiteman et al., 2004; Nasser, 2015a). Although, the studying of coevolution between chewing lice and their host is important, the study of evolutionary events happened to the parasite itself also with the same importance (Schmid-Hempel, 2011).

The present study forms the first work that discussed the role of lice position on the host and host habitat in the evolution of Ischnocera genera. The produced trees of either that depending on morphological and ecological data or that depending on molecular ones show high degree similarity. The first cluster on both trees including only three head chewing lice genera (Campanulotes, Goniodes, and Goniocotes) and the two trees look identical even in the appearance of Goniocotes and Goniodes on the tree as monophyletic cluster while act as paraphyletic toward the third genus Campanulotes. Such appearance refers to the accuracy of analysis in two systems. In the morphological based-tree, the other two genera which infested the bird heads appears as a monophyletic cluster and polyphyletic to the other three heads’ chewing lice genera (Campanulotes, Goniodes and Goniocotes). These results pointed out that the habit of infesting host head appears several times in the evolutionary history of the chewing lice. These results came compatible by recently published work on the South Africa chewing lice phylogeny (Takano et al., 2019). Such conclusion of phylogram produced by morphological and ecological data is supported by the unrooted tree which produced using molecular data as the chewing lice genera appear with long evolutionary distance and separated by chewing lice genera that occurs on the birds’ bodies. The wing chewing lice genera Lipeurus and Columbicola appeared as monophyletic to each other on the morphological based tree while very closely paraphyletic on molecular-based one. This result when combined with the shape of head chewing lice on the tree refers to the role of the position of chewing lice on the host in the radiation of chewing lice forms and branching through the long evolutionary history of these insects (Sweet et al., 2018). The two-produced rooted tree indicated tangle of marine and terrestrial genera, but when analyzing molecular data to a produced unrooted tree which represent the evolutionary distance between genera, the result shows how the marine chewing lice genera are very closely associated in the single line or group with very small evolutionary distance. This result indicated that there is a role in host habitat in shaping the evolutionary routes of lice genera even if its effect is lower than that of the position of lice on the host (Johnson et al., 2018).

In conclusion, both the position of chewing lice on the host and host habitat affect the evolutionary way of chewing lice in their arm race with their hosts for survival. Future studies would give greater confidence in the observed patterns of their association otherwise the missing lice genera possibly leading to misanalysis of evolutionary events in the association between these parasites, but the filling of these gaps by adding the other genera of chewing lice are required in future studies.

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Conflict of Interests
The authors declare that there is no conflict of interest regarding the publication of this paper.

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Phylogenetic Analysis of Ischnocera

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Abstract

The analysis of Phthiraptera (Ischnocera), the only order of lice, is important in understanding the evolution of these insects. The aim of this study was to investigate the phylogenetic relationships of the genera of Ischnocera in Saudi Arabia using morphological, biological, and molecular data. The results showed that the phylogenetic trees based on morphological and biological data and those derived from the analysis of COI and EF-1α gene sequences were highly consistent. A range of genera, including those of Ischnocera and other lice, clustered together in the trees, indicating that the establishment of this insect on the host has occurred independently multiple times. This suggests the importance of the host's location and the species' position on the host, both playing significant roles in the evolution of these insects. The key terms: Ischnocera, lice, Saudi Arabia, zoonotic.