

Chaetotaxy of Three Nymphal Instars of *Menopon Gallinae*

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Abstract: *Phthiraptera* are holometabolous and their nymphal instars resemble to each other to a greater extent, in other words the instars of *phthirapteran* species look alike and are difficult to differentiate. This article presents a comprehensive study of the morphological features of the three nymphal instars of *Menopon gallinae*, an avian louse. Avian lice, scientifically known as *Phthiraptera*, are tiny wingless insects that are permanent ectoparasites infesting warm - blooded vertebrates, such as birds and mammals. The study delves into the intricate details of the head, thorax, and abdomen morphology of these nymphal instars, highlighting their differences in size, sclerotization of mouth parts, and the number of setae on various body parts. The research employs light microscopy and scanning electron microscopy (SEM) to provide a detailed visual analysis. This study contributes valuable insights into the taxonomy and phylogeny of avian lice, shedding light on the developmental changes that occur during their life cycle and aiding in their identification and classification.

Keywords: Chaetotaxy, Phthiraptera, Nymphal Instars, Systematics, Amblycera

1. Introduction

Phthiraptera (formerly called "Mallophaga") or the lice are tiny wingless, dorso - ventrally flattened, permanent obligatory ectoparasitic insects infesting warm blooded vertebrates i. e., the birds and the mammals. Their eyes are reduced; there are ocelli and the antennae remain 3 - to 5 - segmented. Mouth parts are modified biting or sucking type, maxillary palpi 4 - segmented or wanting and labial palpi rudimentary. Prothorax evident and free, Meso - and Meta - thorax often imperfectly separated. Tarsi 1or 2 - segmented, terminated by single or paired claws. Thoracic spiracles are ventral, Cerci absent, metamorphosis absent.

The adaptations of Phthiraptera to an ectoparasitic life include a prognathous head (i. e. the head being horizontal and the mouthparts directed forwards), reduction in the numbers of antennal segments, thoracic and abdominal spiracles, tarsal segments and nymphal stages (Kim, 1985). Biting and sucking lice belong to primitive hemimetabolous group of insects in which the newly hatched insect resembles the adults in most respects.

The life cycle of Phthiraptera is quite simple with an egg, three nymphs and the adult. The sexes are separate with pronounced sexual dimorphism in certain lice. In few cases (mammalian Ischnocera) the males are unknown and may yet to be discovered or the species might be parthenogenic. Generally the female predominate over the male in numbers. All the stages of development of the lice are found on the host, and individual lice soon die if they are lost from their host. Most of the lice are highly host specific i. e., usually a louse species can parasitize only a single host species or a small group of closely related host species (Kim and Ludwig 1978, Emerson and Price, 1985).

Adult female louse glues its eggs on the host hair or feathers. The eggs are called nits and have the incubation period of 4 - 10 days. They require warmth of the host body for the development. There are three nymphal instars in the life

cycle of Phthiraptera studied, so far. The stage which hatches from the eggs is the first instars nymph, the later moults into the second instars. The second instars moults into third instars and the final moult produces the sexually mature adult. The adult appear similar to nymph in most respect, except the size, chitinization, chaetotaxy and the external genital structures. The duration of life cycle is 3 - 4 weeks or one month. Constant conditions on the host's body allow continuous breeding throughout the year, so a louse population could build up very rapidly to high figure.

2. Survey of Literature

The morphological features of adult avian lice are generally used in systematic studies. However, the three nymphal instars of any louse species appear quite similar. They differ only in size. Their identification is a challenging task (nymphal instars of related taxa look alike and it is difficult to differentiate them. Previous workers like Martin (1934); Wilson (1939); Conci (1956 a, b); Arora & Chopra (1959); Agarwal (1967) provided nymphal morphology of selected phthirapterans. On the basis of external morphology of nymphal instars, Mey (1994) has tried to deduce the phylogeny of ischnoceran bird lice. Clay (1958) and Modrejewska & Zlotorzycza (1987) have also performed specific studies on the nymphal morphology of selected Phthiraptera. Agarwal G. P. et al. (2012) study chaetotaxy of three nymphal instars of an ischnoceran louse, *Aegypocercus perspicuous*.

Present study deals with the morphological features of three nymphal instars of *Menopon gallinae* on the basis of number of setae on different part of the body and the morphological features of head, thorax and abdomen.

3. Methodology

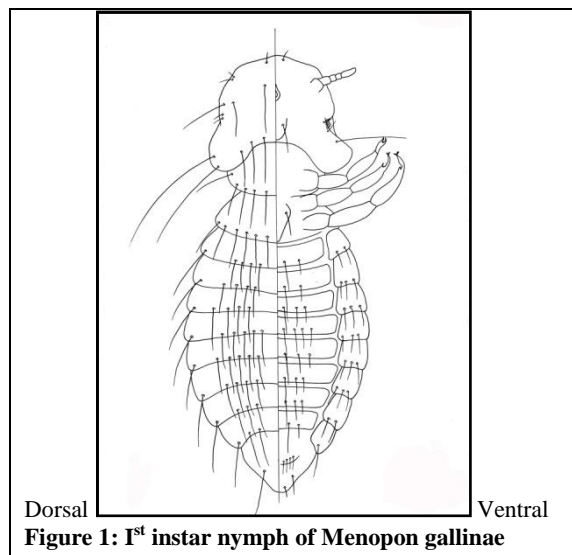
Light microscopy: For light microscopy the preserved/fresh specimens were subjected for preparation of permanent slide. The permanent slide preparation included: **Killing and**

narcotization: Killing and narcotization were done by chloroform or ether. **Fixing:** Fixing was done to antagonize further post - mortem changes. Fixative agents used were absolute alcohol and picric acid. **Washing:** Washing was done thrice in water to remove fixative. **Dehydration:** Lice were first hydrated (passed through 50%, 30% and then in distilled water) and then placed in 10% KOH (cold) for 24 hours, at room temperature to dissolve internal tissues of the body. Next day KOH treated lice were transferred to distilled water and their body contents were removed by puncturing the abdomen and pressing out contents with the help of needle. The punctured specimens were then transferred to acetic acid for half an hour. For dehydration process which is meant for removal of water from the specimen, all stages were passed through 30%, 50%, 70%, 90% and then to 100% for 10 - 30 minutes. **Staining:** Mostly unstained preparations were used for morphological studies on nymphal instars. Occasionally few specimens were stained with Picro - indigo Carmine. **Cleaning:** After dehydration specimens were placed in clove oil or Xylene to remove alcohol and to make the specimens clear. **Mounting:** After clearing, the specimens were mounted in Canada balsam or DPX. **SEM Study:** For SEM study, fresh lice were fixed in 2.5% gluteraldehyde, then passed in phosphate buffer, dehydrated and mounted on double sided tape, gold coated and viewed under SEM for study.

4. Results

Nymphal morphology of *Menopon gallinae* (Figure 1, 2, 3)

First instar nymph (figure 1): First instar nymph consist of head as long as broad, frons curved, eyes invisible, Antennae five segmented IIIrd flagellomere longer than other two,



One long seta present on each abdominal segment, sternal brushes absent, four setae present on vulval margin.

Second instar nymph (figure 2): Second instar nymph increases in length. Head does not show great variation in IInd instar nymph. Eyes feebly seen, lingual sclerite, hypophaynx are clearly visible. Cutting edges of mandibles are brown and sharp, with two short and one long temporal

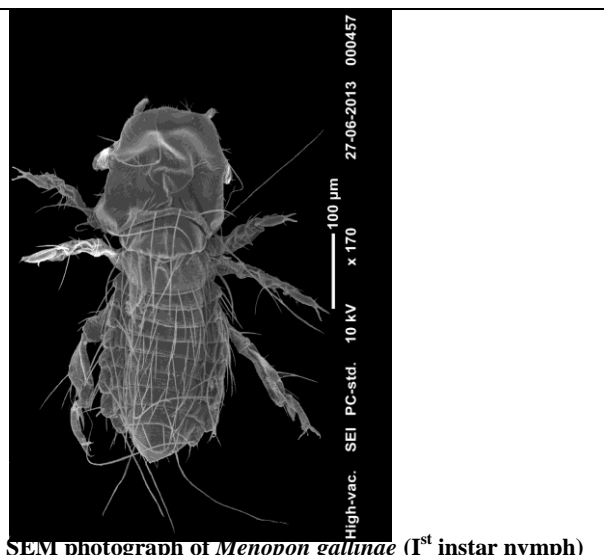
mandibles, lingual sclerite, hypopharynx feebly seen, temporal margin broadly elliptical, with Posterior occipital margin straight and convex on pro - thorax. Gular plate is feebly seen. On the dorsal side three short setae are present on frontal region, one long and two short seta on post antennal region, two long setae on postero - lateral margin on temple and long 21, 22, 23, setae present on posterior occipital margin. On the ventral side, there is one short seta present on upper frontal region, one long post antennal setae and there is one long temporal seta present. There is one long seta present on gular plate, eight fine short temporal brushes present in first instar nymph.

Pro - thorax is broadly concave, posterior margin straight. One long seta is present on postero - lateral corner and four long setae on posterior margin. Meta - thorax as broad as pro - thorax. In first instar nymph there is no clear indication of meso - thorax, lateral margin straight and posterior margin slightly pointed on abdomen, six long setae present on posterior margin with meta - thoracic plate typical menoponid type, there is one long seta present on it, legs well defined, first two legs are equal in length. Five fine femoral brushes are present on third leg. Abdomen is elliptical in shape with clear nine segments, posterior abdominal margin cup - shaped. Tergal, sternal and pleural plates are clearly seen. Abdominal chaetotaxy are as follow:

Tergal chaetotaxy - I - 4, II - 4, III - 4, IV - 5, V - 5 VI - 4, VII - 3, VIII - 2 on each side.

Sternal chaetotaxy - I - 2, II - 2, III - 3, IV - 4, V - 3, VI - 3, VII - 3, VIII - 2, IX - 2,

Pleural chaetotaxy - I - 2, II - 3, III - 3, IV - 2, V - 3, VI - 3, VII - 2, VIII - 1



seta appear on ventral side. Pro - thoracic shape and chaetotaxy are same as in first instar nymph. Meso - thorax is not seen. Meta - thorax is with six long marginal setae. Eight fine micro femoral brushes are present on femur of IIIrd leg. Abdomen is with clear nine segments. All segments are equally broad. Plates are clear. Ninth segment is pear shaped. Abdominal chaetotaxy are as follows:

Tergal chaetotaxy - I - 7, II - 7, III - 7, IV - 7, V - 6, VI - 6, VII - 5, VIII - 4 on each side.

Sternal chaetotaxy - I - 3, II - 3, III - 5, IV - 5, V - 4, VI - 3, VII - 3, VIII - 2 on each side.

Pleural chaetotaxy - I - 3, II - 3, III - 3, IV - 3, V - 3, VI - 3, VII - 3, VIII - 2.

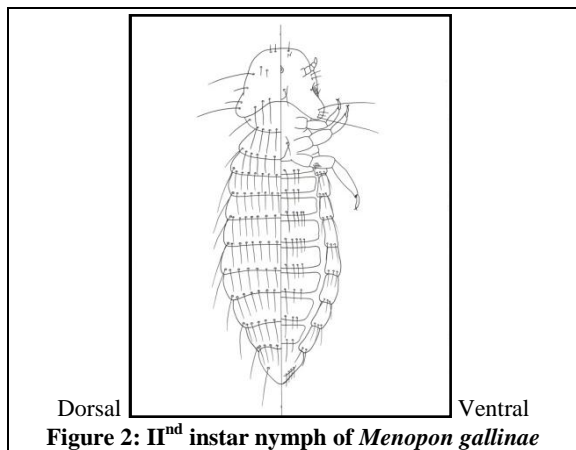
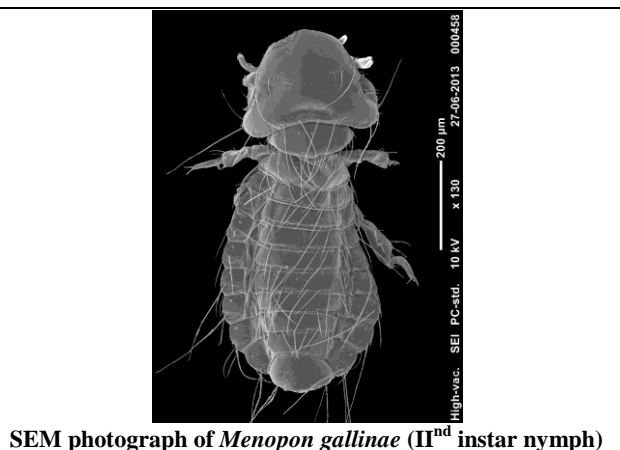


Figure 2: IInd instar nymph of *Menopon gallinae*



SEM photograph of *Menopon gallinae* (IInd instar nymph)

Third instar nymph (figure 3): Third instar nymph shows great variation in chaetotaxy. It increases in length, Eyes are clearly seen. Cutting edges of mandible are brown black in colour. Gular plate is clear with setae, Prothorax is broader than long. Prothoracic lateral margin is small and straight with three spine like setae. Posterior margin is concave with six long and one small seta. Metathoracic lateral margin is straight with four spine like setae and posterior margin bearing eight setae on each side. Metathoracic plate is with two small and one long seta. Femoral brushes are ten in number.

Abdomen increases in length with nine distinct segment posterior margin of VIth and VIIth segment slightly convex. Sternal brush appears in IIIrd instar nymph. Double fringe

vulval margin is having ten and eight setae respectively. Abdominal chaetotaxy are as follows:

Tergal chaetotaxy - I - 10, II - 11, III - 16, IV - 17, V - 16, VI - 14, VII - 10, VIII - 7 on each side.

Sternal chaetotaxy - Anterior setae: I - 4, II - 8, III - 9, IV - 16, V - 11, VI - 8, VII - 5, VIII - 4

marginal setae - I - 5, II - 6, III - 6, IV - 7, V - 6, VI - 5, VII - 6, VIII - 6 (on each side).

Sternal brushes - Anterior setae: II - 4, III - 10, IV - 16, V - 11, VI - 8, VII - 3

Margin setae - II - 4, III - 8, IV - 8, V - 6, VI - 3, VII - 3

Pleural chaetotaxy - Anterior setae: I - 3, II - 2, III - 3, IV - 4, V - 8, VI - 6, VII - 4, VIII - 2

Margin setae: I - 3, II - 4, III - 4, IV - 4, V - 5, VI - 4, VII - 3, VIII - 2.

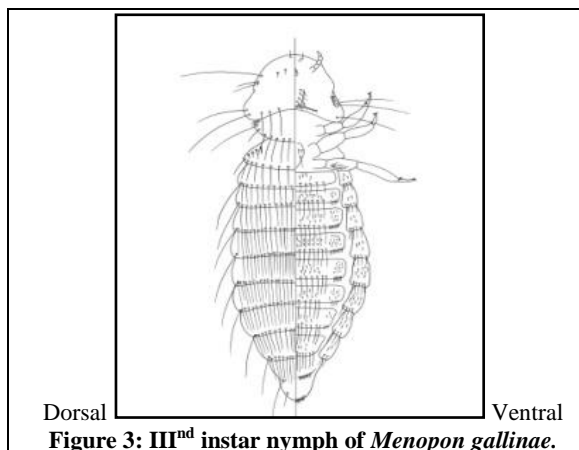
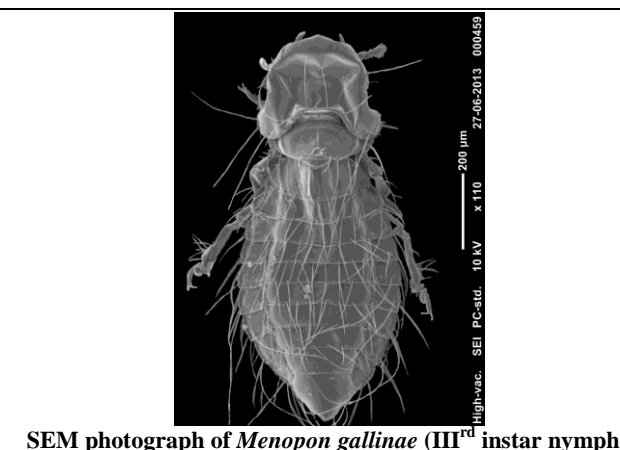
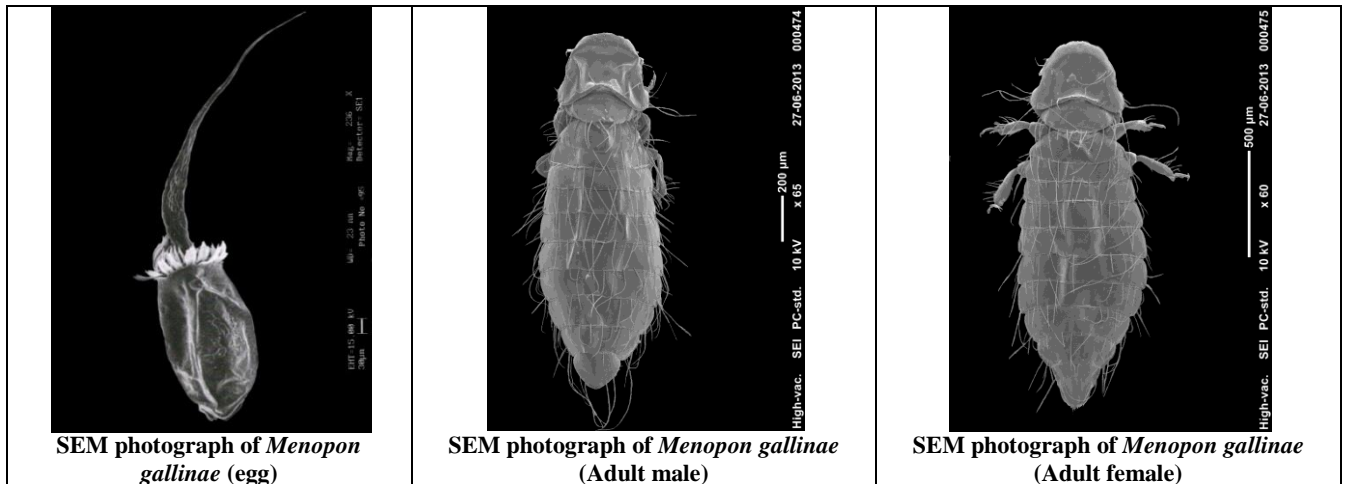


Figure 3: IIIrd instar nymph of *Menopon gallinae*.



SEM photograph of *Menopon gallinae* (IIIrd instar nymph)



5. Discussion

A scrutiny of literature indicates that the nymphal instars of phthirapteran species differ in length of body (dimension of body parts) (Clay, 1958; Conci, 1952, 56a, b; Mey, 1994; Modrejewska and Zlotrzycka, 1987). Sometimes, the nymphs of certain specialized groups can be characterized by the modification of setae (gradual modification in adults). For instance, Eichler (1963) has noted that in *Eulaemoborthion cubense*, in successive instars, number of setae are transformed into thorns on osculum. In certain species (eg, *Columbicola columbae*) clypeal setae develop in to third instar nymph. The gradual appearance of new setae and darkening of sclerite in nymphs have been observed by aforesaid workers. Workers like Clay (1958) and Eichler (1963) have recommended the identification of the nymphal instars through the progression of chaetotaxy. Smith (2001) has performed studies on ontogenic transformation in shape of head of three nymphal instars of four avian lice and emphasized the use of nymphal morphology to establish phylogeny and taxonomy of avian lice.

Further scrutiny of literature alludes that some common transformations which take place during the development of lice are gradual increase in body size, colour of fresh moulted nymph with always pale colour, no regressive changes in color from dark to pale, clypeal carina generally changing from closed to a medio - frontal open type, the ventral carina occurring on either as a complete above the labrum or as two separate strips with at least one post spiracular seta on pronotum.

In case of *M. gallinae*, the three nymphal instars differ not only in size, sclerotization of mouth parts, chitinization of abdominal segments but also in number of setae in different parts of the body. The first instars can be differentiated from second instars due to increase in the number of tergal setae on Ist abdominal segment to VIIIth, pleural setae on Ist, IVth and VIth and sternal setae on Ist to Vth and IXth. Likewise the second instars can be differentiated from third instars by the appearance of sternal brushes on different abdominal segments. Furthermore, there is increase in number of tergal setae on Ist to VIIth, sternal setae on IVth to VIth and pleural setae on IVth to VIIIth. The gular plate bears four minute setae in third instars in comparison to one seta in second

instars. There is number of increase in the setae on pro - and meso - thorax.

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	Orientation	01
	Refresher	03
	Training	01
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