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Micromorphological studies of adult stages of *Haematopinus asini* (Equine sucking lice; Phthiraptera: Haematopinidae) with its eggs from donkeys (*Equus asinus*)

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Abstract
The present study was aimed to determine the ultra-morphological characters of sucking lice, *Haematopinus asini* of equine. During routine examination study of donkeys in Faculty of Veterinary Medicine, Cairo University; in September, 2017, the hairs of infested animals which had visible lice and its nits were combed with fine toothed comb. Lice and its nits (eggs) were subjected to identification process which was scanning electron microscope study. The equine lice eggs were oval in shape, and pearly white in color, measurement of eggs length and width were recorded. The adults male and female were brown in color, with whole length of female was 500 µm ± 0.2, while in male was 410µm ± 0.1. The head was conical in shape and composed of haustellum and labrum which was folded in several membranous. Several sensory structures were described. The thorax length was recorded. The three pairs of legs with its claws were similar in length. Each tibia composed from one strong claw and one thumb like spines with distotibial pad (pullvilli) and empodium which resembles the honeybees comb (pullvilli and empodium). The pullvilli had many sensory sensilla on its surface. Two mesothoracic spiracles present at two lateral sides in mesothorax and six similar spiracular openings in the abdomen which were described with its scales. The abdomen of the male and female were similar except for the genitalia. The nymphs resembles the adult typically but with small size. The whole length of different instars were ranged from 150 - 350 µm ± 0.7, but the genitalia was smaller than adult. All body parts were described in detailed using scanning electron microscope (SEM).

Keywords: *Haematopinus asini*, Scanning electron microscope study, equine sucking lice, ultrastructure

1. Introduction
Haematopinus (Leach, 1815) known as mammalian parasitic lice is represented by 22 species in the genus Haematopinus [1, 2]. These lice may be vectors of a anaplasmosis, African swine fever, hog cholera, Swine-pox [2, 3].
The life cycle of these hematophagous lice is around 1 month and with an incomplete metamorphosis (Kettle) [1]. Every female lays in her lifetime about 50-100 eggs (nits). The female lays about 1-2 eggs per day. It stores them one by one to single hair. The incubation period of the eggs from 4 to 20 days (Kettle) [1]. Nymphs look like adults but are smaller; they take a course of 7 to 21 days to become mature. The life cycle of *Haematopinus asini* takes 3 nymphal instars. Adults lifespan are recorded from 3 to 6 weeks. Lice complete its life cycle on one host; transmission starting with one host then onto the next one is by direct contact, through contaminated equipment, grooming materials (Soulsby) [4].
Lice are one of the most common, highly specific and economically important ectoparasites of domestic animals including horses and donkeys (Soulsby) [4]. The animals which infested with lice suffered from losses in productivity due to unthriftiness, several dermatological lesions, anemia due to sucking of blood, alopecia due to scratching, biting and rubbing with the destruction of the coat and skin infections [5-7].
Studies from other countries showed that family equidae including horses and donkeys are infested by one species of chewing lice, *Bovicola (Werneckiella) equi*, and one species of sucking lice, *Haematopinus asini* [7-9].
The previous record on epidemiology on Iran, song *et al.* [2] and Tafese *et al.* [7] recorded the mitochondrial minichromosome composition of *H. asini*. There is no information about ultrastructures of *H. asini*, sucking lice on horses and donkeys.

Therefore, the present study was designed to determine the different ultra-morphological characters of different stages of lice including the (eggs (nits); adult male and female) using light and scanning electron microscopic studies (SEM).

2. Materials and methods

2.1 Lice collection

During postmortem examination of donkeys in Giza zoo abattoir, the examination was done in September 2017. The recorded temperature and humidity in this month were 45 ^{0}C and 60-70% RH. Carefully attention was paid on the hairs of donkeys; with predilection sites of the lice, which was the mane, head, flank, the base of the tail, and above the hooves [7, 8]. The hairs which had lice and its nits were combed with fine-toothed comb. Careful handling of collected lice by using fine entomological forceps, then it placed in two labeled vials; one containing 70% ethanol for subsequent permanent identification and other vials contains 2.5% glutaraldehyde for scanning electron microscopic study (SEM).

2.2 Identification of collected lice

The identification was carried out following the key and morphological characters recorded by Taylor *et al.* [10]; Soulsby; [4]; Kettle, [1].The morphological parameters from 20 specimens for nymphal instar and adults male and female with eggs were measured under the stereoscopic and light microscope (Labomed X40 and X100).

2.3 Preparation of permanent specimens

For detailed morphological studies, all stages (eggs, nymphs, adults male and female) were washed several times with saline and then, placed to insufficient amount of 5% caustic soda (NaOH) and left at room temperature (37 ^{0}C) for 2 h. The lice were washed with water several times. The lice were dehydrated through passing in ascending serial ethanol degrees 70, 80, 90 and 100% for one hour each. The specimens were cleared in clove oil then put in Xylene for removal of oil for few minutes. The lice stages were mounted in Canada balsam and incubated at 40 °C to dry for 24 hours [11].

2.4 Preparation for ultra-morphological studies using scanning electron microscope (SEM)

Ten specimens from *H. asini* male and female as well as eggs and three nymphal instars were studied using scanning electron microscroscope (SEM) to determine the micromorphological characters of each stage. The samples were washed several times using buffered saline (PH 7.2). The lice prepared by serial washing in saline solution and fixed in 2.5% glutaraldehyde at 4 ^{0}C for 24 h as described by Hilali *et al.* [11]. Lice were dehydrated through the passage in ascending ethanol series (30%, 50%, 70%, 90% and absolute) each concentration for 10 m. Lice were air dried on Whitman no.1 filter paper. Complete dryness occurred in CO2 critical point drier (Autosamdri-815, Germany). Lice specimens were glued on stubs, coated with 20nm gold in (Spi-Module sputter Coater, UK, sputter coater). Specimens photographed with SEM (JSM 5200, Electron prob, Microanalyzer, Jeol, Japan) at Faculty of Agriculture, Cairo University, Egypt.

3. Results

3.1 *Haematopinus asini* eggs or nits

The equine lice eggs were oval in shape, and pearly white in color (Fig.1), measured from 920 µm- 1025 µm (1000µm ± 0.2) in length and 440 µm - 450µm (445± 0.2) in width. The eggs covered with operculum at the anterior end and measured 250 µm - 256 µm (254µm ± 0.1) in width. The eggs usually attached and glued to the base of the hair shaft by cemented materials. The eggs ended by a stalk or pedunculated at the attachment site in hair. The operculum with many aeropyles pores (Fig.2).

3.2 *Haematopinus asini* adults

The adults male and female were brown in color, dorsoventrally compressed, the whole length of female was 490 - 510 µm (500 µm ± 0.2), while in male was 400 - 420 (410µm ± 0.1),SEM investigation showed several micro-morphological.

3.2.1 Head

The head was conical in shape and composed of haustellum anteriorly. There were two pairs of thick setae laterally to haustellum and six short transverse setae dorsal to haustellum. The labrum was folded in several membranous layers ventrally. There were no eyes (Ommatidia) although the ocular processes were prominent. The antenna was composed of five segments, the apex of the distal tip of both male and female antenna were composed of 14 sensory basoconica sensilla and one long seta at the apex (Fig.3). The head was 120-125 µm (124 µm ± 0.2), in length, while the whole length of the antenna was 30 - 34 µm (33 µm ± 0.2).

3.2.3 Thorax

The pro, meso, and metathorax were completely fused and the dorsal surface of thorax had two notal pits on the anterior part and a central notal pit on the middle part, two lateral processes were present at the posterior end (Fig.4). The length of the thorax 105 - 120 µm (115 µm± 0.1) and 55- 60 µm (57 µm ± 0.2) in width.

3.2.4 Legs

There were three pairs of legs with strong claws. The three pairs of legs with its claws were similar in length. The legs composed of coxa, trochanter, femur, tibia, and tarsus. The tibia and tarsus were highly modified to form powerful grasping organ for firmly attachment to the host hairs. Each tibia composed from one strong claw and one thumb like spines with distotibial pad (pullvilli) and empodium which resembles the honeybees comb (pullvilli and empodium). The pullvilli had many sensory sensilla on its surface (Fig.5).

3.2.5 Spiracles

Two mesothoracic spiracles present at two lateral sides in mesothorax. The spiracles were well developed (Fig.4,6). The spiracular lumen was lined with several pentagonal shaped scales. There are six similar spiracular openings in the abdomen from segments 3 to 8.

3.2.6 Abdomen

The abdomen of the male and female were similar except for the genitalia. The abdomen was elongated, with whole length in female 300 - 310 (305 µm ± 0.2), while its width 230-240 (235µm ± 0.5), in male, 280 - 290 (285 µm ± 0.2), while its width 200-210 µm (205 µm ± 0.3). There were a hump like or triangular paratergal plate ventrally (Fig.6).

The genitalia of the male (Fig.7), had two triangular genital plate which carry two groups of long setae. The male posterior ends had an elongated pseudopenis (aedeagus). While in female (Fig.8), the genitalia composed of two broad lobes which was gonopods and genital plate. The genital plate

and two gonopods were supported by numbers of setae. The female had two openings; anal opening and sexual orifice which ended by genital lobes.

3.3 Nymphal instar

The nymphs resembles the adult typically but with small size. The whole length of different instars were ranged from 150 - 350 µm ± 0.7, but the genitalia was smaller than adult.

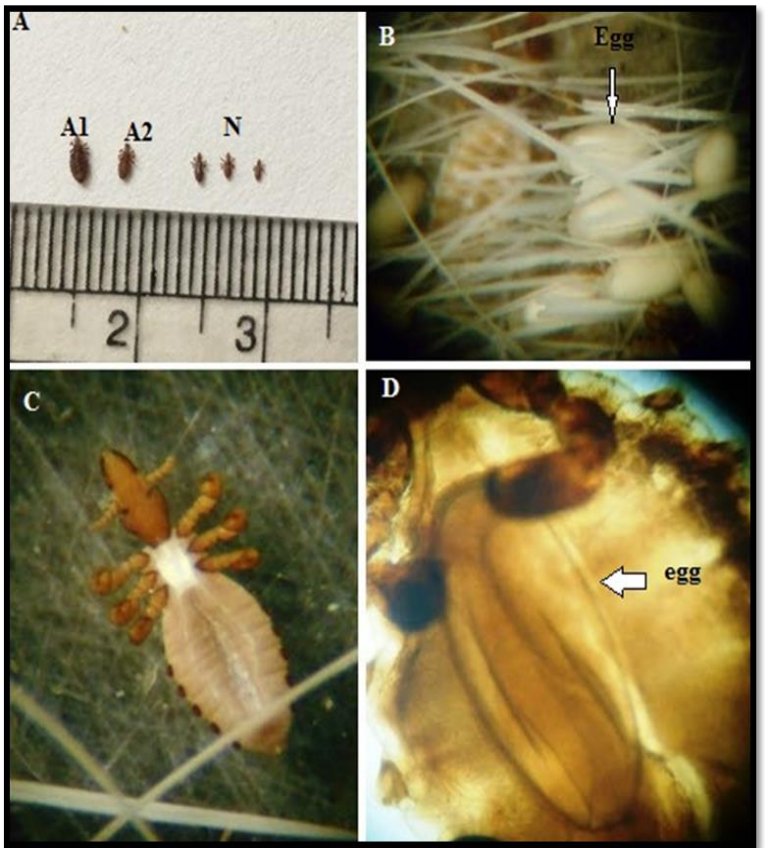

Fig 1: A: different stages from *H. asini*, N: nymphs with different length, A1: adult female, A2: adult male, note; its brownish in color. B: light microscopic study showing the whitish pearly color eggs or nits. C: adults under light microscope. D: light microscopy showing adult female with the eggs inside its uterus.

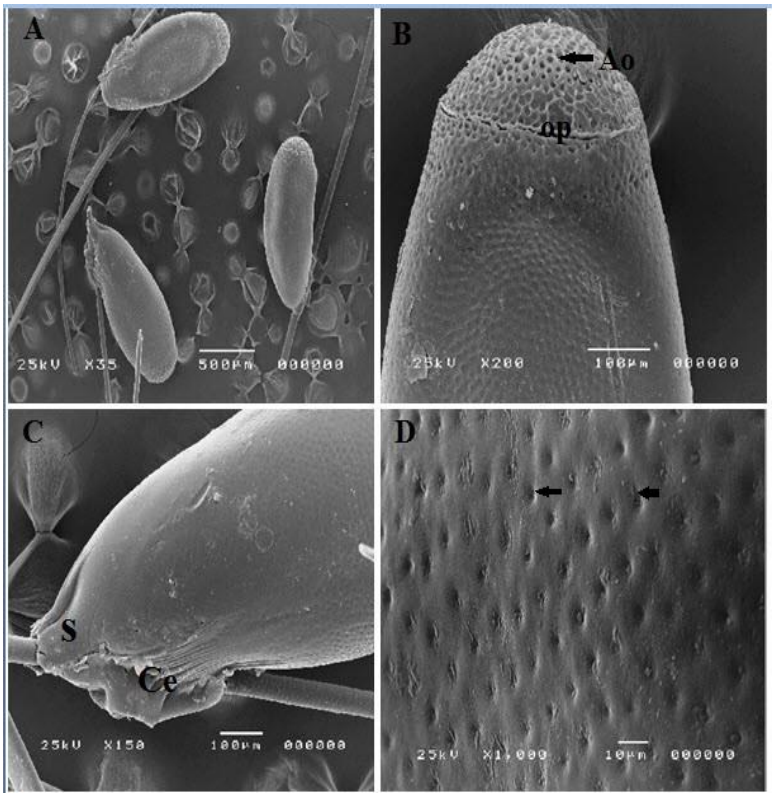

Fig 2: Scanning electron micrograph of *Haematopinus asini* eggs. A: the eggs attached to the hairs firmly. B: showing the operculum (op) and aeropyles pores (Ao) which appears in numerous numbers in operculum. C: showing the distal ends of the eggs with pedunculated ends or stalk (S), the eggs attached firmly to the hairs by cemented materials (Ce). D: surface ultra-structure of the eggs with tiny pores.

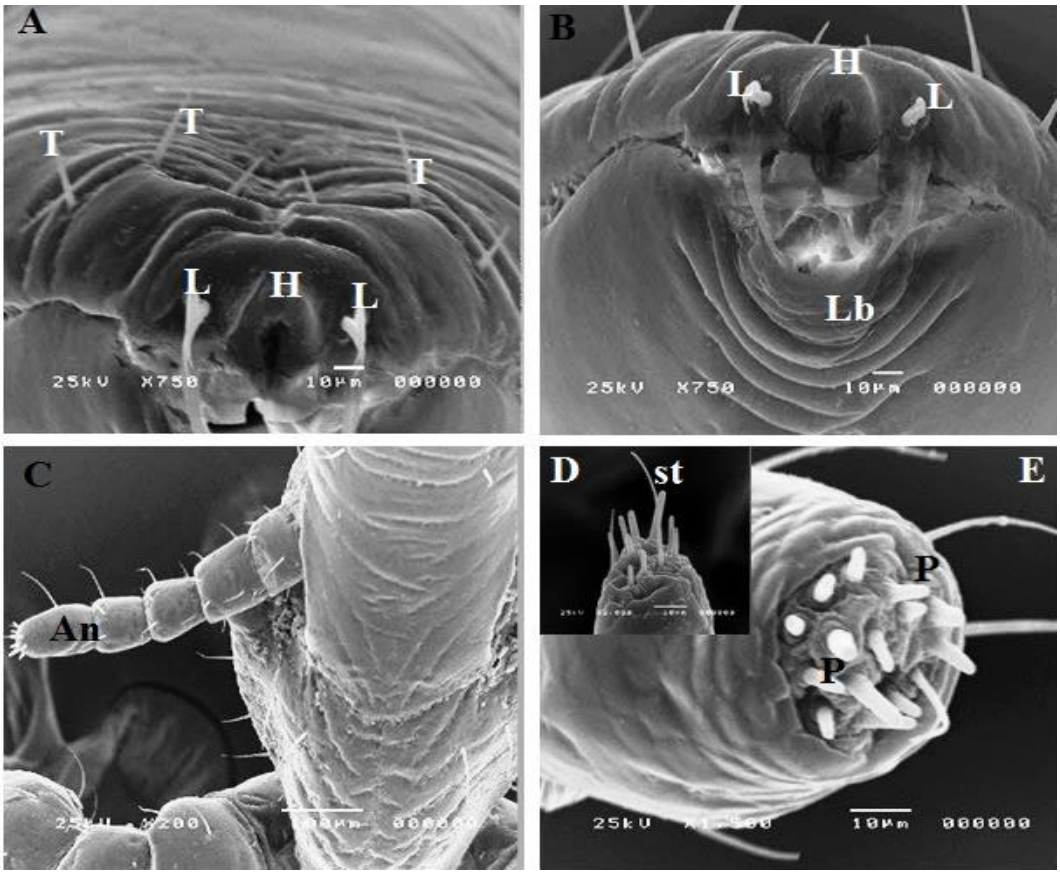

Fig 3: Scanning electron micrograph of *H. asini* head. A: the haustellum (H), with two pairs of laterally (L) situated setae (two in numbers in each side), and six short transverse setae (T). B: showing haustellum (H), with two pairs of laterally (L) situated setae and labrum (Lb) which had several membranous layers. C: the heads having five segmented antenna (An). D and E: last segment of the antenna showing 14 sensory basoconica sensilla or pegs (p) and one long seta (st) at the apex.

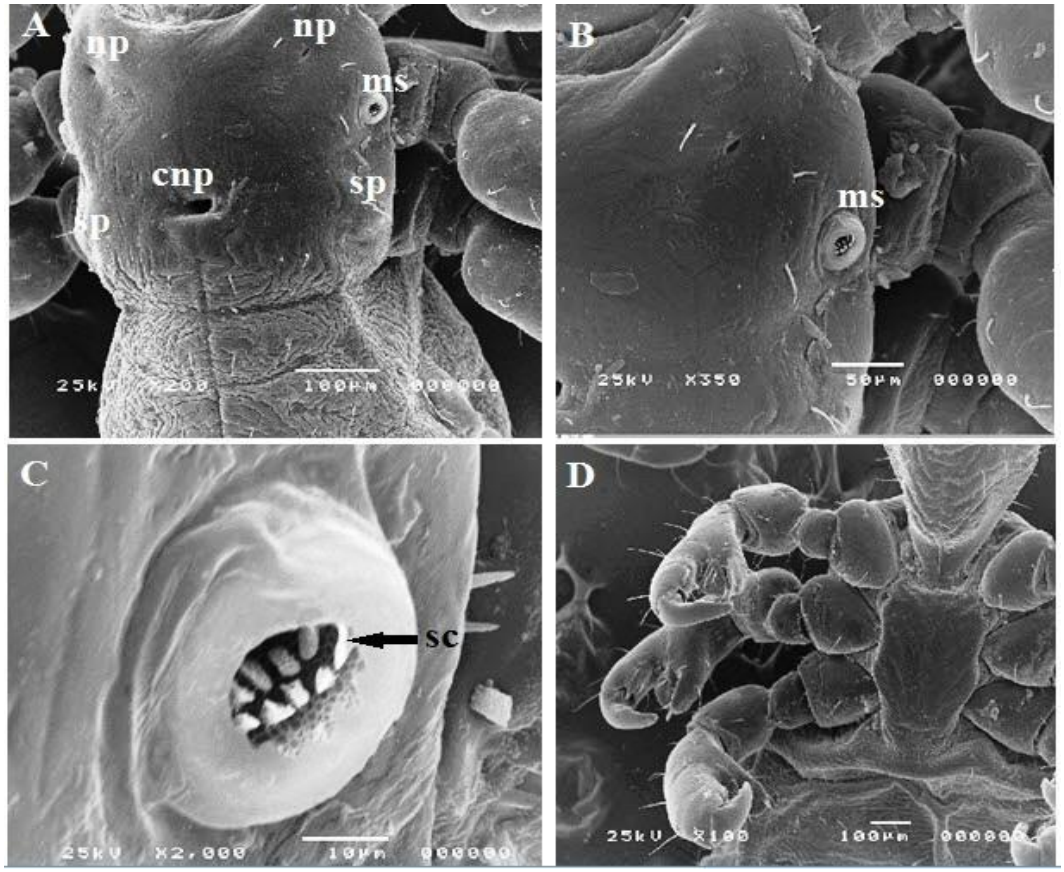

Fig 4: Scanning electron micrograph of *H. asini* thorax. A: dorsal view of the fused thorax showing two notal pits (np) and one central notal pits (cnp) and two sensory process (sp), with one pair of mesothoracic spiracles (ms) which present in higher magnification in Fig. B and C; in Fig. C: showing higher magnification of mesothoracic spiracle showing scales inside it. D: ventral view of the thorax with the three pairs of the legs and its powerful claws and thumb like process.

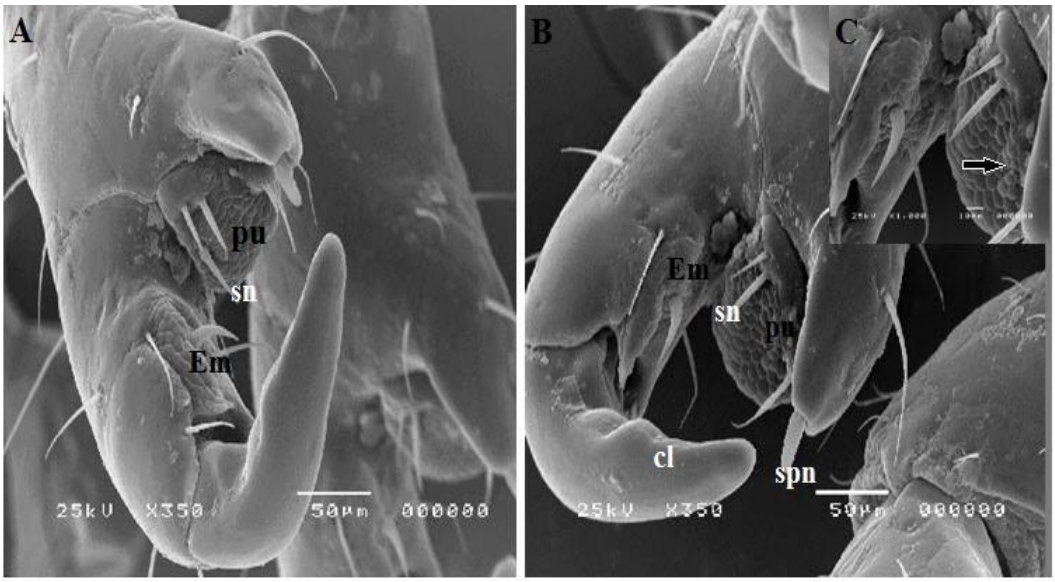

Fig 5: Scanning electron micrograph of *H. asini* legs. A, B: showing the claws (cl) with distal tibial thumb or spine (spn) with pullvilli (pu) and empodium (Em) with sensory sensilla (sn). C: higher magnification of the pullvilli and empodium showing its honeycomb structure.

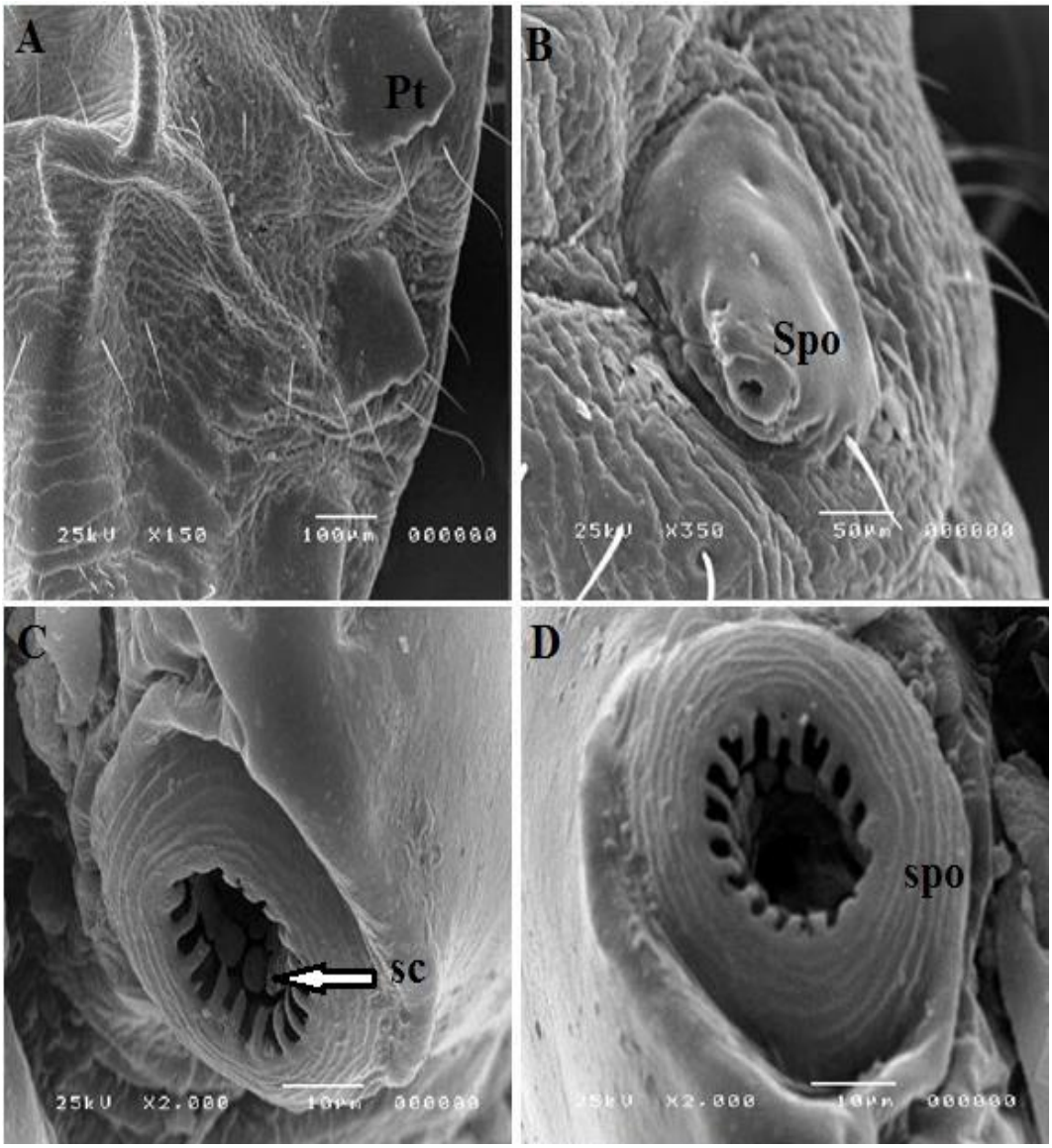

Fig 6: Scanning electron micrograph showing abdomen of *H. asini.* A: ventral surface showing triangular hump of paratergal plates (pt). B, C, D: showing spiracular opening (spo) with different magnification with its scales (sc) inside it.

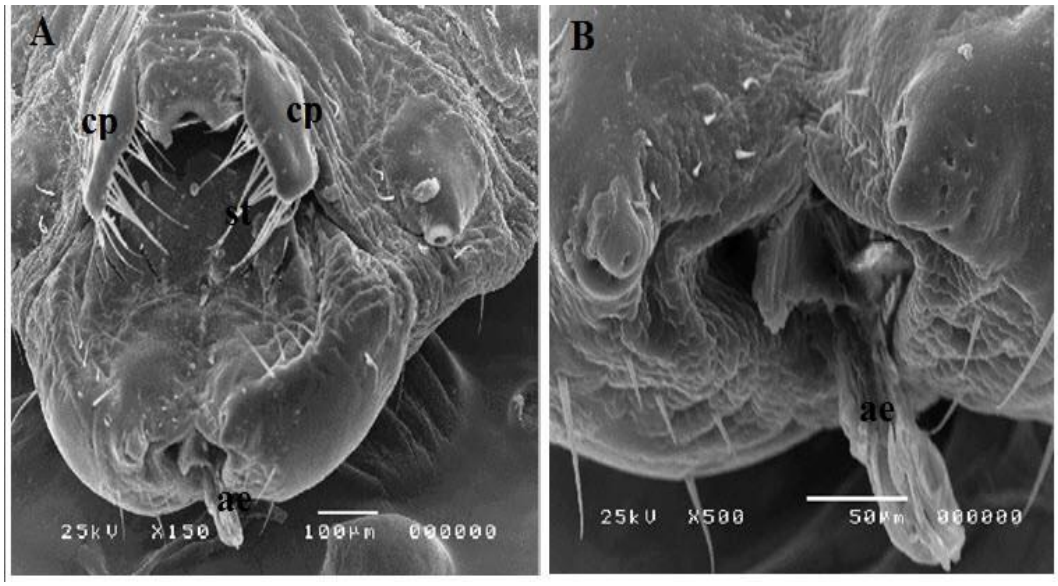

Fig 7: Scanning electron micrograph of male *H. asini*. A, B: showing two claspers (cp) with numbers of long seta (st) and aegeagus (ae).

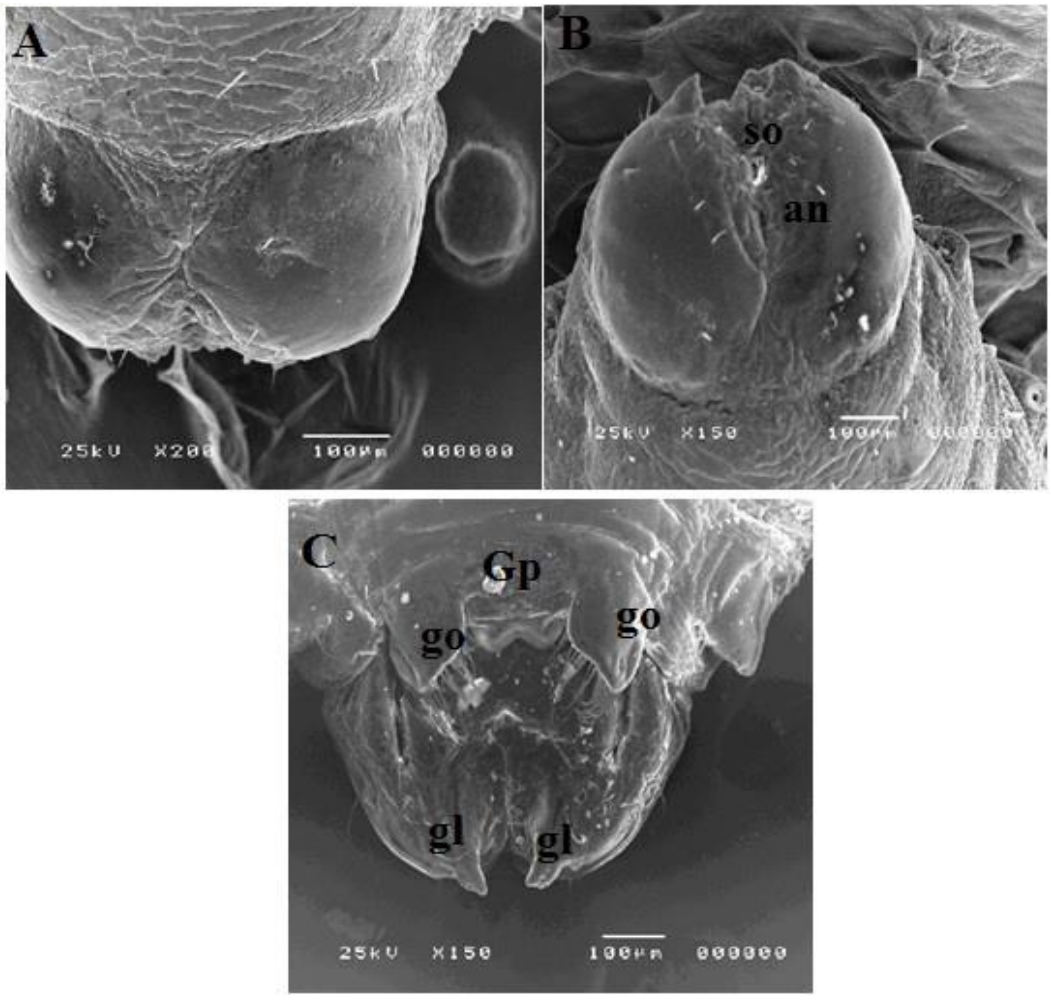

Fig 8: Scanning electron micrograph of *H. asini* female genitalia. A,B: showing the posterior ends of the female dorsal surface which composed from two lobes and anal opening (An); sexual orifice (so). C: ventral surface of female posterior end showing genitalia; Genital plate (Gp), and two gonopods (go) which supported by setae, and two genital lobes (gl).

4. Discussion

Few studies described the morphology of lice [12, 1, 5, 13] but no previous record on the ultra-morphological characters of *H. asini* in details. In our study, some morphological description were similar to *H. bufali* but with different in some characters as; the number of basoconica sensilla which in our morphology 14 in number but in *H. bufali* 12 in number. The shape of empodium differed in the two study. Turner *et al.* [13]; recorded the claspers in female but it must be recorded in male as in this morphological description, which appears with the aedeagus.

Solar Cruz and Martin Mateo, [14] were described the legs of family phthiraptera, from those description, the *H. apri* which differed completely than *H. asini* as the shape of empodium, in *H. apri* striated while in this study was resembled as honeycomb in appearance.

Adhikary and Ghosh, [12]; illustrated different types of sucking lice except *Haematopinus asini*, but it represented a drawing to sucking lice, which taken as a guide for genitalia in male and female in this study. Kettle, [1]; recorded that the genus haematopinus had 4 mm in length with prominent ocular points, all legs with the same length, paratergal plates from 2 or 3-8 abdominal segments, their findings were similar to this research but in this research it illustrated with SEM in all parts of body in male and female. In Kettle, [1], it described the *H. asini* by drawing with Camera Lucida.

5. Conclusion

This study was performed to describe in details the morphological characters of *H. asini*, here, in this research, it could be distinguish this equine sucking louse from other Anoplura lice as well as it can help for epidemiological reasons. In conclusion, it is advisable of using the insecticides to controlling this insects regularly.

6. Acknowledgment

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