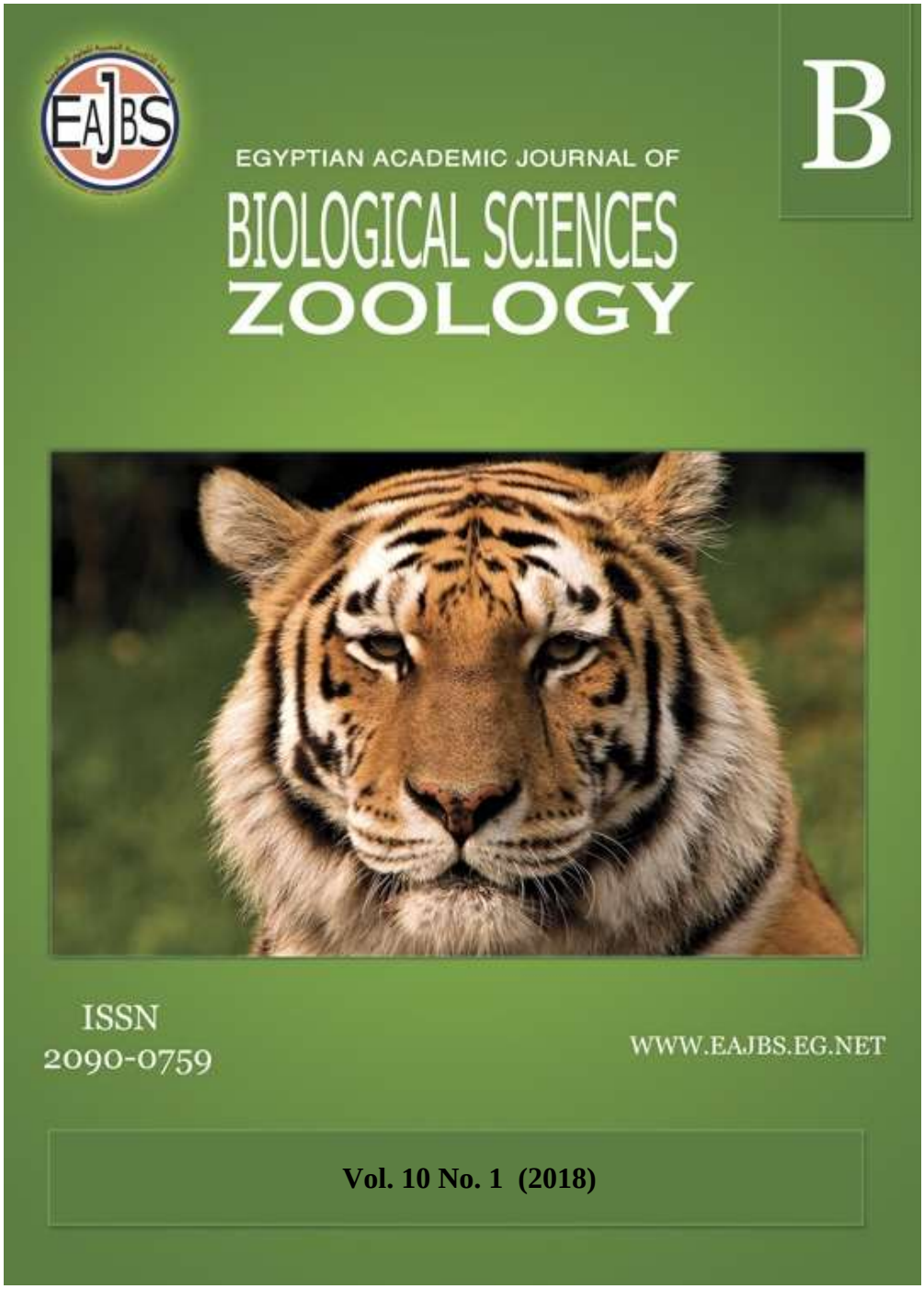

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Morphological Characterization of Arthropod Ectoparasites by Scanning Electron Microscopy

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ABSTRACT

Arthropods constitute a diverse and largest animal phylum of invertebrates. They consist of over 80% of all known animal species and occupying almost every known habitat including marine, freshwater, land and air. Arthropods are important to the ecosystem and to humans. In this context, arthropods ectoparasites cause serious disease to humans and animals and subsequently leading to annual total economic loss. Therefore, an identification of the arthropod ectoparasites is an important tool for the diagnosis and controlling human and animal infections. Hence an identification of the arthropod ectoparasites by electron microscopy needs collecting arthropod ectoparasites, and handling as well as using previous collected data on mite, lice, bed bugs, and fleas. In the present study, the following arthropod ectoparasites: *Demodex canis* ,*Pediculus humanus*, *Haematopinus asini*, *Linognathus africanus*, *Cimex lectularius*, *Ctenocephalides canis* were collectedand 20%(6/30), 3.61%(3/83), 10%(2/20), 46.67%(7/15), 20%(2/10), and 23.33%(7/30) of these arthropod ectoparasite species, respectively were examined.

INTRODUCTION

Arthropods, a diverse assemblage of invertebrates, consisting of nearly 80% of all known animal species and occupying almost every known habitat (Yadav *et al.*, 2017). Arthropods institute the largest phylum in the animal kingdom, and yet a relatively small number of species are related to public health by direct or indirect infection (Fritsche, 2003). Stork (2018) revealed that there are about seven million species of terrestrial arthropods and suggested that 80% stay to be discovered. Arthropods play important role in their relationship with humans and animals and ectoparasites infestation causes a serious loss in health and economy every year (Yadav*et al*., 2017). Importance of medical and public health arthropology seems to be rising (Goddard, 2012), with worldwide certain arthropods (e.g., bed bugs) (Doggett *et al*., 2012.) and others (e.g., mosquitoes and ticks) (Rochlin *et al.*, 2013 and Dekoninck*et al.*,2013).Their public health importance, most notably, they can be biological vectors of disease-causing organisms (Mathison and Pritt, 2014). Arthropods (Insects and mites) are common inhabitants and accidental invaders of

food, and their presence can have both direct and indirect effects on human health, which may be allergenic or even carcinogenic for human (Hubert *et al.,* 2018). Arthropod ectoparasites especially insect infestations are among society health (Rafinejad *et al*., 2006).

Demodex is the parasite classified in the class Arachnida, superorder Acariformes. Several species of *Demodex* have been described by (Gao *et al*, 2005). *Demodex* mites are elongated ectoparasites found on human body hairs surfaces, such as the face, cheeks, forehead, nose, or eyelids and *Demodex canis* infect dogs (Kim *et al*, 2011). Holzchuh *et al.* (2011) explained the meaning of *Demodex* as of Greek origin, demos means wax or fat, and dex means insect. *Demodex* mites, can cause a wide range of dermatological lesions such mild skin irritation, although usually nonpathogenic (Yabsley *et al*., 2013).

Lice are divided into sucking and biting species (Anoplura: Linognathidae and Ischnocera: Trichodectidae), but both biting and sucking lice are ectoparasites (Pandita & Ram, 1990). Bloodsucking lice are tiny insects of the order Diptera that are responsible for biting and sucking the blood of mammals and birds and can survive far from their host body just for a short period of time (Rozendaal, 1997).

Saghafipour *et al* (2017a) determined the prevalence and risk factors associated with head louse (*Pediculus humanus capitis*).

The only genus *Haematopinus* Leach, 1815 of family Haematopinidae of the suborder Anoplura, known as the blood-sucking lice (Durden& Musser ,1994a and Barker, 1994.). Song *et al*. (2014) confirmed that 21 species of the genus *Haematopinus* (blood-sucking lice) parasitizing both even-toed ungulates (pigs, cattle, buffalo, antelopes, camels and deer). Huge economic losses in the yields of meat, milk and wool are caused by lice infestations in goats every year. Lice are known to cause weakening in animals by diminishing body conditions of animals (Uğur *et al.*, 2017). Rashmi & Saxena (2017) investigated the population levels of phthirapteran ectoparasites on the goats and recovered from the goats in district Rampur (Ischnoceran louse, *Bovicola caprae* and the Anopluran, *Linognathus africanus*).

Agnew and Romero (2017) studied the behavioral responses of the common bed bug, *Cimex lectularius*, to insecticide dust and referred to bed bugs. While Campbell *et al* (2017) studied the locomotion inhibition of *Cimex lectularius.*

Fleas are ectoparasitic bloodsucking insects of mammals and birds. They are well known as vectors of pathogens (Gracia *et al*., 2000). Over 2,500 species, and subspecies of fleas (Order: Siphonaptera) exist worldwide, and *Ctenocephalides canis* can act as intermediate hosts for parasitic worms (Durden & Hinkle, 2009). *Ctenocephalides* are the most common ectoparasites infesting dogs and cats worldwide (Andrea *et al*., 2015).

The present work aim to throw light on arthropod ectoparasites and to deal with the following items:

- The morphological features of arthropod ectoparasites using scanning electron microscopy.
- Incidence of these parasitic infections.

MATERIALS AND METHODS

In the present study a total of arthropod ectoparasites collected from village El-Hella, Esna, Luxor, that include: *Demodex canis* and *Ctenocephalides canis* from 30 dogs, *Pediculus humanus* from 83 school children, *Cimex lectularius* from 10 human beds, *Haematopinus asini* from 20 horses, and *Linognathus africanus* from 15 goats. They should be promptly placed into a preservation solution for transportation to the laboratory in order to avoid desiccation or decomposition which will alter morphological features. The best preservative which can be found routinely in most clinical and reference laboratories is 70 to 90% ethanol (Borror *et al*., 1989, Fritsche, 2003, and Garcia, 2007). Formalin is also acceptable, but its use has decreased in recent years due to concerns of its toxicity (Mathison and Pritt, 2014).

Scanning Electron Microscopy had been done in the Electron Microscopy unit, Assiut University. The whole samples were placed on cover slides and fixed in 5% gluteraldehyde in sodium cacodylate buffer for 1.5 hours, rinsed in distilled water and dehydrated in ethanol. Critical point drying was carried out. Samples were mounted on stubs, coated with carbon or gold then examined by Joel JSM 35 Scanning Electron Microscope at 20 kv.

RESULTS

The arthropod ectoparasites *Demodex canis, Pediculus humanus, Haematopinus asini, Linognathus africanus, Cimex lectularius,* and *Ctenocephalides canis* were collected, respectively, on 20% (6/30), 3.61 % (3/83), 10% (2/20), 46.67% (7/15), 20% (2/10) and 23.33% (7/30) of the animals examined (Table 1 and Fig.1).

1. ***Demodex canis*** (Fig. 2, A:C), is mite 120-140µm in length and 200-500µm in width, males somewhat shorter and rounder than females and with abdomen more triangular, invisible to the naked eye, but under the electron microscopy the body is elongated and consists of two fused segments, four pairs of short legs and one pair of pedipalps. The abdomen of both male and female mites was smooth, shiny and had fine cross-striations and the mite has pin-like mouthparts for eating skin cells.
2. ***Pediculus humanus*** (Fig. 2, D:I). The human head louse adult is small 250–320 µm in length and 450-500µm in width, dorsoventrally flattened, wingless, the thoracic segments are fused, but distinct from the head and abdomen, lice are grey in general, but their precise colour varies according to the environment in which they were lived, lice head have one pair of antennae, each with five segments, one pair of eyes are present in all species within the family: Pediculidae and The nymphs and adults all have piercing-sucking mouthparts which pierce the skin and sucking blood. Lice do not have powerful jumping three pairs legs so they move about by clinging to hairs with their claw-like legs. The abdomen is consisted of seven visible segments. The first six segments each has a pair of spiracles, while the last segment contains the anus and the genitalia.
3. ***Haematopinus asini*** (Fig. 3, A:G) The adults with a dorsoventrally flattened body, 3 pairs of dark yellow legs. Dark brown lateral plates, 300-350 µm long. A long narrow head and the mouth are adapted with piercing parts for the sucking of blood. The thoracic sternal part is dark and very well developed in this species. The spiracle shown allows the organism to exchange gas with its environment.
4. ***Linognathus africanus*** (Fig. 3, H :P), the shape of the head with the prominent bulging ocular points posterior to the antennae. The female terminal gonopod of *L. africanus* is rounded and lacks the "tooth-like". The gonopods of the male include a pair of terminal tubercles with setae. The organism is able to achieve

gas exchange by breathing through the spiracle. There is only one thoracic spiracle on either side of the abdomen (lateral symmetry).

5. ***Cimex lectularius*** (Fig. 4, A:D) The bed bug in the adult stage its colour is light-brown to reddish-brown, flat, oval, and wingless. The front wings are vestigial and reduced to pad-like structures. Adults grow to 4.3–5.2 mm long and 1.7–3.4 mm wide. Bed bugs have segmented abdomens with microscopic hairs that give them a banded appearance.

6. ***Ctenocephalides canis*** (Fig. 4, E:I) The adult is 1.6– 4.3 mm in length and dorsoventrally flattened. The abdomen was convex in females whereas, the dorsal surface was more or less flat. It is dark brown in colour, with piercing-sucking mouthparts that support in feeding on the host's blood and the adult *C. canis* has a rounded head.

Table 1: Rate of natural infection with arthropod ectoparasites.

Parameters	*D. canis*	*P. humanus*	*H. asini*	*L. africanus*	*C. lectularius*	*C. canis*
Locality	**Esna**	**Esna**	**Esna**	**Esna**	**Esna**	**Esna**
Host	**Dog**	**Human**	**Horse**	**Goat**	**Human**	**dog**
Microhabitat	**Hair follicle**	**Head**	**Hairs**	**Hairs**	**Bed**	**Hairs**
Total Numbers Examined	**30**	**83**	**20**	**15**	**10**	**30**
Number Infected	**6**	**3**	**2**	**7**	**2**	**7**
Infection %	**20%**	**3.61 %**	**10%**	**46.67%**	**20%**	**23.33%**

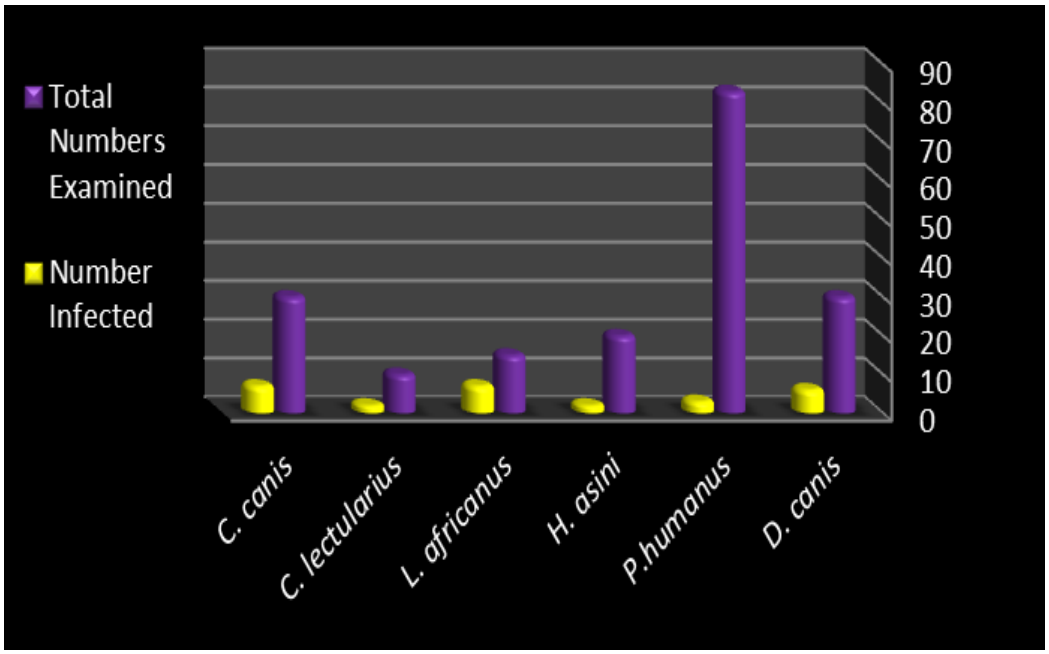

Fig. 1. Variation in relative incidence of infection by arthropod ectoparasites.

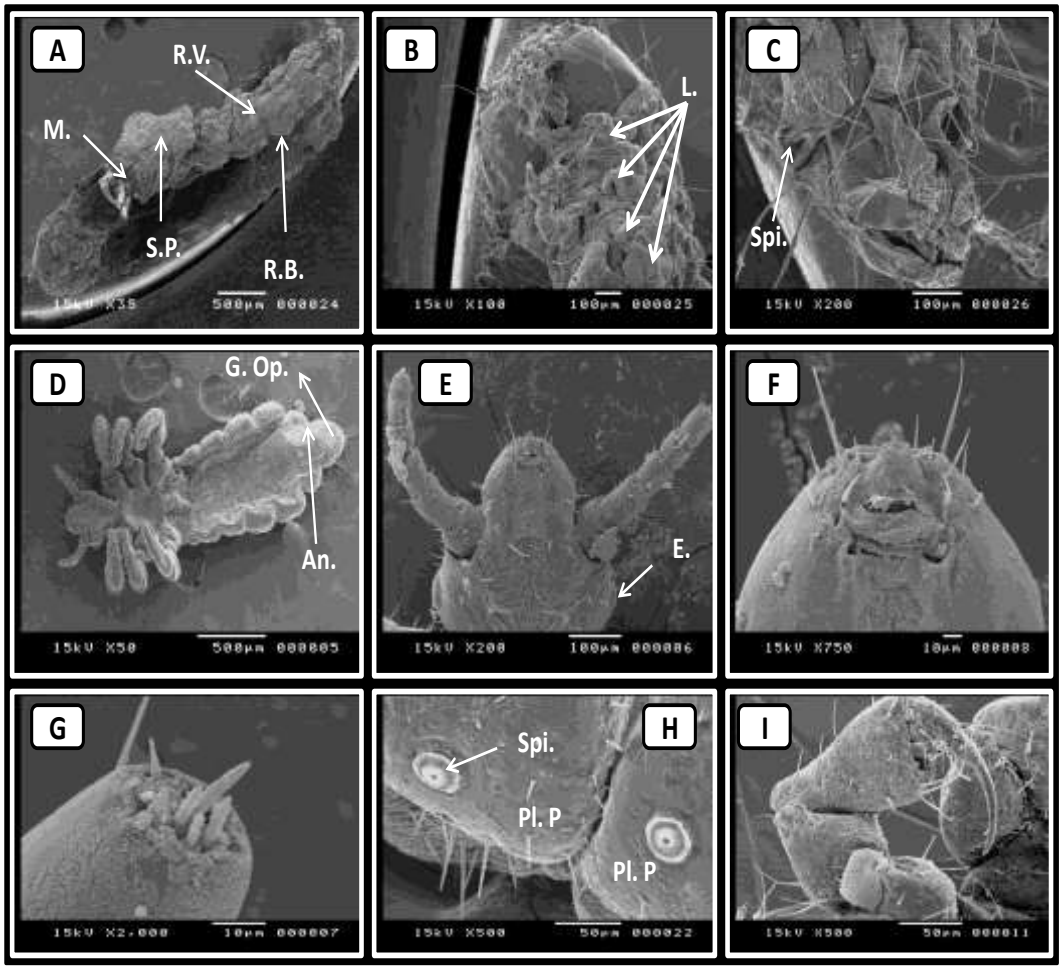

Fig. 2. Scanning Electron Micrograph of A; Dorsal surface of dog mite *Demodex canis* ,B; Ventral surface *D. canis* legs ,C; Enlarged view of the head of *D. canis* showing mouth and spiracle opening ,D; Ventral view of adult human body louse *(Pediculus humanus)*,E; Ventral view of *P. humanus* showing head ,F; Enlarged view of the head of *P. humanus* showing mouth opening,G' Enlarged view of the antennal sensilla of *P. humanus*, H; abdominal spiracle of *P. humanus* and ,I; leg ending of *P. humanus* showing claw.
M.; Mouth, **R.V.;** Round vein ,**S.P.;** Side part, **R.B.;** Rear Body, **L.;** Legs, **Spi.;** spiracle, **G.Op.;** Genital opening, **An.;** Anus, **Pl.P.;** Pleural plate.

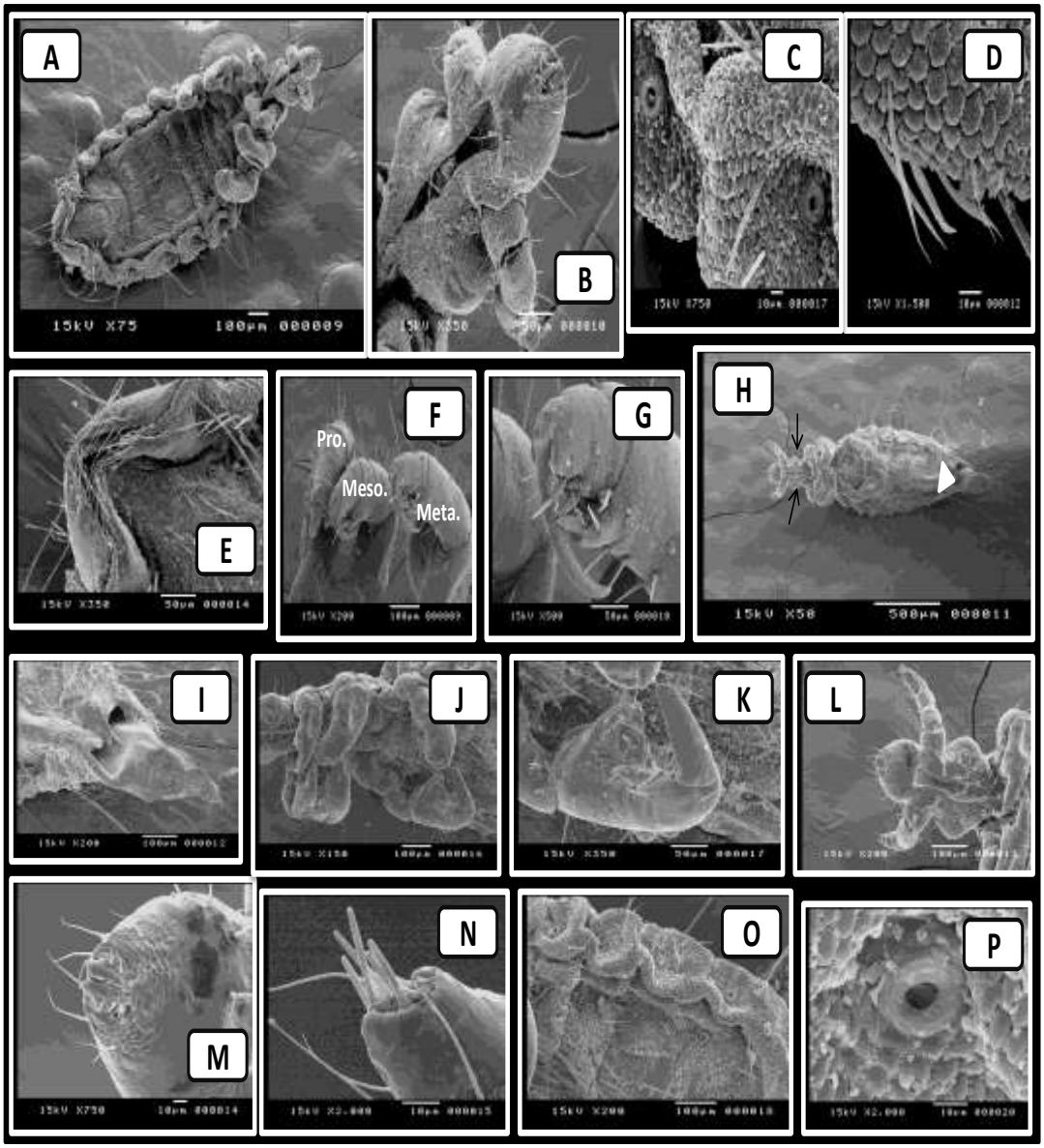

Fig. 3. Scanning Electron Micrograph of A; Ventral view of adult female *Haematopinus asini* ,B; Ventral view of *H. asini* showing head ,C; Ventral view of *H. asini* showing abdominal spiracle, D; Dorsal view of "scaly" surface present on the specimen and singular seta, E; Ventral view of gonopods of *H. asini* ,F; Ventral view of *H. asini* showing legs ,G; Enlarged view of the *H. asini* showing legs ending in large two claws ,H; Ventral view of adult male *Linognathus africanus* showing ocular processes (←) on the head and pseudopenis (White arrow on abdomen),I; Ventral view of male gonopods of *L. africanus* **,**J: Ventral view of *L. africanus* showing legs, K; leg ending of *L. africanus* in large single claw L; Ventral view of *L. africanus* showing head ,M; Enlarged view of the head of *L. africanus* showing mouth opening ,N; Enlarged view of the antennal sensilla of *L. africanus*, O; Ventral view of *L. africanus* showing abdomen and abdomen spiracle and P; Ventral view of *L. africanus* showing thorax spiracle.

Pro.; Proleg, **Meso.;** Mespleg, **Meta.;** Metaleg.

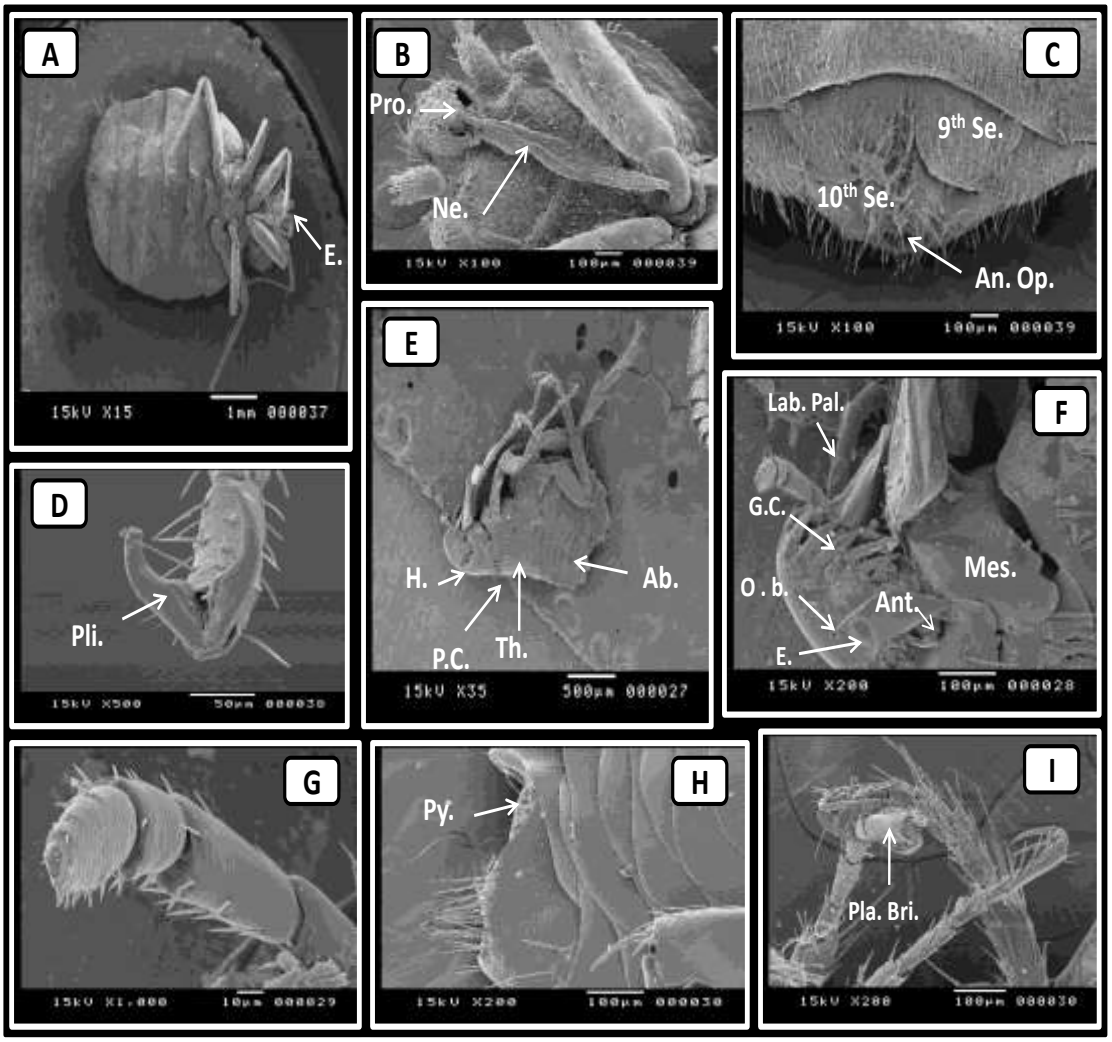

Fig.. Scanning Electron Micrograph of A; Ventral view of adult *Cimex lectularius*, B; anterior part of *C. lectularius* showing Cibarial pump region, C; posterior part of *C. lectularius* showing anus opening, D; Pliers at the end of leg of *C. lectularius*,E; Ventral view of adult *Ctenocephalides canis* ,F; anterior part of *C. canis*, G; maxillary palpus of *C. canis* ,H; posterior part of *C. canis*, I; Ventral view of *C. canis* showing legs.

E.; Eye, **Pro.;** Proboscis, **Ne.;** Needle, **Se.;** Segment, **An. Op.;** Anus opening, **Pli.;** Pliers,**H.;**Head, **P.C.;** Pronotal Comb, **Th.;** Thorax, **Ab.;** Abdomeen, **Lab.Pal.;** Labial palpus, **G.C.;** Genal comb, **O.b.;** Ocular bristle, **Ant.;** Antenna, **Mes.;** Mesopleuron, **Py.;** Pygidium, **Pla.Bri.;** Planter bristles.

DISCUSSION

The main aim of this investigation was to study the morphological features using scanning electron microscopy. Six species of arthropod ectoparasites were observed in the study. Three species of *Demodex* has been reported from different parts of the world (Chesney, 1999; Tamura *et al*., 2001; Desch & Hillier, 2003; Lopez *et al*., 2011; Sreedevi *et al*., 2014; Alice, 2006; Keith, 2003) while one species of mites were observed in the study. Veena *et al.* (2017) Examined the skin of 25 dogs and revealed the presence of *Demodex* mites in nine dogs. *D. canis* and *D. cornei* in 4 dog samples and only *D. canis* was found in 5 dog samples. The present study revealed the presences of *Demodex canis* in six dogs from thirty dogs. The mean total body length of *D. canis* is 215.3µm. The length of *D. canis* is agreeable with (Chesney, 1999; Keith, 2003; Sreedevi *et al*., 2014 and Veena *et al*., 2017).

In the present study regarding arthropod ectoparasites, *Demodex canis, Pediculus humanus*, *Haematopinus asini, Linognathus africanus, Cimex lectularius,* and *Ctenocephalides canis* were collected, respectively, on 20% (6/30), 3.61 % (3/83), 10% (2/20), 46.67% (7/15), 20% (2/10) and 23.33% (7/30) of the animals examined. Previous epidemiological studies have shown different prevalence of *Pediculus humanus;* 4.1% in England (Roberts *et al*., 2000), 8.9% in Belgium (Willems *et al*., 2005), 52% in Ukraine (Kurhanova, 2006), 3.3% in France (Durand *et al*., 2007), 87% in Pakistan (Saddozai & Kakarsulemankhel, 2008), 23.2% in Thailand (Watcharawit& Mayura, 2012), 4.1% in Korea (Oh *et al*., 2010), 42.7% in Brazil (Heukelbach *et al.*, 2005), 9.1% in Peru (Lesshafft*et al*., 2013) 29.35 %. In Central Iran (Saghafipour *et al*., 2017b) while in this present study, the prevalence of head lice infestation is 3.61 %. Probably, the present investigation showed lower in head lice prevalence due to some reasons such as the difference in behavioral habits between countries, the suitability of female's hair as a breeding place for head lice, covering of the female's hair by scarf protect the hair from the infection with lice and so on.

Five species of lice have been described on goats throughout the world (Price & Graham, 1997), but Horak *et al*. (2001) have found mixed infestations of *Bovicola limbatus* and *Linognathus africanus* and Sebei *et al*. (2004) found three species of lice and identified as *Bovicola caprae, Bovicola limbatus* and *Linognathus africanus*. Two species of biting lice and one species of sucking louse while in the present study one species, *Linognathus africanus,* was only found. O'Callaghan *et al.* (1989) mentioned difficulties in identifying species on goats using the light microscopy.

Haematopinus Leach, 1815 is the only genus in the family Haematopinidae of the suborder Anoplura, known as the blood-sucking lice (Meleney & Kim, 1974; Barker,1994 and Durden & Musser, 1994a) In the present investigation *Haematopinus asini* parasitizes horses. Durden & Musser (1994b) disrobed 21 species in the genus *Haematopinus* and described *Haematopinus asini* that parasitizes horses (*Equus caballus),* donkeys (*E. asinus*) and plains zebras (*E. burchelli*).

The dog flea, *Ctenocephalides canis*, may appear to the naked eye as a common human and cat fleas, but closer examination reveals otherwise. In human flea genal and pronotal combs absent, the cat flea has four to five "teeth" on the tibia of all six legs, whereas the dog flea (*Ctenocephalides canis*) has seven to eight teeth on the tibia of all six legs (Kramer and Mencke, 2001). Although the dog and man flea are very similar in appearance, *Ctenocephalides felis* and *Ctenocephalides canis* are very similar in appearance too but the comb on the ventral margin of the head, the genal

ctenidium, is used to help distinguishing between *Ctenocephalides canis* and the cat flea. The first (or outer) genal spine of *Ctenocephalides canis* is much shorter than the second.

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ARABIC SUMMERY

وصف مورفولوجيا الطفيليات الخارجية المفصلية الأرجل باستخدام المجهر الإلكتروني الماسح

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مفصلية الأرجل تشكل مجموعة كبيرة و متنوعة من الحيوانات اللافقارية. فهي تمثل تقريباً أكثر من ٨٠٪ من أنواع الحيوانات المعروفة.تتواجد مفصلية الأرجل في كل البيئات المعروفة مثل المياه المالحة،المياه العذبة،على اليابسة،وفى الهواء.تعتبر مفصلية الأرجل ذات أهمية بيئية وبيولوجية للإنسان. الطفيليات الخارجية المفصلية الأرجل تسبب للإنسان والحيوان الإصابة بأمراض خطيرة و بالتالي تؤدي إلى خسارة اقتصادية كل سنة. ولذلك تهدف الدراسة إلي :معرفة الأنواع موضوع الدراسة ووصف مورفولوجيا الطفيليات الخارجية المفصلية الأرجل باستخدام المجهر الإلكتروني وهذه تعتبر أداة مهمة لتشخيص و مكافحة العدوى البشرية والحيوانية. وتحقيق الأهداف سالفة الذكر يحتاج إلى تجميع الطفيليات الخارجية المفصلية الأرجل،وكذلك التعامل مع البيانات المجمعة على القمل والحلم وبق الفراش والبراغيث. في هذه الدراسة، تم جمع الطفيليات الخارجية المفصلية التالية:

Demodex canis ،*Pediculus humanus* ،*Haematopinus asini* ،*Linognathus africanus* , *Cimex lectularius*,و *Ctenocephalides canis*. ونسب التواجد أثناء التجميع علي التوالي كانت كالآتي:-

٢٠%(٣٠/٦)،٣,٦%(٨٣/٣)،١٠%(٢٠/٢)،٤٦,٦٧%(١٥/٧)،٢٠%(١٠/٢) و٢٣,٣٣%(٣٠/٧).