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Decomposing Rabbit Carrion and It's Associating Insect Fauna in District Peshawar, Khyber Pakhtunkhwa, Pakistan

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ABSTRACT

The aim of the current study was to discover the insects associated with decaying rabbit carrions (*Oryctolaguscuniculus*) used as a model. The study was carried out at Agronomy Research Farm (ARF), The University of Agriculture, Peshawar during 2015-16. Adult insects and larvae were collected at various decomposition stages of the carrions. Samples of the collected larvae were reared to adult stage in the laboratory. Adult insects were killed in killing jar containing cotton swab soaked in Ethyl Acetate. All the insects were identified to species level. The important species in forensic science include the *Chrysomyamegacephala*(Fabricius) (Calliphoridae: Diptera), *Chrysomyarufifacies*(Maquart) (Calliphoridae: Diptera), *Luciliaillustris*(Meigen) (Calliphoridae: Diptera),*Luciliaampullacea*(Villeneuve) (Calliphoridae: Diptera)and*Phormiaregina*(Meigen) (Calliphoridae: Diptera), Sarcophagasp (Diptera: Sarcophagidae), *Musca* domestica Linn. (Diptera: Muscidae), *Necrobiarufipes*Deg (Cleridae: Copleoptera), *Gnathoncus sp.* (Histeridae: Coleoptera),*Saprinus sp.* (Histeridae: Coleoptera), Scarabid sp. (Scarabidae: Coleoptera), *Necrophilarufithorax*(Silphidae: Coleoptera), Staphylinid sp. (Staphylinidae: Coleoptera) and Dermestes maculatus (Dermestidae: Coleoptera). Identification of insects was carried out at Entomological Research Laboratory, Department of Entomology, The University of Agriculture, Peshawar, Pakistan. Key characters for each species are discussed and photographs of each species are provided. All the species recorded in the current study are reported for the first time from District Peshawar Khyber Pakhtunkhwa Pakistan.

Keywords: Forensic insects; Diptera; Coleoptera; Decomposition stages

INTRODUCTION

Forensic entomology is the study of insects associated with a dead body and is mainly used to conclude the time since death (Arnaldos*et al*., 2005). It is generally divided into three main types i.e. medical-legal, urban and stored product. Medico-legal forensic entomology deals with the arthropods involve in serious crimes including murder, rape and suicide. Urban forensic entomology deals with arthropods involve in homes and buildings. Stored product

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commodities forensic entomology deals with the arthropod invading the palatable foodstuffs (Catts and Goff, 1992).

Carrion insects attracted to the specific products of the corpse or may feed on other carrion insects will inhibit the corpse in various stages of decomposition. Blow flies, Flesh flies and House flies are the first among the insects to reach and start laying eggs on soft tissues of the carrion (Kyerematen*et al*., 2012). These flies are considered to be scavengers of the insect world because they have the ability of recognizing decaying bodies over long distances and in any type of vegetation or landscape (Greenberg, 1991). Histerid beetles, Silphid, Staphylinid beetles to feed on larvae of carrion feeding insects (Goff, 1993). During the decomposition process, dermestid beetles arrived late (dry stages), mostly feed on the leftover materials such as skin, hairs etc. (Watson and Carlton, 2003).

After the death, the necrophagous insects arrived on the carrion and starts its life cycle within minutes before any other invertebrates (Haskell *et al.*, 1990; Dillon, 1997). Insect species attracted to the carrion play a key role in the carrion decomposition (Anderson and Cervenka, 2001). These insects using the carrion for shelter, food and its life cycle (Payne, 1965). The insect species mainly belong to Diptera and Coleoptera (Greenberg, 1991). Blowflies and Flesh flies devour major portion of the carrion among all the visiting insects (Early and Goff, 1986). Diverse ecological succession pattern occurs in various insect communities of the decaying body. Each decomposition stage is differentiated by particular group of insects. Each group arrived and occupied the carrion at a specific time (Payne, 1965).

Due to lack of sufficient information regarding taxonomic study of carrion insects, current study was aimed with an objective to collect and identify insect fauna associated withcinsect in District Peshawar. No comprehensive work was performed prior to this study, thus current study will act as a baseline for forensic studies of carrion insects in district Peshawar collected from dead rabbit.

MATERIALS AND METHODS

The current research was conducted at Agronomy Research Farm (ARF), The University of Agriculture Peshawar, during 2015-16 to collect and identify the forensically important insect fauna associated with the rabbit carrion decomposition.

Methodology

During this study a rabbit was slaughtered with a sharp knife. Straight away after death, the rabbit was shifted into a wooden cage covered with steel wire gauze, that allow entrance of the carrions insects while prevented the carrion to be disturbed by other vertebrate scavengers (Wolff *et al.*, 2001). During the study, the carrion was observed twice a day, in morning (9.00am- 10.00am) and evening (5.00pm-6.00pm) for insect's collection. Total 2528 flies and 1143 beetles belonging to different families and species were collected during the study.

Insects Collection

Actively flying insects such as flies were collected through hand net (sweeping) while crawling insects like beetles were picked up with hands, covered with surgical gloves or with forceps from the rabbit carrion. Collected adult insects were killed in killing jar having cotton swab, soaked in Ethyl Acetate. Dead insects were moved to the labeled vials filled with 70 % Ethanol. Each vial was labeled externally with time and date. A similar label was also added

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inside the vial. The specimens were properly pinned with insect pin number 3 and then labelled.

Photographs

Specimens were observed under trinocular stereomicroscope Nikon SMZ 745T. Photographs of diagnostic characters were captured through microscope mounted camera with 5 MP of picture quality.

Identification of the specimens

The collected specimens were identified with the help of available literature i.e. Akbarzadeh*et al.* (2015); Almeida and Mise (2009); Lackner (2010); Ruzicka and Schneider (2011) and Marshall *et al.* (2011).

Repository

All the identified specimens were deposited in the Insect Museum, Department of Entomology, The University of Agriculture, Peshawar, Khyber Pakhtunkhwa, Pakistan.

RESULTS AND DISCUSSION

According to observation of the present study, fourteen insect species (Table 1) visited the rabbit carrion from day 1 till entire flesh was cleaned by carrion feeding insects. From the study, it is evident that, among a total of seven species belonging to the insect order Diptera, family Calliphoridae of this order is represented by five species i.e. *Chrysomyamegacephala*(Fabricius), *Chrysomyarufifacies*(Maquart), *Luciliaillustris*(Meigen), *Luciliaampullacea*(Villeneuve)and*Phormiaregina*(Meigen)whilefamily Muscidae and Sarcophahidae comprises of one species each i.e. *Musca domestica* and *Sarcophaga sp.*respectively. Order coleoptera comprises of seven species. Among them family Histeridae comprises of two species including *Gnathoncus sp.* and*Saprinus sp.*, while family Scarabaeidae,Cleridae,Silphidae, Staphylinidae and Dermestidae is represented by one species each i.e. *Scarabaeid sp., Necrobiarufipes*,*Necrophilarufithorax*,*Staphylinid sp.* and*Dermestes maculatus* respectively.

Table 1.List of Insect species visiting the carrion during the study

Order	**Family**	**Species**	**Stages of decomposition**				
			Fresh	**Bloating**	**Decay**	**Active decay**	**Dry**
Diptera	Calliphoridae	*Chrysomyamegacephala*					
		Chrysomyarufifacies					
		Luciliaillustris					
		Luciliaampullacea					
		Phormiaregina					
	Sarcophagidae	*Sarcophaga sp.*					
	Muscidae	*Musca domestica*					

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Coleoptera	Cleridae	*Necrobiarufipes*			
	Histeridae	*Gnathoncus sp.*			
	Histeridae	*Saprinus sp.*			
	Scarabaeidae	*Scarabaeidsp.*			
	Silphidae	*Necrophilarufithorax*			
	Staphylinidae	*Staphylinid sp.*			
	Dermestidae	*Dermestes maculatus*			

1. Order Diptera

2.

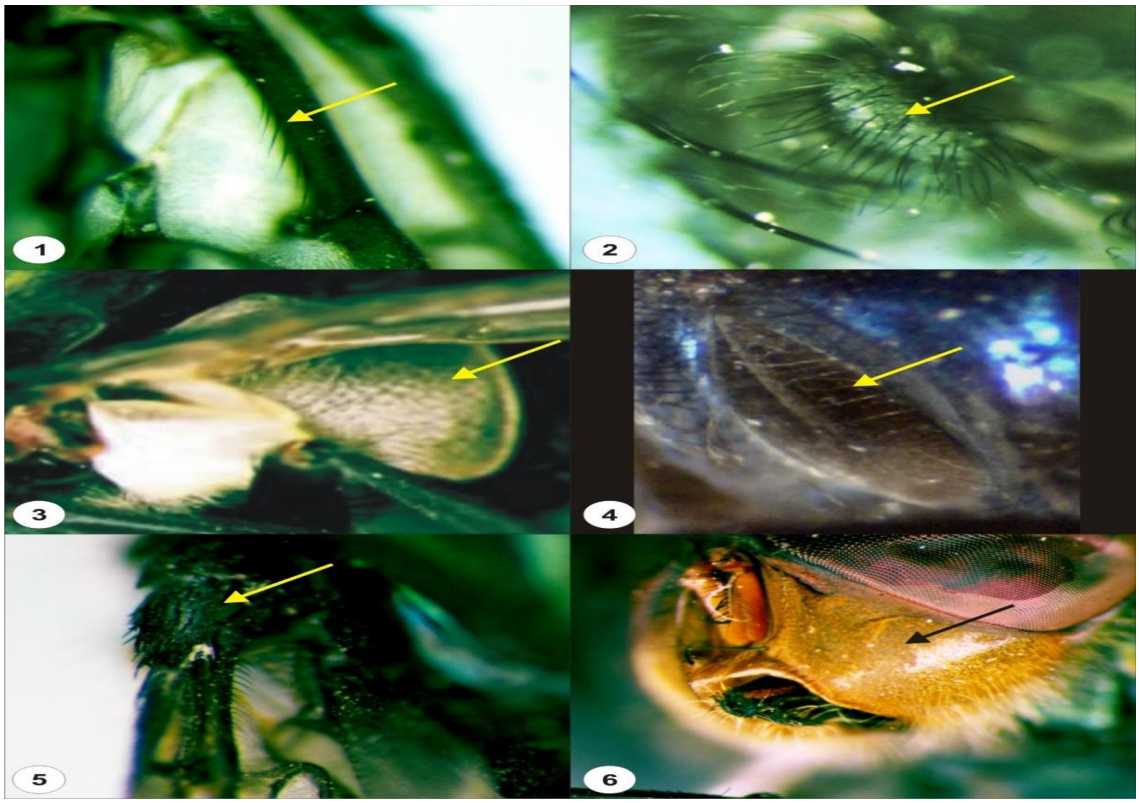
1.1. Family Calliphoridae

1.1.1.*Chrysomyamegacephala*(Fabricius, 1794)

(Fig 1-6)

Diagnosis: Stem-vein with row of hairs dorsally (Fig. 1); greater ampulla with stiff erect hairs (Fig. 2); lower calypter darkened, brownish, with dense hairs at dorsal surface (Fig. 3); Prothoracic spiracle dark, brownish (Fig. 4); basicosta brown or black (Fig. 5);genal dilation and postgena brilliant orange-yellowish, with yellow hairs (Fig. 6).

Remarks:*Chrysomyamegacephala*was the most dominant species. It has a worldwide distribution (Richard and Shearer, 1997). Perveen and Khan (2013) also reported this species from Takht-i-Bahi, Mardan, Khyber Pakhtunkhwa, Pakistan. *C. megacephala* causes myiasis in animals and humans and colonize the carrion rapidly after death (Bunchoo *et al.* 2007).



Figures 1-6. ***Chrysomyamegacephala*1.** Stem vein **2.** Greater ampulla **3.** Lower calypter **4.** Anterior spiracle **5.**Basicosta**6.** Genal dilation and postgena.

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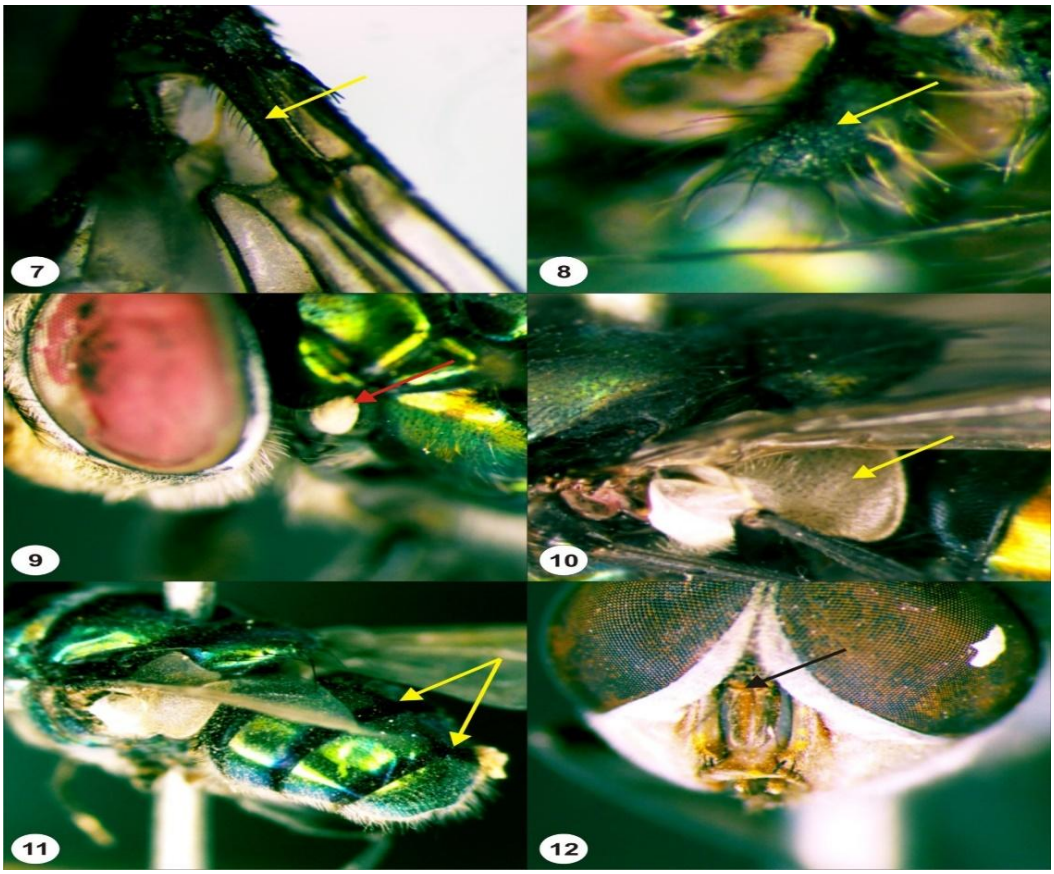
1.1.2. *Chrysomyarufifacies*(Maquart, 1843)

(Fig 7-12)

Diagnosis: Row of hairs on the stem vein, (Fig. 7); greater ampulla with stiff erect hairs (Fig. 8); Pro-thoracic spiracle bright, white-yellowish (Fig. 9); dorsal surface of lower calypter with dense hairs (Fig. 10); anterior wing margin transparent; Katepisternal setae 1+1; Tergite-V laterally with white hairs; thorax shiny, with little dusting dorsally; abdominal segments III and IV with very narrow black transverse bands (Fig.11); female tergite V posteriorly with crevice/incision; third antennal segment pale brown-reddish on inner surface (Fig. 12); proepimeral seta present.

Remarks:*Chrysomyarufifaces*(Macquart) was found to be very common species. The first instar larvae of these flies feed on carrion; third instar are predators of other larvae present on the carrion Akbarzadeh*et al.* (2015). It has worldwide geographical distribution. Cannibalism is common when other food sources are limited.Though this species has been reported from Mardan, Pakistan by Ali *et al.* (2013) This species is however reported for the first time from Peshawar, Pakistan in current study.



Figures 7-12. ***Chrysomyarufifacies*7.** Stem vein **8.** Greater ampulla **9.** Anterior spiracle **10.** Lower calypter **11.**Abdominal segments III and IV **12.** Third antennal segment brown-reddish on inner surface.

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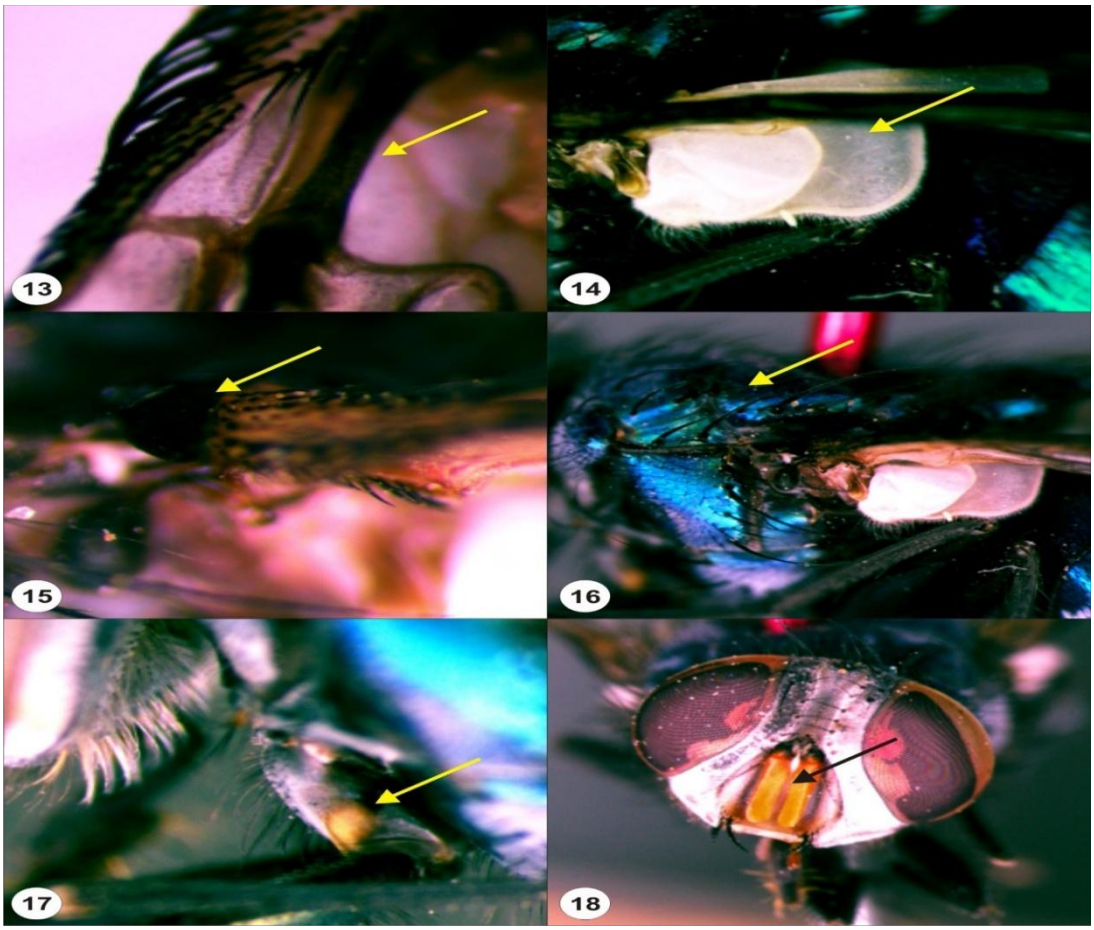
1.1.3.*Luciliaillustris*(Meigen, 1826)

(Fig 13-18)

Diagnosis: These flies were recognized by their bare stem-vein (Fig. 13); lower calypter whitish or slightly darkened, bare dorsally (Fig. 14); Katatergite without hairs or hairy; basicosta dark, brown or black in color (Fig. 15); thorax brilliant green metallic, sometime bluish or cuprous (Fig. 16); thoracic postsutrual area with two pairs of acrostichal setae; subcostal sclerite with black setae ventrally; palpi yellow-orange; coxopleural streak present (Fig. 17); Third antennal segment yellow orange (Fig. 18); female: tergite VI straight in lateral view.

Remarks:*Luciliaillustris*was observed less frequently on the carrion during the study. This species donot colonize the carcass possibly because they could not compete with the two major species of *Chrysomya*, which are abundantly present throughout the study as described by Bharti and Singh (2003). This species was more common on sun exposed carrion than the shady as reported by Campobasso *et al.* (2001).



Figures 13-18. ***Luciliaillustris*13.** Stem-vein **14.**Lower calypter **15.**Basicosta dark, brown or black **16.**Thorax **17.**Coxopleural streak **18.** Third antennal segment yellow orange.

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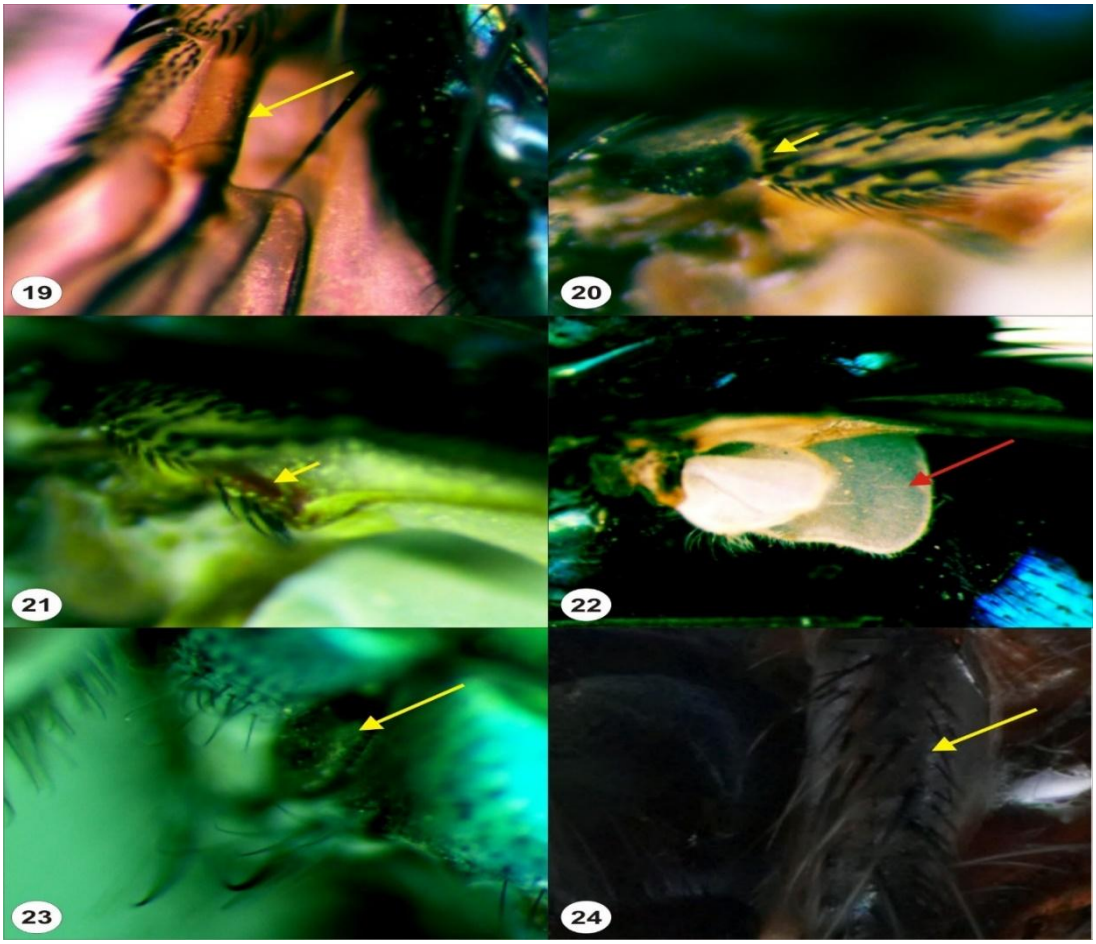
1.1.4. *Luciliaampullacea*(Villeneuve, 1922)

(Fig 19-24)

Diagnosis: Row of hairs onstem-vein absent (Fig. 19); lower calypter bare dorsally; thorax bright metallic green; basicosta brown or black (Fig. 20); ventral surface of subcostal sclerite with black setulae near apex (Fig. 21); palpi yellow-orange; lower calypter white, bare dorsally (Fig. 22); Anterior spiracle dark, brownish or black (Fig. 23); tibia black; body metallic green; Coxopleural streak not present (Fig. 24).

Remarks:*Luciliaampullacea*arrived in the early stages and breed on the carrion (Smith, 1986). They were present in lesser number on the carrion. It is reported for the first time from district Peshawar Khyber Pakhtunkhwa, Pakistan. This species is however reported from Faisalabad, Pakistan by Shah *et al.* (2006).



Figures 19-24. ***Luciliaampullacea*19.** Stem vein **20.**Basicosta**21.**Ventral surface of subcostal sclerite **22.**Lower calypter **23.** Anterior spiracle **24.**Coxopleural streak absent.

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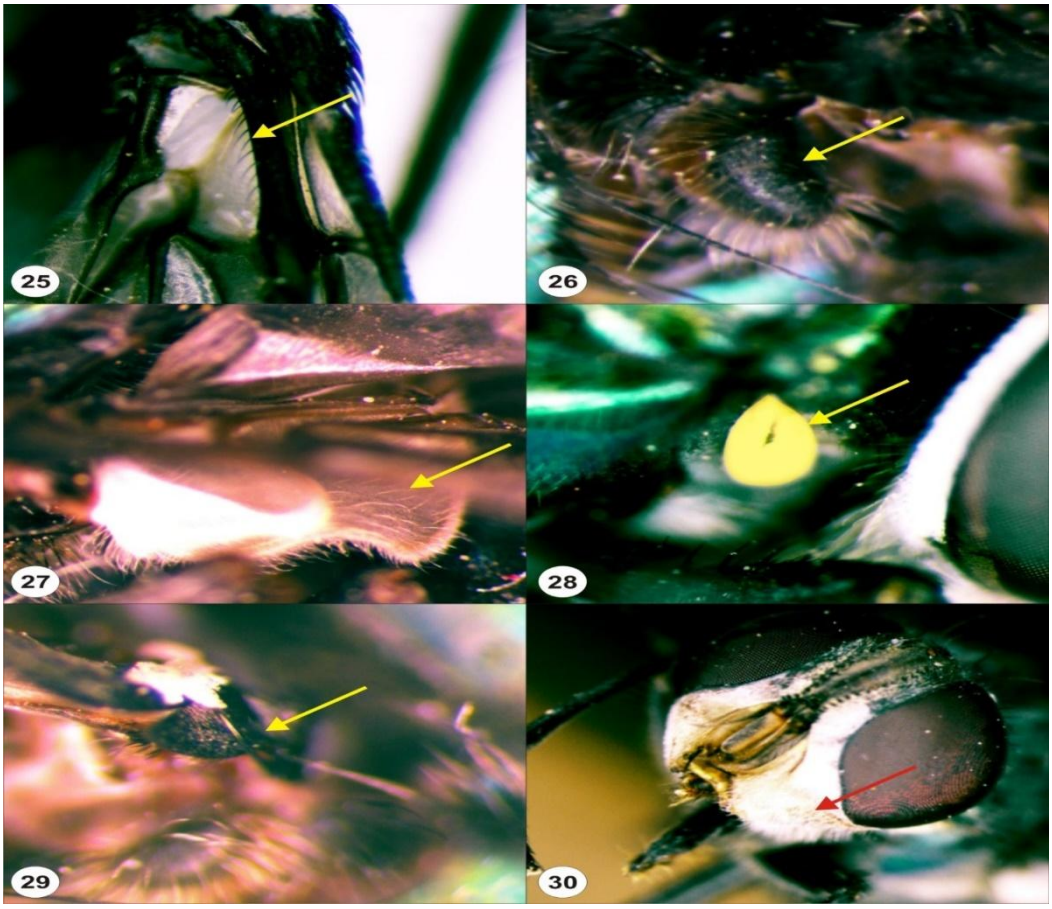
1.1.5. *Phormiaregina*(Meigen, 1826)

(Fig 25-30)

Diagnosis: Stem-vein setose or with row of hairs (Fig. 25); greater ampulla clear or with short fine hairs (Fig. 26); lower calypter bare dorsally or sparsely haired (Fig. 27); Upper and lower calypters bright, yellow or whitish; prothoracic spiracle yellowish (Fig. 28); basicosta dark brown or black (Fig. 29); face and genal dilation whitish (Fig. 30).

Remarks:*Phormiaregina* got attracted to the animal's carrion and causes veterinary medical problems. They also cause secondary myiasis in animals and are serious pests of livestocks. This species is found throughout the world (Marshall *et al.*, 2011).*Phormiaregina* feed on meat, lay eggs on the carrion. Larvae can be used in maggot therapy to promote healing of wounds (Hays, 2007). This species has not been reported from Pakistan before. It is reported for the first time from Peshawar region of Khyber Pakhtunkhwa Pakistan.



Figures 25-30. ***Phormiaregina*25.** Stem vein **26.** Greater ampulla **27.** Lower calypter **28.** Prothoracic spiracle **29.**Basicosta**30.**Face and genal dilation whitish.

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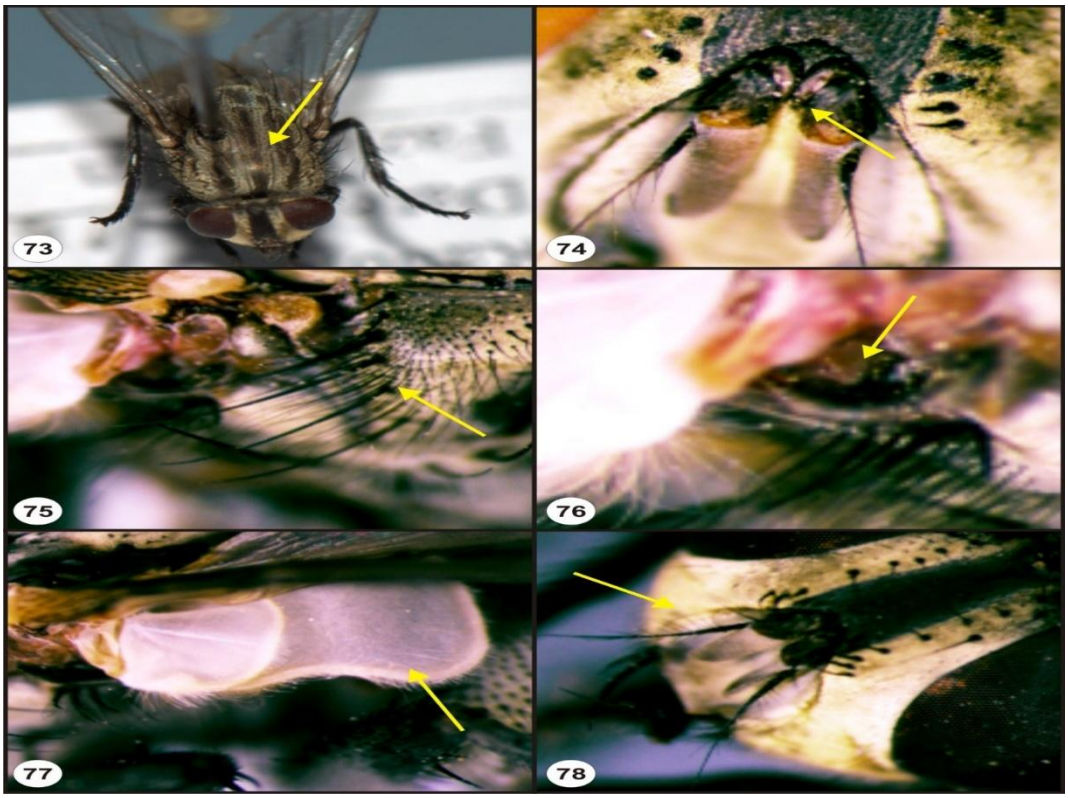
1.2. Family Sarcophagidae

1.2.1. *Sarcophaga sp.*

(Fig. 73-78)

Diagnosis: Thorax with 3 longitudinal black or gray strips (Fig. 73); antennal pedicel with a complete dorsal seam (Fig. 74); meron with a row of setae (Fig. 75); greater ampulla present (Fig. 76); calypter well developed (Fig. 77); ptilinal fissure present (Fig. 78); CuA2 joining A1 near wing base.

Remarks:*Sarcophaga sp.* were recorded more on shady carrion then carrion placed in sunny area as reported by Cranston (2008). They were collected in less number from the carrions. They arrived in early stage shortly after the blow flies.Perveen and Asmat (2013) already reported this species from Mardan, Pakistan. However, from Peshawar Khyber Pakhtunkhwa this species has been reported for the first time. These flies deposited their larvae on the carrion (Richards and Davies, 1977).



Figures 73-78. ***Sarcophaga sp.*****73.**3 Thorax **74.**Antennal pedicel **75.**Meron **76.** Greater ampulla **77.** Calypter well-developed **78.** Ptilinal fissure present.

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1.3. Family Muscidae

1.3.1. *Musca domestica* (Linnaeus 1758)

Diagnosis: Gray thorax, with four longitudinal black strips on dorsal surface; complete transverse suture present on thorax; abdomen yellow ventrally; body covered with hairs; meron without hairs.

Remarks:*Musca domestica* was also recorded during the study but in low number. They were feeding on the fluids that ooze out from the natural openings such as nose, mouth etc. They do not colonize the carrion due to the intense competition with Sarcophagid and Calliphorid species as suggested by Bharti and Singh (2003).

2. Order: Coleoptera

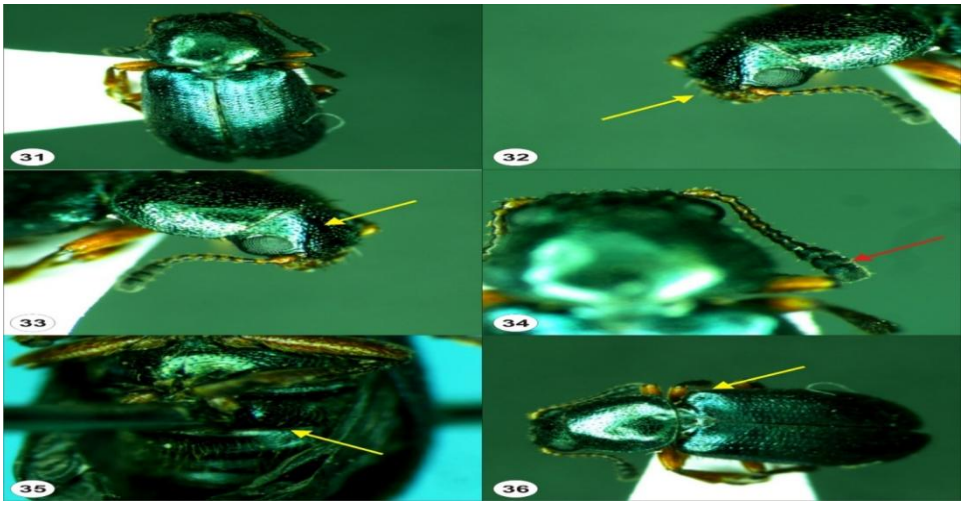
2.1. Family Cleridae

2.1.1. *Necrobiarufipes*(De Geer, 1775)

(Fig. 31-36)

Diagnosis: Shiny metallic green or greenish blue, approximately 5.17 mm. Legs and antennae with red and dark clubs, body elongated, fringed with bristle hairs (Fig. 31); head directed downward (Fig. 32); frons oblique (Fig. 33); eyes large; without Notopleural suture; antennae clubbed shape (Fig. 34); first abdominal sternite entirelyvisible (Fig. 35); Elytra broader than pronotum (Fig. 36); pornotum often nearly cylindrical; procoxae usually conical; tarsi 5 segmented.

Remarks:*Necrobiarufipes*was collected from the carrion in decay and post decay stages preying on the larvae and as well as on the carrion. They were very rear and only a single specimen was collected during the study. Similar results were reported by Bharti and Singh 2003). The adults are mainly surface feeders but the larvae damage the dry meat through bores. These beetles arecommonly known as red-legged ham beetle. This species is already reported from Mardan, Khyber Pakhtunkhwa Pakistan by Parveen and Khan (2013).



Figures 31-36. ***Necrobiarufipes*31.** Body elongated **32.** Head directed downward **33.**Frons **34.** Antennae **35.** First abdominal sternite**36.** Elytra broader than pronotum.

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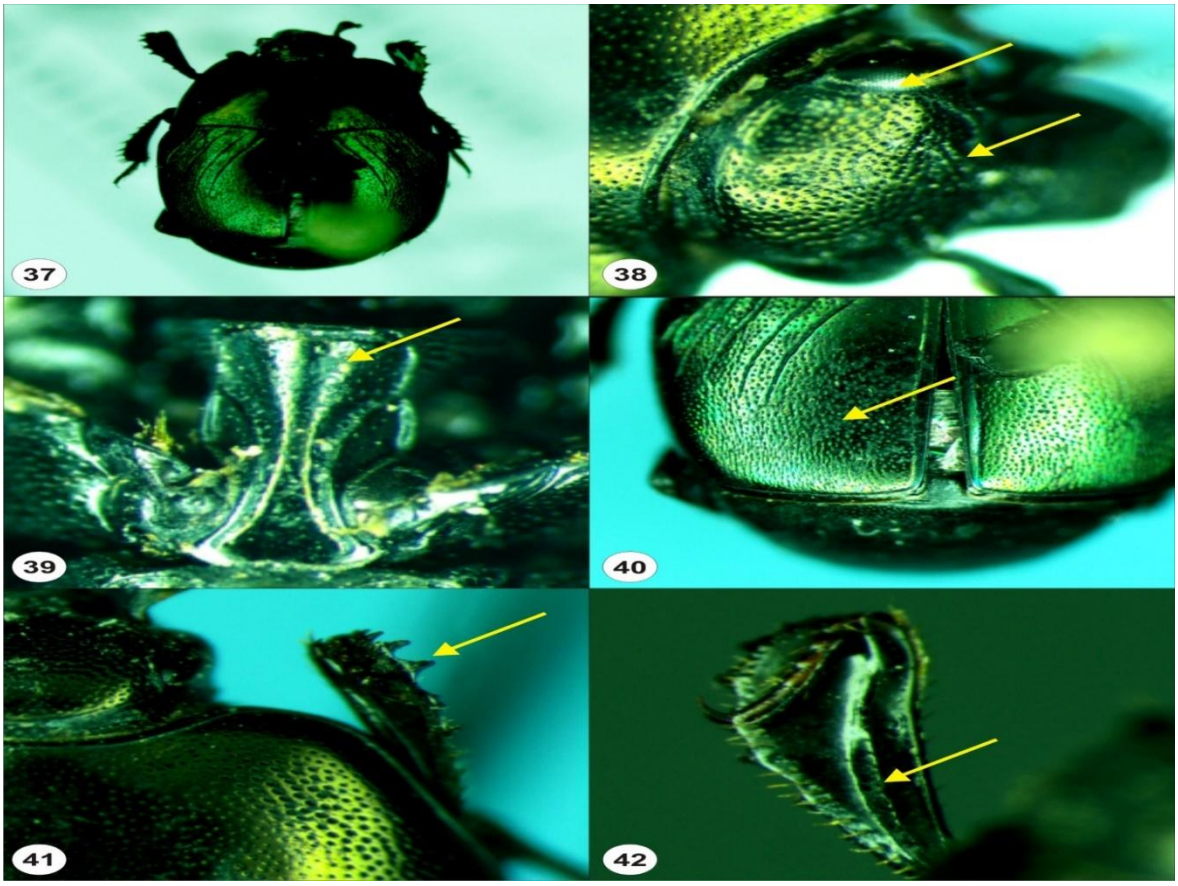
2.2. Family Histeridae

2.2.1. *Gnathoncus sp.* (Jacquelin-Duval, 1858)

(Fig. 38-42)

Diagnosis: Frontal and supraorbital striae completely absent (Fig. 38); pre-apical foveae not present (Fig. 39); Elytral epipleuron bare; Lateral prosternal striae present but strongly reduced; Elytra with prominent punctuation (Fig. 40), a characteristic, short and hooked ap\pendix present between fourth dorsal elytral stria and sutural stria, sutural stria always present but very shortened apically; protibia having denticulate teeth (Fig. 41); protibial groove present (Fig. 42).

Remarks: Fuller (1934) recorded histerid beetles (*Gnathoncus sp.*) during decay and post decay stages as predators on necrophagous insects' larvae and pupae. They were recorded in larger number among the beetles during the present study. It is a new record to District Peshawar, Khyber Pakhtunkhwa.



Figures 37-42. ***Gnathoncus sp.*** **37.** Body oval and flat **38.** Frontal and supraorbital striae absent **39.** Pre-apical foveae absent **40.** Elytra with prominent punctation **41.** Black protibia having denticulate teeth **42.** Protibial groove present.

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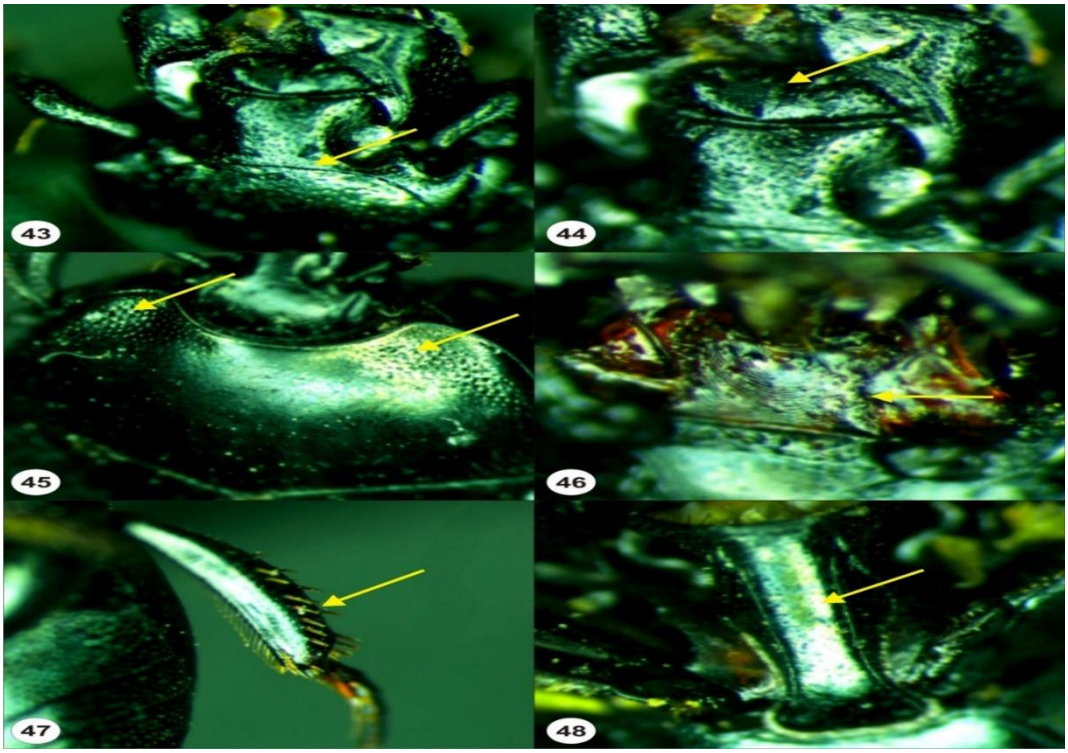
2.2.2. *Saprinus sp.* (Erichson, 1834)

(Fig. 43-48)

Diagnosis: Frontal and supraorbital striae present (Fig. 43), frontal stria often well developed or carinate, in some cases widely interrupted medially and sometime prolonged onto clypeus; semicircular or elongate oval labrum usually smaller than clypeus, convex dorsally, sometimes depressed medially (Fig. 44); Labrum with punctures or coarse structures; Pronotum punctate entirely or at least laterally or in antero-lateral angles (Fig. 45); mentum square shaped, trapezoid or sub-trapezoid, narrowing anteriorly (Fig. 46); hind tibia with two rows of denticles on outer margin (Fig. 47), protibial teeth or denticles usually diminishing in size in proximal direction; elytra entirely punctate or at least on its apical third; prosternal process never knife-like (Fig. 48), flat or slightly convex area between carinal prosternal striae, only rarely somewhat concave; Prosternal process mostly bare; underside of body normally without setae.

Remarks: The *Saprinus sp.* arrive early in the decomposition stages and feeding mainly on the larvae available on the carrion as reported by Payne and Crossley (1966). There finding are in correspondence with our results.



Figures 43-48. ***Saprinus sp.*** **43.** Frontal and supraorbital striae **44.**Labrum semicircular **45.**Pronotum punctate **46.**Mentum square shaped**47.**Hind tibia with two rows of denticles on outer margin **48.**Prosternal process never knife-like.

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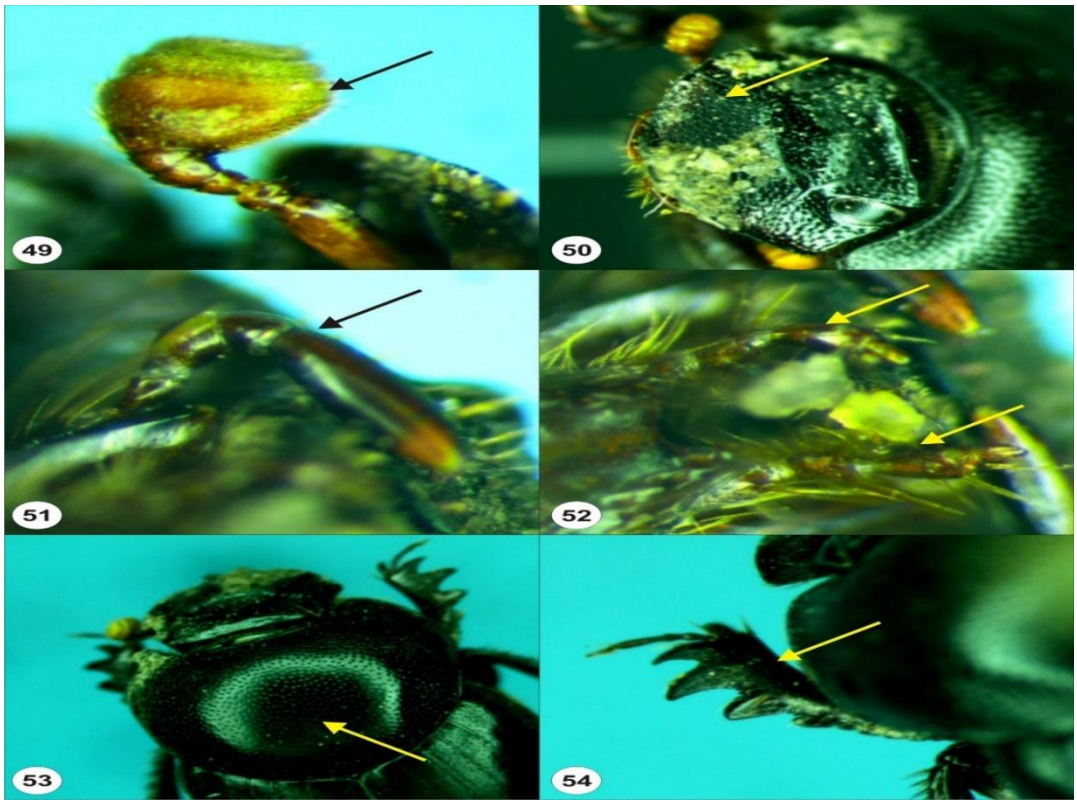
2.3. Family Scarabaeidae

2.3.1. *Scarabaeid sp.*

(Fig. 49-54)

Diagnosis: Lamellate antennae (Fig. 49); Clypeus large covered labrum dorsally (Fig. 50); Mandible not visible from above; Notopleural suture absent; Head weakly deflexed or not deflexed. Antennae 10-segmented; clubbed with apical segments nearly glabrous; maxillae with 4 segmented palpi (Fig. 51); Labium with 3 segmented palpi (Fig. 52); Pronotum variable, with or without horns or tubercles (Fig. 53); Front tarsi denticulate (Fig. 54); Elytra with or without striae.

Remarks: Scarabaeids are the dung beetles and are most important scavengers of the carrion. They arrive early on the dead body and have been noted as early as second day after death. Similar findings have been noted by Catts and Haskell (1990). They were collected from the soil under the carrion during the study. The members of this family dig tunnels and chambers for oviposition near or under the carrion where they deposit pieces of the carrion to feed their larvae as reported by Ururahy-Rodrigues *et al.* (2008).



Figures 49-54. ***Scarabaeid sp.*****49.**Antennae Lamellate**50.**Clypeus **51.**Maxillae **52**. Labium **53.** Pronotum **54.** Front tarsi denticulate

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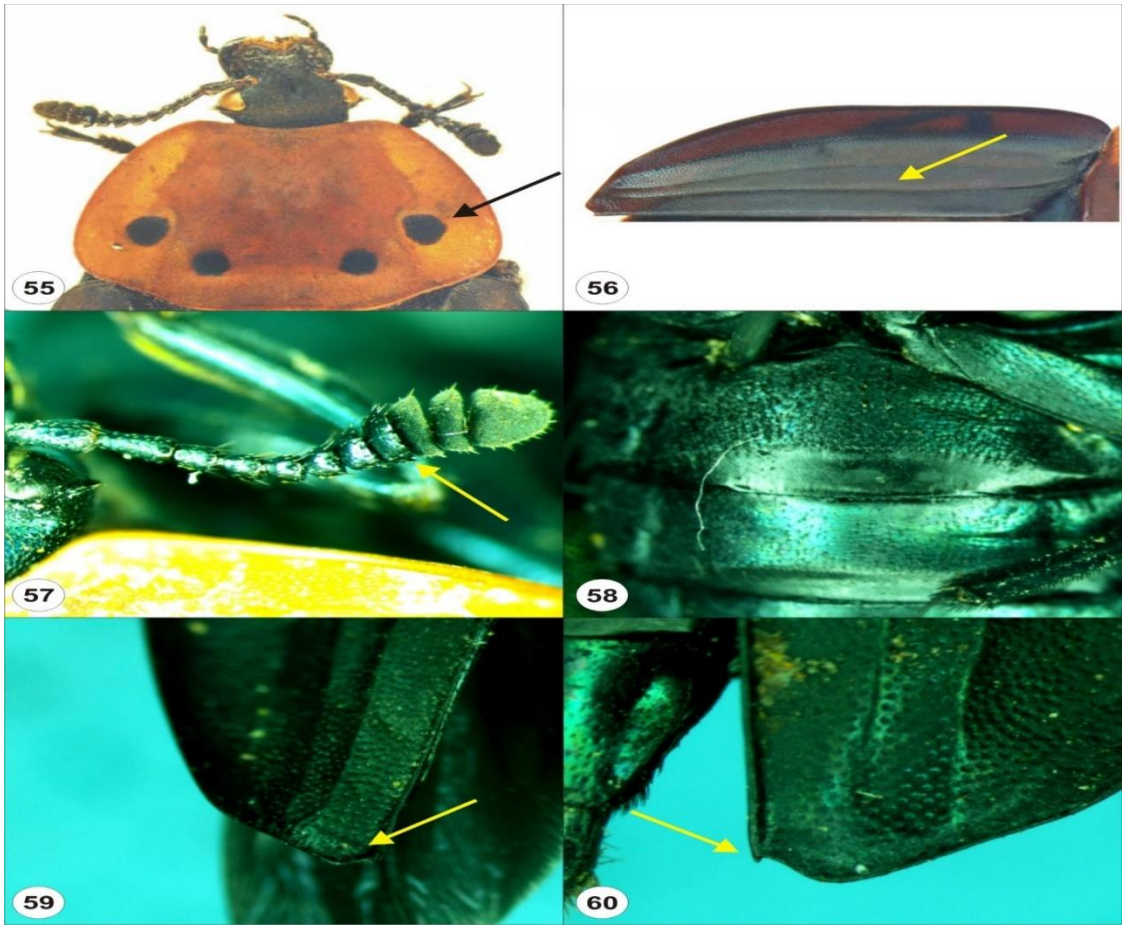
2.4. Family Silphidae

2.4.1. *Necrophilarufithorax*(Wiedemann, 1823)

(Fig. 55-60)

Diagnosis: Body flat, Pronotum orange marked with four black, glabrous spots (Fig. 55), posteriorly arranged in open arc position on disc; Pronotum widely hexagonal in shape; elytra black with longitudinal striae (Fig. 56); 10-11 segmented clubbed antennae, 8th segment cupuliform (Fig. 57); first visible abdominal sternite entire (Fig. 58); Apex of elytron in male truncate, with posterior margin sinuous (Fig. 59); in female the Elytron elongate with convex apex and small, but distinct inner denticle (Fig. 60).

Remarks:*Necrophilarufithorax*were found preying on larvae in decay and post decay stages as observed by other researchers such as Tantawi *et al.* (1996). Only two specimens were collected in this study.



Figures 55-60. ***Necrophilarufithorax*55.** Pronotum **56**. Elytra **57.**Clubbed antennae **58.** First abdominal sternite visible **59.** Apex of elytron **60.**Female Elytron.

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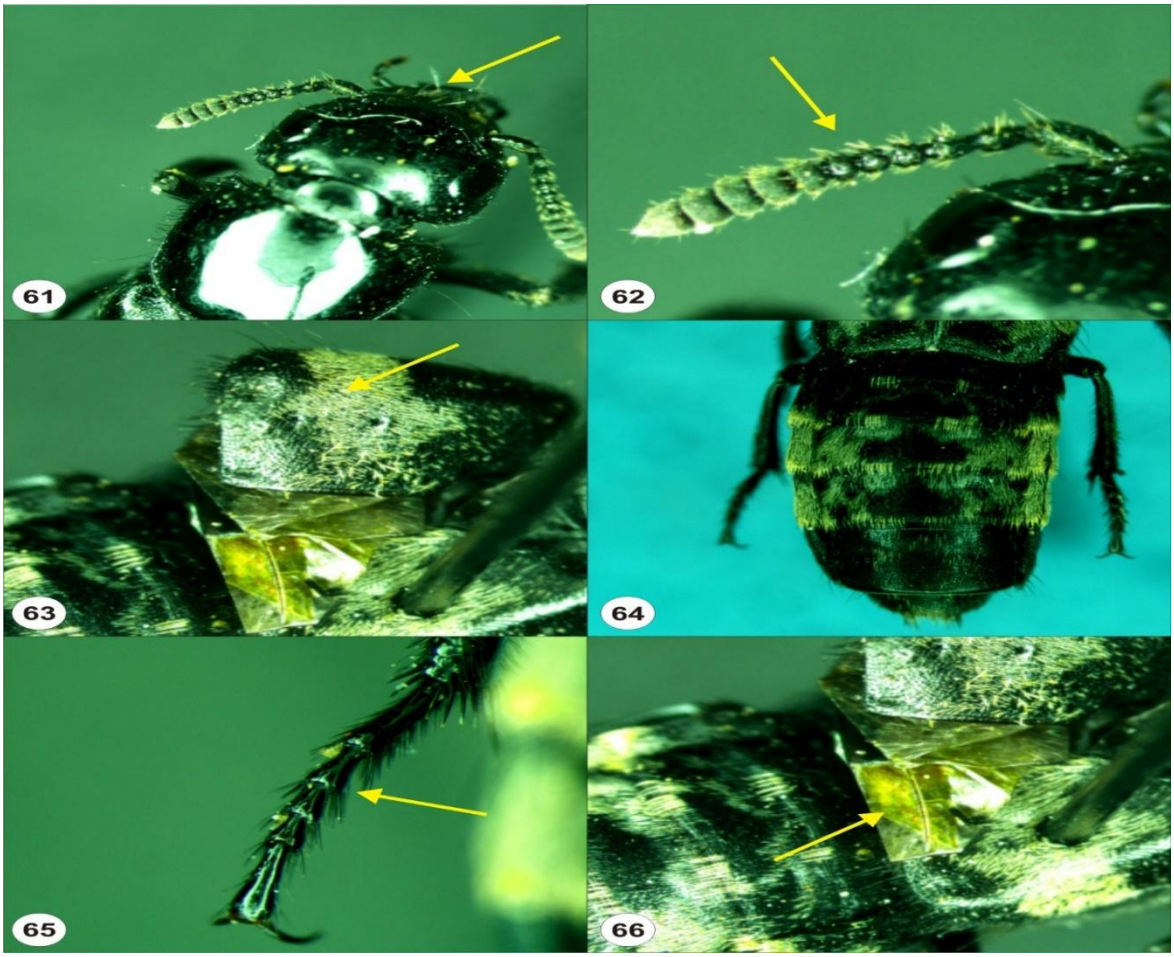
2.5. Family Staphylinidae

2.5.1. *Staphylinid sp.*

(Fig. 61-66)

Diagnosis: Head directed forward (Fig. 61); filiform antennae usually have 11 segments, sometime with weak club (Fig. 62); elytra very short and truncate apically (Fig. 63), exposing more than three abdominal tergites capable of being bend anterio-dorsally (Fig. 64); tarsi 3-5 segmented (Fig. 65); Wings are usually present, completely folded beneath the elytra (Fig. 66).

Remarks: The members of this family occupy almost all moist environments throughout the world. A single staphylinid species was collected during the study. They arrived in decay and post decay stages. They were observed preying on dipteran larvae on decomposing carrion or in the soil which has been observed by other workers as well (Early and Goff, 1986).



Figures 61-66. ***Staphylinid sp.*****61.**Head directed forward **62.** Filiform antennae **63.** Elytra **64.** More than three abdominal tergites exposed **65.** Tarsi 3-5 segmented **66.** Hind wings completely folded beneath the elytra.

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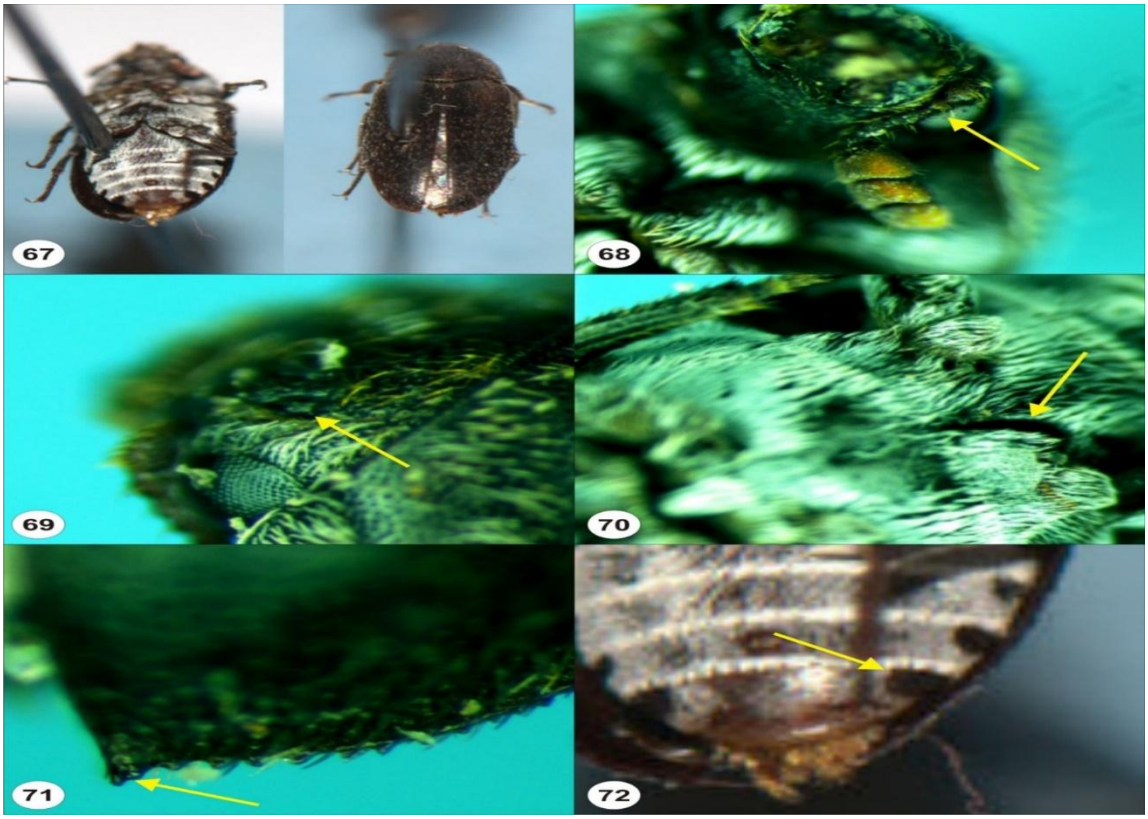
2.6. Family Dermestidae

2.6.1. *Dermestes maculatus*(DeGeer, 1774)

(Fig. 67-72)

Diagnosis: Antennae short and clubbed shape; thorax with white band of hairs on either side; elytra dark brown in color and covered with black, yellow, or white hairs; Elytra serrated at apex terminated with a small spine projecting straight out (Fig. 71),sternites primarily white having black spots at the sides, last sternite has a large black patch (Fig. 72).

Remarks:*Dermestes maculatus* is a cosmopolitan species (Veer *et al.* 1996). *Dermestes maculatus* commonly known as skin beetles were observed in greater number in later stages i.e. post decay and dry stages and were recorded feeding on dried skin, hairs and bones as observed by Richardson and Goff (2001).Archer and Elgar (1998) reported that the exposed pupae of*Dermestes maculatus* arecannibalized by its larvae.



Figures 67-72. ***Dermestes maculatus*** **67.**Body densely covered with scales or setae. **68.**Antennae.**69.**Frons. **70.**Metacoxal grooves **71.**Elytra serrated at apex terminated with a small spine projecting straight out **72.** Last sternite has a large black patch.

CONCLUSION AND RECOMMENDATIONS

Taxonomic information's of 14 necrophagous species from 9 families were reported for the first time from District Peshawar Khyber Pakhtunkhwa, in this study. The carrion feeding insects provide important ecological information about the diversity of species associated with dead organism. The succession of these insects can help the forensic entomologist to estimate the time since death in a particular locality. Studies should be conducted in different

ecological zone of Khyber Pakhtunkhwa to record the diversity of carrion fauna across the province. Various taxonomic attributes of insects feeding on the carrions should further be elaborated for readily identification in future.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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