

Morphotaxonomy of five species of family Sarcophagidae reported for the first time from trans-Himalayan region of Kargil (Ladakh), India

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Abstract

Forensic entomology could offer useful information for the minimal Post-mortem interval (PMI_{min}) estimation as well as other pertinent details, like the reasons and circumstances of death. In the early phases of decomposition, some flesh flies are one of the most prevalent necrophagous insects, showing distinctive biological traits from other necrophagous flies. Additionally, they are major contributors to the development of traumatic myiasis and pose a threat to global health as carriers of numerous harmful microorganisms. Sarcophagid flies are thus regarded crucial for PMI_{min} estimation. However, the inadequate ecological, biological, and taxonomic understanding of sarcophagid flies has severely limited their usefulness. A successional study was conducted to collect and identify diverse necrophagous insect species, including sarcophagids, from various valleys of Kargil (Ladakh). Based on Morphological characters, five species of Sarcophagidae were identified viz. *Sarcophaga (Bercaea) africa* (Wiedemann, 1824), *Sarcophaga (Liopygia) argyrostoma* (Robineau-Desvoidy, 1830), *Sarcophaga (Liosarcophaga) aegyptica* Salem, 1935, *Sarcophaga (Liosarcophaga) portschinskyi* Rohdendorf, 1937 and *Ravinia pernix* (Harris, 1780). Out of these species *Sarcophaga (Liosarcophaga) portschinskyi* Rohdendorf, 1937 is reported for the first time from India and rest of the species are reported for the first time from the region.

Keywords: Forensic entomology, Post-mortem, Sarcophagid flies, Decomposition.

Introduction

Sarcophagidae (Diptera) flies are usually referred as flesh flies. These are essential forensic indicators for determining the rate of human decomposition since they can find a body at several kilometres within a few minutes of its passing (Goff, 2000) [11]. Sarcophagidae distinguished from other Calyptrate by their greyish colour, three stripes running down the dorsal side, the number of notopleural bristles (three to four), and the presence of hypopleural bristles. They have special characteristics that distinguish them different from other fly species and enable them to estimate PMI_{min}. Firstly, many flesh flies are widely recognised for using the ovoviviparous (or ovarviparous) reproduction method, whereby they lay their maggots directly on a body rather than eggs (Byrd and Castner, 2010 [8]; Szpila *et al.*, 2015 [33]; Singh and Bharti, 2018) [30]. Second, due to their larger size, they are more visible (Pape, 1996 [22]; Tomberlin and Benbow, 2015) [35]. Finally, sarcophagid flies are more active during different phases of the decay of corpses (Anderson and VanLaerhoven, 1996 [6]; Povolny and Verves, 1997 [26]; Silahuddin *et al.*, 2015) [29]. Also, given that they are more successful colonists of as compared to blowflies, they might be crucial in the decomposition of buried carrion (Szpilla *et al.*, 2010; Pastula and Merritt, 2013). [25]

The Sarcophagidae family is divided into three subfamilies: Miltogrammatinae, Paramacronychiinae, and Sarcophaginae (Nandi, 2022a) [20, 31]. Sarcophagids are common, with over 400 genera and 31,000 species, throughout all zoogeographic regions of the world (Vairo *et al.*, 2015). From India, 504 species across 50 genera have been described (Nandi, 2002a [20, 31]; Sinha & Nandi, 2002 a, b) [21, 32]. The feeding habits of adult flesh flies vary greatly; they will often consume fruit juice, nectar, and decaying animal waste. Certain species of flesh flies have larvae that are parasitic on different invertebrates, reproduce on carrion,

faeces, or decaying organic matter, and carry numerous pathogens on their body surfaces that can infect humans and animals (Pape, 1987 [23], 1996 [22]; Amoudi *et al.*, 1992 [5]; Al-Misned, 2000; Al-Misned *et al.*, 2001 [3]; Graczyk *et al.*, 2005 [12]; Pape *et al.* 2010 [24]; Al-Khalifa *et al.*, 2020) [1]. Their ability to spread disease is directly impacted by their different feeding strategies, and some species' larvae can infect humans and other animals with myiasis (Zumt, 1965 [38]; Greenberg, 1973 [13]; Guimaraes & Papavero, 1999 [14]; Hagman *et al.*, 2005 [16]; Bermudez *et al.*, 2010 [7]; Mello-Patu & Luna-Dias, 2010 [19]; Kelehear *et al.*, 2020). It is well known that decomposing animal and human carrion attracts a wide variety of sarcophagid flies (Cherix *et al.*, 2012 [9]; Vasconcelos *et al.*, 2014 [37]; Al-Khalifa *et al.*, 2020) [1]. Research on the larval and pupal stages of these insects increases their usefulness in forensic investigations (Pape, 1996 [22]; Wells *et al.*, 2001; Sinha & Mahato, 2016; Samerjai *et al.*, 2020) [28]. For identification of this family male terminalic characters are considered, as these features are species-specific, and scarce information is available on female of this family (Wells *et al.*, 2001).

In present research we are discussing about the species belonging to subfamily Sarcophaginae which are reported for the first time from Kargil (Ladakh). Kargil is a district under the UT Ladakh in India. Its overall area is 14086 km², and its geolocation is 34°34'12.00" N 76°05'60.00" E. Its altitudes range from 2800 to 4500 m above sea level, with numerous Himalayan peaks in Ladakh rising over 6000 m. The average rainfall is less and usually occurring in the form of snow throughout the winter. It is also referred to as a cold arid desert because of the wide range of average temperatures (30 to -25° C) and relative humidity (31% to 64%) and its experience with both arctic and desert climates (Ali *et al.*, 2019 [4], Hussain *et al.*, 2021). Despite these facts this region is very rich in biodiversity. A total of five

species have been identified based on male terminalia viz., *Sarcophaga (Bercaea) africa* (Wiedemann, 1824), *Sarcophaga (Liopygia) argyrostoma* (Robineau-Desvoidy, 1830), *Sarcophaga (Liosarcophaga) aegyptica* Salem, 1935, *Sarcophaga (Liosarcophaga) portschinskyi* Rohdendorf, 1937 and *Ravinia pernix* (Harris,1780). Out of the five species, *Sarcophaga (Liosarcophaga) portschinskyi* Rohdendorf, 1937, is recorded for the first time in the country from Kargil (Ladakh) and all the other species have been documented from India in the past and are often found

at higher elevations. The data generated from this study will offer the information regarding the forensically relevant Sarcophagid species prevail in Ladakh and the data can also be utilised as a basis for future research.

Material and Methods

Study area: Adult male flies were collected using chicken bait from various valleys of Kargil (Ladakh) viz. Suru-valley, Shingo-valley, Drass-valley, Sub-valley (Indus) and Zanskar-valley (Fig. 1).

Table 1: Latitude, longitude, and altitude of localities from where specimens were collected.

Valley	Locality	Latitude	Longitude	Altitude (above mean sea level)
Suru-valley	Shilikchey	34°35'08" N	76°07'30" E	2633m
Shingo-valley	Latoh	34°35'41" N	75°58'32" E	2736m
Sub-valley (Indus)	Chiktan	34°28'57" N	76°27'49" E	3280m
Suru-valley	Kanoor	34°25'11" N	76°03'13" E	2807m
Drass-valley	Gyndial	34°25'54" N	75°47'58" E	3100m
Zanskar-valley	Padum	33°28'10" N	76°52'40" E	3600m

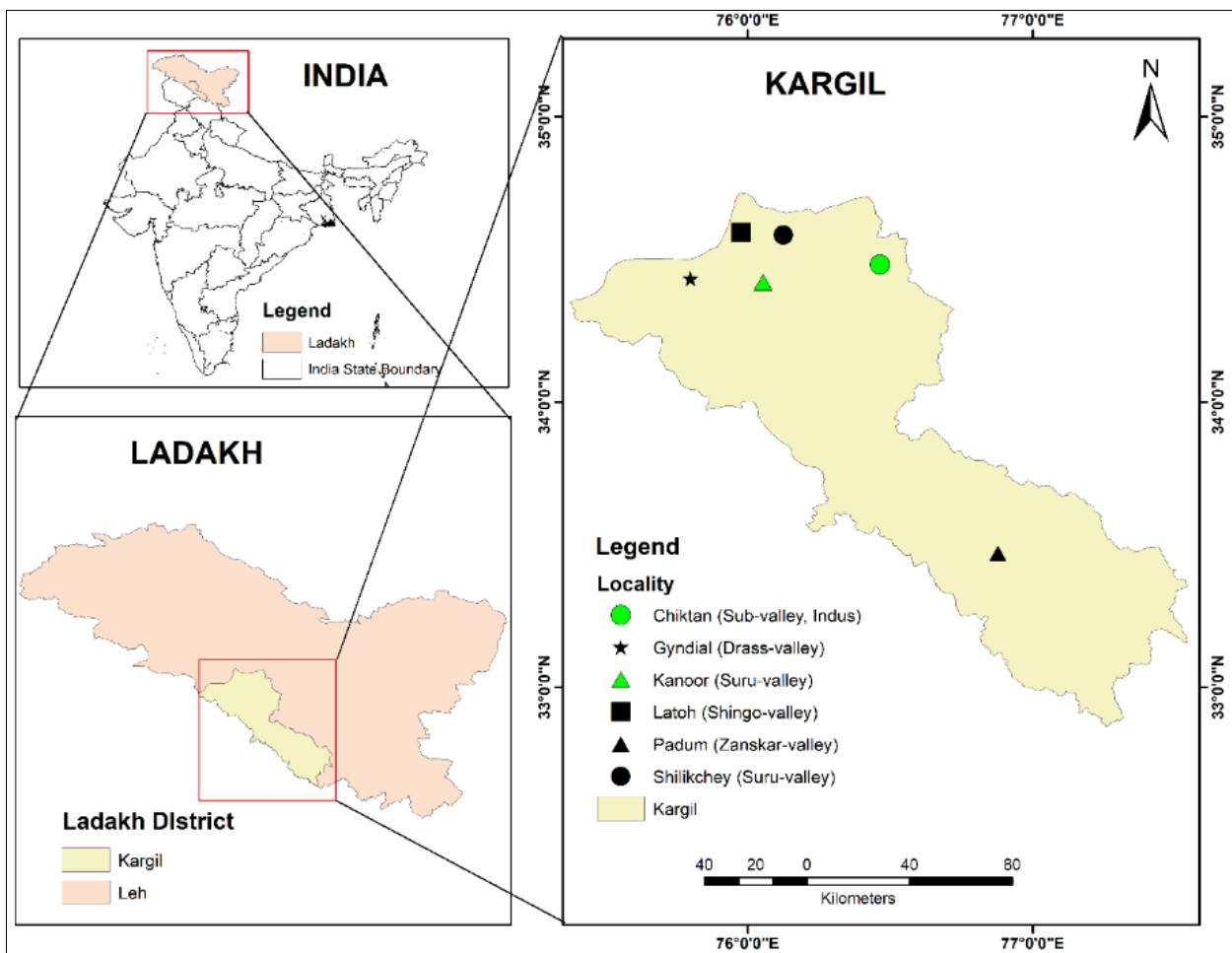


Fig 1: Map depicted the Study area.

Collection: Chicken meat (weighing 1-3 Kg) was used as a bait to attract the forensically relevant insects. Meat was procured from local slaughterhouse. The bait (chicken meat) was placed on the ground and covered with the iron cage (1'x1'x1') with wire mesh having holes of 1 inch for free movements of insects. Cage was used to protect the bait from the attack of scavengers. Collection of flies were done by sweeping hand-net over bait and then collected insects were transferred to killing jar (Jar having ethyl-acetate).

Identification: To identify sarcophagid species, the abdomen of male was detached and soaked in a 10% KOH for 24 hrs. The terminalia was dissected from the abdomen and preserved in the clove oil for clearance purpose and later used for identification and photography. Specimen was identified using the keys given by Nandi (2002a, b) [21, 32]. The images were captured with a Leica microscope at Department of Zoology & Environmental Sciences, Punjabi University Patiala.

Results

The identification of the flesh fly species were carried out following the taxonomic key of Nandi (2002 a, b) [21, 32] and all the identified flesh fly species belonged to the Sarcophaginae sub-family.

Sub-family: Sarcophaginae

In the subfamily Sarcophaginae, first genital segment (GS1) and tergite 5 have been circumvented and fused. Tergite 6 is missing between tergite 5 and GS1 (Nandi 2002a, b) [21, 32]. Notopleuron has 2 strong primary and 2 lesser sub-primary bristles; 2-4 free sternites in the male sternum; hairs on back of the hind coxa..... Sarcophaginae

Genus: Sarcophaga Johnston and Tiegs, 1921

Description of male

Key to studied genera as per (Nandi 2002 a, b) [21, 32]

Apical plate of paraphallus invariably tiny; third abdominal tergite usually lacks long bristles in middle of posterior edge*Sarcophaga*

Morphology: Third antennal segment two to three times that of the second; third abdominal tergite lacks long bristles; anal tergite occasionally red; first genital segment (GS1)

typically without marginal bristles; paraphallus relatively short and heavily sclerotised; apical plate of paraphallus well developed, styli of glans curved, stout, slender and never closely adjacent to apical plate; lateral plate of paraphallus well developed, single but without process; ventralia always present with two pairs of lobes (Nandi 2002a, b) [21, 32].

Key to studied sub-genera as per (Nandi 2002 a, b) [21, 32]

Apical part of paraphallus is small, distinct and projecting forward without a lateral process; lateral plate of paraphallus has two large pointed protruding processes..... *Bercaea* Robineau-Desvoidy

Apical part of paraphallus with lateral process bearing pointed teeth extending forward; lateral plate of paraphallus primitive; posterior section of penis membranous due to separation across median line of basal part of paraphallus.....*Liopygia* Enderlein

Pair of lamellate processes is present on the Lateral plate of paraphallus; the basal portion of the paraphallus is slightly elongated; anal tergite is infrequently red..... *Liosarcophaga* Enderlein

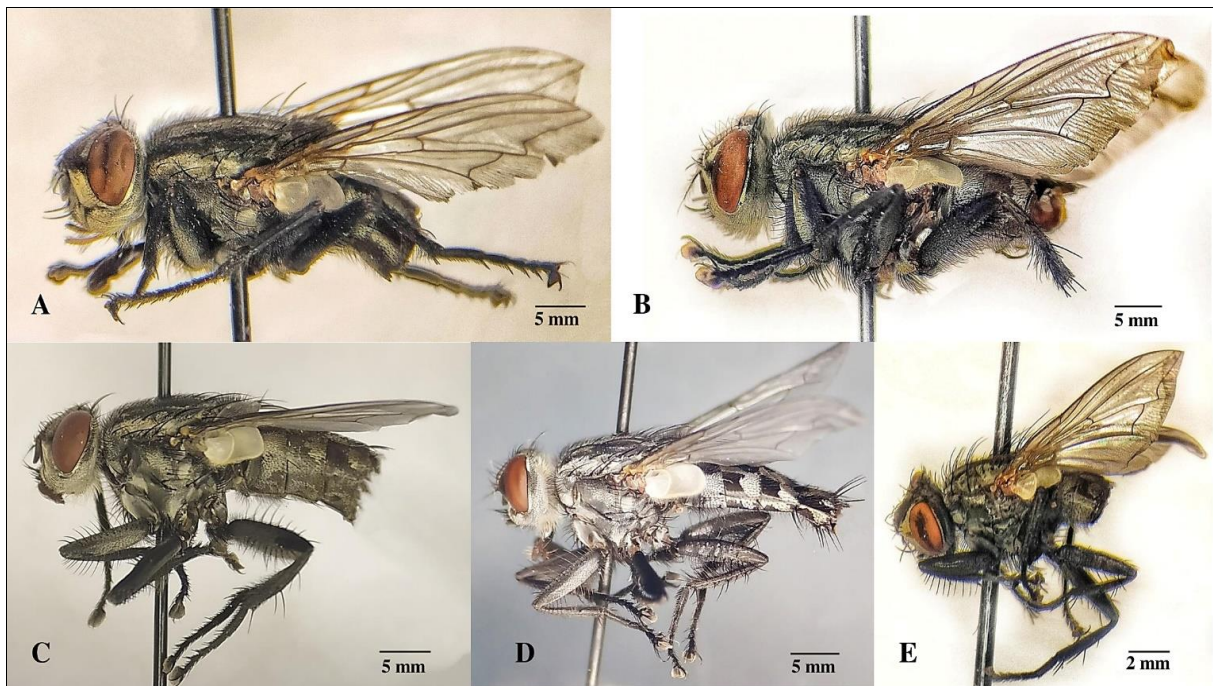


Fig 2: Images of collected adult male specimens (A. *Sarcophaga (Bercea) africa* (Wiedemann, 1824), B. *Sarcophaga (Liopygia) argyrostoma* (Robineau-Desvoidy, 1830), C. *Sarcophaga (Liosarcophaga) aegyptica* Salem, 1935, D. *Sarcophaga (Liosarcophaga) portschinskyi* Rohdendorf, 1937, E. *Ravinia permix* (Harris,1780)).

Description of Male

Sub-genus: Bercaea

Species: Sarcophaga (Bercae) africa (Wiedemann, 1824) (Fig.2.A)

Diagnosis: Male measuring about 1.5cm; head frontal vitta black, parafrontal and parafacial black with golden pollinosity; antennae dark brown, first and second segment blackish while third dark brown with silver pollinosity; arista long, plumose; facial ridge black with golden pollen; vibrissae long; gena black with silvery pollen having black hairs; post genal colour black; palpus slender and black; proboscis black.

Thorax is grey having 3 black longitudinal lines dorsally with golden pollinosity; upper portion of propleura black, barren and containing pollen of white colour; pro-stigmatic and pro-pleural bristles strong and co-occur with short hairs; brown prothoracic and metathoracic spiracles; acrostichal 0+0; dorso-central 6+6 (anterior 4 post dc stout); intra-alar 1+3; pre-sutural 1; humeral 2; post-humeral 3; notopleural 4; post-alar 2; super-alar 4; st 1+1+1; mesopleural 7; hypopleural 11; 1 pair of both apicoscutellar and discoscutellar bristles; 2 pairs lateroscutellar bristles. Wing characteristics include a hyaline sheen with brown veins, bare RI, a row of 9–10 small setae situated dorsally and stretching as much as half the length from the basal node to r–m, prominent costal spines, a black epaulette with

tiny spines, light brown basicostal scale, creamy white squama, and brown halter.

Legs are black; the fore femur has two rows of long bristles on the posterodorsal surface and a series of bristles across the posterior edge of the ventral surface; fore tibia has a row of 3' bristles runs down the basal half of the anterodorsal surface; the mid femur has a line of three to four bristles across the middle portion of the anterolateral surface; the mid tibia has one larger and two shorter bristles along the basal 1/3 of the anterodorsal surface; one bristle each on the anteroventral and posterolateral surfaces; the hind femur has two rows of prolonged bristles running parallel to the anterodorsal surface, a line of shorter bristles along the anteroventral surface, a row of five to six bristles alongside the posteroventral surface, and several long hairs along the ventral surface; The hind tibia has a number of long bristles across the anterodorsal surface and several long hairs alongside the anterior and posterior edges of the ventral surface.

Abdomen is black in colour with a silver-whitish checkered pattern; second and third abdominal tergites with no median marginal bristles however each having 2 sets of lateral marginal bristles, fourth having a couplet of median and 3 sets of lateral marginal bristles, fifth bears a row of 18 bristles along the margin; long hairs on the first and second sternites while short, scattered hairs on the third and fourth sternites.

Terminalia: First genital segment (GS1-7+8) is brownish with two to three pairs of marginal bristles along with long hairs; fifth sternite Y-shaped with broad triangular window and short spines on lateral and short hairs on terminal side of arms (Fig.3.a); inner forceps (IF) long, curved, pointed at apex, somewhat grooved posteriorly, and with tuft of long hairs across basal half (Fig. 3.b); Short outer forceps (OF) apically rounded and hairy (Fig. 3.b); The anterior paramere is long, broad, and sharp apically with no hairs on outside; the posterior paramere is short, apically pointed, and has two small subapical hairs; theca is quite short; the paraphallus (P) is extremely long, deeply sclerotized, but blunt at the end. Its lateral processes are short, bent and sharp; The lateral plate of paraphallus (LP) generates one set of forwarding processes; The styli of glans long and curved; ventralia (V): broad with pointed tip (Fig.3.c).

Material examined: Kargil; Shilikchey (Suru-valley), Lato (Shingo-valley), Kanoor (Suru-valley), Gyndial (Drass-valley), Chiktan (Sub-valley, Indus) and Padum (Zanskar-valley).

Distribution: India; Arunachal Pradesh, Assam, Himachal Pradesh, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim, Uttar Pradesh, and West-Bengal; Bhutan; Nepal; Pakistan; Europe; Afghanistan; Australia; Austria; Canada; China; Costa Rica; Egypt; Iran; Japan; Korea.

Description of Male

Subgenus *Liopygia* Enderlein, 1928

Species: *Sarcophaga (Liopygia) argyrostoma* (Robineau-Desvoidy, 1830) (Fig.2.B)

Diagnosis: Adult male measuring about 1.5cm; head frontal vitta black, parafacial and parafacial black with silver pollinosity; antennae black or dark brown having 1st and 2nd segment reddish-brown while 3rd brownish-black with silver

pollinosity; arista long, plumose; facial ridge black with pollen of golden colour; vibrissae long; black gena with greyish hairs; post genal colour black; palpus slender and black; proboscis black (Fig.2.B).

Thorax is greyish-black having 3 black longitudinal lines dorsally; upper portion of propleura black, barren and featuring pollen of silver colour; pro-stigmatic and propleural bristles well-established and co-occur with short hairs; brown prothoracic and metathoracic spiracles; acrostichal 0+1; dorso-central 1+4; intra-alar 0+3; pre-sutural 1; humeral 4; post-humeral 2; notopleural 3; post-alar 2; super-alar 3; st 1+1+1; mesopleural 6-7; hypopleural 10; 1 pair of both apicoscutellar and discoscutellar bristles; 2 pairs lateroscutellar bristles.

Wing characteristics include a hyaline sheen with brown veins, bare R1, R4+5 comprising of a series of 9–10 small setae located dorsally and stretching as much as 2/3 length from the basal node to r-m, weak costal spines, a black epaulette, light brown basicostal scale, white squama, and brown halter.

Legs are black coloured; the fore femur has two rows of long bristles on the posterodorsal; fore tibia has a row of two to three bristles runs down the basal half of the anterodorsal surface; the mid femur has a set of bristles across the anterolateral surface; the mid tibia has two bristles on mid part of the anterodorsal surface; the hind femur has two rows of bristles running parallel to the anterodorsal surface, a line of bristles across each of anteroventral surface and posteroventral surface; a series of bristles across the anterodorsal surface of hind tibia.

Abdomen is black in colour with grey checkered pattern; second and third abdominal tergites with no median marginal bristles however lateral marginal bristles is present, 2 on second and 1 on third abdominal tergite, fourth having a couplet of median and one lateral marginal bristle, fifth bears a row of 14 to 16 bristles along the margin; long hairs on the second sternite while shorter hairs on the third and fourth sternites.

Terminalia: Both first (GS1-7+8) and second genital (GS2) segments black have short hairs and marginal bristles absent; 5th sternite Y-shaped with transparent broad window and short marginal spines on lateral and short hairs on terminal side of arms (Fig.3.d); inner forceps (IF) linear and broader basally (Fig. 3.e); outer forceps (OF) are oval constituting long hairs (Fig. 3.e); The anterior paramere slightly curved; the posterior paramere is overly curved and has three hairs on the apex; paraphallus (P) is larger than theca and each are sclerotised; apical plate of paraphallus membranous having distinct median part and a duplet of long projecting lateral processes; The lateral plate of paraphallus (LP) curved and generates one set of forwarding processes; wide styli of glans with a set of chitinous lateral processes; ventralia (V): bilobed and fully formed (Fig.3.f).

Material examined: Kargil; Kanoor (Suru-valley).

Distribution in India: Gujrat (Girner Hills, Junagadh), Haryana (Ambala), Rajasthan (Jaipur) and Utrakhand (Mussoorie).

Distribution in world: Afghanistan; Australia; Canada; China; Cuba; Cyprus; Czech Republic; Denmark; Europe; Iran; Iraq; South Africa; Turkey; Turkmenistan; Ukraine; United Kingdom; USA and Uzbekistan.

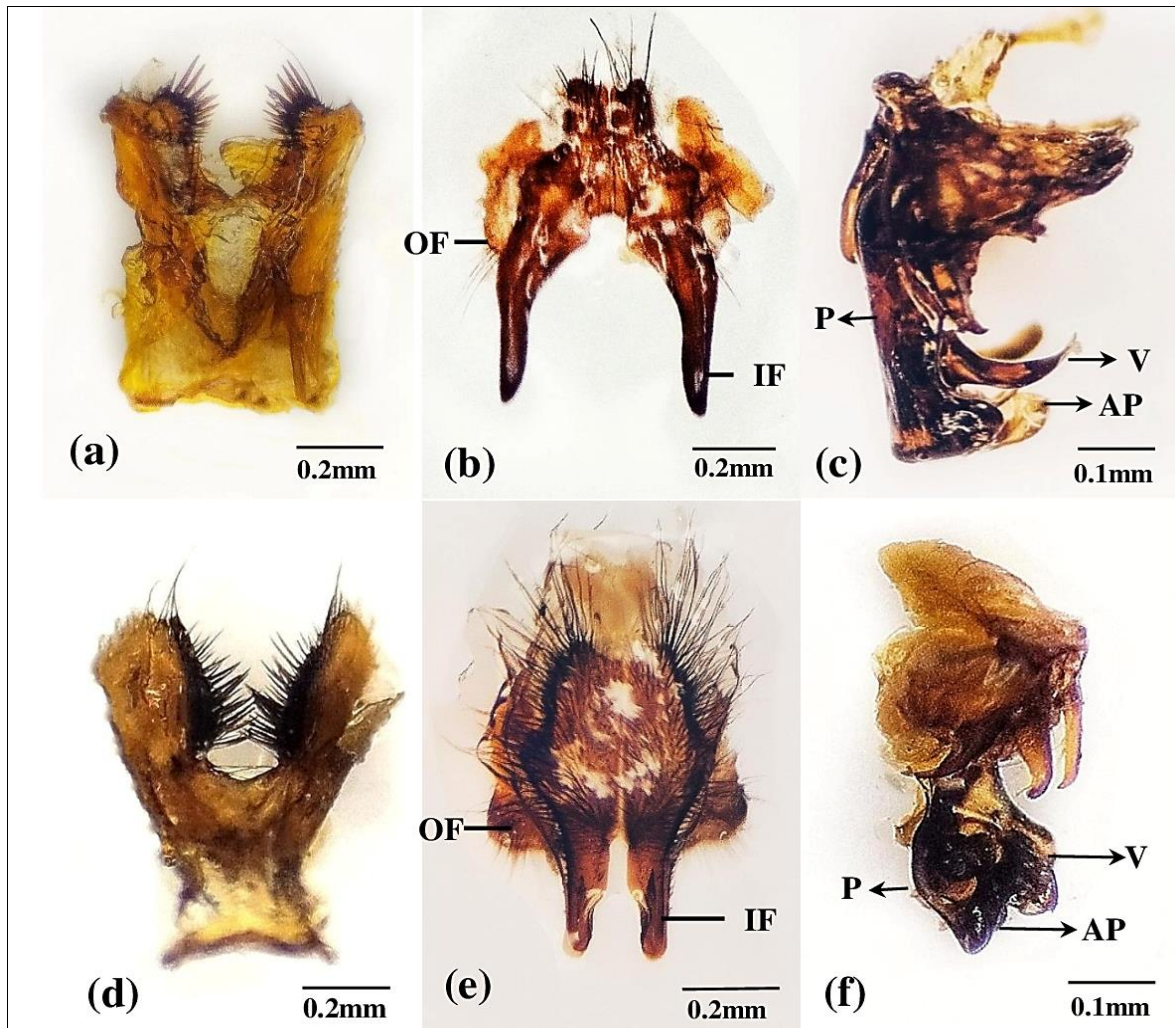


Fig 3: *Sarcophaga (Bercea) africa*, male terminalia. (a) Fifth sternite, ventral view. (b) inner and outer forcep, caudal view. (c) phallus, lateral view. *Sarcophaga (Liopygia) argyrostoma*, male terminalia. (d) Fifth sternite, ventral view. (e) inner and outer forcep, caudal view. (f) phallus, lateral view. Abbreviations: P- paraphallus, AP- apical plate of paraphallus, V- ventrelia.

Description of Male

Sub-genus: *Liosarcophaga* Enderlein, 1928

Key to studied species

Ventralia short, wide and equal to lateral plate of paraphallus; Inner forceps straight and slightly widened at subterminal end; process of apical plate of paraphallus pointed at end *aegyptica* (Salem)

Species: *Sarcophaga (Liosarcophaga) aegyptica* (Salem, 1935) (Fig.2.C)

Adult male measuring about 1.5cm; head frontal vitta black and shiny pollinosity, parafrontal and parafacial black with silver pollinosity and parafrontal consists of short hairs; antennae black-brown having 1st and 2nd segment black with black short hairs while 3rd brownish with silver pollinosity; arista long, crossed; facial ridge brown with pollen of silver colour; black gena with silver pollen; post genal colour black; palpus slender and black; proboscis black-brown in colour (Fig.2.C).

Thorax is greyish-black having 3 black longitudinal lines dorsally; upper portion of propleura black, barren and featuring pollen of silver colour; pro-stigmatic and propleural bristles well-established and co-occur with short hairs; dark brown prothoracic and metathoracic spiracles; acrostichal 0+3; dorso-central 5+5; intra-alar 0+3; pre-sutural 1; humeral 3; post-humeral 2-3; notopleural 4; post-

alar 2; super-alar 3; st 1+1+1; mesopleural 7; hypopleural 12; 1 pair of both apicoscutellar and discoscutellar bristles; 2 pairs lateroscutellar bristles.

Wing characterising of hyaline sheen with brown veins; bare R1; R4+5 comprised of a row of 5–6 small setae situated dorsally and stretching as much as 1/3 of the length from the basal node to r-m; prominent costal spines; creamish-white epaulette with tiny spines; light brown basicostal scale; white squama and brown halter.

Legs are black coloured; the fore femur has two rows of long bristles on the posterodorsal surface; fore tibia has a row of three bristles runs down the anterodorsal surface; the mid femur has a series of three bristles across the mid part of the anterolateral surface also basal half of anteroventral surface is comprised of longer hairs; the mid tibia has a set of two to four bristles on the mid-section of the anterodorsal surface; the hind femur has two rows of bristles running parallel to the anterodorsal surface; a series of bristles across the mid-section of anterodorsal surface of hind tibia.

Abdomen is black in colour having silver checkered pattern; second and third abdominal tergites with no median marginal bristles however one lateral marginal bristle is present on both second and third abdominal tergite, fourth having a couplet of median and two lateral marginal bristle, fifth bears a row of 14 bristles along the margin; shorter hairs on 1st to 4th sternites.

Terminalia: Both first (GS1-7+8) and second genital (GS2) segments jet-brown, having short hairs but marginal bristles absent; 5th sternite Y-shaped with transparent broad window and prominent spines on lateral side of arms (Fig.4.a); inner forceps (IF) more or less linear, anterior margin precisely notched prior to apex (Fig. 4.b); outer forceps (OF) somewhat oval constituting hairs distally (Fig. 4.b); The anterior paramere strong, fully curved & moderately shorter than posterior paramere; the posterior paramere is curved, wide at sub-basal end and has two hairs; paraphallus (P) is larger than theca and each are heavily sclerotised; apical plate of paraphallus having sharp, long projecting lateral processes; The lateral plate of paraphallus (LP) lamellate, long and pellucid; styli of glans elongated and denticulated anteriorly; ventralia (V): broad, pellucid somewhat dissected terminally (Fig.4.c).

Material examined: Kargil; Shilikchey (Suru-valley), Lato (Shingo-valley), and Kanoor (Suru-valley).

Distribution in India: Punjab (Patiala)

Distribution in world: Pakistan; Albania; Armenia; Austria; Azerbaijan; Bulgaria; Czech Republic; Egypt; Ethiopia; Gruzia; Hungary; Iran; Isreal; Kazakhstan; Moldova; Northwest China; Romania; Russia; Slovakia; Tajikistan; Turkmenistan; Ukraine; Uzbekistan and Yugoslavia (former).

Key to species

Ventralia very elongated and shorter than lateral plate of paraphallus.....*portschinskyi* Rohdendorf

Species: *Sarcophaga (Liosarcophaga) portschinskyi* Rohdendorf, 1937 (Fig.2.D)

Diagnosis: Adult male measuring about 1.5cm; head frontal vitta black, parafacial black with silver pollinosity; antennae black having 1st and 2nd segment black but apical section of 2nd segment is reddish-brown, while 3rd black; arista long, plumose; facial ridge black featuring short hairs; vibrissae long; black gena with silver pollinosity having tiny hairs; post genal colour black; palpus elongated and blackish; proboscis shorter, black (Fig.2.D).

Thorax is black having 3 black longitudinal lines dorsally; upper portion of propleura black, barren; pro-stigmatic and pro-pleural bristles well-established and co-occur with short hairs; brown prothoracic and metathoracic spiracles; acrostichal 0+1; dorso-central 5+4; intra-alar 1+3; pre-sutural 1; humeral 4; post-humeral 1; notopleural 3; post-alar 2; super-alar 3; st 1+1+1; mesopleural 5; hypopleural 7; short pair of both apicoscutellar and discoscutellar bristles; 2 pairs lateroscutellar bristles.

Wing characteristics include a hyaline sheen with brown veins; bare R1; R4+5 having a row of 9–10 small setae situated dorsally and stretching greater than half the length from the basal node to r–m; prominent costal spines; a black epaulette; light brown basicostal scale; creamish-white squama; and brown halter.

Legs are black coloured; the fore femur has two rows of long bristles on the posterodorsal surface; fore tibia has a row of three bristles runs down the anterodorsal surface; the mid femur has a series of three bristles across the anteroventral surface; the mid tibia has a single bristle on the anterodorsal surface; the hind femur has two rows of bristles running parallel to the anterodorsal surface; hind tibia: long bristles are present across the posteroventral surface.

Abdomen is black in colour having whitish checkered pattern; second and third abdominal tergites with no median marginal bristles however one lateral marginal bristle is present on both second and third abdominal tergite, fourth having a couplet of median and three lateral marginal bristle, fifth bears a row of 14 bristles along the margin; shorter hairs on 1st to 4th sternites.

Terminalia: Both first (GS1-7+8) and second genital (GS2) segments black in colour and marginal bristles absent; 5th sternite Y-shaped having window and several bristles on lateral side of arms (Fig.4.d); inner forceps (IF) more or less linear, consisting of long hairs on the pointed apex (Fig. 4.e); outer forceps (OF) somewhat triangular constituting of short (Fig. 4.e); The anterior paramere wide, fully curved & bent at tip like a sickle; the posterior paramere is constricted, sharp apically and sub-basal end has two hairs; paraphallus (P) is larger than theca and each are heavily sclerotised; apical plate of paraphallus having a prominent apical, 2 sharp lateral processes; The lateral plate of paraphallus (LP) broad medially, long and pellucid; styli of glans elongated and sclerotised; ventralia (V): base folded, slightly sclerotised, curved anteriorly(Fig.4.f).

Material examined: Kargil; Shilikchey (Suru-valley), Lato (Shingo-valley), Kanoor (Suru-valley), Gyndial (Drass-valley), Chiktan (Sub-valley, Indus) and Padum (Zanskar-valley), first time from India.

Distribution: Pakistan; Albania; Armenia; Austria; Azerbaijan; Bulgaria; China; Czech Republic; Denmark; Estonia; Finland; France; Greece; Gruzia; Hungary; Ireland; Italy; Kazakhstan; Malta; Moldova; Mongolia; Norway; Poland; Romania; Russia; Slovakia; South Sweden; Switzerland; Tajikistan; Turkey; Turkmenistan; Ukraine; Uzbekistan and Yugoslavia (former).

Genus: *Ravinia* Robineau-Desvoidy, 1863

Description of Male

Species: *Ravinia pernix* (Harris, 1780) (Fig.2.E)

Diagnosis: Adult male measuring about 1.5cm; head frontal vitta black, parafacial black with silver pollinosity; antennae densely brown having 1st and 2nd segment brown while 3rd brown with silver pollinosity; arista long, plumose; facial ridge silver pollinosity and brown in colour; featuring short hairs; vibrissae elongated and crossed; black gena with silver pollinosity having long hairs; post genal colour black; palpus elongated and slightly brown-black; proboscis shorter, black (Fig.2.E).

Thorax is blackish-brown having 3 black longitudinal lines dorsally; upper portion of propleura pro-pleural well-established and co-occur with short hairs; dark brown fore and hind spiracles; acrostichal 2+1; dorso-central 3+3; intra-alar 0+3; pre-sutural 1; humeral 3; post-humeral 2; notopleural 3; post-alar 2; supra-alar 3; st 1+1+1; mesopleural 7; hypopleural 8; one pair of both apicoscutellar and discoscutellar bristles; two pairs lateroscutellar bristles.

Wing characteristics include a hyaline sheen with brown veins; bare R1; R4+5 having a row of 9–11 small setae situated dorsally and stretching greater than half the length from the basal node to r–m; shorter costal spines; a black epaulette; light brown basicostal scale; white squama; and brown halter.

Legs are black coloured; the fore femur has two rows of long bristles on the posterodorsal surface; fore tibia having three to four bristles runs down the basal part of

anterolateral surface; the mid femur having two-three bristles across the mid-part of anterolateral surface; the mid tibia having two bristles on the mid-part of anterodorsal surface; the hind femur featuring two rows of bristles on the anterodorsal surface and one row on the anteroventral surface; hind tibia: two long bristles and few shorter bristles are present across the anterodorsal surface.

Abdomen is black in colour having golden checkered pattern; 2nd and 3rd abdominal tergites with no median marginal bristles however one set of lateral marginal bristle is present on both second and third abdominal tergite; fourth having a couplet of median and three lateral marginal bristle, 5th bears a row of 12 to 14 bristles along the margin; shorter hairs of black colour on 1st to 4th sternites.

Terminalia: first genital segment (GS1-7+8) reddish-brown, marginal bristles six in number, second genital (GS2) segment reddish-orange, marginal bristles absent; 5th sternite V-shaped window absent having terminally four to five bristles and short spines on lateral side of arms (Fig.4.g); inner forceps (IF) long and bit curved terminally (Fig. 4.h); outer forceps (OF) somewhat long constituting

long hairs at end (Fig. 4.h); The anterior paramere long and dissected at end; the posterior paramere is long, fully curved at end with short subapical process; paraphallus (P) and theca fused and each are heavily sclerotised; apical plate of paraphallus is blunt terminally; The lateral plate of paraphallus (LP) short and terminally pointed; styli of glans not present; ventralia (V): paired, well-developed and stalked (Fig.4.i).

Material examined: Kargil; Shilikchey (Suru-valley), Lato (Shingo-valley), Kanoor (Suru-valley), Gyndial (Drass-valley), Chiktan (Sub-valley, Indus) and Padum (Zanskar-valley).

Distribution: India; Arunachal Pradesh, Bihar, Himachal Pradesh (Chamba, Dalhousie, Kufri, Manali, Manikaran, Shimla), Jammu and Kashmir (Jhelum Valley, Kogyar, Tangmarg), Uttrakhand (Bardinalath, Mussoorie, Naini Tal, Sahastradhara, Tehri) and West-Bengal (Darjeeling, Jalapahar, Takvar Tea Estate).

Distribution in world: Bangladesh (Dacca); Bhutan; Nepal; Pakistan; China; Europe; Iran; Iraq; Israel; Japan (including Ryukyu Islands); Korea and Switzerland.

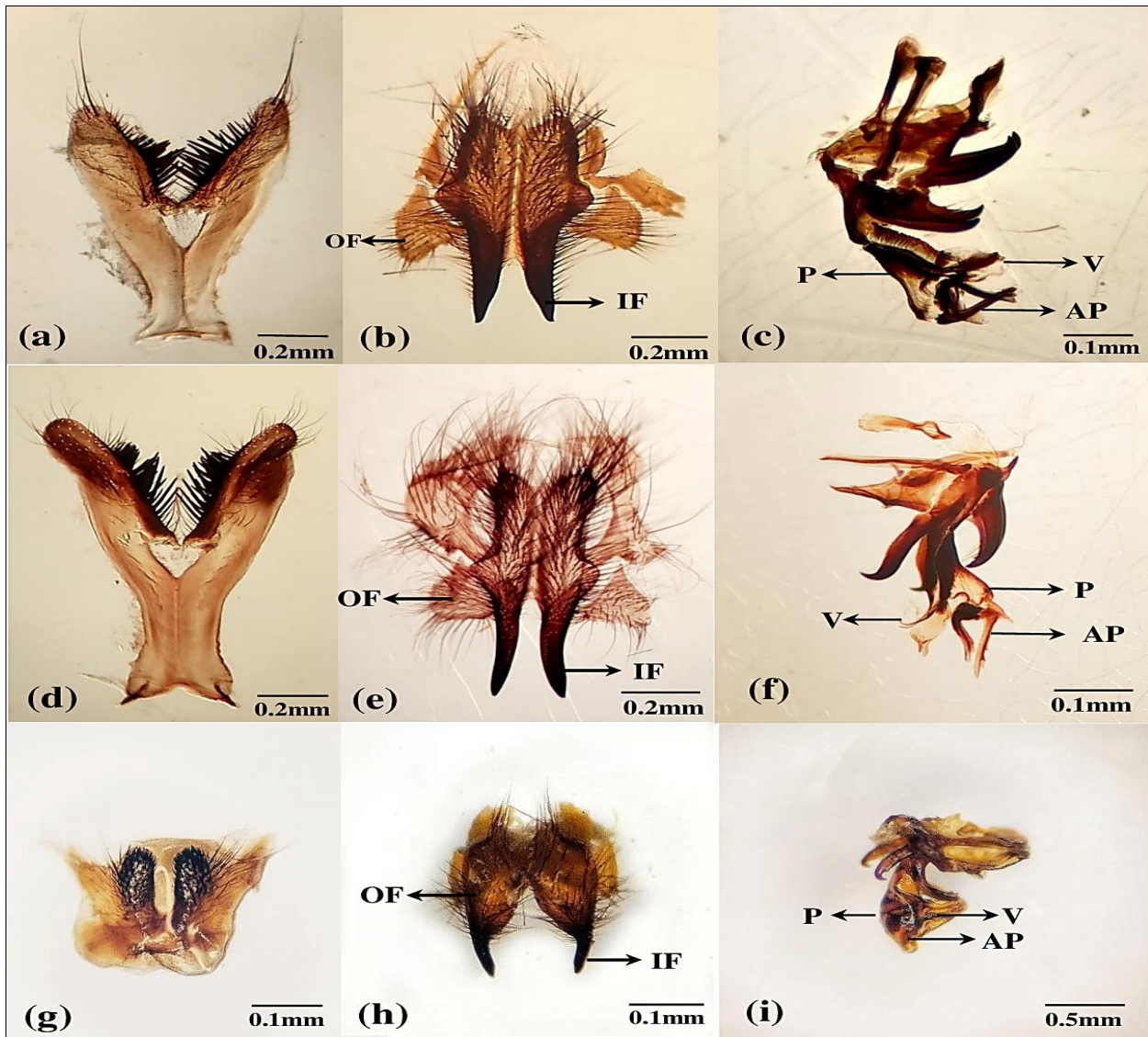


Fig 4: *Sarcophaga (Liosarcophaga) aegyptica*, male terminalia. (a) Fifth sternite, ventral view. (b) inner and outer forcep, caudal view. (c) phallus, lateral view. *Sarcophaga (Liosarcophaga) portschinskyi*, male terminalia. (d) Fifth sternite, ventral view. (e) inner and outer forcep, caudal view. (f) phallus, lateral view. *Ravinia pernix*, male terminalia. (g) Fifth sternite, ventral view. (h) inner and outer forcep, caudal view. (i) phallus, lateral view. Abbreviations: P- paraphallus, AP- apical plate of paraphallus, V- ventrelia.

Conclusion

This study is carried out in the trans-Himalayan region of India, as this part of the country is yet to be explored in terms of scientific research. Five species of flesh flies viz. *Sarcophaga (Berceae) africa* (Wiedemann, 1824), *Sarcophaga (Liopygia) argyrostoma* (Robineau-Desvoidy, 1830), *Sarcophaga (Liosarcophaga) aegyptica* Salem, 1935, *Sarcophaga (Liosarcophaga) portschinskyi* Rohdendorf, 1937 and *Ravinia pernix* (Harris, 1780) belonged to Family Sarcophagidae have been reported for the first time from various river-valleys viz. Suru-valley, Shingo-valley, Drass-valley, part of Indus-valley and Zaskar -valley of Kargil, Ladakh. Since sarcophagids are significant in both medicine and forensics, it is essential to accurately identify them. The species collected are belonging to two Genera viz. *Sarcophaga* and *Ravinia*. In *Sarcophaga*, the first genital segment is without any marginal bristles, styli of glans

present (curved and stout) also theca and paraphallus of penis is distinct while in case of genus-*Ravinia*, the first genital segment is with strong marginal bristles, the styli of glans is absent and there's no distinction between theca and paraphallus of the penis (Nandi, 2002). Out of 5 species, four species are belonging to Sub-genera viz. *Berceae*, *Liosarcophaga* and *Liopygia* under the genus *Sarcophaga*. All these four sub-genera have difference in their Lateral plate of paraphallus (LP), in which Sub-genus *Berceae* comprised with a pair of large sharp LP and in the sub-genus *Liopygia*, LP is rudimentary while in *Liosarcophaga*, LP is with a pair of lamellate processes. In the genus *Ravinia* only one species is collected i.e., *Ravinia pernix*. Several other terminalic characters are also used to differentiate these flesh fly species (as described in the Table 2).

Table 2: Comparison of terminalic characters of flesh fly species.

Species	5 th sternite	Outer-forcep & Inner-forcep	Lateral Plate of Paraphallus (LP)	Styli of glans	Ventrals (V)	Apical Plate of Paraphallus (AP)
<i>Sarcophaga (Berceae) cruentata</i>	Y-shaped Wide window	IF (long, curved, pointed at apex) OF (short and apex; rounded, hairy)	Forms single pair of forwarding processes	Long And curved	Large & Pointed apex	Membranous with a pair of long projecting lateral processes
<i>Sarcophaga (Liopygia) argyrostoma</i>	Y-shaped wide window	IF (straight & wider) OF (oval with long hairs)	Curved with 2 projections	Wide, pair of Strongly sclerotized lateral processes	Bilobed	Heavily sclerotised with short lateral processes
<i>Sarcophaga (Liosarcophaga) aegyptica</i>	Y-shaped prominent window	IF (straight & incised at anterior margin) OF (oval with hairs on distal end)	Leaf like, elongated & transparent	Long and serrated anteriorly	Membranous, wide and slightly Bifurcated at end	With pointed long lateral processes
<i>Sarcophaga (Liosarcophaga) portschinskyi</i>	Y-shaped Prominent window	IF (straight, pointed apically & hairs at sub-basal end) OF (triangular with short hairs)	Transparent, long and wide medially	Sclerotised and long	Sclerotised & curved anteriorly	With distinct apical & 2 pointed lateral processes (One shorter than other)
<i>Ravinia pernix</i>	V-shaped Without window	IF (Elongated and slightly curved at end) OF (elongated and long hairs terminally)	Short and pointed terminally	Absent	Paired and petiolate	Blunt at end

All these species are forensically, and medically important as these species can cause myiasis in both humans and animals and can help in estimating minimum post-mortem interval (PMI_{min}) (Mazen *et al.*, 2007^[18]; Dutto and Bertero, 2010)^[10]. The distribution of these species are altitudinally higher as out of five species, four species viz. *Sarcophaga (Berceae) africa* (Wiedemann, 1824), *Sarcophaga (Liopygia) argyrostoma* (Robineau-Desvoidy, 1830), *Sarcophaga (Liosarcophaga) aegyptica* Salem, 1935, and *Ravinia pernix* (Harris, 1780) are reported from altitudinally higher and colder places of the country in the past viz. Arunachal Pradesh (2432m), Himachal Pradesh (2319m), Jammu and Kashmir (2430m), Sikkim (2019m), Uttarakhand (2012m) and West-Bengal (Darjeeling-2045m) and the species *Sarcophaga (Liosarcophaga) portschinskyi* Rohdendorf, 1937 is reported for the first time from Ladakh (Kargil-2807m) India.

Authentic data of these species are required for the correct identification of flesh fly species. Through this study an initiative has been taken for the correct identification of these five collected species which will provide useful information about the forensically and medically important sarcophagidae species from Ladakh.

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Declarations

Conflict of interest- The authors declare that they have no conflict of interest.

References

1. Al-Khalifa MS, Mashaly AM, Al-Qahtni AH. Insect species colonized indoor and outdoor human corpses in Riyadh, Saudi Arabia. *J King Saud Univ - Sci*, 2020;32(3):1812–1817.
2. Al-Misned FAM. Developmental time, mortality and weight of the immature fleshfly *Berceae cruentata*

- (Diptera: Sarcophagidae) larvae exposed to mercury. *J Agric Sci*,2000:5:63–67.
3. Al-Misned FAM, Amoudi MA, Abou-Fannah SSM. First record of *Sarcophaga* (*Liosarcophaga*) *dux* Thomson, 1868 (Diptera: Sarcophagidae) from Saudi Arabia. *Pak J Zool*,2001:33:313–315.
 4. Ali M, Kumar V, Chandra K. Butterfly Diversity in different Trans-Himalayan Habitats of Ladakh, Jammu and Kashmir. *Proc Himalayan Res Consortium*, 2019, 1-4.
 5. Amoudi MA, Leclercq M, Richet R. Rearing of a newly recorded dipterous fly *Parasarcophaga* (*Liopygia*) *ruficornis* (Fabricius) (Diptera: Sarcophagidae) from Saudi Arabia under laboratory conditions. *Pak J Zool*,1992:24:181–186.
 6. Anderson G, VanLaerhoven SL. Initial studies on insect succession on carrion in Southwestern British Columbia. *J Forensic Sci*,1996:41:617–625.
 7. Bermudez C, Buenaventura R, Couri M, Miranda RJ, Herrera JM. Mixed myiasis by *Philornis glaucinis* (Diptera: Muscidae), *Sarcodexia lambens* (Diptera: Sarcophagidae) and *Lucilia eximia* (Diptera: Calliphoridae) in *Ramphocelus dimidiatus* (Aves: Thraupidae) chicks in Panama. *Bol de la SEA*,2010:47:445-446.
 8. Byrd JH, Castner JL. Forensic entomology – The Utility of Arthropods in Legal Investigation,2nd ed. Boca Raton, FL: CRC Press, 2010:1-16.
 9. Cherix D, Wyss C, Pape T. Occurrences of Flesh Flies (Diptera: Sarcophagidae) on Human cadavers in Switzerland, and their importance as forensic indicators. *Forensic Sci Int*,2012:220:158–163.
 10. Dutto M, Bertero M. Traumatic myiasis from *Sarcophaga* (*Bercaea*) *cruentata* Meigen, 1826 (Diptera, Sarcophagidae) in a hospital environment: reporting of a clinical case following polytrauma. *J Prev Med Hyg*,2010:51:50–52.
 11. Goff ML. A Fly for the Prosecution: Insect Evidence Helps Solving Crimes. London, Cambridge: Harvard University Press, 2000, 1-225.
 12. Graczyk TK, Knight R, Tamang L. Mechanical transmission of human protozoan parasites by insects. *Clin Microbiol Rev*,2005:18:128–132.
 13. Greenberg B. Flies and Disease: Vol. II, Biology and Disease Transmission. New Jersey: Princeton University Press, 1973:1-464.
 14. Guimarães JH, Papavero N. Myiasis in man and animals in the Neotropical Region: Bibliographical database. São Paulo: Editora Plêiades, 1999:1-308.
 15. Mir MH, Aal A, Tak H. New record of *Calliphora* spp. (Diptera: Calliphoridae) from the Trans-Himalayan cold arid desert of Kargil Ladakh. *Munis Entomol Zool*,2021:16(2):1084-1092.
 16. Hagman M, Pape T, Schulte R. Flesh fly myiasis (Diptera: Sarcophagidae) in Peruvian poison frogs genus *Epipedobates* (Anura, Dendrobatidae). *Phyllomedusa: J Herpetol*,2005:4:69–73.
 17. Kurahashi H, Samerjai C. Revised keys to the flesh flies of Thailand, with the establishment of a new genus (Diptera: Sarcophagidae). *Med Entomol Zool*,2018:69(2):67-93.
 18. Mazen NA, Aal AA. A study on some sarcophagid flies (Diptera), causing myiasis in Assiut, Egypt. *Egypt J Exp Biol (Zool)*,2007:3:9-18.
 19. Mello-Patu CA, Luna-Dias C. Myiasis in the Neotropical amphibian *Hypsiboas beckeri* (Anura: Hylidae) by a new species of *Lepidodexia* (Diptera: Sarcophagidae). *J Parasitol*,2010:96:685–688.
 20. Nandi BC. Fauna of India and the adjacent countries-Diptera (Volume X) Sarcophagidae. Kolkata: Director ZSI, 2002a:1-608.
 21. Nandi BC. Fauna of India: Diptera: Sarcophagidae. Kolkata: Zoological Survey of India, 2002b:10:270-274.
 22. Pape T. Catalogue of the Sarcophagidae of the World (Insecta, Diptera). *Mem Entomol Int*,1996:8:1-558.
 23. Pape T. The Sarcophagidae (Diptera) of Fennoscandia and Denmark. *Fauna Entomol Scand*,1987:19:1–203.
 24. Pape T, Dahlem GA, Brown BV, Borkent A, Cumming JM, Wood DM, Woodley NE, Zumbado MA. Sarcophagidae (Flesh flies): Manual of Central American Diptera,2010:2:1297–1335.
 25. Pastula EC, Merritt RW. Insect arrival pattern and succession on buried carrion in Michigan. *J Med Entomol*,2013:50:432–439.
 26. Povolny D, Verves YG. The flesh-flies of central Europe (Insecta, Diptera, Sarcophagidae). *Spixiana Suppl*,1997:24:1–260.
 27. Saigusa K, Takamiya M, Aoki Y. Species identification of the forensically important flies in Iwate prefecture, Japan based on mitochondrial cytochrome oxidase gene subunit I (COI) sequences. *Leg Med*,2005:7:175-178.
 28. Samerjai C, Sukontason KL, Sontgun N, Sukontason K, Klongklaew T, Chareonviriyaphap T, Somboon P. Mitochondrial DNA-Based Identification of Forensically Important Flesh Flies (Diptera: Sarcophagidae) in Thailand. *Insects*,2020:11(1):1-2.
 29. Silahuddin SA, Latif B, Kurahashi H, Walter DE, Heo CC. The Importance of habitat in the ecology of decomposition on rabbit carcasses in Malaysia: implications in forensic entomology. *J Med Entomol*,2015:52:9–23.
 30. Singh D, Bharti M. Some notes on the nocturnal larviposition by two species of *Sarcophaga* (Diptera: Sarcophagidae). *Forensic Sci Int*,2008:177:19–20.
 31. Sinha SK, Nandi BC. *Parasarcophaga* (*Liosarcophaga*) *choudhuryi* sp. nov. (Diptera: Sarcophagidae) from Sagar Island, Sundarbans Biosphere reserve, India. *Rec Zool Surv India*,2002a:100:117–121.
 32. Sinha SK, Nandi BC. A new species of *Lioprocta* *Enderlein* (Diptera: Sarcophagidae) from Sundarbans Biosphere Reserve, India. *Proc Zool Soc Calcutta*,2002b:55(2):39–41.
 33. Szpila K, Mađra A, Jarmusz M, Matuszewski S. Flesh flies (Diptera: Sarcophagidae) colonising large carcasses in Central Europe. *Parasitol Res*,2015:114:2341-2348.
 34. Szpila K, Voss JG, Pape T. A new dipteran forensic indicator in buried bodies. *Med Vet Entomol*,2010:24:278–283.

35. Tomberlin JK, Benbow ME. Forensic entomology international dimensions and frontiers. Boca Raton (FL): CRC Press, 2015:1-468.
36. Vairo KPe, Moura MO, Mello-Patiu CAd. Comparative morphology and identification key for females of nine Sarcophagidae species (Diptera) with forensic importance in Southern Brazil. Rev Bras Entomol,2015;59(3):177-187.
37. Vasconcelos SD, Soares TF, Costa DL. Multiple colonization of a cadaver by insects in an indoor environment: First record of *Fannia trimaculata* (Diptera: Fanniidae) and *Peckia (Peckia) chrysostoma* (Sarcophagidae) as colonizers of a human corpse. Int J Legal Med,2014;128:229–233.
38. Zumpt F. Myiasis in man and animals in the Old World. London: Buterworth's, 1965, 1-267.