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Research Article

The first report of the life cycle of *Sarcophaga (L) dux* on dead reptilian carcass: Their application as forensic indicators

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Abstract: Insects identified on corpses have tremendous application in forensic sciences. In this article, we report, for the first time, in India, the complete life cycle of *Sarcophaga (Liosarcophaga) dux* Thomson (1869), identified on a carrion of the yellow-bellied house gecko (n=5), *Hemidactylus flaviviridis* Ruppel (1835).The life cycle of *S. dux*, on the dead gecko was studied under ambient conditions in the month of May in Kolkata, India, at *38* ± *3*^{0}*C* temperatures, with relative humidity 18-53% and wind speed of 21± 8 km/h. Larval development time to pupation took 200 ± 2 h. The total completion of the life cycle of *S. dux* was studied for ~312 h. The light microscopic morphological study of the adult and the immature males and females confirmed the species *S. dux.* The observed study of the growth and attraction *S. dux* towards the carcass of *H. flaviviridis* could potentially indicate newer ways to prevent reptile poaching. Nearly 6 species of dipterans were identified on the carcass, but this is the first report of *S. dux* on dead gecko. This study will pave its path in the documentation of potential illegal poaching sites of Tokay geckos, being widely traded for extraction of drugs from eastern and north-eastern India.
Keywords: gecko, forensic entomology, reptiles, poaching, Sarcophagidae, India.

INTRODUCTION

Research in the past decade in the field of forensic entomology has advanced, in the identification of insect species on dead organisms. Insects with inbuilt death sensor mechanism, not only are the first species to inhabit the dead organism but also aid in predicting post mortem interval (PMI) in dead. This property of insects has been tremendously exploited in solving human crime as well as wildlife crime control where sometimes other evidences of crime and prediction of PMI is weak.

A forensically important fly is reported to complete its entire life cycle on a dead carcass along with the autolysis, putrefaction, fermentation and diagenesis (dry decay) process in the dead. Carrion-breeding insects, such as flesh flies (Diptera: Sarcophagidae), were less used as evidence in forensic investigations as compared to blow filters. Though there is subdued forensic potential of Sarcophagidae, their use has been limited because its morphological identification at any life stage especially the immature stages, is very challenging.

Forensic importance of fly species including *Sarcophaga (Liosarcophaga) dux* Thomson, *Sarcophaga (Liopygia) ruficornis* (Fabricius), *Sarcophaga albiceps*(Meigen), *Sarcophaga impatiens* (Walker), *Sarcophaga caerulescens* (Zetterstedt), and *Sarcophaga* (Boettcherisca) *peregrina* (Robineau-Desvoidy) have been reported across from Japan, Thailand, Malaysia, Singapore, India, Germany, China, Switzerland, Egypt and Australia [1-6].

Sarcophaga (L) dux Thomson is an arthropod belonging to class Insecta, order Diptera, family Sarcophagidae and subfamily Sarcophaginae. The genus Sarcophaga was first described by Meigen and the subgenus Liosarcophaga was first described by Enderlein. Nandi had re-described the Indian species as *Parasarcophaga (Liosarcophaga) dux* but the present accepted valid name is *Sarcophaga(Liosarcophaga) dux* Thomson [5]. The fly is globally abundant and found in the southern Europe (like France), Oriental region including Thailand, Malaysia, Nepal, Saudi Arabia, Japan, Taiwan, China, Korea, Indonesia), Palaearctic, Australasian and afro-topical regions, neoarctic and neotropical regions all over the world as

well as widely distributed in India (Fig.1) [7-14].This fly is of medical importance as it causes myiasis. It is known to colonize on decomposing human remains, so, it is of forensic importance also. The species has been observed to readily enter human dwellings and larviposit on carrion and garbage [9, 15-17]. *Sarcophaga (L) dux* can be oviparous [14]. *Sarcophaga (L) dux* is synanthropic and breeds in both carrion and faeces in Thailand and is regarded as forensically important fly in Thailand and Japan [7-14, 17].

The growth and development of *Sarcophaga (L) dux* initiating from the first instar larvae, to development into second, third instar larvae, pupae, imago and finally emergence of the adult fly (Figs 1, 2; Table 1) on dead carrion of the yellow-bellied house gecko (n=5), *Hemidactylus flaviviridis* Ruppel is being reported for the first time from India thus establishing the role of *Sarcophaga (L) dux* as a fly of forensic importance from India.

There was multiple colonization of dead carcass *H flaviviridis*. There was colonized by six species of Diptera, three from family Calliphoridae, one from Sarcophagidae and two from Muscidae (unpublished observations). Some adventitious species were also found in and around the dead body. The most abundant species were *Sarcophaga (L) dux* (72 ± 5 % of all emerged adults). We provide here the first record of *Sarcophaga (L) dux* colonizing yellow bellied gecko corpses. We also report the first case of cadaver colonization by *Sarcophaga (L) dux* in India. Information on the development time of *Sarcophaga (L) dux* was used for estimation of the PMI (in minutes) (unpublished observations).

MATERIAL AND METHODS

a. Observation of the dead body and its successive colonization by insect species

Dead yellow-bellied house geckos (n=5), *Hemidactylus flaviviridis* Ruppel found in the Zoological Survey (ZSI) of India, Kolkata premises, was kept in the ambient laboratory conditions. All the five dead geckos were collected from ZSI premises and were maintained in same experimental conditions. The local climate was hot and humid in the month of May. Temperature and relative humidity was measured after each 30 minutes interval. During the experiments, the measured temperature ranges from 25-45^{0}C, relative humidity ranging from 36-40% and wind speed was 13-29 km/h (Table 1) simulating natural environment conditions in Kolkata, India. The rationale of our study was to observe and monitor the growth and development of the forensic flies (especially Sarcophagidae) on the dead organism in the natural environmental conditions. Strikingly, *S. dux* was observed to complete its life cycle on the dead geckos in same experimental condition. The dead geckos (n=5) were monitored for ~330 h even after the completion of the total life cycle of *Sarcophaga (L) dux*, starting from the observation of first instar larvae to the development of adult from pupae. Different stages of insects growing on the geckos were monitored and photographed using the Nikon Coolpix camera model P520.

b. Collection of the fly specimen

Malise-insect trap were used to collect the adult fly. Different developmental stages were observed each hour and specimens were collected in labeled vials. The collected adult insects and their developmental stages were killed with ether and preserved in 70% alcohol. The experiments, taxonomic and morphological identification and quantification were carried out in the above mentioned Laboratory of Diptera Section, Kolkata, India. The meteorological data was collected from the Meterological Department, Alipore, Kolkata.

c. Morphological identification of the different developmental stages of *Sarcophaga (L) dux*

The morphology of *Sarcophaga (L) dux* was observed under light microscope (LM) at each stage in its life cycle. To observe the anatomical feature of the genitalia, both male and female flies were dissected and examined under LM [18].The male genitalia, are the most important characteristic feature used to differentiate different flesh fly species. The abdominal segments between 3rd and 4th segments of the flies were dissected on the clean glass slide using a sharp blade, transferred to a mixture of 10% potassium hydroxide and 95% ethanol for 3 days and following procedures were done as detailed in ref.14, 18. The genitalia and ovipositor were observed under Leica M205 Stereo-zoom dissecting microscope and photographs taken by the allied Leica camera.

RESULTS

The growth and development of *Sarcophaga (L) dux* initiating from the first instar larvae, to development into second, third instar larvae, pupae, imago and finally emergence of the adult fly (Figs. 4, 5) on dead carrion of the yellow-bellied house geckos (n=5) is being reported for the first time from India thus establishing the role of *Sarcophaga (L) dux* as a fly of forensic importance from India. Some unpublished observations under the same experiments were also reported from the same group of authors.

The characteristic features of eggs of S. dux were reported [14]. In our experiments, *Sarcophaga (L) dux* has been observed to larviposit on the carcass and its life cycle is completed in~312h at temperature ranging from 35-41^{0}C, relative humidity ranging from 18-51%, wind speed from 11-29km/h (Table 1) simulating natural environmental conditions in the month of May in Kolkata, indicating that normal growth and developmental rate of stages of the fly on carrion under uncontrolled natural conditions.

We observed that at 26 ± 1h, the first instar larvae emerged from the dead body, emergence of the second instar larvae from the first instar larvae took additional 30 ±1 h, while it took another 100 ± 2 h for the emergence of the third instar larvae. The pupae and imago was observed after 44 ± 2 hand 151 ± 3 h from the third instar larvae (Figs. 1, 2).The entire life cycle of *Sarcophaga (L) dux* on dead geckos were completed in ~312 h.

In order to characterize the identified fly, we have taken LM images of the third instar larvae and the male genitalia (Fig. 3). Features of morphology, genitalia, wings, fore arms of both male and female species confirms the identity of *Sarcophaga (L) dux* (Figs. 3, 4).

Morphology-based identification

As numerous flesh flies species exist in India, information related to morphology of flesh flies is significant for comparison with species, particularly those of medical and forensic importance [7-14]. The wide distribution of *Sarcophaga (L) dux* in different states of India were shown in Fig. 5.The primary step of forensic investigation, involves the gathering of information of all stages *in Sarcophaga (L) dux's* life cycle for proper identification of this organism. Most of the morphological traits come from LM (Figs. 3, 4) and SEM observations (unpublished observations).

a. Description of the morphology of the male and female adult fly

Males were 12 ± 2 mm long [14, 17, 19]. The width of the frons in the head was about 2/3rd that of one eye. Frontal vittae, para-frontal and para-facial were black in colour with silvery to golden dust on it (Fig. 3).Antennae brownish with silvery pollen - like appearance.The1st and 2nd segments of the antennae were blackish-brown, tip of the 2nd segment was reddish and 3rd segment brown in color. Vibrissae and arista were long, plumose along basal 2/3rd of facial ridge (Fig. 3).Palpi slender and black, proboscis long and black. Greyish colored thorax with three longitudinal black stripes. Propleural and prostigamatic bristles well developed with short hairs. Pro- and meso-thoracic spiracles brown; latero-scutellar, apico-scutellar and disco-scutellar bristles were 2, 1, and 1 pair each respectively.

Hyaline wings with brown veins; R_1 bare ;R_{4+5} with a row of 9-10 short setae located dorsally and 3-4 short setae along the ventrally placed basal node. Basicostal scale was yellowish, squama white and brown haltere (Fig. 3). Blackish colored legs, the fore femur with rows of bristles along the postero-dorsal surface and posterior margin of ventral surface. Hind femur has rows of bristles along the antero-dorsal and antero-ventral surface. Mid femur has a row of 4-5 bristles along middle portion of antero-dorsal surface. Fore tibia with a row of 3 bristles, hind tibia with 2 rows of short bristles along antero-dorsal surface (Fig. 3).

Abdomen has blackish and silvery checkered pattern. 1st and 2nd sternites with long hairs, whereas 3rd and 4th sternites with short bristles and hairs (Fig. 3). The v-shaped 5th sternite has short bristles laterally and 1 long hair terminally on arms (Fig.3).

The 1st and 2nd genital segments were reddish in colour with short hairs but without marginal bristles. The inner forceps at the mouth of the genital aperture was almost straight, pointed and slightly curved at end. In contrast, the outer forceps was nearly oval with hairs on distal end. The end of the apical plate of paraphallus was unequal with straight lateral processes. The apically pointed lateral plate of paraphallus was wide and abrupt [14, 17, 19].The male genitalia of *Sarcophaga (L) dux* was observed using SEM for identification of the species (unpublished observations).

Females were 9 ± 2 mm long [14, 17, 19]. The width of the frons in the head was almost same in measurement as in male (Fig. 4).

Apico-scutellar bristles were absent in females. The appearance of the wings and legs were more or less similar as in males. The 2nd and 4th abdominal sternites each has 4 rows of bristles. The 3rd, 5th and 6th abdominal sternite has 6, 8 and 18 rows of bristles respectively. The 7th and 8th abdominal sternite has weakly developed bristles. The nut-shaped anal sternite has short hairs.The 7th tergite has undivided with strong bristles (Fig.4) [14, 17, 19].

b. Description of the morphology of the developmental stages of *Sarcophaga (L). dux*

Morphological features of immature *Sarcophaga (L) dux* revealed distinct morphological parameters of larvae. Ultrastructure of the larval instars of *Sarcophaga (L) dux* was observed using SEM (unpublished observations). To identify the larval stages, special attention was given to the ultrastructure of anterior and posterior spiracles. These spiracles were the important features used to differentiate between different species of Sarcophagidae [14, 17].Other important distinguishing characters noted in SEM were the cephalic region (terminal organs, dorsal organs and ventral organs) and the ventrally curved mouth hooks. The structure of the ventral mouth hooks supports that this fly species as being necrophagous thus capable of causing myiasis. The antero-dorsal process of the cephalopharyngeal skeleton of the first instar larvae was observed under LM. In the first instar larvae, the length of the ventral cornua was less than the dorsal cornua. In the second instar, the length of the ventral cornua was much less than the dorsal cornua. The dental sclerite in the first, second and third instar larvae gradually reduce in size. In third instar larvae, the LM distinguishing

features were the dorsal spines between the first and second thoracic segments [14,17].

Puparia of *Sarcophaga (L). dux* were cylindrical in shape, measuring 9 ± 2 mm in length and 3.5 ± 0.5 mm in width. The pupal respiratory horn and the posterior spiracular disc were observed as distinguishing features in SEM (unpublished observations).

However, no reports exist on the complete life cycle of *Sarcophaga (L) dux* on any dead corpse, carcass or carrion in India. In this research article, for the first time, we report, the complete life cycle of *Sarcophaga (L) dux*, at *~312 h* inhabiting dead geckos, thus stating the forensic importance of the fly species in India.

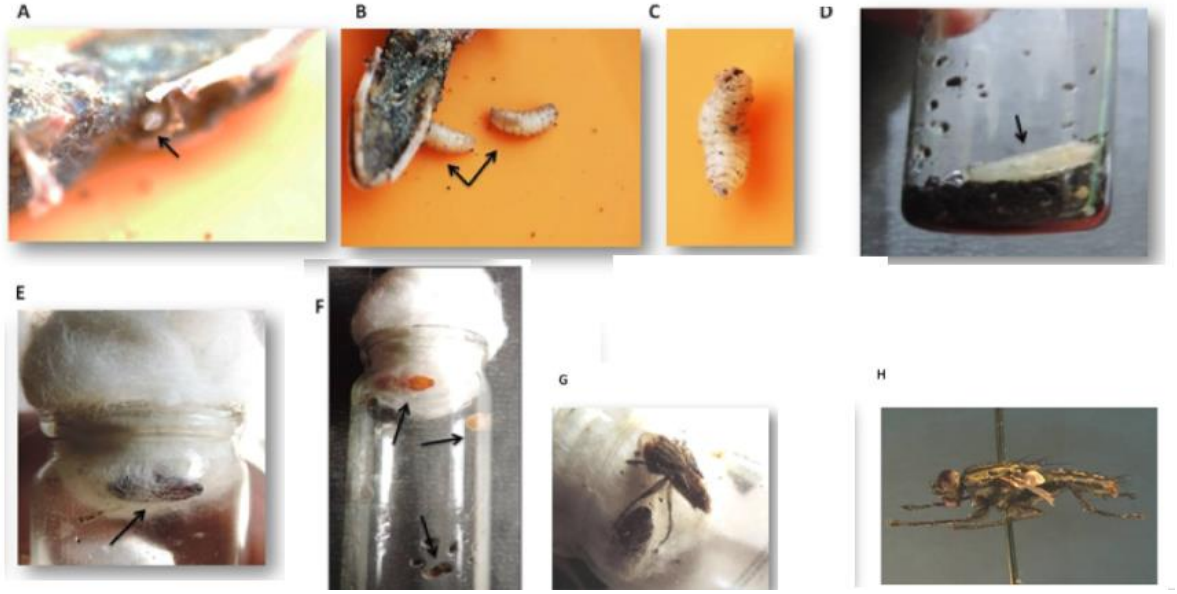

Fig. 1. Different developmental stages of *Sarcophaga (Liosarcophaga) dux* on dead gecko.

A. Emerging 1st instar larva from the dead gecko as marked by arrow, B. 2nd instar larva, C. 3rd instar larva and D. 3rd instar larva kept in a glass vial, E. Pre-pupa kept in a glass vial, F. Pupa kept in a glass vial, G. Imago, H. Emerging adult male were pinned and observed under LM (side view). D-F .All live developmental stages were shown in the glass vial for further monitoring of development in controlled environment. These specimens were preserved for further studies.

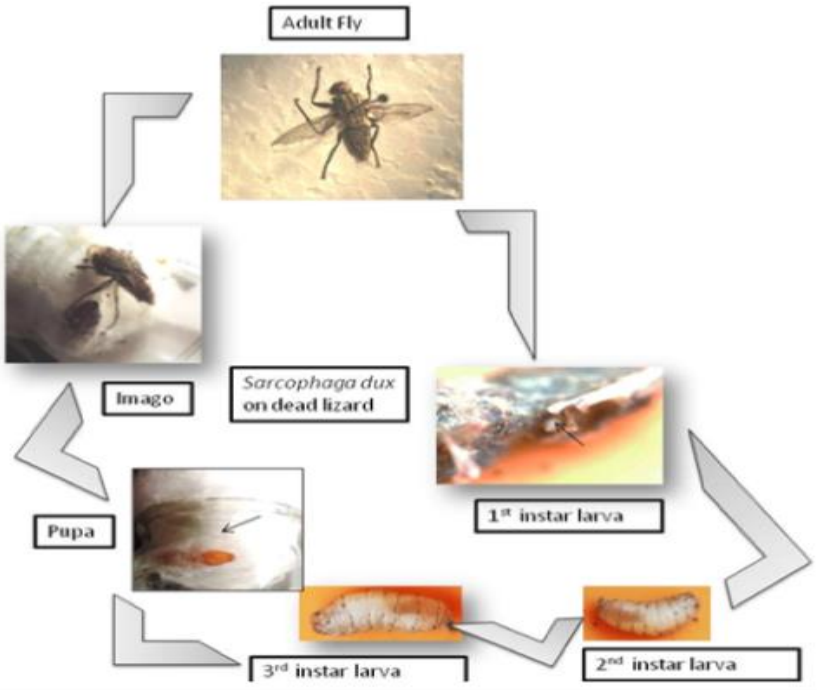

Fig. 2. Life cycle of *Sarcophaga(Liosarcophaga) dux.*

The figure represents the life cycle starting from adult, to different instars, pupae and then imago in a cyclical way.

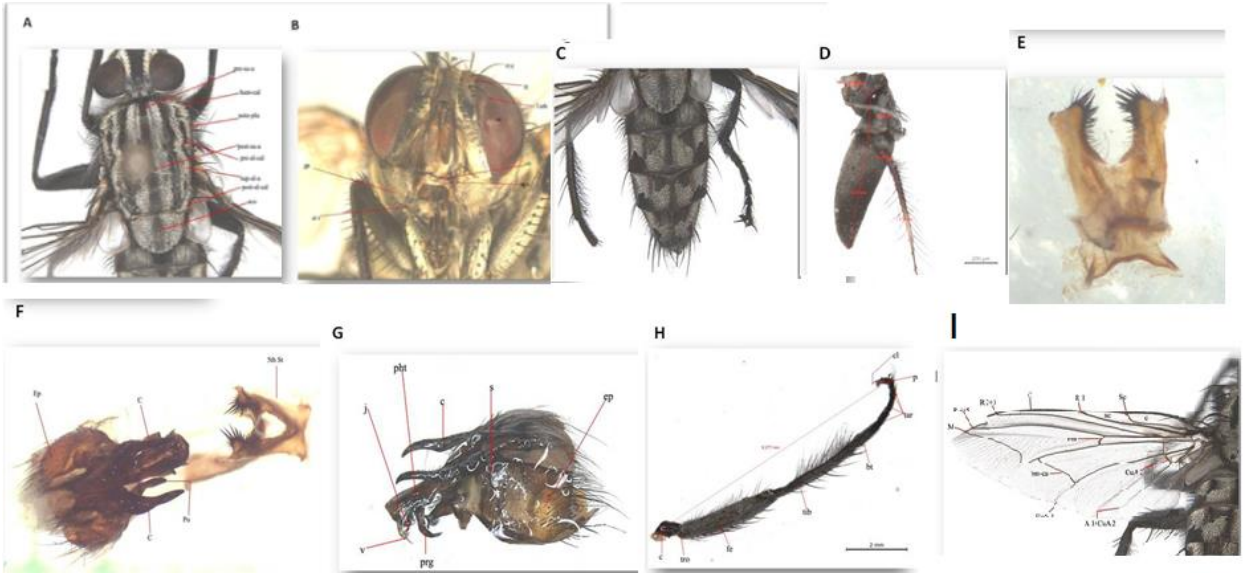

Fig. 3. Morphology of the adult male of *Sarcophaga (Liosarcophaga)dux*

A:Dorsal view of the male *Sarcophaga (Liosarcophaga)dux* :The figure shows the head, thorax, a part of the abdomen and legs of an adult male. **B: Anterior view of the head and mouth parts of the male *Sarcophaga (Liosarcophaga) dux*:** The two prominent ommatidia with antennae and frons, sucking mouth parts are shown in the figure. po: post ocellar; oc : ocellar; vte : outer verticle; fr: interfrontal; f orb: fronto orbital, vb: vibrissae; sp v : supra vibrissal; gn: genal; st v : sub vibrissal. **C: Dorsal view of the abdomen of an adult male:** Variable abdominal markings were visible due to light reflections with light incidence. **D. Antenna of an adult male:** The antenna of an adult male was dissected out with the arista as shown in the figure with measurements as marked. Arista plumose on basal 2/3rd, Sternopleurals 1:1:1. **E-F. Adult male 5thsternite and genital capsule:**The V-shaped 5thsternite of the adult male was dissected out. Bristle like projections are coming out of the 5thsternite as shown in the figure, which is also a confirmatory identifying character of *S. dux* as shown by LM. (**E**).Dissected out genital capsule along with 5thsternite was shown. Praeputium with upper prong of apical fork longer and stronger than lower fork. Surstylus; distiphallus, basiphallus was also observed (**F**) P: phallus;,C:cercus; Ep:epandrium; Po:postgonite. **G. Side view of an adult male Genitalia:** The genitalia of an adult male was dissected and processed as mentioned in material and methods, viewed under LM. c:cercus;ep:epandrium; prg:pregonite; j:juxta; v:vesica ; s:surstylus; pht: phallic tube. **H. Right fore leg of an adult male:**The fore leg was dissected and viewed under LM. c: coxa; tro: trochanter; fe: femur; tib: tibia; bt: basitarsus or first tarsomer, tar: tarsomer (2,3,4,5); pul : pulvillius; cl :claw. **I.Left fore wing of an adult male:**The fore wing was visualized under LM and different parts were noted. c: costal cell; sc: sub costal; Sc: Sub Costal vein; C: Costal vein; R_1: anterior branch of radius; R_{2+3}: radial vein; R_{4+5}: radial vein 4+5; M: media; r-m: radial-medial; bm-cu: basal-medial-cubital; CuA_1: anterior cubital 1; CuA_2: anterior cubital 2; A_1+CuA_2: Ist anal vein+ anterior cubital 2.

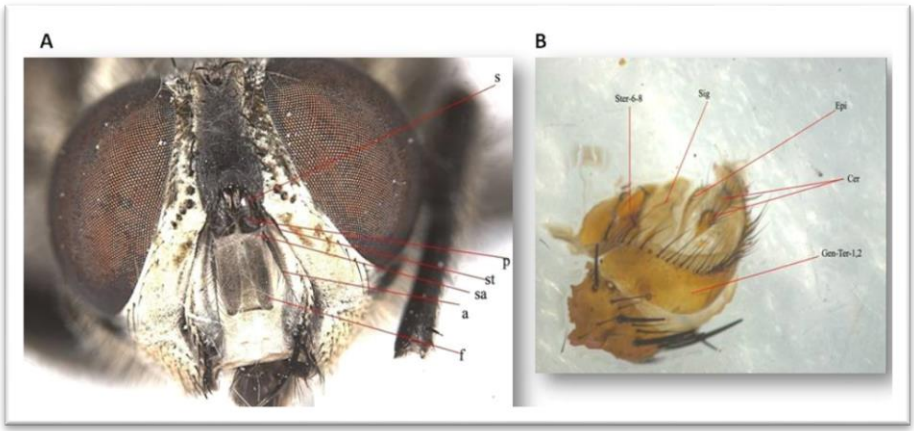

Figure 4: Morphology of the adult female of *Sarcophaga (Liosarcophaga)dux*

A: Front and anterior view of the female *Sarcophaga (Liosarcophaga)dux* head and mouth parts:The front view of the head of an adult female was visualized by LM. f: first flagellomere; a: arista; s:scape; p: pedicel; st: bristle; sa: antennal seam. **B.Female Genitalia:**The female genitalia was dissected out and processed as mentioned in materials and methods. Sternites 6,7and 8,signum,epiproct,cerci and genital tergites 1 and 2 are shown in the figure.Ster-6-8: Sternites 6-8; Sig:Signum; Epi:Epiproct; Cer: Cerci; Gen-Ter-1, 2: Genital tergites 1-2.

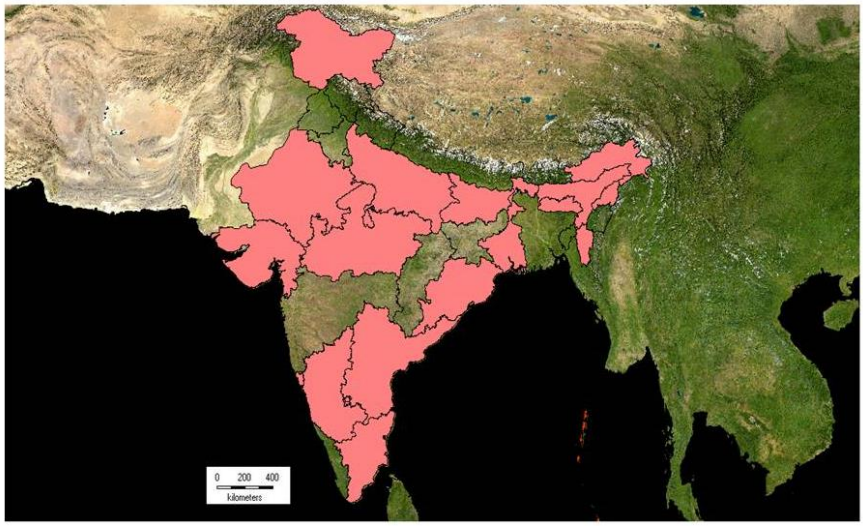

Fig. 5.Distribution of *Sarcophaga (Liosarcophaga) dux* in India.

Sarcophaga (Liosarcophaga) dux is found in the states of West Bengal, Orissa, Rajasthan, Jammu and Kashmir, NE India and in other states as noted by the pink colour in the physical map of India.

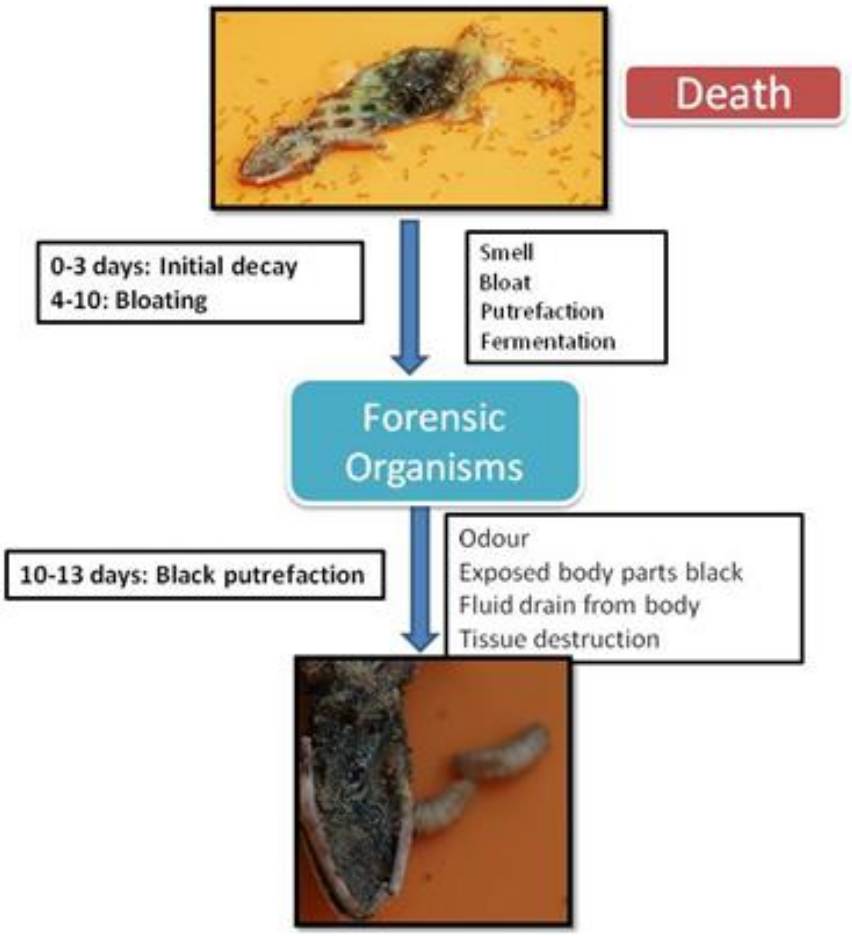

Fig.6. Death and decomposition of dead gecko

The stages of decomposition of a representative dead gecko (n=5) was shown in the figure starting from fresh/initial decay (autolysis), putrefaction or bloating and black putrefaction occurs with the advent of forensic flies.

Table: 1.[*] Meteorological Data and development of *Sarcophaga dux* on dead lizard (n = 5).

NO OF DAYS	TIME (h)	AVERAGE TEMPURATURE (°C)	AVERAGE HUMIDITY %	PRECIPATATION (mm of Hg)	WIND SPEED (Km/h)	DEVELOPMENTAL STAGES
1	24	38 ± 2	44± 1	0	29± 0	No instar larvae seen
2	48	36± 1	49± 2	0	27± 1	1st instar larvae-26 ± 1 h
3	72	37± 1	45± 1	0	22±1	2nd instar larvae-56 ± 1 h
4	96	39± 2	39± 2	0	13±2	3rd instar larvae - 156 ± 2 h
5	120	38± 2	37± 1	0	24±3	
6	144	36± 1	32± 2	0	21±1	
7	168	38± 1	26± 3	0	19±2	
8	192	38± 2	27± 2	0	19±1	Pupae200 ± 2h
9	216	39± 3	30± 1	0	19± 2	
10	240	40± 1	26± 1	0	14±3	Imago 307 ± 3h
11	264	40± 1	28± 1	0	13± 0	
12	288	39± 2	19± 1	0	18±1	Adult 312 ± 3 h
13	312	38± 1	25± 2	0	22± 2	

[*] *S. dux* at 38 ± 3 °C took ~13 days to complete its life cycle.

DISCUSSION

Nearly 2500 fly species comprise the Sarcophagidae family worldwide. The use of dipteran Sarcophagidae for PMI estimation is restricted in forensic entomology as compared to Calliphoridae because morphological identification is often dampened because of similar features in the larval, pupal, and even adult stages. For more accurate and reliable species identification, the DNA-based identification should be tagged with morphological identifications [13-14, 17](unpublished observations).

A decomposing body with its huge resources attracts and colonized by both animals and plants (Fig. 6) and is totally decayed. The body not only provides a food source, but also serves as the niche to reproduce in ambient conditions. However there are only a few species of obligate forensic flies who complete their life cycle on a corpse or a carcass. Our study on the carcass of the yellow-bellied house geckos confirmed for the first time that *Sarcophaga (L) dux* in India was not only attracted by a dead reptile (Figs. 1, 2, 3, 4, 6) but also could complete its life cycle on the reptile carrion under natural conditions. The study also hinted the volatile organic compounds released from the dead gecko could be sensed by the sarcophagid flies; however the nature of the volatiles remains a future scope of this study.

Morphologically and developmentally, the adult male characteristics and the third instar larvae respectively are key characters to identify this species [1, 14, 17]. In Thailand, *Sarcophaga (L) dux* has been reported to be synanthropic, breeding on both faeces and carrion [8]. The ventral mouth hooks of larvae observed under SEM [14] revealed the importance of this necrophagous fly as a myiasis-causing agent (unpublished observations). The sarcophagid, *Blaesoxipha plinthopyga* was first reported from a human corpse in U.S.A [17].

However different observations have been reported for the developmental rates of the S. dux. Life cycle of *Sarcophaga (L) dux* from the first instar to the adult is 312.0 ± 3.0 h under un-controlled indoor temperatures has been reported in Malaysia [20].Reports from a study in north Thailand in the year 2002-2003 under natural ambient temperature (approximately 24-28^{0}C) and a natural light/dark photoperiod (~ 12:12 h), indicated seasonal variation in development of fly with rapid larval development in summer, at around 72 h, while it took 72-96 h in the rainy season, and 96 h in winter [1,21]. Reports from Spain on the life cycle of Sarcophaga cultellata Pandellé, 1896 studied at 25^{o}C and 50% relative humidity indicated a total development time from larviposition to first adult emergence for 330 ± 12 h [22]. The only report from India on the development of larvae of Sarcophaga (Liosarcophaga) tibialis Macquart (1851), raised on chicken liver indicated maximum development between 15-30°C [12,14,19,23-25].

Even though numerous flesh fly species exist in India, we report for the first time, one species of Sarcophagidae to complete a life cycle on a reptilian carcass. Morphological characterization and information on all the different immature stages of the life cycle and observation under LM of genitalia, wings, fore limbs, antenna of both male and female adult stages confirms the identification of *Sarcophaga (L) dux* (Figs. 3, 4),which is the primary step in a forensic investigation.

The rationale behind our study was to observe whether *Sarcophaga (L) dux* is a mere visitor on a carcass or an obligate species of forensic importance who could sense death in a reptile under natural circumstances. We report the complete life cycle of *Sarcophaga (L) dux* in ~312h, (Fig.6) under normal environment conditions in the month of May in Kolkata, India. Our observations provoked further

questions which remains the future scope of the study. The challenge ahead seems that since Sarchophaga (L) dux is the first and the only sarcophagid observed arouses questions like- is it a species of restricted attraction, i.e., could it be the only flesh fly that senses reptilian death, if so then, are there any specific volatile chemical compounds which draws the *Sarcophaga (L) dux* to the reptile carcass and not any other commonly abundant sarcophagids? This remains the scope of our future studies.

Our observations would find application in forensic studies on the Tokay *Gecko (*Gekko gecko *Linneaus 1758)* which is one of the largest geckos in the country being illegally traded in the international market for its medicinal properties from the eastern and north-eastern India. The rapidly depleting Tokay gecko population due to extensive poaching and illegal trading in the eastern and north eastern part of India has already triggered concern in the Wildlife Crime Control Bureau, India. Our current observations may throw light upon identification of probable poaching sites and specify the time of death during their illegal transit thus helping in wildlife crime control.

Conflict of Interest: The authors hereby declare no conflict of interest.

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