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# Occurrence of necrophagous flies of forensic importance in medico-legal cases in Tamil Nadu State, India

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## Abstract

**Background:** The present study is based on the necrophagous fly samples collected from 24 medico-legal cases between the year 2011 and 2018 in Tamil Nadu State, India. The fly life stages were identified based on morphological features. Pre-autopsy condition of the human corpse colonized by necrophagous flies and indoor/outdoor occurrence of the flies were recorded.

**Results:** *Chrysomya megacephala*, *Chrysomya rufifacies*, *Sarcophaga* spp, and *Musca domestica* life stages were collected from the human corpses. *Chrysomya megacephala* was the most prevalent (70.8%) insects of forensic importance and found both in indoor and outdoor environments. Drowned and burnt human corpses were found to be only colonized by *C. megacephala*. *Chrysomya rufifacies* was found only in outdoor environments and *Sarcophaga* spp was found only in indoor environment. There was a fair agreement between the percentage occurrence of necrophagous flies in human corpse in the present study in Tamil Nadu State and percentage historical occurrence of necrophagous flies in human corpse in India.

**Conclusions:** *Chrysomya megacephala* was the predominant blowfly species found to colonize corpses in Tamil Nadu State, India. *Chrysomya megacephala* was the only blowfly species found to colonize both burnt and floating corpses and corpses located indoor and outdoor.

**Keywords:** Forensic entomology, Necrophagous flies, *Chrysomya megacephala*, *Chrysomya rufifacies*, *Sarcophaga* spp, and *Musca domestica*

## Background

Life stages of necrophagous flies collected from human corpses are being used for estimating minimum post-mortem interval (PMI<sub>min</sub>) (Kashyap and Pillai 1989; Wells and LaMotte 2001). Colonizing the corpse or carcass for survival and breeding are the natural tendency of necrophagous flies. The common necrophagous flies are

placed under the order: Diptera, including Calliphoridae, Sarcophagidae, and Muscidae families, which are well known as forensically important flies (Anderson and VanLaerhoven 1996; Byrd and Castner 2010). As insects of forensic importance differ across geographical regions, various seasons, indoor and outdoor locations, the insect fauna collected from various death scenes add to datasets that can be applied to countries with similar landscape or climatic conditions. There are few reports on incidence of insects of forensic importance in India; however, the reports from Tamil Nadu State are scarce (Singh and Bharti 2000; Nandi 2002; Ramaraj et al. 2014; Bharti and Singh 2017; Shinde et al. 2021; Babu et al. 2022). Further,

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systematic prevalence study on necrophagous flies of forensic importance collected from medico-legal cases or human corpses are not available in India. There were few medico-legal case reports associated with necrophagous flies published in India (Kashyap and Pillai 1989; Singh et al. 2004; Suri Babu et al. 2013; Sharma and Bala 2016; Bala and Sharma 2016; Sharma et al. 2018; Babu et al. 2022). However, there are no published reports on necrophagous flies colonizing human corpse available from several Indian states, including Tamil Nadu. Tamil Nadu is the southernmost state of India which is bordered by the states Andhra Pradesh to the north, Karnataka to the northwest, and Kerala to the west and the Bay of Bengal and Indian Ocean to the east and south. Tamil Nadu is the only state in India that has both the Western Ghat and the Eastern Ghat mountain ranges meet at one point. The climate of Tamil Nadu is essentially tropical with hot and humid summer months. Documentation of various insect species of forensic importance in this geographical region will strengthen the forensic insect database and help the forensic entomologists to understand the prevalence of different insect species native to this region as well as invasive insects. This report is the first comprehensive report on the occurrence of different necrophagous flies of forensic importance collected from various medico-legal cases in Tamil Nadu, India, that were referred to Department of Veterinary Parasitology, Madras Veterinary College, Chennai, between the year 2011 and 2018.

## Methods

### Insect specimen

Different life stages of necrophagous flies were collected from medico-legal cases by a forensic pathologist from Department of Forensic Medicine, Government Kilpauk Medical College, Chennai, Tamil Nadu. All the samples were collected between the year 2011 and 2018. Dead insect samples in 80% ethanol and live insect stages in a glass jar with muslin cloth covering the open end were transported to Department of Veterinary Parasitology, Madras Veterinary College, Chennai, in environmental temperature within 1 to 6 h of sample collection.

### Identification of necrophagous fly life stages

The larval stages were identified based on the posterior and anterior spiracle structures, and spines on body (Sawaby et al. 2018). The adult flies were identified based on the morphological features such as unique abdominal pattern, body color, color of anterior thoracic spiracle, pattern of eye facets, wing venation, proepisternal seta, and vertical setae on head (Sawaby et al. 2018). The larval stage of *Chrysomya megacephala* was identified based on the posterior spiracle with incomplete peritreme and

three straight stigmal slits and anterior spiracle with 11–13 fingers like projections (Sukontason et al. 2004). The adult *C. megacephala* was identified based on dark brown color anterior thoracic spiracle and smaller facets in the lower quarter of the eye of adult male flies (Sawaby et al. 2018; Prins 1982). The eggs of *Chrysomya* spp fly were identified by the presence of narrow 'Y'-shaped plastron. The larval stages of *Chrysomya rufifacies* were identified based on spiny maggots, posterior spiracle with incomplete peritreme and three straight stigma slits and anterior spiracle with 10 fingers like projections (Sukontason et al. 2004). The adult *C. rufifacies* was identified based on pale or white color anterior thoracic spiracle, presence of proepisternal seta, presence of inner and outer vertical setae, and absence of clear line of demarcation between larger and smaller sized facets in the eye of adult male flies (Sawaby et al. 2018; Silva et al. 2012; Grella et al. 2015). *Sarcophaga* spp adult flies were identified by the checkerboard appearance on the abdomen of the adult flies and larval stages were identified by kidney-shaped posterior spiracle with straight stigma slits directed away from peritreme opening (Jordaens et al. 2013; Ren et al. 2018). L3 larva of *Musca domestica* was identified based on 'D'-shaped posterior spiracles and 'M'-shaped stigma slits (Shinde et al. 2021). When immature life stages were sampled, the identification of the fly life stages was doubly confirmed morphologically by identifying the larval stages and the emerged adult flies.

### Laboratory rearing of necrophagous fly life stages

The live insect stages, like larval and pupal stages were reared at room temperature. The immature larval stages such as L2 and feeding stage of L3 were placed on a 90 mm petri dish containing minced beef of 5 mm thick and the petri dish was placed inside a 2000-ml glass beaker (Borosil®) half filled with hot air oven sterilized dry garden soil and covered with muslin cloth. Every 24 h, a layer of fresh minced beef was added into the petri dish to prevent drying of the larval diet. The petri dish with immature larval stages and minced beef were placed on the dry soil inside the glass beaker in order to facilitate the movement of post-feeding L3 stage larvae in to the dry soil for pupation. The samples received as pupal stages were directly placed inside the dry soil and maintained until they emerged as adult flies. The immature life stages of the necrophagous flies were reared in the laboratory until the emergence of adult fly.

### Historical distribution of necrophagous fly species in India and agreement statistics

Published reports on the occurrence of necrophagous fly species in human corpses in India associated with

medico-legal cases were collected and summarized. The location, time of collection, indoor/outdoor occurrence, gender and age of the human corpse and the fly species were extracted from seven published reports and the historical percentage occurrence of the necrophagous fly species in India was calculated.

Agreement statistics between the percentage occurrence of necrophagous fly species in human corpses in Tamil Nadu State (2011–2018) and historical percentage occurrence of necrophagous fly species in human corpses in India (1985–2018) and the inter-rater agreement were calculated using online statistical software (<http://vassarstats.net/>).

## Results

### Morphological identification of necrophagous fly species

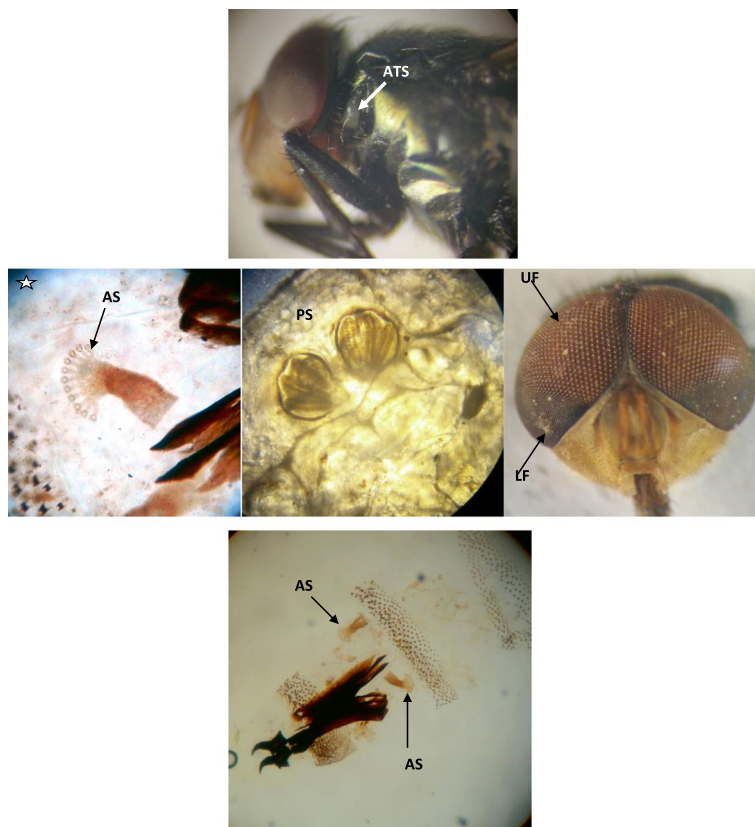
*Chrysomya megacephala*, *C. rufifacies*, *Sarcophaga* spp, and *Musca domestica* life stages were collected from the human corpses between the year 2011 and 2018. The collected fly life stages were identified based on gross and microscopic morphological features (Figs. 1, 2, 3, 4, and 5). Details on location coordinates, life stage of

necrophagous flies collected from medico-legal cases, and pre-autopsy conditions of corpse are given in Table 1.

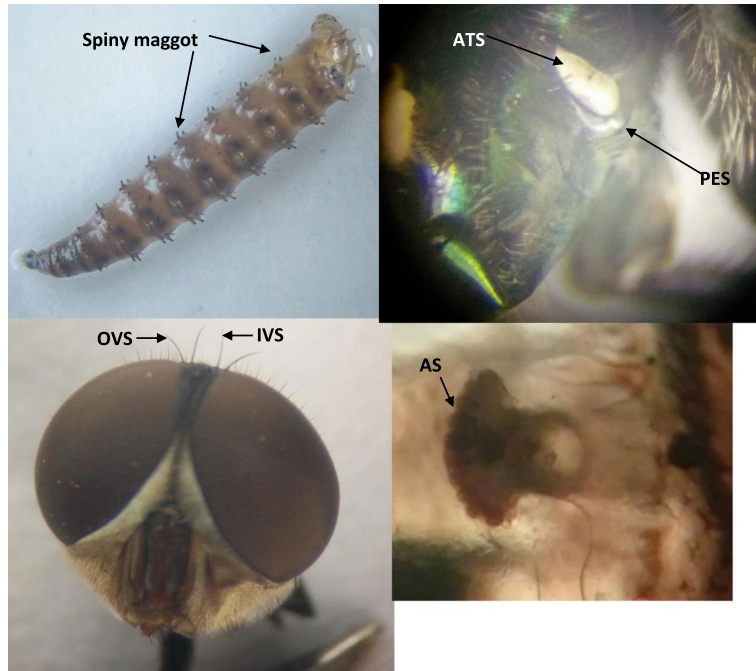
### Frequency of occurrence of necrophagous fly species

*Chrysomya megacephala* was the most prevalent (70.80%) necrophagous flies of forensic importance found in Tamil Nadu State, India, followed by *C. rufifacies* (12.5%), *Sarcophaga* spp (12.5%), and *M. domestica* (4.2%) (Fig. 6). *Chrysomya rufifacies* fly samples were found in only in outdoor environment. *Sarcophaga* spp and *M. domestica* flies were found only in indoor environment. *Chrysomya megacephala* fly samples were found in both indoor and outdoor environments. However, among the *C. megacephala* fly samples, 65% of them were found only in indoor environments (Fig. 7) (Table 2).

Drowned/floating and burnt human corpses were found to be only colonized by *C. megacephala*. *Chrysomya megacephala* was found in human corpses of diverse pre-autopsy conditions such as corpses with traumatic injury, drowned/floating corpses, corpses in hanging condition, burnt, and highly decomposed corpses (Fig. 8) (Table 2).



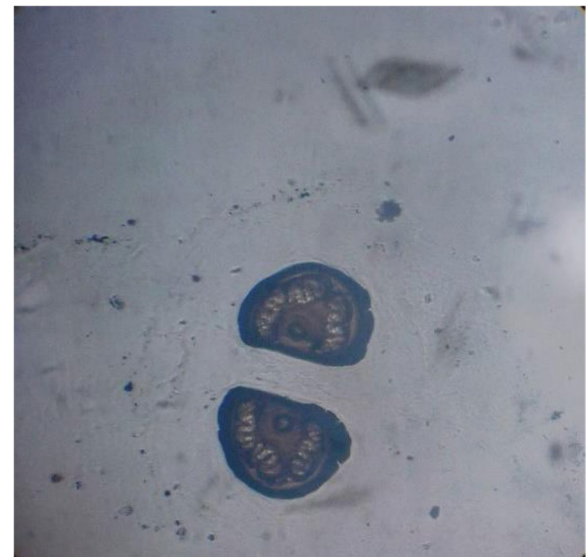
**Fig. 1** Gross and microscopic morphological features of Male *Chrysomya megacephala* (all the pictures were  $\times 100$  magnified except the star marked picture  $\times 400$  magnification\*). ATS—anterior thoracic spiracle (dark brown); AS—anterior spiracle (11 finger projections); PS—posterior spiracle (incomplete preitreme, straight stigmal slits, no button); LF—lower eye facets; UF—upper eye facets



**Fig. 2** Gross and microscopic morphological features of male *Chrysomya ruffacies* (× 100 Magnification). ATS—anterior thoracic spiracle (pale or white); PES—proepisternal seta; OVS—outer vertical seta; IVS—inner vertical seta; AS—anterior spiracle (10 finger projections)



**Fig. 3** Microscopic features of posterior spiracle of *Sarcophaga* spp (× 100 magnification). Arrow mark: posterior spiracle inside deep cavity; incomplete peritreme lacks button; stigmatal slits directed away from peritreme opening



**Fig. 4** Microscopic features of posterior spiracle of *Musca domestica* (× 100 Magnification). 'D'-shaped posterior spiracle with "M" shaped stigmatal slits

**Agreement between present study and the historical prevalence data**

Total 32 necrophagous fly species life stages were reportedly collected from 26 human corpses associated



**Fig. 5** Microscopic features of *Chrysomya megacephala* eggs found on the hair of a corpse ( $\times 400$  magnification). Y—'Y'-shaped and narrow plastron of the *Chrysomya megacephala* egg

with medico-legal cases between 1985 and 2018 (Table 3). These samples were collected from 5 Indian states viz. Andhra Pradesh, Karnataka, Punjab, Madhya Pradesh and Chhattisgarh. The percentage occurrence of necrophagous fly species in human corpse viz., *Calliphora erythrocephala*, *Chrysomya megacephala*, *Chrysomya rufifacies*, *Sarcophaga* spp, *Chrysomya albiceps*, and *Phormia regina* from published reports was 25%, 21.9%, 21.9%, 21.9%, 6.2%, and 3.1%, respectively (Table 3).

Occurrence of *Musca* spp in human corpses was not reported in India in the published reports. Occurrence of *Calliphora erythrocephala*, *Chrysomya albiceps*, and *Phormia regina* life stages in human corpses was reported in the published studies. However, these fly species were not found in the present study in Tamil Nadu State. The inter-rater agreement between the occurrence of necrophagous fly species in Tamil Nadu (2011–2018) and historical occurrence in India (1985–2018) is 75%. Out of 4 necrophagous fly species reported in the present study, 3 fly species were reported in historical necrophagous fly species distribution data. There was a fair agreement ( $\kappa = 0.273 \pm 0.051$ ) between the percentage occurrence of necrophagous fly species in human corpses

in the present study (2011–2018) and published reports (Table 4).

## Discussion

Collection and identification of insects of forensic importance, prevalence, and regional distribution of insects of forensic importance, ethology of insects of forensic importance, entomotoxicology, and estimation of post-mortem interval are the components of forensic entomology that help the investigation agencies not only to estimate the date and time of death, but also provide other evidences to trace the cause of death and the perpetrator of the crime. Knowledge on the biodiversity of the insect fauna of forensic importance helps the entomologists to identify the insects quickly and to know the invasive insects of forensic importance (Jeong et al. 2022). Understanding the necrophagous flies colonizing human corpses in Tamil Nadu State which has a unique geographical location and tropical climate would be a very useful information for the forensic entomologists. Understanding the local distribution pattern of insect fauna of forensic importance also helps in medico-legal cases where the corpse was relocated from the site of death. Although relocation of corpse from the scene of crime or site of death can be identified by sampling non-native insect species from the corpse, wider distribution of blowfly species across several agro-climatic regions and occurrence of blowfly species outside the preferred ecological niche make it highly unlikely (Charabidze et al. 2017; Zabala et al. 2014).

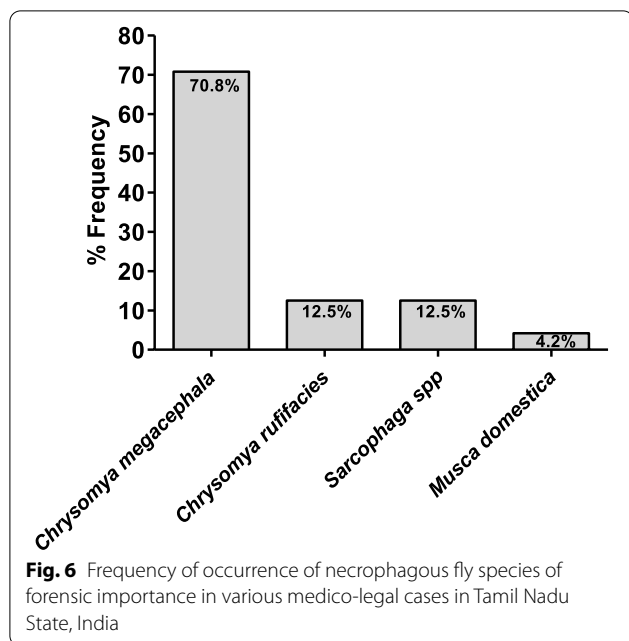
*Chrysomya rufifacies* adult flies were morphologically distinguished from related species such as *C. albiceps* and *C. putoria* by the presence of proepisternal seta and outer vertical seta, respectively (Silva et al. 2012). *Chrysomya megacephala* was found to be the most predominant necrophagous fly species of forensic importance in Tamil Nadu State, India. This finding is in agreement with the other published reports in India and elsewhere. A study by Kumara and co-workers found that *C. megacephala* was the most prominent fly species of forensic importance in Malaysia followed by *C. rufifacies* and *Sarcophaga* spp and that matches with the findings of our study in India (Kumara et al. 2012). *Chrysomya megacephala* and *C. rufifacies* fly species were the widely distributed insects of forensic importance in India (Wall and Howard 2001; Verma 2013; Bala and Singh 2015). *Chrysomya megacephala* was reported to be one of the most common blowfly species of forensic importance in many parts of the world as well as in India. It has been reported in Asia, Africa, Australia, North America, South America, Central America, and parts of Europe (Wells 1991; Kumara et al. 2012; Ramaraj et al. 2014; Bala and Sharma 2016; Sontigun et al. 2018). *Chrysomya megacephala*

**Table 1** Details on location coordinates, month and season of collection, oldest stage of necrophagous flies collected from medico-legal cases, and pre-autopsy conditions of corpse

Case no.	Blowfly species (location coordinates)	Month, year of sample collection and season	Oldest life stage of fly collected	Indoor/outdoor	Pre-autopsy status of corpse
1.	<i>Chrysomya megacephala</i> 13.1210° N, 80.2326° E	June, 2011/Southwest monsoon	L3	Outdoor	Drowned/floating
2.	<i>Chrysomya megacephala</i> 13.1488° N, 80.2306° E	November, 2014/Northeast monsoon	L3	Outdoor	Traumatic injury
3.	<i>Chrysomya megacephala</i> 12.9249° N, 80.1000° E	December, 2014/ Northeast monsoon	L3	Indoor	Traumatic injury
4.	<i>Chrysomya megacephala</i> 13.0850° N, 80.2101° E	January, 2015/Winter	L2	Indoor	Hanging
5.	<i>Chrysomya megacephala</i> 13.0850° N, 80.2101° E	January, 2015/Winter	L2	Indoor	Hanging
6.	<i>Chrysomya rufifacies</i> 13.1067° N, 80.0970° E	December, 2014/Northeast monsoon	Pre-emergent adult	Outdoor	Hanging
7.	<i>Chrysomya megacephala</i> 13.0270° N, 80.1107° E	March, 2015/Summer	L3	Indoor	Hanging
8.	<i>Chrysomya megacephala</i> 13.0101° N, 80.1492° E	April, 2015/Summer	L3	Outdoor	Traumatic injury
9.	<i>Chrysomya megacephala</i> 13.0698° N, 80.2245° E	May, 2015/Summer	L3	Indoor	Burnt/ charred
10.	<i>Chrysomya megacephala</i> 11.3806° N, 77.8944° E	June, 2015/Southwest monsoon	L3	Outdoor	Traumatic injury
11.	<i>Musca domestica</i> 13.0837° N, 80.1750° E	July, 2015/Southwest monsoon	Pupa	Indoor	Highly decomposed
12.	<i>Sarcophaga</i> spp 13.0837° N, 80.1750° E	July, 2015/Southwest monsoon	Pupa	Indoor	Highly decomposed
13.	<i>Sarcophaga</i> spp 13.0656° N, 80.1608° E	June, 2015/Southwest monsoon	L3	Indoor	Highly decomposed
14.	<i>Chrysomya rufifacies</i> 12.8866° N, 80.0216° E	November, 2016/Northeast monsoon	L3	Outdoor	Traumatic injury
15.	<i>Sarcophaga</i> spp 13.0656° N, 80.1608° E	November, 2016/Northeast monsoon	L3	Indoor	Traumatic injury
16.	<i>Chrysomya megacephala</i> 12.9802° N, 80.1265° E	February, 2017/Winter	L3	Outdoor	Burnt/charred
17.	<i>Chrysomya megacephala</i> 13.1067° N, 80.0970° E	November, 2014/Northeast monsoon	Pupa	Outdoor	Traumatic injury
18.	<i>Chrysomya megacephala</i> 13.0576° N, 80.1545° E	January, 2016/Winter	L3	Outdoor	Drowned/floating
19.	<i>Chrysomya megacephala</i> 13.0925° N, 80.1372° E	April, 2016/Summer	L3	Outdoor	Drowned/floating
20.	<i>Chrysomya megacephala</i> 13.1014° N, 80.2704° E	December, 2016/Northeast monsoon	Pupa	Outdoor	Drowned/floating
21.	<i>Chrysomya megacephala</i> 13.1143° N, 80.1548° E	April, 2017/Summer	L3	Outdoor	Drowned/floating
22.	<i>Chrysomya megacephala</i> 13.0837° N, 80.1750° E	July, 2017/Southwest monsoon	Pre-pupa	Indoor	Highly decomposed
23.	<i>Chrysomya rufifacies</i> 13.1210° N, 80.2326° E	December, 2017/Northeast monsoon	L3	Outdoor	Traumatic injury
24.	<i>Chrysomya megacephala</i> 13.0925° N, 80.1372° E	April, 2018/Summer	L3	Outdoor	Drowned/floating

reportedly inhabits tropical and temperate climates and was found to be distributed between Eastern Siberia, at about 50°N to Argentina at about 50°S (Badenhorst and Villet 2018).

*Chrysomya megacephala* was sampled in both indoor and outdoor environments. Though the number of samples was less, *C. rufifacies* samples were found only in outdoor and *Sarcophaga* spp and *M. domestica* samples



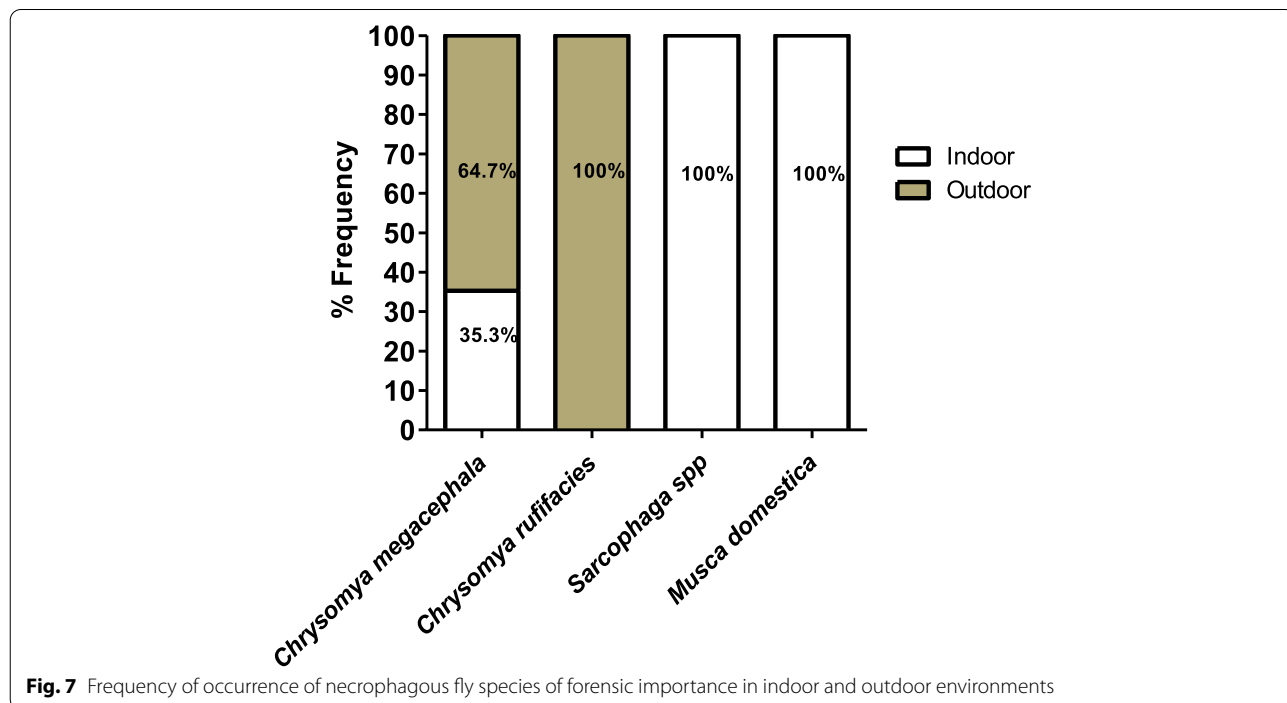
**Fig. 6** Frequency of occurrence of necrophagous fly species of forensic importance in various medico-legal cases in Tamil Nadu State, India

were found only in indoor environments. There were no previous studies in India that investigated the insect species colonizing human corpse/animal cadaver indoor and outdoor conditions. The frequency and dominance of occurrence of *Sarcophaga* spp flies on human corpse were recorded as high in indoor environments compared to outdoor in Malaysia which suggests the preference of

indoor colonization of corpse by *Sarcophaga* spp flies in the tropical climate countries such as India and Malaysia (Kumara et al. 2012). Several experimental studies using animal cadavers suggested more blowfly species colonizing outdoor cadaver than indoor cadaver and delay in blowfly colonization was found in the indoor conditions (Cainé et al. 2009; Reibe and Madea 2010; Cammack et al. 2016). Though there are a greater number of insect species found colonizing the cadaver in outdoor compared to indoor, none of the insect species considered exclusively indoor (Frost et al. 2010).

Blowfly species were reported to colonize the burnt or charred cadavers in same time as the non-burnt control cadavers suggesting PMI estimation for burnt corpses/cadavers is no different from non-burnt corpse/cadavers. *Chrysomya megacephala* was the only fly species found to colonize the burnt human corpses in this study (Vanin et al. 2013; Mahat et al. 2016). *Chrysomya megacephala* flies were reported to preferably colonize burnt pig cadaver compared to unburnt cadaver and *C. megacephala* and *C. rufifacies* were found to colonize burnt human corpse, burnt pig and rabbit cadavers (Pai et al. 2007; Oliveira-Costa et al. 2014; Mahat et al. 2016).

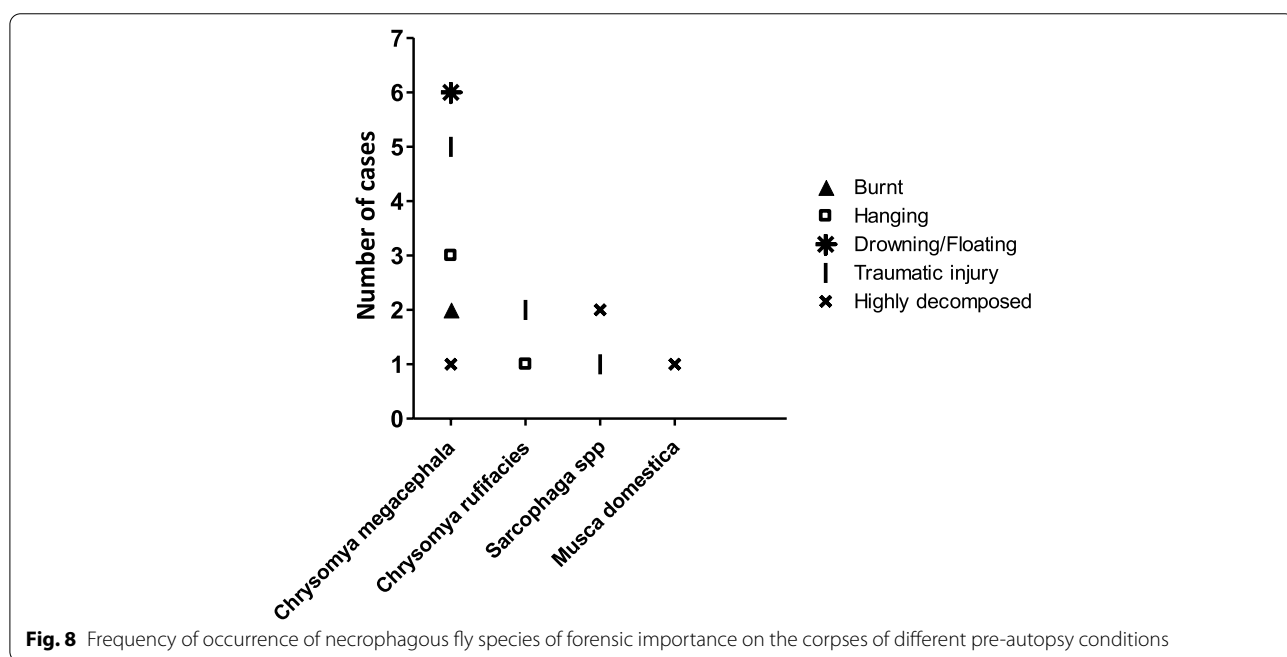
In the present study, *C. megacephala* was the only fly species sampled from floating human corpses. Floating human corpses were readily colonized by *C. megacephala*, *C. rufifacies* and *Sarcophaga peregrina* and floating pig cadaver was colonized by *C. megacephala*, *C. rufifacies*, and *M. domestica* (Chin et al. 2008; Syamsa



**Fig. 7** Frequency of occurrence of necrophagous fly species of forensic importance in indoor and outdoor environments

**Table 2** Frequency of occurrence of necrophagous flies of forensic importance from various medico-legal cases in Tamil Nadu State, India

Blow fly species	Overall % occurrence	Indoor/outdoor % occurrence		Frequency of occurrence of blowfly species on corpses of different pre-autopsy conditions				
		Indoor occurrence (%)	Outdoor occurrence (%)	Traumatic injury	Floating in water	Hanging	Burnt	Highly decomposed
<i>Chrysomya megacephala</i>	17 (70.8%)	6 (35.3%)	11 (64.7%)	5	6	3	2	1
<i>Chrysomya rufifacies</i>	3 (12.5%)	0 (0%)	3 (100%)	2	0	1	0	0
<i>Sarcophaga spp</i>	3 (12.5%)	3 (100%)	0 (0%)	1	0	0	0	2
<i>Musca domestica</i>	1 (4.2%)	1 (100%)	0 (0%)	0	0	0	0	1



**Fig. 8** Frequency of occurrence of necrophagous fly species of forensic importance on the corpses of different pre-autopsy conditions

et al. 2010; Ying et al. 2013; Sharma et al. 2018). The reason for finding only certain species of necrophagous fly or mono-species colonization in the present study could be attributed to the selection of oldest life stages of the insect samples for the identification and PMImin estimation. Report on occurrence of *Calliphora erythrocephala*, *C. megacephala*, *C. rufifacies*, *C. albiceps*, and *P. regina* in Punjab, a North-western state of India suggesting the difference in occurrence of necrophagous fly species in Southern and northern parts of India and the diversity of entomofauna associated with human corpse (Kashyap and Pillai 1989; Sharma et al. 2018).

*Calliphora erythrocephala* was the highest prevalent necrophagous fly species colonizing human corpse in India as per the historical necrophagous fly species distribution data. However, there was no medico-legal case

report in India involving *C. erythrocephala* published after 1989 (Singh et al. 2004; Suri Babu et al. 2013; Sharma and Bala 2016; Bala and Sharma 2016; Sharma et al. 2018; Babu et al. 2022). It seemed that the presently predominant necrophagous fly species *C. megacephala* and *C. rufifacies* had replaced *C. erythrocephala* fly colony in India and which could be attributed to global climate change (Amendt 2021). *Musca* spp was reported as one of the necrophagous fly species colonizing human corpse in the present study. However, *Musca* spp life stage could not be found in the published reports on necrophagous flies colonizing human corpse in India. Similarly, *Calliphora erythrocephala*, *Chrysomya albiceps* and *Phormia regina* life stages in human corpses in India were reported in the published studies (Kashyap and Pillai 1989; Sharma



**Table 3** Historical distribution of necrophagous fly species colonizing human corpses in India as per the published reports between 1985 and 2018

S.no.	Location	Time of collection	Indoor/outdoor	Gender and age of human corpse	Necrophagous fly species	Reference
1.	Andhra Pradesh and Karnataka, India	May, 1985 to September, 1985	Unknown	Unknown	<i>Calliphora erythrocephala</i>	Kashyap and Pillai 1989
2.					<i>Calliphora erythrocephala</i>	
3.					<i>Calliphora erythrocephala</i>	
4.					<i>Calliphora erythrocephala</i>	
5.					<i>Calliphora erythrocephala</i>	
6.					<i>Calliphora erythrocephala</i>	
7.					<i>Calliphora erythrocephala</i>	
8.					<i>Calliphora erythrocephala</i>	
9.					<i>Chrysomya rufifacies</i>	
10.					<i>Chrysomya rufifacies</i>	
11.					<i>Sarcophaga camaria</i>	
12.					<i>Sarcophaga camaria</i>	
13.					<i>Sarcophaga camaria</i>	
14.					<i>Sarcophaga camaria</i>	
15.					<i>Sarcophaga camaria</i>	
16.					<i>Sarcophaga haemorrhoidalis</i>	
17.	Bhopal, Madhya Pradesh, India	20 June 2003	Outdoor (drowning)	Male, 10 years	<i>Chrysomya megacephala</i> (Fabricius)	Singh et al. 2004
18.	Bhopal, Madhya Pradesh, India	07 August 2003	Outdoor	Male, 30 years	<i>Sarcophaga</i> spp	Singh et al. 2004
19.	Jagdarpur, Chhattisgarh, India	07 July 2012	Outdoor (nursery)	Male, 9 months	<i>Chrysomya rufifacies</i> (Macquart)	Suri Babu et al. 2013
20.	Kakrala, Patiala, Punjab, India	26 October 2014	Outdoor (paddy field)	Female, 23 years	<i>Chrysomya megacephala</i> (Fabricius), <i>Chrysomya rufifacies</i> (Macquart)	Bala and Sharma 2016
21.	Ludhiana, Punjab, India	30 March 2015	Outdoor	Male, 40 years	<i>Chrysomya megacephala</i> (Fabricius)	Sharma et al. 2018
	Ludhiana	05 April 2015	Indoor (inside house)	Male, 45 years	<i>Chrysomya megacephala</i> (Fabricius, 1794)	Sharma and Bala 2016
22.	Patiala, Punjab, India	22 April 2015	Indoor (inside house)	Male, 26 years	<i>Chrysomya rufifacies</i> (Macquart)	Sharma et al. 2018
23.	Bhakra canal, Patiala, Punjab	22 April 2015	Outdoor (canal)	Female, 52 years	<i>Chrysomya megacephala</i> (Fabricius)	Sharma et al. 2018
24.	Dholanwala bridge, Bhatthalan, Punjab	06 July 2015	Outdoor (drainage)	Male, 32 years	<i>Chrysomya megacephala</i> (Fabricius), <i>Chrysomya rufifacies</i> (Macquart), <i>Phormia regina</i> and <i>Chrysomya albiceps</i>	Sharma et al. 2018
25.	Pathanmajra, Punjab, India	05 July 2015	Outdoor (paddy field)	Male, 50 years	<i>Chrysomya albiceps</i>	Sharma et al. 2018

**Table 3** (continued)

S.no.	Location	Time of collection	Indoor/outdoor	Gender and age of human corpse	Necrophagous fly species	Reference
26.	Modakpal, Bijapur, Chhattisgarh, India	15 June 2018	Outdoor (drainage)	Male, 45 years	<i>Chrysomya megacephala</i> (Fabricius), <i>Chrysomya ruffiacies</i> (Macquart)	Babu et al. 2022
	Total number of samples in each necrophagous fly species and their percentage of occurrence in bracket	#Total 32 necrophagous fly species life stages were reportedly collected from 26 human corpses associated with medico-legal cases from 5 Indian states between 1985 and 2018			<ul style="list-style-type: none"> <li>• <i>Calliphora erythrocephala</i> (8, 25%)</li> <li>• <i>Chrysomya megacephala</i> (7, 21.9%)</li> <li>• <i>Chrysomya ruffiacies</i> (7, 21.9%)</li> <li>• <i>Sarcophaga</i> spp (7, 21.9%)</li> <li>• <i>Chrysomya albiceps</i> (2, 6.2%)</li> <li>• <i>Phormia regina</i> (1, 3.1%)</li> </ul>	

**Table 4** Agreement statistics between percentage occurrence of necrophagous fly species in human corpses in Tamil Nadu (2011–2018) and historical occurrence in India (1985–2018)

Necrophagous fly species	% Occurrence in Tamil Nadu State (2011–2018) –present study [A]	% Historical occurrence in India (1985–2018) [B]	Agreement statistics between [A] and [B]
<i>Chrysomya megacephala</i>	70.8	21.9	Kappa = 0.273 ± 0.051 <sup>a</sup> Fair agreement between the % occurrence between [A] and [B]
<i>Chrysomya ruffiacies</i>	12.5	21.9	
<i>Sarcophaga</i> spp	12.5	21.9	
<sup>b</sup> <i>Musca</i> spp	4.2	0	

<sup>a</sup> Standard error of kappa value

<sup>b</sup> Not included in the kappa statistics as not reported in the historical distribution data

The inter-rater agreement between the occurrence of necrophagous fly species in Tamil Nadu (2011–2018) and historical occurrence in India (1985–2018) is 75%. Out of 4 necrophagous fly species reported in the present study, 3 fly species were reported in historical distribution data

et al. 2018). However, these fly species were not found in the present study in Tamil Nadu State. The reason for this disagreement between the published reports and the present study could be attributed to small sample size, opportunistic, and non-random sampling in medico-legal cases, spatial, and temporal variation in sampling and dynamic change in the diversity of the necrophagous fly population.

High level of inter-rater agreement (0.75) between the occurrence of necrophagous fly species in Tamil Nadu and historical occurrence in India confirms that the 75% of the necrophagous fly species reported in Tamil Nadu State had also been reported from other parts of India. The agreement between the percentage occurrence of necrophagous fly species in human corpses in the present study and published reports from India was low (kappa = 0.273 ± 0.051). The reason for this could be attributed to spatial and temporal variation in sampling and dynamic change in the diversity of the necrophagous fly population. Further, sampling necrophagous flies in medico-legal cases is an opportunistic sampling and not a representative sampling.

## Conclusions

Knowledge on diversity and eco-geographical distribution of insects of forensic importance is important in identification of insects of forensic importance, detection of invasive insect species and as evidence of relocation of corpses and cadavers. *Chrysomya megacephala* was the predominant blowfly species found to colonizing corpses in Tamil Nadu State, India, which is a tropical climate region with no drastic swing in temperature pattern. *Chrysomya megacephala* was the only blowfly species found to colonize both burnt and floating corpses and corpses located indoor and outdoor.

## Abbreviations

PMImin: Minimum post-mortem interval; PMI: Post-mortem interval; L3 larva: Third stage larva.

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#### Authors' contributions

Azhahianambi Palavesam: conceptualization, methodology, formal analysis, investigation, visualization, writing—original draft. Selvakumar, R: resources, methodology, investigation, writing—review and editing. Soundararajan, C: formal analysis, data curation. Jyothimol, G: methodology, investigation, data curation. Bhaskaran Ravi Latha and Harikrishnan, T.J: writing—review and editing, project administration, supervision. The author(s) read and approved the final manuscript.

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#### Availability of data and materials

Not applicable.

#### Declarations

##### Ethics approval and consent to participate

All the insect samples collected during the autopsy were studied as per the recommendations of Institutional Ethics Committee, Government Kilpauk Medical College, Chennai, Tamil Nadu, India. Name, address, and contact details of the deceased persons were not disclosed in the manuscript. Experimental animals were not used in this study. This study was conducted in accordance with code of ethics of the World Medical Association.

##### Consent for publication

All the authors of this manuscript unanimously agree to publish the data.

##### Competing interests

The authors declare that they have no competing interests.

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