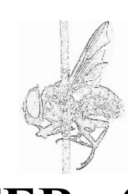

CHAPTER - 2

REVIEW OF LITERATURE

REVIEW OF LITERATURE

2.1. International

The first report, where blowflies were used for criminal investigation perspectives was in China during the 13th century, is mentioned in Sung Tzu's *The Washing Away of Wrongs*. According to the aforesaid incident, a farmer was murdered with a sharp weapon in a field and the investigators said that after the murder people placed their sickles in the field. Among the various sickles placed in the field, only one of the sickles attracted blow flies, since the sickle concerned had a trace of blood that cannot be seen by the naked eye. This extraordinary and scientific view has opened the way towards the confession of the murderer. This marked the beginning of forensic entomology.

The application of the above said perspective in front of the court, especially in the the modern world was witnessed in France during the 18th century where the data from entomological evidence was recognized as valid proof for clearing the existing inhabitants at a home.

According to Patton, (1931), the size of the larvae of blow flies differed according to the temperatures they inhabited and larvae contracted quickly just prior to the pupariation. The drawings and illustrations of *C. megacephala* with special inference to the conventional taxonomy were unveiled (James, 1947).

Zumpt, (1965) in his treatise mentioned that the rapid dissemination of *C. megacephala* all over the world was witnessed in the past few epochs. Moreover, many studies also unveiled certain significant records indicating the geographical distribution of the same in Indian regions. The adult flies of *C. megacephala* were usually attracted to decaying cadavers and reach within a few hours of death of the animal.The literature concerning the comparative investigations that would reveal the conventional identification of *C. megacephala* using eggs was found scanty in the current scenario. Typically, the eggs of *C. megacephala* are sausage-shaped with whitish cream in colour measuring 1.5–1.6 mm.

James, (1971) was of the view that some of the previous literature found that the identification of *C. megacephala* for forensic needs exhibited some degree of difficulty since it may be markedly confused with other species which have common identification features with closely related *C. megacephala.* The *Chrysomya saffranea* is known for the aforesaid perspective all over the world. In this regard, it is essential to remember the fact that maximum care should be engaged while verifying the taxonomic information of every insect which has forensic significance including *C. megacephala.*

C. megacephala is recognized to have a diverse habitat, and in addition to the human inhabiting regions, they have also been reported from different ecotypes, rural and natural forested provinces, and straddling urban and peri-urban regions (Tumrasvin et al., 1979).

Kurahashi, (1979) described *Chrysomya chani* from Singapore. The information on life cycle, the influence of various factors in its development, life cycle and forensic importance followed by molecular analysis of *C. chani* were limited. According to Kurahashi, (1982), *C. megacephala* was found to be initially reported from Australasian regions while considering the most abundant blow flies.

Smith, (1986) discussed about the oviposition preferences of blow flies in open wounds and also categorized the group of invertebrates infesting on carrion. Tullis & Goff, (1987) reported that measurement of larval length, width and weight can be used for the age determination of particular instar for the PMI estimation.

Liu and Greenberg (1989) provided the key for egg and all the larval instars of flies of forensic significance. In their study they also suggested that need for identification of larvae becomes significant when larval specimens are presented. So rapid and accurate identification will be possible through the ultrastructural examination of the larvae.

Erzinclioglu, (1990) did a study on the morphology of larval instars of *Chrysominae* species and tried to compare them through the spinulation characteristics on the larval body.

A study by Goodbrod & Goff, (1990) revealed that the rate of development of insect larvae inhabiting the cadaver may depend on the environmental temperature, which suggests the role of temperature in forensic entomology studies. In addition to this, it was also evident that each stage of insect development has a specific temperature and other factors' needs. Each insect species that has forensic significance has its own specific days or degree hours to complete the various developmental stages during its development.

While reviewing the geographical distribution of *C. megacephala*, a study by Kurahashi, (1991) reported the presence of the same from the Australasian regions including New Caledonia, New Guinea and Samoa.

Medico legal cases world over have reported the forensic relevance of *C. megacephala* (Goff and Flynn, 1991, Amendt et al., 2004, Richards and Villet, 2009, Gruner et al., 2017). The age of the different species of the blow flies like *C. megacephala*

(Fabricius), can be used to estimate the PMI from the development rate linked with the environmental temperature. Since blowflies regularly find a corpse and promptly lay eggs on the same without delay (Turner & Howard, 1992), they are noted to be ideal candidates for estimating the PMI.

C. rufifacies was originally described in 1978 from Central America and have been reported from the various provinces like Arizona, Texas, California, Guatemala and Florida during 1986 to 1991. A silent invasion of the same in Puerto Rico was also reported in 1991 (Baumgartner, 1993).

When death happens, the cell's enzyme digestion, as well as the cell death takes place in the cadavers, thereby offering the habitat for the insect species. According to Ashworth & Wall, (1994), once the body starts to decompose, the microbial consortia living in the dead body start to destroy the tissues thereby resulting in the production of liquids as well as the following gases; carbon dioxide, hydrogen, sulphur dioxide, apneumones, methane, hydrogen sulphide and ammonia. Thus formed chemical elements and gaseous candidates escaping from the dead body may invite the insect species to the decomposing matter. Each stage of the decomposition may witness the presence of a plethora of chemical constituents and the scientific community all over the world is trying to identify such constituents.

The earlier experiments conducted by Wells & Kurahashi, (1994) at 16L: 8D light conditions and a temperature of 27°C, unveiled the pupariation of the studied species *C. megacephala* at 144 h.

The identification of *C. megacephala* species-group with special inference to their adult has been published by Wells & Kurahashi, (1996). The various drawings and photographs of the adult *C. megacephala* along with the shape of female frons and eyes of male have also been discussed by James, (1947) and Zumpt, (1965).

Many previous investigations have already studied the developmental stages of *C.megacephala*, which include development at regular temperatures, and changing temperatures followed by outcomes of different feeding matters. The results from such studies primarily display the developmental plasticity in *C.megacephala*, and that extraordinary populations have differences in development according to the environment as well as the geographic location (Wells & Kurahashi, 1994, Ma et al., 1998) prominently depends on their surrounding temperature.

The morphological identification of insects having forensic significance, especially *C. megacephala,* has been previously reported (Wells & Kurahashi, 1996). To get the data that can be used to estimate the PMI in the future, it is essential to investigate the developmental rate of forensically significant fly species like *C. megacephala* with special inference to the various environmental factors that have been found in the death scene (Queiroz, 1996). If so, such studies can be used to compare the forensically important blow flies collected from death scenes or environments as stated in the former sections.

The developmental rate of *Chrysomya* species in context with the various natural temperatures and cyclic temperatures, results in an extensive difference ranging from 4h to 38 h (Byrd & Butler, 1997). Many investigations have been performed previously by them to analyze the pattern of the various developmental stages of *C. rufifacies* for forensic needs. They investigated the growth curve for the various developmental stages of *C. rufifacies* including the larva, and pupa under a constant temperature of 25°C and at average cyclic temperatures of 15.6°C, 21.1°C, 26.7°C, and 35.0°C.

In addition to the forensic significance shown by the *H. ligurriens,* these flies were also found to make nuisances in gardens and markets. Moreover the adult flies of *H. ligurriens* are also responsible for the transmission of a plethora of pathogens to humans as well and they were also attracted to the human excreta usually found near human-inhabited surroundings (Kurahashi, 1997).

Earlier studies have reported that in addition to the adult flies obtained from the carcass, the various developmental stages of the same, specifically the eggs, and larva flowed by puparia can also be used to determine the postmortem interval (Anderson, 1999; Smith, 1986)

A fact sheet published by CABI in 2001 revealed that the following countries reported the presence of *C. rufifacies*: Bangladesh, Fiji, Papua, China, Arizona, Nebraska, Texas, Saudi Arabia, Alabama, New South Wales, New Caledonia, Sri Lanka, Australia, Western Australia, Costa Rica, Malaysia, India (Kerala, Karnataka, West Bengal and Tamil Nadu),, Pakistan, Philippines, New Zealand, Argentina Vanuatu, Arkansas, Hawaii, Louisiana, Thailand, Queensland, New Guinea, and Mexico (CABI, 2001).

Likewise the, fact sheet by CABI, 2001 revealed that *C. chani* was reported from the following regions over the world; Saudi Arabia, Sri Lanka, Australia, Bangladesh, Fiji, Papua, New South Wales, New Caledonia, Argentina Western Australia, China, Arizona, Nebraska, Texas, Philippines, Costa Rica, Arkansas, Hawaii, Louisiana, Thailand, Malaysia, New Zealand, Alabama, Vanuatu, Queensland, New Guinea, Mexico and Pakistan.

H. ligurriens was known for its prominent contribution to forensic investigations and are primarily distributed in the following countries; Australia, The Philippines, India, Malaysia, Papua New Guinea, Laos, Indonesia, Taiwan, Korea, China, Thailand, Singapore, and Sri Lanka (Tumrasvin et al., 1979, Kurahashi and Chowanadisai, 2001).

C. rufifacies larvae usually exhibit an unfamiliar activity. For example, they start to prey on other larvae once they have attained the second instar stage. Hence, they can alter the population composition on the cadavers, occasionally imposing other *Chrysomya* sp. to attain the wandering stage in a quick manner. In accordance with the various contributing factors, the rate of *C. rufifacies* larval weight was previously known to possess extensive statistical significance towards temperatures and time (Madeira, 2001).

According to Amorim & Ribeiro (2001), the mouth hooks of the mature larvae which can also be seen sticking inside the enclosed puparium, contribute to the identification of the same at the species level.

The prominent characteristic feature used for the identification of *C. chani* is the densely sclerotized spiracle on the posterior part, complete peritreme followed by the presence of accessory sclerite (Greenberg & Kunich, 2002).

Von Aesch et al., (2003) conducted study on the abundance and diversity of blow flies in the outdoor to relate the influence of climatic factors on the fly activity and also to check the level of attraction of the blow flies to different stages of decomposition.

Lertthamnongtham et al., (2003) have investigated the seasonal activity of *C. megacephala* from the Chiang Mai Province, the northern region of Thailand and also validated the same perspective in terms of seasonal fluctuations in blowfly populations. The seasonal fecundity of the same insect was also analyzed in their study and found that the ovarioles number statistically differed between the various seasons studied, and there exists a significant increase of the same in the rainy season than in the summer and winter seasons.

Sukontason et al., (2003) clearly depicted the major characteristic features of the ultrastructure of the larvae of *C. megacephala* for species identification using Scanning Electron Microscopy. The authors emphasized about the significance of hairy nature of *C. rufifacies* that can be used to differentiate it from *C. megacephala*. Their study also revealed about the importance of spiracular hairs in the posterior region of the *C. rufifacies* to differentiate its various developmental stages.

The establishment of forensic entomology with sufficient scientific evidence was initially done by Yovanovich and Megnin's evaluation concerning the succession of insects on the cadavers. The presence of insect species belonging to the above said group can deliver significant information for the estimation of PMI. For this, the precise identification of the insect specimen inhabiting the cadaver could be recognized as the initial step. In order to consider the insect specimen as significant evidence for the criminal investigation, especially for the estimation of PMI, the insect larvae need to be determined in terms of their length, width, growth rate and other influencing factors (Amendt et al., 2004).

Amendt et al., (2007) discussed in detail about the good methods in forensic entomological work and stipulated certain standards and guidelines with respect to collection, preservation, and transportation of entomological evidences.

From a forensic entomology perspective and also based on the many investigations carried out, the construction of the life table for a particular species of fly like *C. rufifacie*s and *C. megacephala* could be noted as a significant study in the current scenario (Sukontason et al., 2007).

While considering the same perspective in an international concern, specifically in the Brazilian context, it was observed that there exists a constant increase in the abundance of *C. megacephala* found between the end of spring, October and February (all of summer) and attained its maximum level in December (Mello et al., 2007). The study unveiled that the fluctuation in its abundance and diversity is usually specific to its habitat and location which are noted to be altered according to various seasons including pre-monsoon, monsoon and post-monsoon.

Studies focusing the development of *C. megacepehala* has been done previously (Sukontason et al., 2008; Niederegger et al., 2010; Zhang et al., 2018). *C. megacephala* has been considered as an important fly for the determination of minimum postmortem interval (Wang et al., 2008). Many of the studies performed from various regions all over the world including Malaysia have reported the forensic significance of *C. rufifacies*. The predacious activity exhibited by *C. rufifacies* larvae is recognized to be the major reason behind the same. One of the major points while discussing the life cycle stages of *C. rufifacies* is the influence of *C. megacephala* in stimulating the oviposition activities. This essentially means that the *C. rufifacie*s consume the larvae of *C. megacephala* (Shiao & Yeh, 2008).

Due to the various complications linked with the conventional taxonomic analysis of the *C. megacephala*, a study by Stevens et al., (2008) has suggested the use of the whole mitochondrial genome of *C. megacephala* in species identification.

Wang et al., (2008) reported that the presence of three slits in the posterior spiracles and 11–13 branched anterior spiracles on the third larval instar of *C. megacephala* could be used for distinguishing it from the first and second instar larvae. According to them, as a predatory and necrophagous species, the immature stages of *C. megacephala* have been found on almost all cadavers reported from Southern China. *C. rufifacies* are found on carcasses almost all year usually in warmer areas while they are mainly found during the warmer months in the temperate zone of China.

According to a study by Evaldo et al., (2008), there exists a lot of environmental factors such as rainfall, relative humidity, temperature and altitude including the land use types. They have a significant role in the rapid distribution and extensive abundance of flies belonging to the Calliphoridae.

Sukontason et al., (2008) reported the morphological characteristic features of the *C. rufifacies* from Thailand. They primarily compared the morphological characteristic features of the *C. rufifacies* and *C. megacephala* with special reference to the cephalopharyngeal skeleton, posterior spiracle, cuticular spines and appearance of the body.

Among the various previously studied flies, *C. megacephala* has relevance to medical entomology, forensics, and public health in various provinces over the world especially in Asia, South America, Africa and Australia (Richards & Villet, 2009). The authors also reviewed the rate of development and influence of various parameters including the environmental temperature, physiological age and surrounding environmental condition of *C. megacephala*.

Estimates of postmortem interval (PMI) based on the known characteristics of the infesting fauna in the natural conditions of the specific geographical location are very important (Sukontason et al., 2008). Niederegger et al., (2010) suggested that negligence of fluctuating temperatures in legal cases can lead to distinctly wrong estimates of the PMI.

Recent studies have validated the use of molecular strategies for the identification of blow flies (Tan et al., 2009).

According to the findings by Ahmad et al., (2010), it was evident that they principally used the light microscope for the examination of the morphological structures (anterior spiracles, posterior spiracles and cephalopharyngeal skeleton) of second and third instar larvae of *Hypopygiopsis* from Malaysia. Also the developmental data generated by the authors really helps the scientific community to estimate the PMI of corpses from the study region of Malaysia.

According to Gallagher et al., (2010), to implement the approaches targeting the insect for forensic entomology, the study concerning all the immature stages followed by the developmental rate in relation to different parameters including the temperatures and the humidity in geographically distinct populations is having great forensic significance, because such insect studies will be useful for the precise estimation of PMI in the specific region.

The morphology of various developmental stages of the *H. ligurriens* was revealed through the light microscope and SEM by Firdaus et al., (2010); Sukontason et al., (2004), justifies the use of SEM for the identification of *H. ligurriens*.

As mentioned by Mendonça et al., (2010), the identification of insect specimen using conventional approaches, especially based on the morphological features has some advantages but it has certain limitations when applied to immature stages. Moreover, when damaged specimens were obtained from the field or crime scene, DNA-barcoding, specifically the mitochondrial COI genome has great potential for the identification of insect specimens particularly Calliphoridae. Mendonça et al., (2010) also validated the efficacy of SEM to study the morphological characteristic features of flies belonging to the Calliphoridae.

Studies involving microscopic investigation of larval instars of blow flies were reported by Ahmad et al., (2010) from Malaysia. The presence of *C. chani* in outdoor and indoor circumstances was witnessed by a study that used *Macaca fascicularis* Raffles as a candidate animal. From their observations, the adults of *C. chani* were collected from 6 to 13 days, which are generally known for the decomposition stages. While considering an indoor perspective, it was clear that the *Chrysomya chani* was sampled from 4 to 30 days, which justifies a preference for distended to extensive decay (Ahmad et al., 2011).

Sukontason et al., (2011) tried to identify the larval instars of *C. megacephala* using Scanning Electron Microscopy (SEM). According to Olea et al., (2011), the appearances of *C. megacephala* in new regions draw attention to the current scenario; also reminds us of the increased rate of the spread of the same from one region to another. They are able to travel about 2-3 km/day and recent studies indicated that the *C. megacephala* extended its habitat to a region that is 500 km far from its original habitat. Brundage et al., (2011) investigated the abundance and distribution of insects belonging to the Calliphoridae with special reference to seasons and habitats for two years.

SEM studies on ultrastructure of *C. megacephala* larvae have been reported by Mendonça et al., (2012). They claimed that the spinulations present on the body integuments and the cluster of spines having either one or two tips on the cephalic region can distinguish *C. megacephala* from various *Chrysomya* species.

Yang and Shiao, (2012) studied about the oviposition preferences of *C. rufifacies* and *C. megacephala* in context with the possibility of using them as prominent candidates for the estimation of PMI. In addition to this, they have also validated that the adult female of *C. rufifacies* and *C. megacephala* usually arrive on corpses immediately less than an hour of their presence, and are typically found to lay about 220–325 eggs at a time. The influence of various parameters like temperature, humidity from their surroundings on rate of development was also recommended by them in forensic investigations.

. In addition to the above-reviewed species, the blow flies belonging to *Hemipyrellia* have also exhibited sturdy significance in forensic entomology. Among the various investigated blow flies, *H. ligurriens*, were found to have prominent significance in forensic entomology as stated in various studies previously from Malaysia and Thailand.

A study by Bunchu et al., (2012) revealed the geographical distribution of *H. ligurriens* over the following regions; Taiwan, Korea, China Papua New Guinea, Australia, The Philippines, India, Thailand, Singapore, Sri Lanka Malaysia, Laos and Indonesia.

Hemipyrellia is represented by four species in the Oriental region. In India, it is represented by two species; *H. pulchra and H. ligurriens* (Senior-White et al., 1940, Nandi, 2004., Bharti, 2011). *H. ligurriens* was reported on decomposing human cadavers in Malaysia (Rajagopal, 2013), Thailand (Moophayak et al., 2014) and other regions (Chen et al., 2004, Lee et al., 2004, Sukontason, 2007) and also has significant forensic importance. The development studies of *H.ligurriens* has been done by a few researchers (Sinha and Nandi, 2007, Sukontason et al., 2008, Sukontason et al., 2010, Bunchu et al., 2012, Yang et al., 2015).

The metallic copper green coloured adult, possesses about 12.23 mm in length and 2.85 mm in width. The head of the male and female were holoptic and dichoptic respectively (Bunchu et al., 2012). There exists a whitish coloured squama and the thorax was noted with postsutural acrostichal setae. All the above said morphological characteristic features are reported to be the major contributing factors in *H. ligurriens* species identification.

As a comparison perspective against the findings of Sukontason et al., (2008), Bunchu et al., (2012) verified the forensic significance of *H. ligurriens* in context with the developmental rate and the morphology of all stages. In their study, they maintained natural ambient settings for their experiments and used a light microscope for identification purposes. The final findings of them clearly validated that *H. ligurriens* took about 270.71 h for completing its life cycle. Some of the immature stages of *H. ligurriens* larvae, specifically the third instar larvae have been investigated using the light microscope.

Szpila et al., (2013) have developed keys to identify the larvae up to species level from Europe and Mediterranean regions. They also reported that in the larval instars of *C. megacephala,* cuticular spines in the dorsal region between the mesothorax and the prothorax are organized close together in a single form. They also pointed that various ranges of temperatures have really affected the developmental process and growth rate *C. megacephala*.

Successional studies conducted on the in *Oryctolagus cuniculus*, the New Zealand white rabbit, illustrated that *C. chani* adults are typically active in the bloated stage of decomposition. The larvae of *C. chani* at second and third developmental stages were also collected during active decay. However, the larvae at third developmental stages were also reported from 6 to 8 days of decay, which is usually referred to as the advanced stages of decay (Silahuddin et al., 2015).

The information concerning the influence of various parameters including the temperature humidity and other factors towards the growth rate and the development of other *Chrysomya* sp. except *C. chani* were reported from many countries. Jordaens et al., (2013) appended the mitochondrial COI gene analysis of *C. megacephala* for species identification.

Moophayak et al., (2014) sampled 2,115 blow flies belonging to six genera including *H. ligurriens*, and found that there exists a prominent link between the studied seasons and the frequency of occurrence. Furthermore, they concluded that many of the insect species in their study had shown their peak during the summer seasons, as found in various literatures reported in Thailand previously.

According to the perspective of Owings et al., (2014), it was evident that the variations in the abundance and life cycle of blow flies may probably be due to the vast differences in the in population variation, study sites sampling strategies, and the sampling period.

A study by Zabala et al., (2014) sampled 28,507 adult calliphorids and analyzed for various forensic perspectives with special inference on the influence of various seasons including summer, winter and monsoon. According to their findings, it was evident that *C. vomitoria* (6530) and *C. vicina* (9883) were the most abundant species in winter season. In addition to this, *L. ampullacea* and *L. caesar* were abundant. Some of the following species reported in their study also possessed significant seasonal abundance and exhibited a maximum peak in the summer season like *C. albiceps, L. sericata* and *L. illustris.*

Studies conducted by Whitaker, (2014) proved that same species of blow flies were attracted to both pig and human cadaver and comparable results were obtained regarding the oviposition sites, larval development which reinstated the claim for pigs as a better substitute for forensic entomological studies.

A study performed by Akbarzadeh et al., (2015) clearly defined the various morphological structures and specific pattern which can be used for the identification of *C. rufifacies.*

Yang et al., (2015) while studying the PMI estimation, suggested that it is essential to highlight the significance of implementing the various technologies and concepts to get enough knowledge concerning the biology of the various developmental stages of the native species belonging to the Calliphoridae with special inference on the diverse affinity toward the geographical regions. In a previous study, they uncovered the possibilities of constructing growth curve models for *H. ligurriens* using different constant temperature regimes.

C. megacephala was originally reported from Australasian regions including the New Caledonia, New Guinea and Samoa. The dramatic spread of the same has resulted in the record of the species grew from various other regions such as Mauritius, Rodriguez, and India (Akbarzadeh et al., 2015).

The larval development rates of *C. rufifacies* within the South-East Asia region were reported by Yanmanee et al., (2016) and found that the different geographic locations have a prominent role in the rate of development of *C. rufifacies*. The results clearly validated that the developmental times varied among different temperatures from egg to adult stage of *C. rufifacies*.

Yang et al., (2016) and Gruner et al., (2017) have validated the probable use of the thermal accumulation models for forensic entomology research. The aforementioned thermal accumulation models are primarily focused on either the landmarks or developmental events, which were typically followed by maintaining the samples of live insects for forensic needs.

It has been observed that *C. megacephala* lay eggs at night in certain circumstances where their surrounding environment is found to be warm (Williams et al., 2017). Previous research has shown that *C. megacephala* is one of the first reported insects to arrive at a cadaver, and its exceedingly huge population size makes them unquestionably the dominant species on the remains (Wang et al., 2017 & Sukontason et al., 2022). The observation of Sontigun et al., (2018) validates that the ecological niches inhabited by the *C. megacephala* may influence the fecundity of the same.

Mendonça et al., (2010), used mitochondrial COI gene to identify the blow flies; *C. bezziana, C. megacephala, H. ligurriens, C. nigripes, C. chani, C. villeneuvi, C. pinguis, Lucilia porphyrina, C. rufifacies, L. papuensis, Hypopygiopsis infumata, H. tumrasvini C. thanomthini, L. sinensis, L. cuprina,* and *H. pulchra.* Many of the previous studies have primarily used the applicability of DNA-based strategies, especially the use of mitochondrial COI gene to identify blow flies (Qiu et al., 2017, Yusseff and Agnarsson ., 2017, Sukontason et al., 2008).

The influence of seasonal variations including the summer, monsoon and winter on blow flies (Dipter: Calliphoridae) was reported by Sontigun et al., (2018). According to them, the various environmental factors including the relative humidity, altitude, rainfall, land use types and temperature can influence the abundance and the distribution of insects belonging to Calliphoridae. In this regard, they sampled and investigated about 88,273 flies belonging to *Chrysomya* and found that among the various analyzed insects, *C.*

megacephala was found to be predominant in summer and the abundance got gradually weakened in rainy and winter seasons.

Sukontason et al., (2018) have verified that *C. rufifacies* and *C. chani* had been known to illustrate their prominent significance in forensic entomology. Based on the analysis, *C. megacephala, C. rufifacies* and *C. chani* were the predominant insect species that have prominent significance in forensic entomology and confirmed that PMI was six days. The authors examined the larval instars of *C. chani* and suggested that the elongate tubercles with innumerable small spines on body, sclerotized spine bands and heavily sclerotized peritreme of the spiracles were characteristic and could help in identifying the species. Their study also validated that the biological information was found to be limited. The authors also suggested that phylogenetic analysis using COI and COII genes can be employed to distinguish *C. chani* from closely resembling *Chrysomya pinguis* and *C. megacephala.* They described larval instars of *C. rufifacies* using the light microscopy and mentioned the presence of large tubercles on abdominal segments with spines on the tip can be used for the identification of the species.

The identification of *C. megacephala*, along with its biology and geographical distribution were reviewed by Badenhorst and Villet, (2018) from South African provinces to get an extensive background for various strategies that might be used for this fly for forensic needs.

Irish et al, (2014) and Badenhorst and Villet, (2018) studied the various morphological features of *C. megacephala* using photographs and drawings along with the ultrastructure analysis of first, second and third instar larval stages. According to them, first, second, and third instar larvae can attain 1.7–3.5 mm, 6–8 mm, and 16 mm long respectively. They treated *Lucilia flaviceps, Musca flaviceps, Musca remuria, Pollenia basalis, Somomyia dives, Somomyia cyaneocincta, Somomya cyaneocincta, Musca bata, Musca combrea, Musca megacephala, Somomya pfefferi,* and *Somomyia cyaneocincta* as synonyms of *C. megacephala.*

A study by Sontigun et al., (2018) revealed the various findings concerned with the seasonal variation perspectives of *C. megacephala* activity with special inference on the various developmental stages of the same collected from the Chiang Mai area of Northern Thailand. They reported that the highest abundance of *C. megacephala* was found to be in summer and the abundance gradually diminished in the rainy and winter seasons.

The growth and development patterns of *C. megacephala* from Yangtze River Delta region of China was studied by Zhang et al., (2018). They have constructed three models; isomegalen diagram, thermal summation model and isomorphen diagram for PMI estimation. They found that various development stages of *C. megacephala* such as eggs, first instar second instar and third instar larvae, followed by the pupae were reported with diminishing duration of development with higher temperatures. The total developmental duration was also found to be diminished to 171.8 h at 34.0 °C from 794.8 h at 16.0 °C, justifying the significant link between the temperature and developmental duration in *C. megacephala*.

Klong-Klaew et al., (2018) studied geographical and ecological information of insects having forensic significance with special inference on the PMI estimation. They collected nearly 1298 flies belonging to Chrysomyinae and *C. chani* was predominant in their study and the maximum abundance of the fly was observed during summer with temperatures ranging from 25–30 °C.While the rainy and winter seasons witnessed a drastic reduction in their numbers.

A study from China by Li et al., (2016) also found the similar observations as reported by Klong-Klaew et al., (2018). However, certain kinds of conflict were also found in some previous studies by Moophayak et al., (2014).

Regarding seasonality, according to Klong-Klaew et al., (2018), *H. ligurriens* species was predominantly reported during the late summer and the diversity was drastically reduced in the rainy season. In addition, the population diversity of *H. ligurriens* was reported as reduced in the winter season. There exists a positive correlation between relative humidity and increased temperature in the above-said contexts.

In agreement with the findings reported by Sukontason et al., (2018); Singh et al., (2011), validated the applicability of 2386 bp of combined nuclear carbamoylphosphate synthetase and COI gene in the molecular characterization of *C. chani*. In contradiction to their above findings, the use of 28S nuclear rRNA gene along with a size of 1000 bp placed *C. chani* into the hairy maggot blow flies group indicating the significance in choosing the particular gene for specific analysis. One of the major reasons behind the group change may be the prominent range of variations that happened in the different genes. Such movements will definitely result in the alteration of the taxa arrangements (Zajac et al., 2016).

Zhang et al., (2019) investigated the thermal summation, development duration and larval growth of *Chrysomya* sp. under a specific range of temperatures typically within the range of 16–34°C and found a prominent range of links between the developmental rate and temperature. They also suggested that the data obtained from their findings can be used to build developmental models and that geographically isolated populations of insects belonging to *Chrysomya* genus can differ in their developmental time. This is in conformity with studies by Grassberger & Reiter, (2002).

Constant range of temperature primarily at 25°C formed the pupation times and adult emergence of *C. rufifacies* from 134 h to 162 h and 237 to 289 h respectively (Hu et al., 2019). The most preferable temperature determined for the larva of *C. rufifacies* while using the gradient system was 35.1°C. The various benefits including the slight larval length variation and extremely foreseeable developmental time followed by low cohort variation highlight *C. rufifacies* as a highly predominant candidate for PMI determinations based on forensic entomology.

Similar to *C. megacephala*, the adult and immature stages of *C. rufifacies* collected from corpses have been primarily used as forensic evidence in various investigations mainly to estimate the PMI (Herrera et al., 2021). Nigoghosian et al., (2021) recently proposed a two-step protocol for the mounting of cephalopahryngeal skeleton of calliphoridae larvae for the microscopic examination.

According Lutz et al., (2021), in instances where entomological evidence was collected and insects identified (n = 279), many of the dead bodies have been inhabited by one or two species with almost 10 unique species colonizing a carcass. Collectively, mono-colonization is found more regularly interior than outdoor environment. It cannot be forgotten that this particular pattern was based upon numerous factors along with the person who was incharge for the sample collection (medical expert, forensic entomologist, crime scene technician, or police), the sampling area (scene of death, place of autopsy) or specific pattern for sample collection.

A recent study by Acosta et al., (2021, 2022), reported the fact that humidity and controlled temperature have a direct impact on the life cycle of various flies belonging to Calliphoridae.

The early 1900s witnessed the first report of *C. rufifacies* from Hawaii as a new fly and the drastic dissemination of the same towards various regions across the world were reported since the ships and aircraft that link abroad paid them clear route for the dissemination. Due to this, in 1958, *C. rufifacies* was reported from Japan which was far about 6,611 km from Hawaii, thereby justifying the role of international travel and migration approaches in the extensive spread and diversity of *C. rufifacies* from various parts all over the world (Baumgartner, 1993; Dawson, et al., 2022). A recent study by Jeong et al., (2022) revealed the distribution of blowflies having forensic significance in terms of various seasons such as spring, summer and autumn followed by winter.

Pelletti et al., (2022) validated and concluded that SEM can be used for the morphological characteristics-based identification of *C. vomitoria.* The identification of any insect which has forensic significance needs a proper identification approach to validate its significance in forensic entomology (Abdullah et al.,, 2022; Apasrawirote et al., 2022; Greenberg & Ibrahim, 2022). The data concerning the various developmental stages and life cycles of *C. megacephala* was studied by many of the authors (Xu et al., 2022).

2.2. National

While considering the review of perspectives for the study area, it was evident that some of the studies (Subramanian & Mohan, 1980) have analyzed the biology of flies belonging to *Chrysomya* and the fact is that many of the studies have focused on *C.*

rufifacies and *C. megacephala*. But the aforesaid species were not reported with forensic significance from the current study region except in this study.

Kulshrestha & Chandra (1987) detailed 25 case studies with inferences drawn on the PMI estimation based on the data of developmental stages of blow flies obtained through rearing in the prevailing conditions.

Kashyap and Pillay (1989) checked the efficacy of entomological method over other conventional medico legal approaches for the estimation of PMI by studying 16 insect infested dead bodies. In their study they found that the entomological method was superior to other methods.

Senior-White et al., (1940), Nandi, (2004), Singh & Sidhu, (2004) and Bharti, (2011) recorded 30 genera and 119 species of Callophoridae from India. Among the various genera found in the Indian region, *Chrysomya* was noted to be the most predominant genus in Calliphoridae with forensic, medical and veterinary significance (Bharti, 2011). Bharti & Kurahashi, (2009), revealed that the feral derived form (fdf) of *C. megacephala* was reported from the Himalayan forests in India.

The various blow files belonging to Calliphoridae with forensic significance were enlisted by Singh & Bharti, (2001). While considering the environmental factors, especially the influence of various seasons, Aggarwal, (2005) performed an investigation in Punjab in the context of the autumn, rainy, summer, spring, and winter seasons.

Study by Wall et al., (2001) discussed that the abundance of *C. megacephala* is directly and positively linked with the relative humidity. However, there exists a negative pattern in the abundance of *C. megacephala* while rainfall and temperature are considered as parameters. Their findings revealed that the trimodal peaks of *C. megacephala* supported the above said inferences in which the highest abundance was reported in January, followed by September and June. According to their findings, the population rate of *C. megacephala* was extensively augmented in the rainy season and the dry hot season was reported with relatively low population rate.

Bharti et al., (2003) conducted an insect succession study on the decaying rabbit carcass in Punjab and found that among the insect species recorded, Calliphoridae were the first to arrive on the carcass in all seasons.

A study by Sinha & Nandi, (2004) reported that the synanthropic form of the aforementioned species is usually found common in the human-inhabited regions which allow direct interaction with the human population. The occurrence of *C. megacephala* from fruits, carcasses, dead fish, human excrement and sweet was previously reported by the authors which justifies the significance of *C. megacephala* in forensic entomology in Indian scenario.

Singh and Sidhu, (2004) prepared the check list of blowflies of North-West India and reported the presence of *Idiella mandarina* (Wiedemann, 1830) and *Chrysomya pinguis* (Walker, 1858).

One of the most predominant contributions to the blow flies checklist was done by the Occasional Paper NO. 231, Zoological Survey of India (Nandi, 2004). According to the list, it was evident that the following species were primarily reported from Kerala; *Bengalia jejuna* (Fabricius, 1787) (Cochin, Trivandrum and Valayar), *Bengalia surcoufi* Senior-White 1924 (Cochin, Kerala), *Lucilia ampullacea* (Malabar Coast)), *Lucilia papuensis* (Malabar Coast)), *I. mantlarina* (Wiedemann), 1830 (Trivandrum), *Rilinia unicolor* (Townsend), 1917 (Cochin), *Cosmina bicolor* (Walker, 1856) (Kerala), *Cosmina* *simplex* (Walker), 1858), *Strongylura strongylura* (Van Hasselt, 1823) (Chalakudy, Kerala) and *Borbororhinia bivittata* (Walker, 1856) (Cochin, Kerala). In addition to this, many of the studies have reported the presence of blow flies from the study region and such data has been provided below with special inference to the source and distribution of the acquired data. The data concerning the sources have also been added in the reference part.

Studies focusing the development of *C. megacepehala* has been previously studied in India by few authors (Subramanian and Mohan, 1980; Bharti et al., 2007; Verma and Reject Paul, 2013; Bala and Singh, 2015).

Age grading studies on immature stages of *C. megacephala* at different temperatures in the laboratory has been done at Punjab, India where the fly took 6.3 days for development from egg to adult stage at 30°C (Bharti et al., 2007; Bala and Singh, 2015).

An investigation by Bharti and Singh, (2007) reported the fact that the humidity and controlled temperatures have a direct impact on the life cycle of various flies belonging to Calliphoridae.The pattern of various stages in the lifecycle of the blowfly *C. megacephala* was analyzed (Bharti et al., 2007) with the following constant temperatures; 22 °C, 25 °C, 28 °C, and 30 °C. The findings from their study revealed that the range of temperature was inversely related to the development periods (63 days to 155 days).

The presence of *C. megacephala* has been reported in some regions in India and only three following studies had discussed its predominant significance in forensic entomology; (Bharti & Kurahashi, 2009; Roy & Dasgupta, 1975; Wall et al., 2001). According to Bharti & Kurahashi, (2009), the Indian form (fdf) of the blow fly *C. megacephala* may usually be recognized as a prominent form and they were typically found all over the year in the warmer conditions. However, the abundance may drastically be diminished in winter seasons.

The Indian scenario has witnessed the presence of about 119 species belonging to 30 genera for the Calliphoridae cosmopolitan group (Bharti, 2011). Radhakrishnan et al., (2012) reported the presence of maggots of *C. albiceps* from the cadaver of sambar deer from Kerala. *C. albiceps* was previously reported from North Africa, Madagascar, Rodriguez, Africa, Cape Verde, Socotra, United States, Afro-tropical Islands of Aldabra, South America, Palearctic region, and north-west India, which justified the significance of the same in an international perspective.

A study by Verma and Reject Paul, (2013) verified that the rate of insect development especially the various developmental processes in *Chrysomya* are strongly influenced by various environmental factors including temperature and humidity.Among the various studied flies, *Chrysomya* was noted to be one of the most predominant genera under Calliphoridae having veterinary, forensic and medical significances (Ramaraj et al., 2014).

According to Ramaraj et al., (2014), the presence of synanthropic form of *C. megacephala* and its diversity analysis in the various regions of South Indian region especially Tamil Nadu indicate the significance and scope of forensic entomology studies in the South Indian region. The authors validated for the first time that the aforesaid region witnessed an extensive abundance of the blow fly *C. megacephala.* The output gathered from their study can be used to identify the same species in their region for forensic entomology needs, especially for PMI estimation. For the identification of the blow fly *C.*

megacephala, they have used the following studies: Sinha & Nandi, (2004); Siriwattanarungsee et al., (2005) and Sukontason et al., (2008).

While considering the above-said perspectives for *C. chani*, a few international articles were found, and of which many of them cited that *C. chani* was reported from India. In addition, certain studies from India, Bharti et al., (2014) and Kurahashi, (1997) have revealed the habitat preferences of *C. chani* with special reference to the tropical and secondary forests. However, based on the previous literatures from an Indian perspective, it is clear that none of the studies had reported the developmental rate and life cycle studies of *C. chani*, and this justifies the significance of investigating the same for the current study.

While considering the geographic distribution of *C. chani*, it was reported that the following regions were found to be endemic for the aforesaid species that include Bangladesh (Chittagong), China (Hainan, Guangdong), India, Indonesia (Kalimantan), Malaysia (Perak, Selangor, Kuala Lumpur, Pahang), Philippines, Nepal (Mindanao, Samar), Sri Lanka, Vietnam Singapore, and Thailand. The natural forested areas were predominantly known to possess their extensive diversity in northern Thailand while adults have also been reported from the secondary forest in Indian regions (Bharti, 2014).

Abd Al Galil and Zambare, (2015) investigated the morphological parameters along with the life cycle duration of *C. rufifacies* and observed that the fly took longer duration for completion of its life cycle during winter in comparison to rainy and summer seasons.

Bharti and Singh, (2017), while reviewing the Forensic entomology perspective in the Indian context, one of the major things that has been found from the literature is that reliable and specific keys for the identification of various immature stages of flies using morphological characteristics are found to be either incomplete or unavailable in the current scenario. The authors have enlisted the *Chrysmoya* species along with their locations from Kerala, India and are as follows; *C. rufifacies* (Calicut, Kerala; Patiala, Punjab), and *C. megacephala* (Calicut, Kerala; Patiala, Punjab; Paonta Sahib, Himachal Pradesh;. *C. nigripes* (Calicut, Kerala); *C. chani* (Calicut, Kerala). Based on the aforesaid studies and various other literatures from the international perspective, *C. megacephala, C. rufifacies, C. chani* and *H. ligurriens* have prominent significance in the Indian forensic scenario.

Siddiki and Zambare, (2017) studied the life cycle of *C. megacephala* and *C. rufifacies* and observed a shorter life cycle duration in summer when compared to winter season. Gruner et al., (2017), in their study mentioned that the age of larvae would be helpful for the determination of minimum postmortem intervals.

Bharti and Singh, (2017) have used the mitochondrial COI gene to identify blow flies from India. The presence of *C. megacephala* in the Indian region was also reported by Badenhorst & Villet, (2018). Bharti, (2019) provided a revised key for the identification of known Indian *Chrysomya* species.

None have piloted the study in South India concerning the forensic implications on *H. ligurriens* and *C. megacephala* with special inference on the development rate and life cycle during different seasons except few recent studies (Reject Paul & Binoy, 2021 and 2022). Another study concerning the ultra structural details of larval instar using SEM on *H. ligurriens* with special inference on forensic significance was reported by Reject Paul and Binoy, (2021) for the first time from India.