



## **APPENDIX**

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# I. PUBLICATIONS

1.

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## Forensic implications of the seasonal changes in the rate of development of the blowfly, *Chrysomya megacephala* (Fabricius) (Diptera, Calliphoridae)

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**ABSTRACT:** Studies on the development rate of *Chrysomya megacephala* (Fabricius) suggested that the blowfly as a significant candidate for forensic investigations. Under natural ambient conditions development rate of *C. megacephala* in monsoon, winter and summer seasons indicated significant differences among seasons. The larvae began pupation at 92<sup>nd</sup> h in summer, 157<sup>th</sup> h in the monsoon season and 191<sup>st</sup> h in winter. Rapid larval growth in terms of length was observed in summer. During summer, the length of the larvae increased to a maximum of 13.9 mm at 54<sup>th</sup> h. Time taken for the emergence of the adult fly was 164, 249 and 311 h in summer, monsoon and winter seasons respectively. Life table studies were conducted to assess the percentage survival and mortality by recording the survival rate of different development stages. Molecular diagnosis of species was done using COI gene. The analysis included molecular sequences of other samples of the same species from different regions of India. The neighbor-joining method allowed us to identify the species at molecular level with precision and accuracy.

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**KEYWORDS:** Larval growth, pupation, adult emergence, seasonal differences, life table, molecular diagnosis

### INTRODUCTION

The blowfly, *Chrysomya megacephala* (Fabricius) (Diptera, Calliphoridae), a synanthropic fly, commonly known as oriental latrine fly inhabiting human settlements and commonly seen on decomposing cadavers, fish, carrion, human feces and sweet materials; indicating its medical, veterinary and forensic significance. Due to their cosmopolitan distribution, ubiquity and abundance, *C. megacephala* is recognized as the one of the most important species of insects in forensic entomology (Badenhorst and Villet, 2018). The larvae feed and grow on soft tissues of living and

dead vertebrates especially mammals, birds and fish (Yang and Shiao, 2012). The adult flies were usually attracted to decaying cadavers and reach within a few hours of death of the animal (Zumpt, 1965), and it has been considered as an important fly for the determination of minimum postmortem interval (Wang *et al.*, 2008). Medico legal cases world over have reported the forensic relevance of *C. megacephala* (Gruner *et al.*, 2017; Richards and Villet, 2009; Amendt *et al.*, 2004; Goff and Flynn, 1991). For the determination of minimum postmortem intervals, age of larvae will be helpful (Gruner *et al.*, 2017). Studies focusing the

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# Life cycle and development rate of *Hemipyrellia ligurriens* (Wiedemann) (Diptera: Calliphoridae) during monsoon season in South India: applications in estimation of postmortem interval

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## Abstract

*Hemipyrellia ligurriens*, considered as one of the forensically important blow fly species, has a wide distribution in many countries including India. To conduct forensic entomological investigations involving deaths of livestock, human beings and wild animals, standard life cycle data should be prepared for the local blow fly species under various weather conditions. Reliable forensic entomological data specific to geographic locations in India are not available presently to assist the post mortem interval assessment. In this study, life cycle and the rate of development of *H. ligurriens* was determined during monsoon season in Kerala, South India. Survival rate observed from egg to adult emergence was 44.68 %. Total duration of development of the species from oviposition till adult emergence was 462.57 h. Growth curves based on the age, specific length parameter and time taken for development of each larval stage was constructed. This development model would be helpful for the medical, veterinary and law enforcement officials in forensic estimation of post mortem interval by analyzing the length parameters of larvae collected from decomposed dead bodies of humans, cadavers of wild animals and livestock.

**Keywords:** *Hemipyrellia ligurriens*, development stages, postmortem interval, veterinary forensics

The *Hemipyrellia* genus 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), the latter being 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 has significant forensic importance. These species are widely distributed

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## Ultra structure of second instar larva of *Hemipyrellia ligurriens* (Wiedemann) (Diptera: Calliphoridae), a forensically important blow fly species from India

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**ABSTRACT:** Ultra structural characters of second instar larvae of *Hemipyrellia ligurriens* are elucidated through micrographs (Scanning Electron Microscope). Morphological details of maxillary palpi, antennae, oral cirri, facial mask, labial lobe, spinulations, and papillae of anal segment are described. Oral cirri are ten in number, arranged bilaterally on each side of the functional mouth opening and gently curved medially. The labial lobes are distinctively demarcated with fleshy projections antero-ventrally and have a characteristic shape. Thoracic spines have a bulbous base, slender sharp tips and are directed backwards. Prominent dorsal and ventral anal papillae with projected tips and broad conical base were present surrounded by microtrichia. The ultrastructure details of *H. ligurriens* would help in the rapid and accurate identification of the species in forensic investigations and to estimate time since death in medico legal cases. This is the first report on the ultra-structural features of *H. ligurriens*. © 2021 Association for Advancement of Entomology

**KEYWORDS:** *Hemipyrellia ligurriens*, identification, micrograph, scanning electron microscope

Forensic examinations involving decomposed dead bodies need a careful scrutiny of the entomological evidence as the latter being very significant in calculating time of death when the natural postmortem signs of body hold no significance beyond certain level of putrefaction. Studies on insects of forensic significance is very much rudimentary in India except for a few reports on selective species (Bala and Singh, 2015; Bharti and Singh, 2003; Kulshrestha and Chandra, 1987; Rao *et al.*, 1984). *Hemipyrellia ligurriens* (Calliphoridae: Luciliinae) seems to be a synanthrope found in close association with human habitats, garbage dumps, decaying animal bodies and cadavers. The adult flies are generally

considered as the vectors of many enteric pathogens (Sinha and Nandi, 2007). Kano and Sato (1952) reared this species on raw fish in Japan and described the larval stages. Ishijima (1967) described the third instar larvae of *H. ligurriens* while Bunchu *et al.* (2012) studied the morphological characters of larval stages of the species using light and stereo microscopy.

The oldest descriptions about all the three larval instars of *H. ligurriens* were provided by Tao (1927) and Knipling (1939). The keys provided in these works were of limited application owing to lack of species specific details. In recent works, blowfly species were identified based on the

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## II. NCBI GenBank submission details.

Sl.No	Species	Gene	Accession Numer
1	<i>C. megacephala</i>	Mitochondrial COI	MW522614.1
2	<i>C. rufifacies</i>	Mitochondrial COI	OM019083.1
3	<i>C. chani</i>	Mitochondrial COI	MW600494.1
4	<i>H. ligurriens</i>	Mitochondrial COI	MN831480.1

### III.

#### Seasonal life cycle data of *C. megacephala*, *C. ruffifacies*, *C. chani* and *H. ligurriens*

Species	Season	Pre-oviposition period (days)	Eggs laid in a day (nos.)	Periodicity of egg laying (days)	Eggs laid during life span (nos.)	Duration of different life cycle stages (hrs)						Total life cycle period from egg till the emergence of adult fly
						Incubation period of eggs (hrs)	Instar I	Instar II	Instar III	Post feeding Stage	Pupation stage	
<i>Chrysomya megacephala</i>	Monsoon	9.33 ± 1.00	374.67 ± 8.53	4.33 ± 0.35	2796.33 ± 114.39	15.67 ± 2.52	16.67 ± 1.53	22.33 ± 2.08	45.33 ± 13.32	32.00 ± 5.29	95.00 ± 3.61	227.00 ± 22.52
	Summer	8.44 ± 0.88	344.78 ± 14.73	4.17 ± 0.25	2442.89 ± 80.12	12.00 ± 2.00	12.00 ± 0	17.00 ± 1.00	27.00 ± 4.36	23.00 ± 4.58	77.00 ± 5.57	168.00 ± 5.29
	Winter	10.33 ± 0.71	317.00 ± 7.16	4.67 ± 0.35	2217.11 ± 69.91	24.00 ± 2.65	23.00 ± 6.08	28.00 ± 5.29	49.00 ± 6.25	36.33 ± 10.69	125.67 ± 7.37	286.00 ± 23.26
<i>Chrysomya ruffifacies</i>	Monsoon	7.56 ± 0.53	281.00 ± 5.45	4.50 ± 0.35	1953.89 ± 22.70	17.00 ± 3.00	17.67 ± 2.08	20.00 ± 4.58	50.00 ± 4.58	29.33 ± 4.04b	89.00 ± 1.00	223.00 ± 13.45
	Summer	7.67 ± 0.71	246.89 ± 11.68	4.17 ± 0.25	1849.11 ± 26.05	12.00 ± 1.73	16.67 ± 2.52	15.67 ± 3.51	15.00 ± 5.00	16.00 ± 4.00	73.00 ± 2.65	148.33 ± 6.43
	Winter	9.22 ± 0.67	215.33 ± 5.87	4.67 ± 0.35	1723.78 ± 12.18	18.33 ± 5.03	21.33 ± 3.51	32.67 ± 3.51	46.67 ± 7.02	36.67 ± 1.53	111.33 ± 3.79	267.00 ± 18.68
<i>Chrysomya chani</i>	Monsoon	7.78 ± 0.67	265.00 ± 6.27	4.56 ± 0.33	1735.11 ± 12.62	21.67 ± 4.51	20.33 ± 4.04	23.67 ± 5.13	42.00 ± 11.14	30.33 ± 10.21	108.00 ± 3.61	246 ± 20.08
	Summer	8.33 ± 1.00	234.44 ± 5.75	4.44 ± 0.33	1640.22 ± 5.40	15.67 ± 3.51	15.33 ± 2.89	16.67 ± 4.51	22.67 ± 4.51	25.33 ± 11.93	96.67 ± 1.53	192.33 ± 8.15
	Winter	10.11 ± 0.60	221.00 ± 5.72	4.78 ± 0.26	1627.22 ± 7.82	27.00 ± 5.57	18.67 ± 2.52	27.33 ± 2.52	42.00 ± 6.56	52.00 ± 10.58	153.33 ± 5.51	320.33 ± 24.03
<i>Hemipyrella ligurriens</i>	Monsoon	9.44 ± 0.53	238.78 ± 3.96	3.67 ± 0.35	1531.56 ± 16.01a	27.33 ± 4.04	18.00 ± 2.00	29.33 ± 5.51	62.00 ± 1.73	139.33 ± 14.64	154.33 ± 10.6	430.33 ± 33.5
	Summer	8.89 ± 0.78	221.33 ± 4.98	3.78 ± 0.36	1481.67 ± 9.99	17.67 ± 2.31	16.00 ± 1.00	16.33 ± 1.53	55.33 ± 5.69	81.00 ± 9.85	113.67 ± 8.15	300.00 ± 23.07
	Winter	10.44 ± 0.53	187.11 ± 4.46	3.56 ± 0.39	1340.56 ± 18.3	35.00 ± 5.29	16.67 ± 1.53	28.67 ± 4.51	59.67 ± 3.51	125 ± 46.18	192.33 ± 9.61	457.33 ± 54.31