

First record of *Chrysomya saffrana* (Diptera: Calliphoridae) of forensic importance in India

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Abstract. Many of the blow flies (Calliphoridae) are of economic importance because of their effects on public health and the economy by causing myiasis in human beings and animals. Blow flies are also, however, forensic bioindicators since they can be used to determine the Post-Mortem Interval (PMI). *Chrysomya saffrana* (Bigot 1877), a dipteran calliphoridae of forensic and economic importance is currently endemic and recorded only in Australia and New Guinea. Here, the occurrence of *C. saffrana* is reported for the first time from Aurangabad City, Maharashtra State in India. Fully grown third instar larvae of *C. saffrana* were collected from decaying cats. The larvae were reared under laboratory conditions and adult flies freshly emerged from pupae were collected and identified by their morphological features.

INTRODUCTION

There are about 1500 species of flies in the family Calliphoridae (Carvalho & Melo-Patiu, 2008). The Indian representatives of this family include 63 species under 18 genera (Nandi, 2004; Singh & Sidhu, 2004; 2007). *Chrysomya* is one of the most significant genera of Calliphoridae with great medical, veterinary and forensic importance. *C. saffrana* is a metallic greenish-blue blowfly with a black anterior spiracle and an appearance very similar to that of *C. megacephala* (James, 1971).

Ten species of the genus *Chrysomya* have been recorded in India (Bharti, 2011). Some species, such as those from the genus *Chrysomya* Robineau-Desvoidy, 1830, have medical relevance as vectors of pathogens, including bacteria, protozoans, and helminths, and as causal agents of myiasis (Greenberg, 1973). A study performed in Ethiopia showed that *C. ruffacies* acted as a

vector of at least five helminth parasites, including *Ascaris lumbricoides*, and four species of protozoan parasites: *Entamoeba histolytica* dispar, *Entamoeba coli*, *Giardia lamblia*, and *Cryptosporidium* sp. (Getachew *et al.*, 2007). Many Calliphorids are reported to be colonizers of human and animal corpses (Carvalho & Melo-Patiu, 2008). Indeed, they are one of the first colonizer insects of cadavers since they are attracted to the body often within minutes of death (Amendt *et al.*, 2004; Moretti *et al.*, 2011) and lay the eggs in natural orifices or wounds. The eggs develop through maggots and pupae from which adults then emerge. Observation of the later stages of development and the colonization sites of maggots can assist in determining the manner of death, the Post Mortem Interval (PMI) and any movement of the corpse from one site to another after death (Moretti *et al.*, 2011).

In order to interpret a crime scene, it is essential to identify the type of species of

insects invading the body, their behaviours and environmental requirements (Lord & Burger, 1983). During the process of decomposition, carcasses provide a staged series of habitats for insects, each characterized by a particular group of insects (Amendt *et al.*, 2004; Megnin, 1894; Fuller, 1934; Payne, 1965; Sukontason *et al.*, 2007). The process of infestation can be divided into three stages, entailing primary, secondary and tertiary colonizers (Fuller, 1934), although some of the secondary colonizer insects have the capability to act as a primary colonizer, such as *C. rufifacies* (Macquart), *C. saffranaea* (Bigot) and *C. varipes* (Macquart). Studies into the ecological distribution of Calliphoridae explore whether certain species are more associated with particular ecological habitats (e.g. urban, forest, swamp, etc.). This article reports the first observation of *C. saffranaea* in India.

MATERIALS AND METHODS

1. Collection, rearing and morphological identification

The specimens analysed in this study were collected from the garden of the Zoology Department at Dr Ambedkar Marathwada University Reserve (19° 52' 48.3" N, 75° 19' 12.3" E), Aurangabad City, Maharashtra State in India. The adult flies and maggots were collected in July, 2012 in suspended traps placed on the body of cat died in an accident. The site was visited daily. On the second day a huge number of ants attacked the eggs and maggots and hence small amount of larvae were collected, but on the third and fourth days after its death about 40 -50 larvae which came on surface were collected.

Both the adults and the maggots were reared in the laboratory in rearing boxes until the second generation. The flies were then identified by studying their morphology using a 4 stereoscope (ERMA Optical works, Tokyo, No. 44883), and a light microscope (Magnus Trinocular Microscope MLX-DX, Olympus (India) PVT. LTD. No. 4B525145).

2. Morphological description

Blowfly specimens were removed from the traps daily (at 24 hour intervals), preserved in 70% alcohol in the field and then mounted on pins. The abdomens were removed from both males and females and were then bleached unheated in 70% KOH for 24 hours, before being transferred to glycerine for further dissection and examination. Bleached abdomens and genitalia were stored in micro vials with glycerine and pinned beneath the respective source specimen.

The procedure adopted to identify the morphological features and the terminology used in the description were based on the identification key provided in (James, 1971; White *et al.*, 1940; O'lynn & Moorhouse, 1980; Spradbery, 2002), with some modifications, which described below in the keys. The identification characters for larvae and adults are given below. The adults and maggots were photographed using a digital Sony cyber-shot camera 16.1 MP. 5X optical zoom.

3. Key to larvae

- 1 - Tracheal trunks not heavily pigmented. Posterior margin of segment 11 with dorsal spines. Bands of pale brown to dark brown spines with 1 or 2 or more teeth, occurring singly or in files. Peritreme open. Button indistinct and in open area of peritreme (Fig. 1)..... 2



Figure 1. *C. saffranaea* posterior spiracle shows open peritreme with indistinct button like structure

- Tracheal trunks not heavily pigmented, posterior margin of segment 11 without dorsal spines.....
..... *Cochliomyia macellaria*

- 2 - Spine bands composed of elongate bifid or trifid spines anteriorly, tending to become single pointed posteriorly, whole band grading laterally into sharp thorn-like spines. Posterior sub-spiracular area bare. Anal protuberance with short files of setae along its posterior surface. Anterior spiracles with 10-11 papillae (Fig. 2)..... *Chrysomya saffranaea*

- Spine bands sparse having short blunt, simple spines mostly tending to form files of 2-5, some elongate bifid structures present in lateral regions. Posterior sub-spiracular region densely setulose. Setae forming distinct polygonal blocks which extend down posterior surface of anal protuberance. Anterior spiracles with about 13 papillae.....
..... *Chrysomya megacephala*

4. *Keys to Adult*

- 1 - Greater ampulla, in addition to the pubescence, with hairs that are longer than the height of the ampulla. Humeri not contrasting in colour with mesonotum and abdominal terga not marked with reddish yellow dorsally..... 2
- 2 - Anterior spiracle black or blackish (Fig. 3). Gena orange to reddish yellow, with yellow hairs..... 3
- 3 - No black setulae on facial or parafacial around the vibrissa (Fig. 4), or, rarely, 2 or 3 present. Frontal stripe of female not broader at middle of frons, parallel-sided (Fig. 5). Facets of eye of male larger above than below, but without any distinct line of demarcation (Fig. 6). Squamae brown..... *Chrysomya saffranaea* (Bigot)
- At least several, usually many, black setulae around vibrissa (Fig. 7), on face and parafacial..... 4

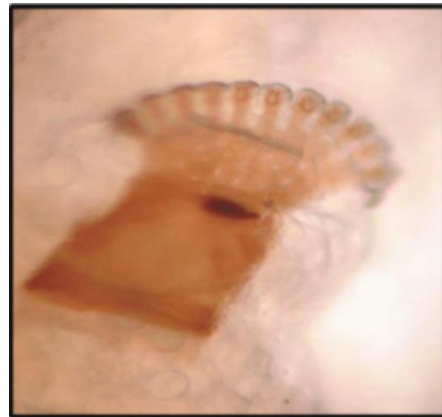


Figure 2. Anterior spiracle of *C. saffranaea* larvae show 11 papillae

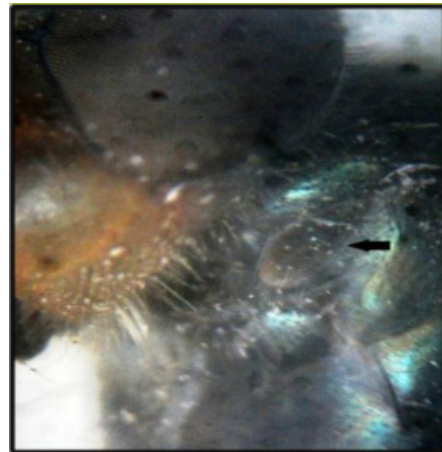


Figure 3. Anterior spiracle of adult of *C. saffranaea*, black in color

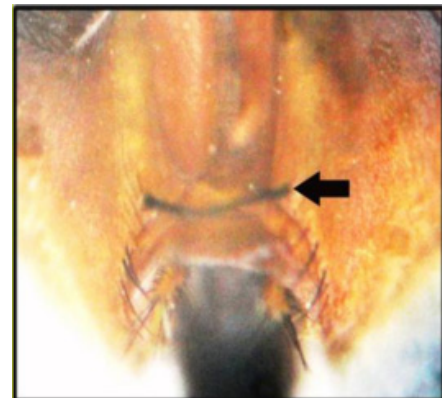


Figure 4. Female head of *C. saffranaea* did not shows any black setulae on facial or parafacial around the vibrissa

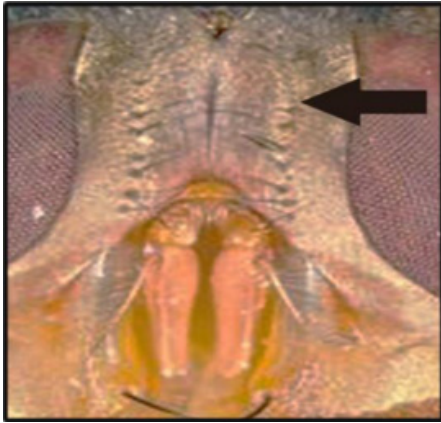


Figure 5. Female head of *C. saffranae* with parallel frontal stripe



Figure 8. Male head of *C. megacephala* shows demarcation between the upper and lower facets of eyes



Figure 6. Male head of *C. saffranae* without any distinct line of demarcation



Figure 9. Female head of *C. megacephala* shows broader frontal stripe at middle of frons

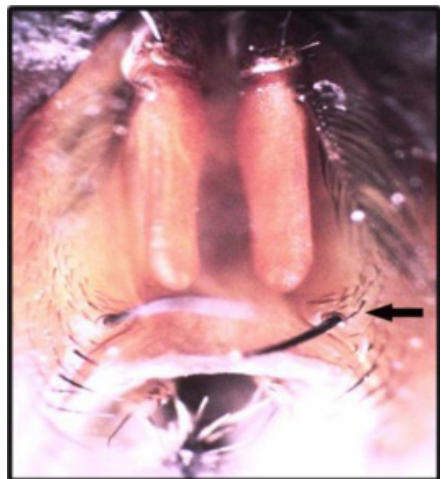


Figure 7. Female head of *C. megacephala* shows many black setulae around vibrissa

- 4 - Squamae brown; facets of eye much enlarged above and sharply demarcated from the area of the smaller facets below (Fig. 8). Frontal stripe of female broader at middle of frons, not parallel-sided (Fig. 9) [19,20].....
Chrysomya megacephala (Fabricius)

RESULTS

The site was visited till the body get dried however rupture of the abdomen on fourth day released large number of the third instar maggots which crawled on the surface and

then were collected. The bodies of the larvae were smooth without prominent papillae; and few papillae were present on the posterior segment. Posterior spiracles (Fig. 1) were flush with the face of the posterior segment. The peritreme was open, showing the button indistinctly in the open area of the peritreme, especially in third instar and prepupae. Tracheal trunks were not heavily pigmented. Anterior spiracles (Fig. 2) had 10-11 papillae, with these papillae sometimes varying from one larva to another, and sometimes varying in the same larva. Spines were present on the dorsal surface of at least a few segments, other than the abdominal segment, and there were complete bands of spines on the anterior margins of segments to at least segment VI. The abdomen exhibited broad, complete bands of spines on II-VIII; a narrower band on IX; and spines that were more lightly pigmented on X. On the posterior margin of segment XI the band was very narrow and often absent. Most spines were bifid or trifid anteriorly. These larvae were identified as larvae of *C. saffranaea*.

Adults appeared superficially similar to *C. bezziana* and very similar to *C. megacephala* (Fabricius). Their colour was metallic blue, and there was a row of bristles at the base of the stem vein dorsally. Lower (posterior) squamae were covered with fine hairs above, and a greater ampulla, in addition to the pubescence. Abdominal terga were not marked with reddish yellow dorsally while lower squamae were blackish brown to dirty grey. The frontal stripe (Fig. 5) of females was broader in the middle, parallel sided, with a relatively long ovipositor and no marked 7 indentation in the cheek. The anterior spiracle was black or blackish; gena orange to reddish with yellow hairs. Black setulae (Fig. 4) on facial or parafacial around the vibrissa were absent, although rarely, two or three black very small ones were present. The eye facets of males were larger above than below, but without any distinct line of demarcation. Adults were identified as of *C. saffranaea*.

DISCUSSION

Calliphoridae flies are commonly known as blow flies or green bottle flies. They are small to large metallic blue-green, robustly-built, and are covered with varying degrees of dusting. Adults frequent vegetation and excrement, or feed on decaying plant and animal matter. The larvae, meanwhile, are omnivorous, carnivorous, or parasitic. Calliphorids are known as primary scavengers feeding on carrion. Although (Wells *et al.*, 2004) could not distinguish *C. putoria* from *C. chlorogpya* and *C. megacephala* from *C. saffranaea*, these are four forensically important blowflies; therefore correct identification is a key criterion for PMI estimations when these flies are present.

Nandi (2004) give the first checklist record of Calliphoridae and Rhiniidae flies in India, they record about 63 species under 18 genera of Calliphoridae and 57 species under 12 genera of Rhiniidae. This checklist of Calliphoridae was updated (Bharti, 2011) by giving the Rhiniidae as one of 9 subfamilies known in India. These subfamilies contain 30 genera and 119 species, which have been recorded from all over India.

The observations reported in this study represent the first record of *C. saffranaea* in India, with this species having previously been reported only in Australia (Spradbery 2002) and in New Guinea (James, 1971). Also called the Australian blowfly, the species has infrequently been found breeding in the necrotic tissue associated with *C. bezziana* (Villeneuve) causing myiasis, although it typically breeds on carrion. Wherever the geographic location, it has been accepted that the most significant and the first insect colonizers on carrion are typically species of necrophagous flies in the family's Calliphoridae (blowflies) and Sarcophagidae (flesh flies) (Anderson & Van Laerhoven, 1996; Tabor *et al.*, 2005). But the species involved will vary according to the biogeographic region This habit of feeding

on carrion means that *C. saffrana* is useful for PMI determination in criminal investigations. Many myiasis cases have been reported in India in different metropolises caused by *C. bezziana*, *C. megacephala* and *C. albiceps* (Kumar *et al.*, 2011; Kulkarni *et al.*, 2012; Sankari & Ramakrishnan, 2010; Radhakrishnan *et al.*, 2012; Das *et al.*, 1979).

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