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The forensic significance of *Sarcophaga ruficornis*: A brief review

Irfaana Chowdhary, Mohammad Amir*
Department of Zoology, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

Abstract
Forensic entomology, the application of insect biology to legal investigations, has become an indispensable tool in estimating the postmortem interval (PMI) and understanding the circumstances surrounding death. Among the various insect families studied, the Sarcophagidae family, particularly *Sarcophaga ruficornis*, has garnered significant attention due to its unique biological characteristics and ecological roles. This review synthesizes current knowledge on *S. ruficornis*, focusing on its biological and ecological traits, forensic applications, and health implications. *S. ruficornis* is an early colonizer of decomposing remains, often arriving within 48 hours post-mortem, and its larvae develop rapidly under optimal conditions, making it a valuable indicator for PMI estimation. The species exhibits a larviparous reproductive strategy, giving birth to live larvae, which enhances its survival in competitive environment. Morphologically, *S. ruficornis* larvae possess distinct features that aid in their identification, such as anterior spiracles with 11-15 papillae and posterior spiracles with inner projections. The species has a broad geographic distribution, thriving in both urban and rural settings, and has been documented in regions such as Malaysia, Thailand, Brazil, and Kuwait. In addition to its forensic applications. *S. ruficornis* is associated with traumatic myiasis, a condition where larvae invade living tissues, posing health risks to humans and animals. Molecular techniques, such as DNA barcoding, have enhanced the accuracy of species identification, further solidifying its role in forensic investigations. This review underscores the importance of *S. ruficornis* in forensic science and highlights the need for continued research to improve methodologies and expand databases on flesh flies.

Keywords: Dipteran, forensic entomology, flesh flies, *sarcophaga ruficornis*, sarcophagidae

Introduction

Forensic entomology, the application of insect biology to legal investigations, plays a crucial role in the field of forensic science. It leverages the life cycle and behaviour of insects to provide valuable information regarding the circumstances surrounding death, particularly in estimating the postmortem interval (PMI) [1]. Among the various insect families studied within this discipline, the Sarcophagidae family, commonly known as flesh flies, has garnered significant attention due to its unique biological characteristics and ecological roles [2]. Notably, *Sarcophaga ruficornis*, a species within this family, has emerged as an important subject of study due to its implications in forensic investigations [3].

Insects are often among the first organisms to arrive at a decomposing body, making them vital indicators of time since death. Studies have shown that necrophagous insects, particularly those from the Sarcophagidae family, can colonize carcasses within hours of death [4, 5]. For instance, research indicates that flesh flies can complete their life cycle in as little as 7 to 14 days under optimal conditions. This rapid development is critical for determining PMI in homicide investigations [6]. Specifically, *S. ruficornis* has been documented to appear during the fresh to mid-decay stages of decomposition, highlighting its relevance in forensic contexts [7]. *S. ruficornis* is particularly noteworthy for several reasons. Unlike many other necrophagous flies that lay eggs on carrion, flesh flies exhibit a reproductive strategy known as ovoviviparity. This means that they give birth to live larvae rather than laying eggs, allowingthem to exploit decomposing remains more effectively and reducing the risk of egg predation [8]. The larvae of *S. ruficornis* are known to develop rapidly under suitable environmental conditions; studies have shown that they can reach maturity within 7 to 14 days depending on temperature and humidity levels [9]. Research conducted in various regions has demonstrated that *S. ruficornis* is capable of colonizing carcasses within 48 hours after death. For example, in Kuwait, first larvae were observed two days post-mortem and remained until the last rabbits were collected after 14 days. The species has also been recorded on human cadavers in Malaysia and pig carrions in Thailand and Brazil, indicating its widespread presence and forensic importance [10, 11]. Morphologically, *S. ruficornis* larvae possess distinct features that aid in their identification: anterior spiracles with 11-15 papillae arranged in a single row and posterior spiracles exhibiting inner projections between spiracular slits. The adult flies are characterized by their greyish bodies with black longitudinal bands and yellowish-orange antennae [12].

This review aims to synthesize current knowledge regarding the forensic implications of *S. ruficornis*. It will explore their biological and ecological characteristics, their applications in estimating PMI, and their potential health implications due to their association with human remains. Additionally, this article discusses the challenges faced in identifying Sarcophagid species and highlight future research directions that could enhance their utility in forensic entomology. By delving into these aspects, this article seeks to underscore the importance of *S. ruficornis* in forensic science and encourage further exploration into its role as a vital indicator in criminal investigations. Through a comprehensive understanding of this species, forensic professionals can improve investigative methodologies and contribute to more accurate determinations of death timelines. The establishment of detailed databases on flesh flies and their thermo-biological histories is essential for advancing forensic entomology.

Biological and Ecological Characteristics

S. ruficornis, a member of the Sarcophagidae family, exhibits a complex life cycle characterized by distinct developmental stages: egg, larva (with three instars), pupa, and adult (Figure 1). Notably, this species is primarily larviparous, meaning it gives birth to live larvae rather than laying eggs, a reproductive strategy that enhances survival rates in competitive environments. Research indicates that *S. ruficornis* can produce between 40 to 80 first-instar larvae per reproductive event, which are capable of feeding on decomposing organic matter almost immediately upon birth [13, 14]. The developmental duration of *S. ruficornis* larvae is significantly influenced by environmental factors such as temperature and humidity. Studies have shown that under optimal conditions (20-35°C), the larvae can complete their development in approximately 7 to 14 days. For instance, at temperatures of 22°C, 27°C, and 32°C, the emergence times for adults were recorded at 440 hours, 272 hours, and 232 hours, respectively [15, 16]. This rapid development makes *S. ruficornis* particularly useful in forensic investigations for estimating the postmortem interval (PMI).

The morphological features of *S. ruficornis* are integral to its identification and understanding its role in forensic entomology. The larvae possess distinct anatomical structures that are critical for their classification:

- **First Instar Larvae:** Approximately 6.8 mm in length.
- **Second Instar Larvae:** Approximately 11.8 mm.
- **Third Instar Larvae:** Approximately 16.9 mm.
- **Puparia:** About 11.7 mm in length with a short respiratory horn located dorsolaterally on the first abdominal segment

The anterior spiracles of the larvae have between 11 to 15 papillae arranged in a single row, while the posterior spiracles exhibit distinct inner projections between the spiracular slits. These morphological traits are crucial for differentiating *S. ruficornis* from other forensically significant species within the Sarcophagidae family [17]. *S. ruficornis* is known to be an early colonizer of decomposing remains, often arriving on carcasses within hours after death [18]. First-instar larvae were observed as early as two days post-mortem and remained active until approximately two weeks later. This rapid colonization is essential for forensic entomologists when assessing PMI [19]. Additionally, *S. ruficornis* demonstrates a preference for specific habitats that are conducive to its reproductive strategies. It thrives in warm climates and is frequently found in urban areas where decaying organic matter is readily available. Its adaptability to various environmental conditions enhances its potential as a forensic indicator [20, 21].

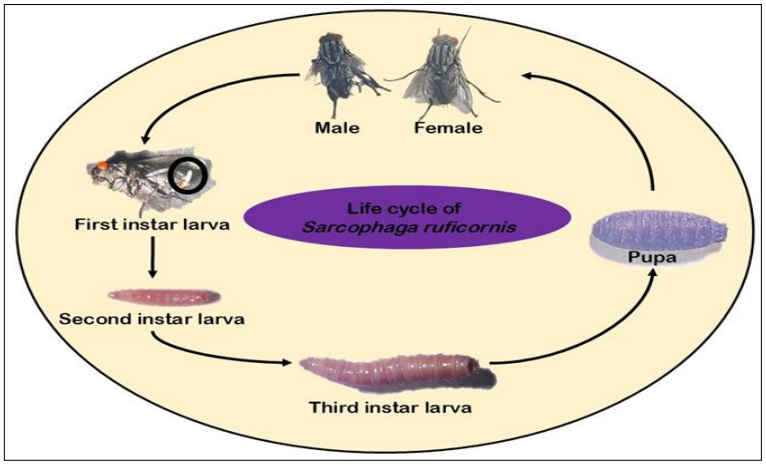

Figure 1: Life cycle of *Sarcophaga ruficornis*

Geographic Distribution and Habitat Preferences

S. ruficornis, a species within the Sarcophagidae family, exhibits a broad geographic distribution across multiple biogeographic regions. It has been recorded in the Afrotropical, Australasian, Nearctic, Neotropical, Oriental, and Palaearctic regions, demonstrating its adaptability to diverse environments and climates. This extensive distribution indicates that *S. ruficornis* is not only prevalent in tropical areas but also in temperate zones, making it a significant species for forensic studies globally. Recent studies have documented the presence of *S. ruficornis* in various countries, including Malaysia, Thailand, Brazil, and Kuwait. For instance, it was reported as the most abundant flesh fly species in Kuwait, with first-instar larvae appearing just two days after death on carcasses [10]. Notably, the first record of *S. ruficornis* in Egypt was confirmed through morphological and molecular techniques, expanding its known range further into the African continent [22]. This species' ability to thrive in both urban and rural settings underscores its ecological versatility. *S. ruficornis* exhibits specific habitat preferences that influence its distribution and colonization patterns. Typically, this species is found in environments where decaying organic matter is readily available, such as urban areas, rural settings, and even forested regions [23]. In urban settings, the presence of waste and decomposing materials provides abundant breeding sites for *S. ruficornis*, allowing it to thrive in cities where carrion may be more accessible. In rural areas, it can be found on animal carcasses in agricultural fields or near livestock facilities, where the availability of large animal remains enhances its reproductive success. Although less common, this species can also inhabit forested areas where natural animal mortality occurs; the shade provided by trees may offer suitable microclimates for larval development [24, 25]. Several environmental factors play a critical role in shaping the distribution of *S. ruficornis*. Temperature significantly impacts the developmental rates of this species; warmer climates facilitate faster growth and reproduction,

allowing populations to thrive. Additionally, high humidity levels are conducive to larval survival and development, as moist environments reduce desiccation risk for larvae and provide favourable conditions for decomposition processes [21]. The presence of vegetation can also influence microhabitats available for colonization; areas with dense vegetation may support a higher diversity of necrophagous insects due to increased animal activity and mortality [26].

Forensic Applications

The forensic applications of *S. ruficornis* are significant, particularly in estimating the postmortem interval (PMI), which is crucial in criminal investigations. As a necrophagous insect, *S. ruficornis* is often one of the first species to colonize decomposing bodies, making it an important indicator for determining the time since death [27]. In a study conducted in Kuwait, first-instar larvae were observed as early as two days after death, and they remained active on the carcass for up to 14 days. The ability of *S. ruficornis* to complete its life cycle in approximately 7 to 14 days under optimal conditions allows forensic entomologists to estimate the PMI with considerable accuracy [19].Research indicates that optimal temperatures for larval development range from 20°C to 35°C; at these temperatures, the larvae can mature quickly, facilitating timely estimations of PMI [28]. In one study, it was reported that at 22°C, larvae took about 440 hours to develop into adults, while at 32°C, this duration decreased to approximately 232 hours [29]. Such precise developmental data are invaluable for forensic investigations where determining the time of death is essential. In addition to its role in estimating PMI, *S. ruficornis* is also significant due to its association with traumatic myiasis. This condition occurs when larvae invade living tissue, leading to health complications in humans and animals [30]. Cases of myiasis caused by *S. ruficornis* have been documented, including a notable instance involving a five-month-old infant who suffered from oral myiasis due to the presence of larvae in the mouth. The diagnosis was confirmed through both morphological examination and molecular techniques, specifically by analyzing the cytochrome oxidase subunit I (COI) gene [12]. This highlights not only the medical implications of *S. ruficornis* but also its importance in forensic contexts where understanding health risks associated with deceased individuals is crucial. Molecular identification techniques have further enhanced the forensic applications of *S. ruficornis*. DNA barcoding using the COI gene has proven effective in confirming species identity, which is essential given the morphological similarities among various flesh fly species [31]. For instance, a recent study confirmed the identity of *S. ruficornis* through an integrative approach combining morphological and molecular methods. The COI gene was amplified and sequenced, yielding an approximately 817 base pair fragment that was compared against public databases like GenBank and BOLD (Barcode of Life Data System) [22]. This molecular confirmation supports accurate species identification and enhances the reliability of PMI estimations. Moreover, entomotoxicology—the study of toxins accumulated by insects feeding on decomposing remains—represents another emerging application of *S. ruficornis* in forensic science. As these necrophagous insects consume tissues contaminated with drugs or toxins, they can provide critical information regarding substances present at the time of death [32]. Research has indicated that *S. ruficornis*, like other flesh flies, can accumulate various toxic substances during its life cycle. Analysing these toxins within insect samples may yield more reliable results than traditional analyses performed on decomposed tissues [33].

Relation between traumatic myiasis and flesh flies

Myiasis is the invasion of tissues and organs both in humans and animals by dint of the larvae of saprophagous flies. Those larvae feed on the host tissues, body fluids, or ingested food as parasites in the skin, subcutaneous tissues, mouth, stomach, eyes, nose, ears, intestines, urinogenital system, and other soft tissues of humans and warm-blooded vertebrate animals [34, 35]. Relevant cases were mainly reported in Europe and Asia at present. In humans and animals, Sarcophagid species have been reported to cause myiasis in ophthalmic, nasal, urinogenital, aural, cutaneous, oral and gastrointestinal cases. Accordingly, it is crucial to exclude traumatic myiasis in the PMI estimation based on the development of saprophagous flies. Investigations illustrated that the most common species causing traumatic myiasis is *Wohlfahrtia magnifica* Schiner, Wohlfahrt's wound myiasis fly, the third of the most important obligatory traumatic myiasis agents. Besides, the common Sarcophagid species causing myiasis also includes *S. africa*, *S. argyrostoma*, *S. crassipalpis* and *S. ruficornis* [36, 42]. Traumatic myiasis caused by Sarcophagid species is extensively reported as the consequence of ignorance and can be used as an indicator of wound care neglect, either by oneself or by the nurses. However, criminal investigations require more researches involving various fly species and means of administration to establish a systematic database [35].

Conclusion

S. ruficornis has emerged as a critical species in forensic entomology due to its rapid colonization of decomposing remains and its ability to provide accurate estimates of the postmortem interval (PMI). Its unique reproductive strategy, larviparity, allow it to exploit carrion efficiently, while its distinct morphological features facilitate reliable identification. The species' broad geographic distribution and adaptability to various environments further enhance its utility in forensic investigations across different regions. Additionally, *S. ruficornis* is implicated in traumatic myiasis, highlighting its medical significance and the need for careful consideration in forensic contexts. Advances in molecular identification techniques, such as DNA barcoding, have improved the accuracy of species identification, while entomotoxicology offers new avenues for detecting toxins in decomposing tissues. Despite these advancements, challenges remain in differentiating Sarcophagid species and understanding their developmental biology under varying environmental conditions. Future research should focus on expanding databases on flesh flies, refining molecular techniques, and exploring the ecological factors that influence their behaviour and development. By deepening our understanding of *S. ruficornis* and other necrophagous insects, forensic entomologists can enhance their investigative methodologies, contributing to more accurate and reliable determinations of PMI and advancing the field of forensic science.

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