

الجمهورية الجزائرية الديمقراطية الشعبية
PEOPLE'S DEMOCRATIC REPUBLIC OF ALGERIA
وزارة التعليم العالي و البحث العلمي
MINISTRY OF HIGHER EDUCATION AND SCIENTIFIC RESEARCH
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A Dissertation Submitted in Fulfilment of the Requirements for
Master Degree in Biological Sciences
Speciality: Parasitology

**Determination of primary necrophagous insects
from different biogeographic regions in Algeria in
consideration of forensic and medical application**

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**Academic year
2020-2021**

ACKNOWLEDGEMENTS

At the end of this work, I would like to thank above all the good Lord Almighty who gave me the will, the courage and the patience to do this modest job.

*I would like to thank profoundly **Dr. Taleb M.**, for her scientific guidance and corrections, suggestions and advice, pertinent criticism and pragmatism, and particularly for her hard work and patience, I'm very grateful to her, for without her help an important part of this research would have been missed.*

*My sincere acknowledgements go also to **Dr. makhlouf** and **Dr. Saighi H.** who have been so kind to read my work. I thank both of them for being a member of the jury.*

DEDICATIONS

I dedicate this humble work to first of all my beloved parents and all the family members.

To the Volleyball Family Team and all team mates I ever practiced with in volleyball.

And last but not least to the Hive speaking club for being a fascinating club my biggest wishes for them for success.

Determination of primary necrophagous insects from different biogeographic regions in Algeria in consideration of forensic and medical application

ABSTRACT

Necrophagous Diptera have a significant role in medical, veterinary, and forensic entomology. In addition to being potential mechanical vectors of several microorganisms, these species may be parasitic as maggots are capable of invading living tissues causing myiasis. Furthermore, fly larvae are effective in treating chronic wounds.

The establishment of a national catalogue of forensically and medically important Diptera is crucial for the application of these species. Therefore, we investigated the effectiveness of several baits in trapping necrophagous Diptera and determined the species composition and relative abundance in urban habitats in different biogeographical regions of Algeria.

Specimens were collected in October 2020 and March, April, and May 2021 using baited traps in the Mediterranean, semi-arid, and desert regions. The samples were morphologically identified with the help of dichotomous keys.

The main forensically important Algerian blow fly species, namely *Calliphora vicina* (Robineau-Desvoidy), *Chrysomya albiceps* (Wiedemann), and *Lucilia sericata* (Meigen) were widely represented among the sampled populations. The biodiversity of the species in the Mediterranean zone was the highest compared to the semi-arid and the desert regions. *C. vicina* and *L. sericata* were present in all the regions. During early autumn and spring *L. sericata* was the most abundant species while *C. vicina* was less frequent. *Ch. albiceps* and *Ch. megacephala* occurred only on fish and chicken meat baits.

In conclusion, the most frequent species found on decomposed tissue belong to the family Calliphoridae. Classified as forensically important species, the blow flies were widely represented among the sampled populations.

Keywords: Diptera, necrophagous flies, forensic entomology, biogeographic regions, Algeria.

Détermination des insectes nécrophages primaires de différentes régions biogéographiques de l'Algérie compte tenu de leur application forensique et médicale

RESUME

Les Diptères nécrophages ont un rôle important en entomologie médicale, vétérinaire et médico-légale. En plus d'être des vecteurs mécaniques potentiels de plusieurs micro-organismes, ces espèces peuvent être parasitaires étant donné que les asticots sont capables d'envahir les tissus vivants provoquant une myiase. De plus, les larves de mouches sont efficaces dans le traitement des plaies chroniques.

L'établissement d'un catalogue national des diptères d'importance forensique et médicale est indispensable pour l'application de ces espèces. Par conséquent, nous avons étudié l'efficacité de plusieurs appâts pour piéger les Diptères nécrophages et déterminé la composition des espèces et leur abondance relative dans les habitats urbains de différentes régions biogéographiques d'Algérie.

Des spécimens ont été collectés en octobre 2020 et en mars, avril et mai 2021 à l'aide de pièges appâtés dans les régions méditerranéennes, semi-arides et désertiques. Les échantillons ont été identifiés morphologiquement à l'aide de clés dichotomiques.

Les principales espèces de mouches nécrophages algériennes importantes en médecine et en criminalistiques, à savoir *Calliphora vicina* Robineau-Desvoidy, *Chrysomya albiceps* (Wiedemann) et *Lucilia sericata* (Meigen), étaient largement représentées parmi les populations échantillonnées. La biodiversité de l'espèce dans la zone méditerranéenne était la plus élevée par rapport aux régions semi-arides et désertiques. *C. vicina* et *L. sericata* étaient présentes dans toutes les régions. Au début de l'automne et au printemps. *L. sericata* était l'espèce la plus abondante tandis que *C. vicina* était moins fréquente. *Ch. albiceps* et *Ch. megacephala* n'ont été observées que sur des appâts à base de poisson et de viande de poulet.

En conclusion, les espèces les plus fréquemment trouvés sur les tissus en décomposition appartiennent à la famille des Calliphoridae. Classée comme espèce d'importance médico-légale, les mouches à viande étaient largement représentées parmi les échantillons collectés.

Mots-clés : Diptères, mouches nécrophages, entomologie médico-légale, Régions biogéographiques, Algérie.

تحديد الحشرات الجبرية الأولية من مناطق جغرافية حيوية مختلفة في الجزائر في ضوء تطبيقها في الطب الشرعي والطب

الملخص

تلعب ثنائيات الأجنحة الرميّة دورًا مهمًا في علم الحشرات الطبي والبيطري والطب الشرعي. بالإضافة إلى كونها نواقل ميكانيكية محتملة للعديد من الكائنات الحية الدقيقة، يمكن أن تكون هذه الأنواع طفيلية لأن الديدان قادرة على غزو الأنسجة الحية مسببة النغف. بالإضافة إلى ذلك، فإن يرقات الذباب فعالة في علاج الجروح المزمنة.

يعد إنشاء فهرس وطني لثنائيات الأجنحة الرميّة أمرًا بالغ الأهمية لاستعمال هذه الأنواع. لذلك قمنا بدراسة فعالية العديد من الطعوم في اصطياد ثنائيات الأجنحة الرميّة وتحديد تكوين الأنواع ووفرتها في الموائل الحضرية في مختلف المناطق الجغرافية الحيوية في الجزائر.

تم جمع العينات في أكتوبر 2020 ومارس وأبريل ومايو 2021 في مناطق البحر الأبيض المتوسط وشبه القاحلة والصحراوية. تم تحديد العينات شكليًا باستخدام مفاتيح ثنائية التفرع.

تم تحديد أنواع ثنائيات الأجنحة الرميّة الجزائرية الرئيسية ذات الأهمية في الطب الشرعي، وهي *Lucilia* و *Chrysomya albiceps* (Widemann) و *Calliphora vicina* Robineau-Desvoidy (*sericata* (Meigen) على نطاق واسع. كان التنوع البيولوجي في منطقة البحر الأبيض المتوسط أعلى مقارنة بالمناطق شبه القاحلة والصحراوية. كانت *C. vicina* و *L. sericata* موجودتان في جميع المناطق في أوائل الخريف والربيع. كانت *L. sericata* أكثر الأنواع وفرة بينما كانت *C. vicina* أقل انتشارًا. تم ملاحظة *Ch. albiceps* و *Ch. megacephala* فقط على لحوم الأسماك والدجاج.

في الختام، تنتمي الأنواع الأكثر تواجداً في الأنسجة المتحللة إلى عائلة Calliphoridae. حيث تم تصنيف ذباب اللحم على أنه ذو أهمية في الطب الشرعي، ولقد لوحظ على نطاق واسع بين العينات التي تم جمعها.

الكلمات المفتاحية: ثنائيات الأجنحة، ذباب رمّي، ذباب، علم الحشرات الشرعي، مناطق جغرافية حيوية، الجزائر

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INTRODUCTION

Introduction

Insects are count to be the most important creatures on the planet Earth, composed over than 86,000 known species due to their impact on the human kind and nature (Byrd and Castner, 2010). Diptera is one of the major orders that present flies and mosquitoes with over than 153,000 species distributed in approximately 180 families (Brown et al, 2009). Flies are important as scavengers, removing decomposing plant and animal material from the environment. Some are predators and parasites of other insect species, and others aid in the pollination of plants. Furthermore, considering the fact that they are important decomposers, the presence of flies in medical entomology and the medical field is inevitable (Byrd and Castner, 2010).

The proper identification of the insect and arthropod species of forensic importance is the most crucial element in the field of forensic entomology. It is the species identification that allows the proper developmental data and distribution ranges to be applied to an investigation. If the species determination is incorrect, the PMI is invalid (Byrd and Castner, 2010).

Likewise, the use of maggots in wound healing is among the best studies of direct medical applications of invertebrates. For centuries maggots, and various invertebrate based medicinal products and treatments have been used in traditional medical practices worldwide. There is evidence for the medical use of maggots dating back to the compared to non-maggot bearing wounds (Jordan et al, 2018).

Nevertheless these maggots have also the ability to cause a certain condition known as myiasis. It has been defined as the infestation of live vertebrates (humans and/or animals) with dipterous larvae flies causing myiasis are still some of the world's most devastating insects, responsible for severe losses in animal husbandry, with significant economic losses. In mammals (including humans), dipterous larvae can feed on the host's living or dead tissue, liquid body substance, or ingested food and can cause a broad range of infestations, depending on the body location and the relationship of the larvae with the host. The distribution of human myiasis is worldwide, with more species and greater abundance in poor socioeconomic regions of tropical and subtropical countries. In countries where it is not endemic, myiasis is an important condition, where it can represent the fourth most common travel associated skin disease (Francesconi and Lupi, 2012).

Forensic entomology was first established in Algeria by the National Institute for Criminalistics and Criminology of the National Gendarmerie (NICC) in 2010 (Taleb, 2019). However, the identification of the species causing myiasis is rarely carried out in Algeria. Epidemiological data on human myiasis are rare and registration of cases are usually not mandatory.

For this reason, the establishment of a national catalogue of forensically and medically important Diptera is crucial for the application of these species. Therefore, we investigated the effectiveness of several baits in trapping necrophagous Diptera and determined the species composition and relative abundance in urban habitats in different biogeographical regions of Algeria.

The current study is divided into three chapters; Chapter 1 consists of a literature review on necrophagous Diptera and their applications, Chapter 2 presents the materials and methods of our surveys, and finally Chapter 3 describes the results of the study as well as their discussion. In the end, a conclusion and perspectives are presented.

CHAPTER I: LITERATURE REVIEW

1 Overview of necrophagous Diptera

1.1 Definitions

Diptera are holometabolous insects, distinguished from others by the presence of a single pair of functional wings in adults. This feature is what refers to the word Diptera: “two” (di-) and “wings” (ptera), it comes from the fact that they lost a pair of wings that got replaced by small structures known as halteres. (Fusari et al., 2018).

This order is a house of myriad species, the representative of flies and mosquitoes with over than 153,000 species distributed in approximately 180 families. It is by far the most important order due to the morphology and the ecology of insects hence the presence of flies and mosquitoes in the medical entomology and veterinary is inevitable (Brown et al., 2009).

Necrophagous insects are arthropods that forage directly on decomposing organisms, or the fluids released from these organisms during the decomposition process. Often, necrophagous blowfly species are the first to reach and colonize decomposing organisms for oviposition as such are considered the most important insects for post-mortem interval estimations (Tsifoanya et al., 2017).

Flies are important as scavengers, removing decomposing plant and animal material from the environment. Some are predators and parasites of other insect species, and others aid in the pollination of plants, furthermore, there’s only some of these fly species that could be used in forensics considering the fact that they are important decomposers (Castner, 2001).

1.2 Taxonomy

There are two major suborders of Diptera: the Nematocera and Brachycera. We can distinguish these two suborders by the antennae, which is thin and multi-segmented in Nematocera and shorter with less than six segments in Brachycera (Wyss and Cherix, 2006; Lemonnier and Reguardati, 2012). Necrophagous flies are classified among the Brachycera suborder.

a. Nematocera

Nematocera means threathorn and refers to the long and uniform multis-segmented antenna they are typified by mosquitoes and other flies with conspicuously long antennae.

There are almost 35 families and 50,000 species of Nematocera (Wyss and Cherix, 2006).

The number of recognized families ranges from five to eight it includes the mosquitoes (culicidae), black flies (simuliidae) and biting midges (Ceratopogonidae) etc.

b. Brachycera

The name refers to short antenna of this large and extremely varied suborder consisting of 113 families and 100,000, this suborder is divided into two groups (Wyss and Cherix, 2006):

- Orthorrhaphs (23 families and 35,000 species) are represented by only the Tabanidae or horseflies.
- Cyclorrhaphs (90 families and more than 65,000 species) such as Calliphoridae, Muscidae, Syrphidae, Sarcophagidae *etc.*

Table 1: Taxonomic classification and families of Diptera interest to medical and veterinary entpmologists.

Higher taxa	Family	Common names
Suborder Nematocera	Tipulidae	Crane flies
	Bibionidae	March flies
	Psychodidae	Moth flies, sand flies
	Chaoboridae	Phantom midges
	Culicidae	Mosquitoes
	Simuliidae	Black flies
	Ceratopogonidae	Biting midges
Suborder Brachycera	Tabanidae	Horse flies, deer flies
	Muscidae	House flies, stable flies, and allies
	Glossinidae	Tsetse
	Calliphoridae	Blow flies
	Sarcophagidae	Flesh flies

1.3 Morphology

The external body of a fly may appear in different shapes, size and colours. Nevertheless, flies can be found in almost any habitat and are characterized by having only one pair of wings. The second pair of wings is reduced to only knob-like organs called halteres, which are used to stabilize the insect in flight. Flies have large compound eyes with mouthparts of various types. However, most flies associated with carrion have sponging mouthparts (Figure 1). The larvae of flies are called maggots and most are cream coloured, soft, legless, and lack a visible head (Figure 2) (Byrd and James Castner, 2010).

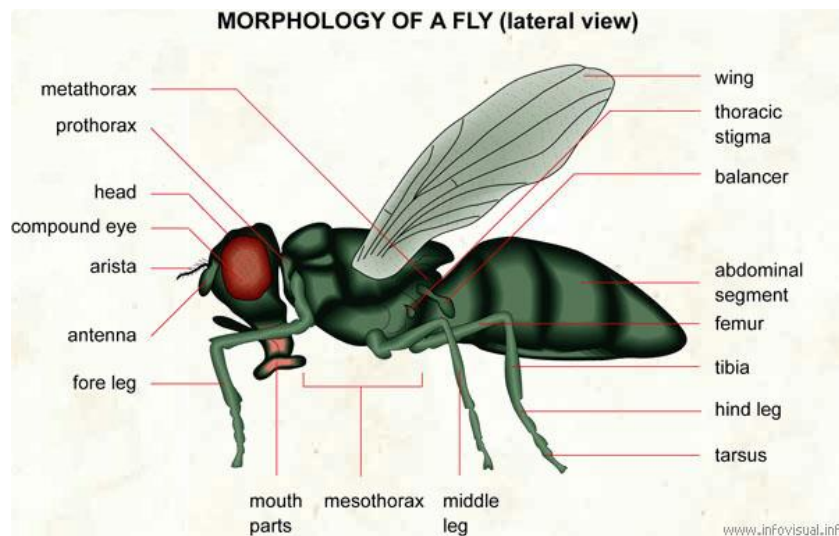


Figure 1: General morphology of fly (drey, 2007).

1.4 Morphology and biology of the main families with forensic and medical importance

a. Family Calliphoridae

Known as blow flies, this is an extremely large family of medium-sized flies that contains more than 1000 species. Calliphoridae adults usually range from 6 to 14 mm in length. The majority of species are metallic in appearance (Figure 3), with colours ranging from green, blue, bronze, or black. In some species, a covering of fine powder or dust masks the bright metallic coloration of the fly body. Adults have three segmented antennae with a hair or arista on the last segment, this arista is plumose or hairy throughout its entire length. They are generally the first insects to colonize bodies. Mature blow fly larvae range from 8 to 23 mm in length. They are mostly white or cream coloured. The last segment of the larval

body typically has six or more cone-shaped tubercles; this segment also contains the posterior spiracles, which are the primary breathing apparatus of the larva (Figure 2) (Mcalister, 2015).

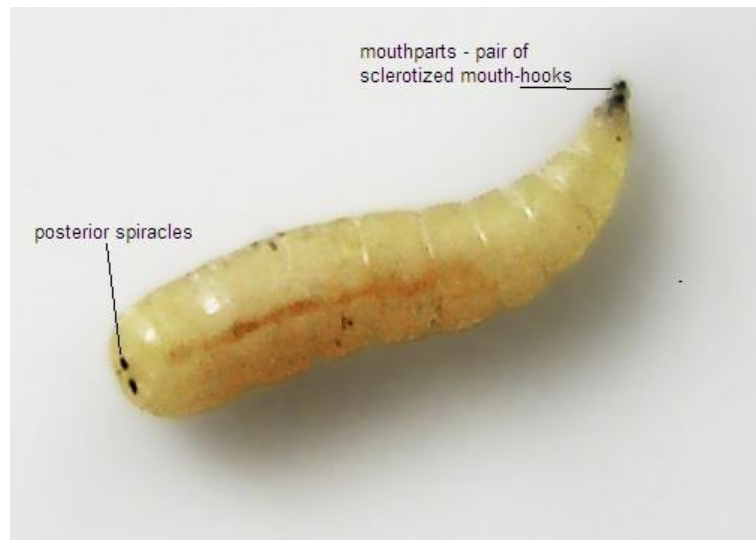


Figure 2: The larvae morphology (Mcalister, 2015).

Calliphoridae are the most important species that provide information relating to the accurate estimation of the postmortem interval. Calliphorid flies are attracted to carrion and excrement, with some species use open wounds causing myiasis. This family includes the familiar green bottle flies (*genus Sericata* also called *Phaenicia*) and blue bottle flies (*genus Calliphora*), as well as the screwworm flies (*genus Cochliomyia*) (Byrd and James Castner, 2010).



Figure 3: *Chrysomya megacephala* known as blow fly (Cimon, 2018).

In experimental studies, calliphorid flies have been recorded arriving at carcasses within minutes of their exposure. The telescoping segments of the tip of the female's abdomen extend to form an ovipositor, which is used for egg laying. Large numbers of eggs are commonly placed in the natural cavity of the human body such as nose and mouth, as well as other openings in the body that are exposed. Areas with open wounds also are selected for egg placement. Thus, subsequent maggot mass formations and uneven defleshing of bodies can be indicative of premortem or perimortem trauma. It is a good practice to have both a forensic pathologist and a forensic anthropologist closely examine these sites. (Arnett and Jacques, 1981; Bland and Jacques, 1978; Borrer and White, 1970; Borrer et al., 1989; Castner et al, 1995; Hall and Doisy, 1993; Hogue, 1993; James and Harwood, 1969; Liu and Greenberg, 1989; Peterson, 1979; Shewell, 1987).

b. Family Sarcophagidae

Commonly called flesh flies, Sarcophagidae comprise a large family with over 2000 species, approximately 327 of which occur in either tropical or warm temperate regions. Flesh flies are medium-sized and range in length from 2 to 14 mm. The adults commonly have gray and black longitudinal stripes on the thorax and have a tessellated (checkerboard) pattern on the abdomen. The bodies of sarcophagids tend to be bristly and the eyes are fairly widely separated in both sexes. In some species the eyes are bright red in colour, as are the highly visible genitalia at the tip of the abdomen (Figure 4). The larvae of flesh flies have the posterior spiracles located in a pit or depression at the tip of the abdomen, which is edged with fleshy tubercles. This characteristic can be used to differentiate between flesh fly and blow fly larvae.

Adults are common and often found on flowers where they are attracted to nectar. The adult flies feed on other sweet substances as well, including sap and honeydew. This family's Latin name means "flesh eating" and essentially refers to the larvae or maggots that typically feed on some sort of animal material. In addition to carrion, they also may feed on excrement or exposed meats. They have been known to cause myiasis and may be involved in the mechanical transmission of diseases. Many sarcophagid species or flesh flies are parasitoids of other insects, especially bees and wasps (Aldrich, 1916; Arnett and Jacques, 1981; Bland and Jacques, 1978; Borror and White, 1970; Borror et al., 1989; Castner et al., 1995; Greenberg, 1971; Hall and Doisy, 1993; Hogue, 1993; James, 1947; James and Harwood, 1969; Knipling, 1936; Payne, 1965; Peterson, 1978; Shewell, 1987; Smith, 1956, 1975, 1986).



Figure 4: *Sarcophaga bercaea* known as flesh fly (Martiré, 2011).

c. Family Muscidae

Muscid flies belong to a large family that is worldwide in distribution, many species are ubiquitous and synanthropic (found closely associated with man). The propensity with which muscid flies are found in domestic situations contributes to both their medical and forensic importance. Common members of this family are the house fly, stable fly, horn fly, and latrine fly. Muscid flies are small to medium-sized, typically ranging from 3 to 10 mm in length. They tend to be dull gray to dark in colour, although a few species have a metallic sheen (Figure 5). Most muscid larvae conform to the typical cylindrical shape associated with a maggot, tapering from the tail end towards the head and mouth. Mature larvae range from 5 to

12 mm in length and are white, yellow, or cream-colored. The surface of the maggot is smooth in most species (Byrd and James Castner, 2000).

The biology and habits of muscid flies are quite varied as the adults may feed on decaying plant and animal material, dung or excrement, pollen, or even blood.. Other species that breed and feed on garbage, sewage, and human waste can be responsible for the mechanical transmission of diseases. These include species such as the house flies which regurgitate digestive fluid directly onto food materials as part of their feeding process (Byrd and James Castner, 2000).



Figure 5: *Hydroteia dantipes* known as dumb fly (Vikhrev, 2015).

Muscid flies are of great forensic importance due to their wide distribution, ubiquitous nature, and close association with man. They tend to arrive at bodies after the flesh flies and blow flies. They often lay their eggs in natural body openings, at wound sites, or in fluid-soaked clothing. Larvae feed directly on carrion, but in some species exhibit predacious behavior as they mature. In such cases, muscid larvae may affect the faunal composition on a set of remains by preying on the eggs and larvae of other carrion-feeding flies. (Arnett and Jacques, 1981; Bland and Jacques, 1978; Borror and White, 1970; Borror et al., 1989; Castner et al., 1995; Hogue, 1993; Hockett and Vockeroth, 1987; James and Harwood, 1969; Peterson, 1979).

d. Family Piophilidae

Also the skipper flies comprise a small family of only 69 species. Adults are metallic blue or black in colour and typically range from 2.5 to 4.5 mm in length (Figure 6). Piophilidae or skipper flies are found in a variety of habitats that may include carrion, human waste, bones, skin, and fur. They are common and usually associated with protein-rich food sources that are dry in nature.

The common name of “skipper” comes from an unusual behaviour exhibited by the larvae of some species, the larvae will grasp small protrusions on the anal segment with the mouth hooks and suddenly release their grip, this action flings the larva into the air 7 to 10 cm, and laterally over a distance of 15 to 20 cm. This “jumping” behaviour is used as an effective “escape” mechanism and it also is utilized extensively during larval migration. However, they also move in the more traditional creeping manner exhibited by most fly larvae (Arnett and Jacques, 1981; Bland and Jacques, 1978; Borror and White, 1970; Borror et al., 1989; Castner et al., 1995; Hogue, 1993; James and Harwood, 1969; McAlpine, 1987; Peterson, 1979).



Figure 6: Piophilidae species also known as skipper fly (maimon, 2011)

e. Family Scathophagidae

The Latin name for this family means “dung-eating,” There are over 250 species, almost all of which are found in the Holarctic region. Approximately 150 species occur in temperate North America with the greatest diversity found in southern Canada. The most common species of dung flies are red or yellow and densely hairy (Figure 7). Some species are attracted to excrement or decaying plant material, and their larvae are most commonly found in excrement. Other species are dark as adults and have larvae that are leaf miners, pests of flower heads, parasitic, predacious, and aquatic. The larvae of the genus *Scatophaga* feed on dung, but also are often found on decaying seaweed. (Arnett and Jacques, 1981; Bland and Jacques, 1978; Borror and White, 1970; Borror et al., 1989; Castner et al., 1995; Hogue, 1993; James and Harwood, 1969; Peterson, 1978; Vockeroth, 1987.)



Figure 7: *Scathophaga stercoraria* known as golden dung fly (Prpspect Park, 2007).

f. Family Sepsidae

Black scavenger flies or sepsids are worldwide in distribution and represented by at least 240 species. The adults are small, shining black, purple, or red flies that are usually no more than 3.5 mm in length. They have a characteristic shape due to a head that is noticeably rounded and a constriction or narrowing of the abdomen at the base (Figure 8). Despite their small size, they are easily identified by the behavioural characteristic of flicking their wings

outward as they walk. This habit of wing waving also has given them the common name of “waggle flies.” These flies often occur in large numbers and are very common on dung. Sepsid fly larvae develop in a variety of decomposing organic matter, including carrion and excrement. Some species are found in decaying seaweed. Mature larvae are small, ranging from 3 to 6 mm in length (Arnett and Jacques, 1981; Bland and Jacques, 1978; Borror and White, 1970; Borror et al., 1989; Castner et al., 1995; Hogue, 1993; James and Harwood, 1969; Streyskal, 1987; Peterson, 1979).



Figure 8: *Sepsidae* sp known as black scavenger (Perk, 2019).

g. Family Sphaeroceridae

The small dung flies are a group with cosmopolitan distribution; they are small flies, ranging from 1 to 5 mm in length and typically a dull black or brown colour (Figure 9). Sphaerocerid fly adults and larvae are found in association with dung and excrement. They are a common part of the insect fauna that inhabits cow dung, and the transport of which is probably largely responsible for the spread of certain species. They are often found with the larger dung flies (family Scathophagidae) and the black scavenger flies (family Sepsidae) (Arnett and Jacques, 1981; Bland and Jacques, 1978; Borror and White, 1970; Borror et al., 1989; Castner et al., 1995; Hogue, 1993; James and Harwood, 1969; Marshall and Richards, 1987; Peterson, 1979).



Figure 9: Sphaeroceridae male post abdomen laterally (Roracek, 2016)

h. Family Stratiomyidae

Soldier flies encompass more than 250 described species that range in size from 5 to 20 mm. The colour of adults varies, although many of the most common species are wasp-like in appearance (Figure 10). The antennae of soldier flies consist of three segments, the last of which is either elongated or rounded with a long hair protruding from it. Adults are often found at flowers and occur near woods and on vegetation in wet areas, as well as on almost any decomposing plant or animal tissue. Mature larvae vary greatly in length from 4 to 40 mm, depending upon the species. Some are purely aquatic, while others are found in terrestrial habitats. The terrestrial larvae usually develop in decomposing plant or animal material, although in some cases they may be predacious. Rotting wood, compost piles, and excrement are typical habitats. The aquatic forms sometimes can be found in surprisingly hostile environments such as hot springs or extremely saline waters (Arnett and Jacques, 1981; Bland and Jacques, 1978; Borror and White, 1970, Borror et *al.*, 1989; Castner et *al.*, 1995; Hogue, 1993; James, 1981; James and Harwood, 1969; Peterson, 1979).



Figure 10: Stratiomyidae also known as soldiers fly (Vladimirov, 2013).

i. Family Phoridae

The humpbacked flies are a large family with more than 2500 species worldwide. Nearly half of these species belong to the single genus *Megaselia*. Phorid flies are small, ranging in length from about 1.5 to 6 mm. They are easily recognized by their humpbacked appearance that is especially noticeable when the flies are viewed in profile (Figure 11). Humpbacked flies may be black, brown, or yellow in colour.

Adult phorid flies are found in a variety of situations. They are commonly associated with decaying plant matter and are often unwanted yet ubiquitous pests where live insect colonies are maintained. The adult insect will run in a very characteristic swift and erratic manner, which has earned them the common name of “scuttle flies.” The larvae typically develop in any decomposing organic matter, whether human, animal, or vegetative in origin. The puparia are easily recognized as they are dorsoventrally flattened, with a pair of horns or “breathing trumpets” emerging from the anterior end. Some species are predacious while others are parasitic. A few are commensals in the nests of ants and termites, while other larvae develop in fungi. (Arnett and Jacques, 1981; Bland and Jacques, 1978; Borror and White, 1970; Borror et al., 1989; Castner et al., 1995; Hogue, 1993; James and Harwood, 1969; Peterson, 1979, 1987.)



Figure 11: A phoridae also known as the humpbacked flies (Vladmirov, 2015).

1.5 Life cycle

Necrophagous flies go through different stages while developing in a complete metamorphosis that refers to morphological and physiological changes consisting of four stages, namely egg, larva, pupa, and adult (Figure12) (Rognes, 1991).

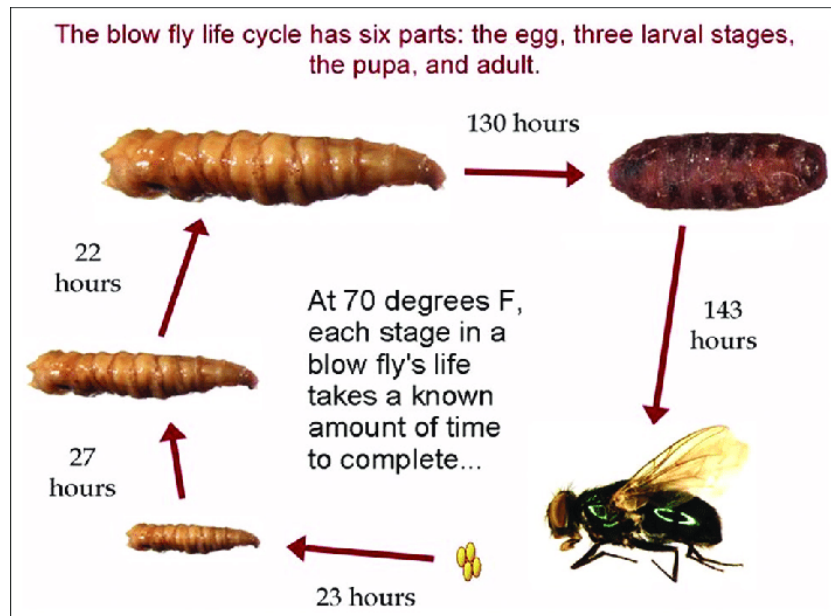


Figure 12: Life cycle of blowfly (Cleveland museum of natural history, 2011).

1.5.1 Egg stage

The general number of eggs laid by a necrophagous fly is around 150-200 egg. They tend to lay them in batches in places on corps where protein, moisture and food are provided. The blow fly egg is usually very shiny and white, ranging in size from around 0.9 mm to over 1.50 mm long and 0.3–0.4 mm wide. The outer, textured coating of the egg is termed the chorion. It could appear as reticulate or spotty, by using this data and observing the surface of the egg it could be used as an identification key to at least genus, of the fly species which has colonized the body. The end of the egg has a hole in it, called the micropyle (Figure 13). The emergence of the first larva from the egg is called eclosion, although this term has also been used to describe any form of hatching (Rognes, 1991).

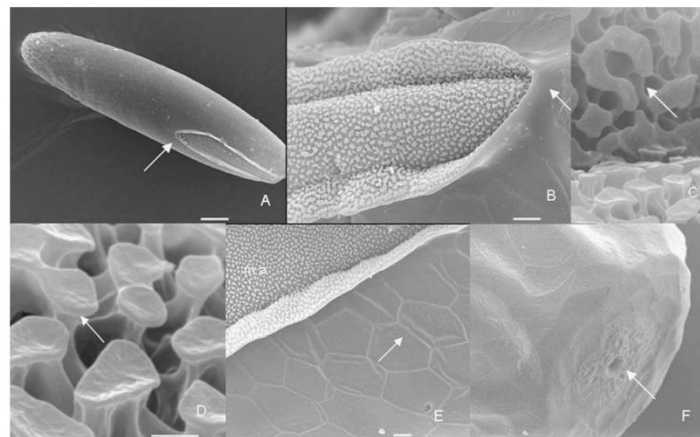


Figure 13: Scanning electron micrographs of the *L. cuprina* eggshell. (A) Dorsal view, anterior at right and median area (ma) not continuous; (B) dorsal view showing exochorion with smooth edges; (C) islands in the median area with anastomosis; (D) details of the island without holes; (E) dorsal view showing exochorion with smooth edges; (F) micropyle (Mendocin, 2008).

1.5.2 Larval stage

The larva has 12 segments and a pointed anterior end, all that remains of the head capsule found in other insect larvae, with a black structure comprising mandibles and related sclerites and ending in mouth hooks (the cephalopharyngeal skeleton) (Figure 14).

The fly larval goes to three stages, the specific life stage of the larva can be identified by the number of slits present in each posterior spiracle (Figure 14). Larvae in the third instar are the largest, and half-way through this stage they stop feeding and become migratory, seeking a place for pupariation (the final developmental stage of metamorphosis into the adult stage). This is called the larval post-feeding stage. Larvae move away from the body, towards dark and somewhat cooler areas. In the post-feeding stage the contents of the crop begin to reduce, until finally there is no obvious dark line of crop material visible through the white larval cuticle. Cragg (1955) suggests that the postfeeding larvae may move up to 6.4 metres from the carcass. On concrete floors, such as might be found in buildings, post-feeding larvae have been known to migrate up to 30 metres from the body (Green, 1951). Usually the post-feeding larva attempts to bury itself in soil or some other dark location. They may be found by searching in the first 2–3 cm depth of soil outdoor crime scenes (Byrd and James Castner, 2010).

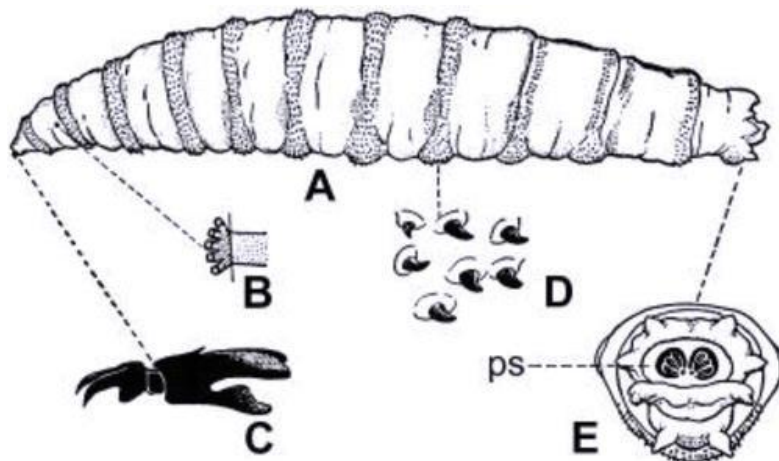


Figure 14: Blowfly larva, *Chrysomya bezziana*. (A) Complete larva; (B) anterior spiracle; (C) cephalo pharyngeal skeleton; (D) spines; (E) caudal end with pair of spiracular plates (Badii, 2019).

1.5.3 Pupal stage

The puparial case changes colour over time, becoming an oval object resembling an uncut cigar, coloured somewhere between reddish-brown and a dark brown or black, this case maintains all of the features of the third instar (Figure 15). Some attempts have been made to relate the state of colouration development of the puparium to post mortem interval, but to date the methods have not shown great accuracy beyond the first 24 hours (Greenberg, 1991).



Figure 15: Pupal form of diptera (Badii 2019).

1.5.4 Adult stage

Emergence of the adult, at the end of the life cycle, is achieved by its pushing the cap (operculum) off the puparium, using a blood-inflated region on the head called a ptilinum (Figure 16). Once out the fly ‘dries out’ and eventually its wings expand and the greyish-coloured fly becomes recognizably pigmented as, for example, a bluebottle or greenbottle (Greenberg, 1991).



Figure 16: Blowfly Emerging From Pupa (Nuridsany, 2018).

2 Forensic applications

2.1 Definition forensic entomology

Forensic entomology is a branch of scientific study that has been founded by a number of biology developments between the thirteenth and nineteenth century, in which arthropods are used as evidence to draw a conclusion when investigating legal cases related to human or wild life and importantly solving crimes.

Forensic entomology is also known as medicocriminal entomology due to its several uses in biological crime scenes; often focuses on violent crimes, it may include deliberate homicide or an assault although the use of insects found on the corps may lead to answerers to typical questions posed by the medicocriminal entomologist involve estimates of the time a decedent has been dead (the “postmortem interval” or PMI) and less frequently, the place where death occurred (Hall, 1990) further more cases with possible sudden death, traffic accidents with no immediately obvious cause and detection of toxins, drugs, or even the DNA of the victim through analysis of the insect, larvae are also included.

2.1.1 History of forensic entomology

The relationship between flies and body decomposition was first observed in China. In the book *Compendium of Materia Medica* (Li, 1596), Li clarified this relationship by stating, “Flies are everywhere, they come out in summer, hide in winter. They like warm and hate cold, maggots burrow into the subsoil and become flies as silkworm.” There were numerous cases where insects were used as evidence in criminal investigations. Furthermore, the first forensic entomology case was declared by the Chinese lawyer and death investigator Sung tzu in his book *Washing Away of Wrongs* (Tz’u, 1247) in which he reported a case of stabbing near to a rice field (Beneck, 2012).

The first modern forensic entomology case report to include estimation of post-mortem interval (PMI) was given by the French doctor Bergeret in 1855. The case was dealt with blow fly pupae and larval moth, on the other hand, it was purposed that Reinhard and Hofman as co-founder of this discipline in the late 1880s (Beneck, 2012).

Forensic entomology was introduced to Algeria in 2010 by the National Institute for Criminalities and Criminology of the National Gendarmerie (NICC). Since then PMI has been estimated using the entomological evidence when available. Research work related to forensic entomology has also been carried out in multiple universities in Algeria (Taleb, 2019).

2.1.2 Estimation of the postmortem interval

The postmortem interval (PMI) is the time elapsed between death and the discovery of the body. After three days, it is impossible to predict the PMI using classical medical methods. After a short time of death, insects colonise cadavers as soon as body decomposition begins. Estimation of insect activity helps the investigators to determine the PMI (Wyss and Cherix, 2006).

2.1.3 Entomotoxicology

Entomotoxicology is the analysis of toxins in carrion feeding arthropods. It also examines the effects of drugs or toxins on the insects. Diptera and other arthropods can be utilized as alternate samples for toxicological analysis where no viable specimens are available (Joshi, and Kumar, 2020). Flies are the most commonly used insect in entomotoxicological studies as they are the first to colonize the corpse.

2.2 DNA in forensic entomology

There are few published reports on the use of DNA techniques by forensic entomologists. However, there are several ongoing research programs that will soon expand the available applications. Most efforts have been directed at improving our ability to identify the insect specimens. It is also possible to identify the gut contents of blood or carrion feeding arthropods and, thereby, associate an insect with a living or dead human even when contact between the two is not observed (Byrd and castner, 2001).

2.3 Child or care-dependant person neglect

Until now insect evidence has been helpful in determine PMI to link a suspect to the crime scene to approve moving of the corpse to different location or determine drug level in a deceased person. Blow fly larvae, and pupae can also give information on how long a person was neglected. The examination of maggot fauna found on dead body can provide the information about the death if it was caused by neglecting (Bencke, 2001).

3 Medical importance of flies

3.1 Myiasis

3.1.1 Definition

Derived from greek “mya” or fly, was first proposed to define diseases of humans caused by dipterous larvae, as opposed to those caused by insect larvae in general, Myiasis has since been defined as the infestation of humans or animals with larvae. The distribution of human myiasis is worldwide, with more species and greater abundance in poor socioeconomic regions of tropical and subtropical countries. In countries where it is not endemic, myiasis is an important condition, where it can represent the fourth most common travel associated skin disease (Francesconi and Lupi, 2012).

3.1.2 Myiasis classification

There are two main systems for categorizing myiasis: anatomical (clinically) and ecological (entomologically) classifications. Since a single species can be assigned to more than one anatomical location, and the same location can be infested by different species, a classification system based on the degree of parasitism shown by the fly is also used (Francesconi and Lupi, 2012).

a. Anatomical Classification (clinically)

The anatomical system of classification, first proposed by Bishopp, is considered useful for practical diagnosis and to classify the infestation in relation to the location on the host. It is basically based on these proposed classifications sanguinivorous or bloodsucking, cutaneous myiasis, furuncular and migratory, wound myiasis and cavitary myiasis, where the infestation receives the name of the affected organ, e.g., cerebral myiasis, aural myiasis, nasal myiasis, and ophthalmomyiasis Table 2 (Francesconi and Lupi, 2012).

The anatomical classification system was first proposed by Bishop, modified later by James and by Zumpt. Each of these authors used terms different in the same direction (BENBRAHIM, 2015).

Table 2: Anatomical classification of myiasis (Francesconi and Lupi, 2012).

Bishopp (1922)	James (1947)	Zumpt (1965)
Bloodsucking	Bloodsucking	Sanguinivorous
Tissue-destroying Subdermal migratory	Furuncular Creeping Traumatic/wound Anal/vaginal	Dermal/subdermal
Infestation of the head passages	Nose, mouth, sinuses Aural Ocular	Nasopharyngeal
Intestinal/urogenital	Enteric Anal/vaginal	Intestinal
Intestinal/urogenital	Bladder, urinary passages Anal/vagina	Urogenital

j. Ecological Classification (entomologically)

This classification takes into account the level of parasitism of the parasite and the host. It is necessary to consider the ecological classification together with the species life cycle Table 3 (Francesconi and Lupi, 2012).

Table 3: Ecological classification of myiasis (Francesconi and Lupi, 2012).

Ecological classification	Description
Specific/obligatory	▪ Parasite dependent on host for part of its life cycle
Semispecific/facultative Primary Secondary Tertiary	▪ Free living and may initiate myiasis ▪ Free living and unable to initiate myiasis; may be involved once animal is infested by other. ▪ Free living and unable to initiate myiasis; may be involved when host is near death.
Accidental/pseudomyiasis	▪ Free-living larva and not able to complete its life cycle; causes pathological reaction when accidentally in contact with the host.

i. Specific or obligatory or primary myiasis

In obligatory myiasis, it is essential for the fly maggots to live on a live host for at least a certain part of their life for their development for example, larvae of *Chrysomaya bezziana* and *Dermatobia hominis* (Alajmi, 2015).

ii. Accidental myiasis

Accidental myiasis results when fly egg or larvae contaminate foods ingested by a host or come in contact with the genitourinary tract. Fly species involved include those which are free-living in all stages and rarely are parasitic. In most cases these flies pass unharmed through the host's alimentary tract, but they can cause discomfort, nausea, and diarrhea. In some cases symptoms can be severe (Alajmi, 2015).

iii. Facultative myiasis

In facultative myiasis larvae are normally free-living, but under certain conditions they may infect living tissues. Several types of fly, including species of *Calliphora*, *Lucilia* and *Sarcophaga*, which normally breed in meat or carrion, may cause myiasis in people by infecting festering sores and wounds (Alajmi, 2015). Three types of facultative myiasis are recognized by Kettle (1995):

- 1) Primary myiasis: Involving species which can initiate myiasis.
- 2) Secondary myiasis: Involving species which continue myiasis.
- 3) Tertiary myiasis: Involving species which join the primary and secondary species just prior to host death.

3.1.3 Diagnosis

The diagnosis of myiasis is made by the finding of fly larvae in tissue and identifying them. Identification to the genus or species level involves comparing certain morphological structures on the larvae, including the anterior and posterior spiracles, mouthparts and cephalopharyngeal skeleton and cuticular spines. Travel history can also be helpful for genus or species-level identification (Alfred, 2017).

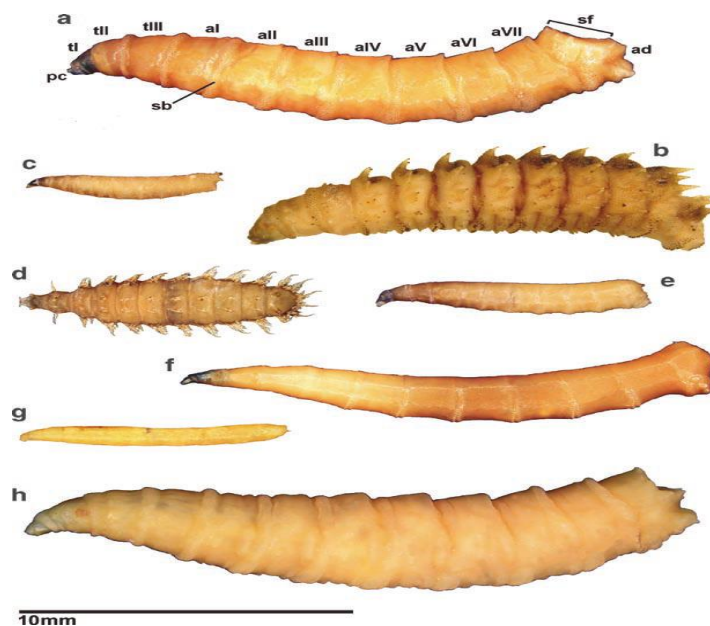


Figure 17: Third instars of necrophagous flies a: *Calliphora vomitoria*; b: *Chrysomya albiceps*; c: *Nemopoda nitidula*; d: *Fannia coracina*, dorsal view; e: Heleomyzidae; f: *Hydrotaea dentipes*; g: *Stearibia foveolata*; h: *Sarcophaga caerulescens*. Abbreviations: ad – anal division, aI-VII – abdominal segments, pc – pseudocephalon, sb – spinose band, sf – spiracular field, tI-III – thoracic segments (szpila, 2010).

3.2 Factors causing myiasis

It is genuinely known that poor hygiene and low socioeconomic status are the most important risk factors for acquiring myiasis. Poor sanitation is probably the most important risk factor for human myiasis. Low socioeconomic status, especially in poor countries, has an intimate relationship with the lack of basic sanitation and inadequate garbage disposal, leaving organic material exposed, which attracts insects and small animals, creating a sustainable cycle of filth.

In addition, the important factor is an abundance of exposed pre-existing supportive lesions that attract and stimulate the deposit of eggs by the female insect. As much as health care professionals judge myiasis to be a disease of minor importance, it also must be taken as a serious problem of public health due to the severe diseases caused by such as cutaneous myiasis, cavitary myiasis, intestinal myiasis, umbilical cord myiasis and other rare cases (Francesconi and Lupi, 2012)

3.2.1 Prevention

Individual actions should also be implemented and include emptying and steam cleaning dumpsters on a regular schedule, washing food and making a visual inspection of the food before consumption, storing food in adequate receptacles, making sure wounds are cleaned and dressed regularly, and more. Good sanitation can avert many myiasis cases. Field control of flies is extremely important. All available methods should be used, including aerial sprays, destruction of animal carcasses, elementary sanitary and hygiene practices, and clearing of debris and rubbish near houses (Francesconi and Lupi, 2012).

3.3 Maggot debridement therapy

3.3.1 Definition

Maggot therapy is essentially an artificially induced myiasis performed in a controlled environment by experienced medical practitioners. The selection of a suitable fly species for use in maggot therapy is of paramount importance, as it determines both the safety and success of the treatment. It is imperative to select a species that feeds almost exclusively on necrotic tissue. *Lucilia sericata* is considered the most suitable species for maggot therapy. The larvae must be prepared and maintained sterile before clinical use (Cumber *et al.*, 2016).

3.3.2 History

During World War I soldiers used to have infected wounds with maggots, it is where William Baer noticed how the maggot-laden wounds of soldiers when removed often appeared clean, once the larvae were wiped away. After that Baer first presented his results in 1929. By 1935, thousands of physicians and surgeons had embraced this practice. Many hospitals maintained their own therapeutic fly colonies; other practitioners obtained maggots from pharmaceutical companies. Maggot therapy disappeared during the 1940s. The reasons for this probably include the development of antibiotics and the refinement of surgical techniques that came about during World War II. Over the next several decades, therapeutic myiasis was performed only rarely, and only as a last resort, in patients who failed to respond to aggressive surgical and antibiotic treatments. But the 1980s brought about the realization that surgery and antibiotics could not cure all wounds. Many microbes were by now resistant to the once omnipotent antimicrobials. In the 1990s, maggot therapy was reintroduced as a treatment for non-healing wounds, after a series of controlled clinical studies demonstrated

better responses to maggot therapy than to conventional medical and surgical treatments (Sherman, 2014).

3.3.3 Mechanisms of action

Maggot therapy has the following three core beneficial effects on a wound: debridement, disinfection, and enhanced healing.

Debridement is the removal of cellular debris and nonviable necrotic tissue from the wound bed. The removal of necrotic tissue, which acts as a microbial substrate, may also reduce the risk of infection. Maggots debride wounds quickly and effectively, without damage to viable tissue. Maggots are photophobic and will naturally move into the deep crevices that may be beyond the reach of a surgeon's scalpel. Reports have been published marvelling at the benefits of maggot debridement therapy in all sorts of wounds (Francesconi and Lupi, 2012).

Several mechanisms have been suggested for disinfection, including the simple mechanical irrigation of the wound by increased secretions/excretions produced by larvae, the action of the midgut commensal *Proteus mirabilis* on digested bacteria, and the elimination of antibacterial products from living maggots (Francesconi and Lupi, 2012).

Enhanced healing is started by the proteolytic digestion of necrotic tissue and disinfection promoted by the maggots. Maggots encourage wound healing, centred on their ability to physically stimulate viable tissue and to enhance tissue oxygenation in chronic wounds (Francesconi and Lupi, 2012).

3.3.4 Clinical application

Over the last decade, maggot debridement therapy (MDT) has been recognized by many clinicians as a potential adjunct to conventional therapy, and many patients with non-healing, chronic ulcers have been treated (Figure 18). Numerous case reports and case series have described the successful use of MDT in a variety of ulcers such as diabetic foot ulcer and pyoderma gangrenosum which is also a cutaneous ulceration (din, 2019).

The studies report MDT as being significantly more effective than hydrogel or a mixture of conventional therapy modalities, including hydrocolloid, hydrogel and saline moistened gauze. However, the design of the studies was suboptimal, with important differences in the use of other therapies, such as compression, that may influence both debridement and healing between the compared groups, as well as inappropriately short follow-up times (zerachi and jemec, 2012).



Figure 18: A shown ulcer before and after maggot therapy A: before; B: during and C: one year after maggot debridement therapy (Sherman, 2009).



MATERIALS AND METHODS

CHAPTER II: MATERIALS AND METHODS:

Consist of field trials and laboratory work. The field trials were conducted in early autumn (October 2020) in Tipaza and Blida, therefore Ghardia and tiaret in spring (March 2021) to collect necrophagous Diptera. Laboratory work was performed at the university laboratory (laboratoire PFE), in which the collected specimens were identified, it was carried out from May to June 2021.

1 Objective

The present study aims to:

- Investigate the effectiveness of several baits in trapping necrophagous Diptera.
- Determine species composition and relative abundance of early carrion colonizers in an urban environment in different biogeography regions in Algeria during warm seasons.

2 Study area

Algeria is the largest country in Africa located in the subtropical zone of North Africa with climate zone characterized by hot and humid summer and cold to mild winters. According to the National Climate Centre, it is of the Mediterranean type all over the northern fringe which encompasses the Tellian Atlas and coastlines, semi-arid in the high plains in the centre of the country, and desert after crossing the chain of the Saharan Atlas (Figure 19).

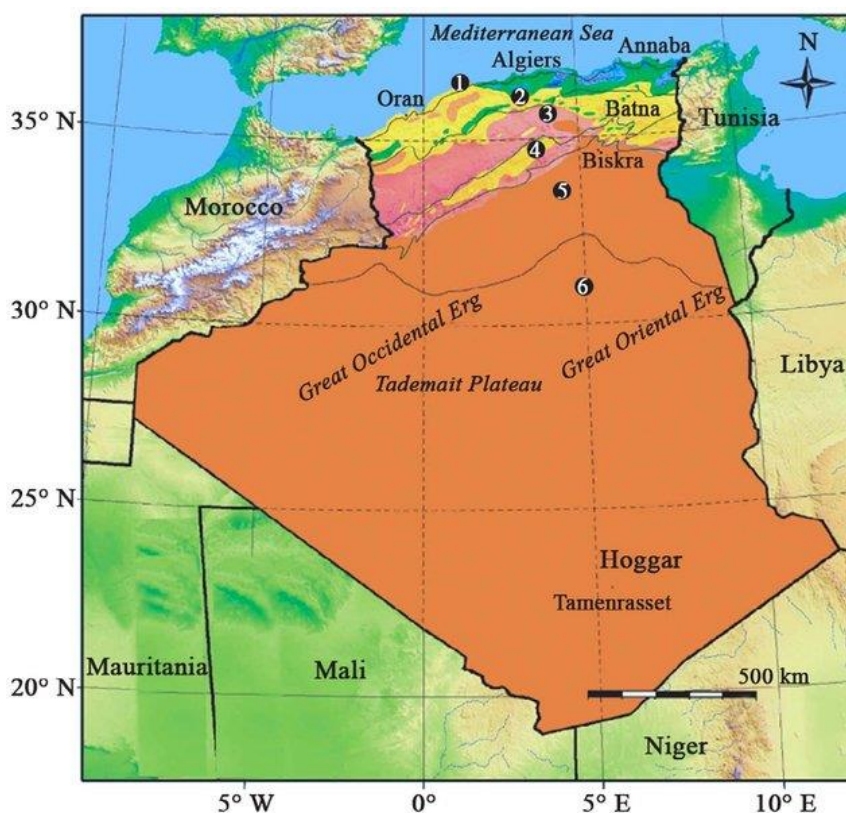


Figure 19: Bio geographic and bioclimatic zones of Algeria (Reproduced from Mohamed Sahnoun et al. (2010), *Zootaxa* 2432 © 2010 Magnolia Press, with permission). Bio geographic zones: 1. Shoreline; 2. Tellian atlas; 3. High Plateaux; 4. Saharan atlas; 5. northern Sahara; 6. central Sahara. Bioclimatic zones (perhumid zone not shown): blue, humid; green, subhumid; pink, arid; yellow, semi-arid; orange, Saharan.

3 Methods

a. Field work

Traps consisted of 2-L plastic bottles cut into two halves, at the bottom, an amount of sand on which 250 g of bait were placed. After that, the top of bottle was inverted inside the bottom half and the two halves were attached together (figure 20).



Figure 20: An example of a liver trap.

During the first experiment, attractiveness of different baits was tested (*i.e.* chicken liver, chicken meat, and fish) in the Mediterranean zone at localities in Blida and Tipaza. Insects were collected using forceps, killed in ethyl acetate vapours and preserved in 70% ethanol.

In the second experiment, sampling was carried out in the three different biogeographic zones at each locality a number of three trap replicates were used in:

- Mediterranean zone: Tipaza ($36^{\circ}34'59.99''$ N $2^{\circ}25'59.99''$ E) and Blida ($36^{\circ}28'47.525''$ N, $2^{\circ}48'2.045''$ E).
- Semi-arid zone : Tiaret ($35^{\circ} 23' 17''$ N, $1^{\circ} 19' 22''$ E).
- Dessert: Ghardaia ($32^{\circ}29'27''$ N, $3^{\circ}40'24''$ E).


b. Laboratory work

At the laboratory, specimens were pinned using stainless pin through their thoraces. Each individual was morphologically identified with the help of dichotomous keys (Genard et al, 2012; Szpila, 2012). And reference collections using a stereomicroscope and a cold light source. After identification, specimens were labelled with the following information: species name, gender, place and date of collection, date of identification, and name of collector and identifier.

c. Data analysis

Fisher's exact test was used to test for differences in the composition of the captured species collected on the baits and from the different regions at the 5% significance level using the software IBM SPSS Statistics 28.

Arthropod species abundance and richness of each bait and region were recorded. To estimate species diversity, Dominance D , Simpson's Index of Diversity $1-D$, Shannon Diversity Index H , and equitability based on Pielou's Evenness J were all calculated. Beta diversity was also estimated using Bray-Curtis Similarity Index. All data on biodiversity were analysed in PAST 4.04.



**RESULTS AND
DISCUSSION**

CHAPTER III: RESULTS AND DISCUSSION.

1 Temperature data

Temperature data at each location during the first and second trials are depicted in Table 4. Average daily temperatures ranged between 17 and 24°C. The experimental period was dominated by a temperate climate. Recorded temperature ranges were between the minimum and maximum temperature thresholds required for the activity and development of most of insects of forensic importance (Marchenko, 2001; Gennard, 2012). As expected, temperatures were mild and no precipitations were recorded during this study.

Table 4: Average daily temperature data recorded in the first and second trial at different locations.

Trial	Location	Date	Average daily temperature (°C)
1	Tipaza	20/10/2021	22
	Blida		24
2	Tipaza	10/04/2021	19
		11/04/2021	17
		22/05/2021	18
	Blida	20/05/2020	22
	Ghardaia	29/03/2021	20
	Tiaret	31/03/2021	19

2 Attractiveness of different bait types

2.1 Fly composition and abundance

A total of 294 specimens, which to belong to two families and six species were sampled (Table 5 and Figure 23). The results illustrate the abundance of fly species collected on each bait type during early autumn in the Mediterranean region. According to Fisher's exact test, fly species composition differed significantly between the baits ($p = 0.001$).

Lucilia sericata was the most abundant species representing of the total catches 37.1 and 75% on the fish and the liver baits respectively. However, this species was absent on the chicken meat bait. *Chrysomya albiceps* and *Chrysomya megacephala* were more attracted to

the chicken meat than the fish bait. The abundance of *Chrysomya albiceps* and *Chrysomya megacephala* on the chicken meat was 33.33% and 44.44% respectively while their abundance on the fish bait was 5.7% and 11.4% respectively. These species were totally absent on the liver bait. *Calliphora vicina* was collected only on the fish bait representing 11.4% of the total trapped flies. As for the Sarcophagidae, *Sarcophaga* sp. was more abundant on the fish bait (34.2%) than the chicken liver (25%) and chicken meat (22.22%).

The absence of *Ch. albiceps* on liver baits in Algeria has also been noted (Taleb et al. 2018a). From the results we noticed that the most preferable bait for the necrophagous flies was fish whereas the least attracting one was chicken meat. Liver could also attract an acceptable number of flies.

The main forensically important Algerian blow fly species, namely *Calliphora vicina*, *Chrysomya albiceps*, and *Lucilia sericata* are widely represented among the sampled populations on both treatments. Most species that were collected in the present study has also been collected previously in Algeria (Taleb et al, 2018a), Lisbon, Portugal (Prado e Castro et al. 2012), and Murcia, southeast Spain (Arnaldo et al, 2012)(Arnaldos et al., 2001, 2005), environmental conditions of which are similar to those of Algeria.

Table 5: Abundance of fly species collected on each bait type during early autumn in the Mediterranean region (Blida and Tipaza).

Family	Species	Chicken meat		Fish		Chicken liver	
		N	%	N	%	N	%
Calliphoridae	<i>Caliphora vicina</i>	0	0	12	11.4	0	0
	<i>Chrysomya albiceps</i>	9	33.33	6	5.7	0	0
	<i>Chysomya megacephala</i>	12	44.44	12	11.4	0	0
	<i>Lucilia sericata</i>	0	0	39	37.1	27	75
Sarcophagidae	<i>Sarcophaga (Liopyga) sp.</i>	0	0	6	5.7	0	0
	<i>Sarcophaga sp.</i>	6	22.22	30	28.5	9	25
Total		27	100	105	100	36	100

N: Absolute abundance; %, Relative abundance.

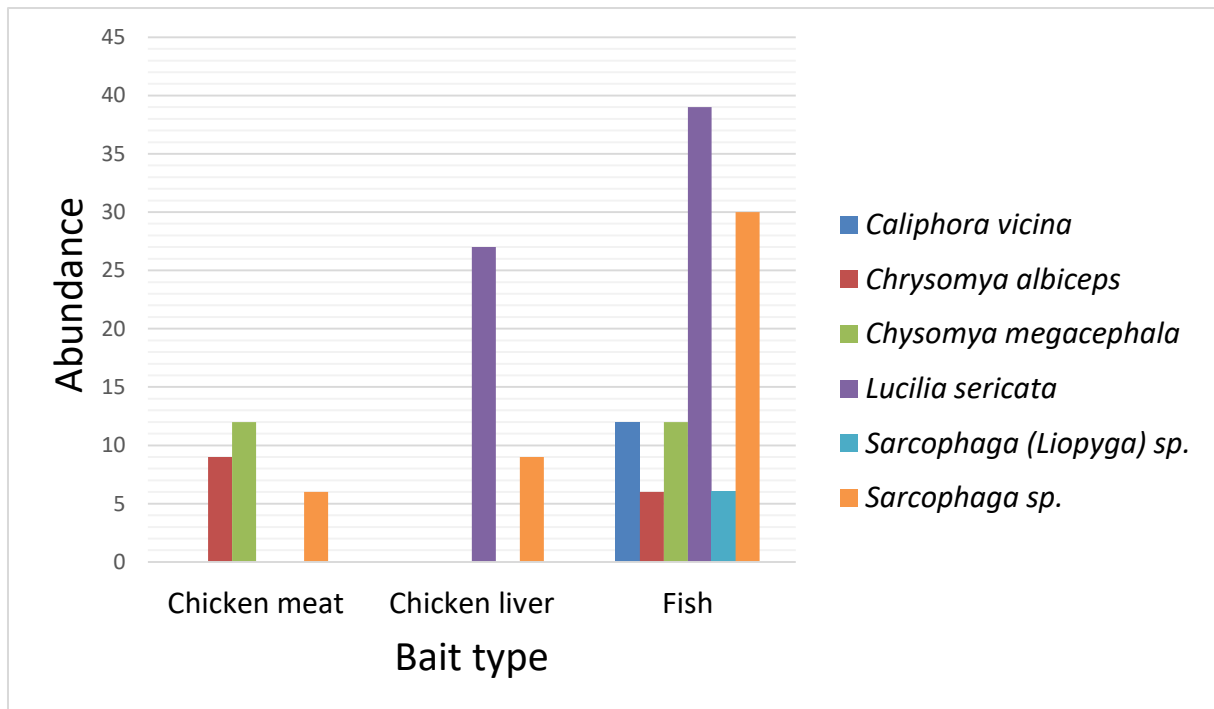


Figure 21: Bar graphs of the distribution of fly species collected on chicken meat, chicken liver, and fish during early autumn in the Mediterranean region

2.2 Biodiversity results

Alpha biodiversity indices are shown in Table 6. Species richness, Abundance, Simpson's Index of Diversity, and Shannon Diversity Index of the fish bait were the highest. Yet, in term of Equitability (Pielou's Evenness) chicken liver was noted to be the highest. The fish bait had a greater number of species; however, the individuals collected on chicken meat baits were distributed more equitably among the species.

Table 6 represents the similarity analysis results between the baits according to Bray-Curtis index. The result of Bray-Curtis cluster analysis of similarity between the chicken meat and the liver baits (19%) and the chicken meat and the fish baits (36%) appears to be low, indicating that these baits do not share a lot of similar species. Nevertheless, similarity between the liver and the fish baits was higher (51%).

Table 6: Alpha diversity indices of fly assemblages associated with different bait types during early autumn in the Mediterranean region of Algeria.

Index	Chicken meat	Chicken liver	Fish
Species richness S	3	2	6
Abundance N	27	36	105
Dominance D	0.358	0.625	0.252
Simpson's Index of Diversity $1-D$	0.642	0.375	0.748
Shannon Diversity Index H	1.061	0.5623	1.549
Equitability J (Pielou's Evenness)	0.963	0.877	0.784

Table 7: Similarity matrix of fly assemblages associated with different bait types during early autumn in the Mediterranean region of Algeria according to Bray-Curtis similarity index.

Bait type	Chicken meat	Chicken liver	Fish
Chicken meat	1	0.19	0.36
Chicken liver	0.19	1	0.51
Fish	0.36	0.51	1

3 Early fly colonizers in different bio geographical regions during spring

3.1 Fly composition and abundance

A total of 294 specimens, which to belong to two families and six species were sampled (Table 8 and Figure 24). The results illustrate the abundance of fly species collected on each bait type during early autumn in the Mediterranean region. According to Fisher's exact test, fly species composition differed significantly between the baits ($p = 0.001$).

Lucilia sericata was the most abundant species representing of the total catches 37.1 and 75% on the fish and the liver baits respectively. However, this species was absent on the chicken meat bait. *Chrysomya albiceps* and *Chrysomya megacephala* were more attracted to the chicken meat than the fish bait. The abundance of *Chrysomya albiceps* and *Chrysomya megacephala* on the chicken meat was 33.33% and 44.44% respectively while their abundance on the fish bait was 5.7% and 11.4% respectively. These species were totally absent on the liver bait. *Calliphora vicina* was collected only on the fish bait representing

11.4% of the total trapped flies. As for the Sarcophagidae, *Sarcophaga* sp. was more abundant on the fish bait (34.2%) than the chicken liver (25%) and chicken meat (22.22%).

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Table 8: Abundance of fly species collected on each bait type during early autumn in the Mediterranean region.

Family	Species	Chicken meat		Fish		Chicken liver	
		N	%	N	%	N	%
Calliphoridae	<i>Caliphora vicina</i>	0	0	12	11.4	0	0
	<i>Chrysomya albiceps</i>	9	33.33	6	5.7	0	0
	<i>Chysomya megacephala</i>	12	44.44	12	11.4	0	0
	<i>Lucilia sericata</i>	0	0	39	37.1	27	75
Sarcophagidae	<i>Sarcophaga (Liopyga)</i> sp.	0	0	6	5.7	0	0
	<i>Sarcophaga</i> sp.	6	22.22	30	28.5	9	25
Total		27	100	105	100	36	100

N: Absolute abundance; %, Relative abundance.

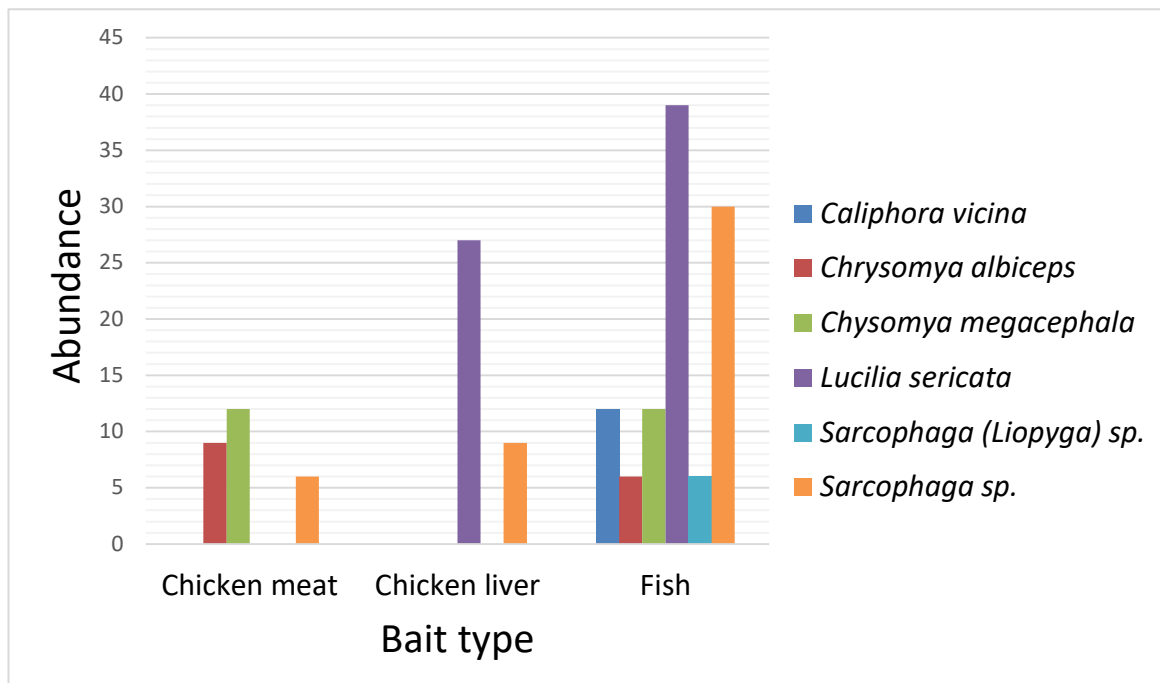


Figure 22: Bar graphs of the distribution of fly species collected on chicken meat, chicken liver, and fish during early autumn in the Mediterranean region

3.2 Biodiversity results

Alpha biodiversity indices are shown in Table 9. Species richness, Abundance, Simpson's Index of Diversity, and Shannon Diversity Index of the fish bait were the highest. Yet, in term of Equitability (Pielou's Evenness) chicken liver was noted to be the highest. The fish bait had a greater number of species; however, the individuals collected on chicken meat baits were distributed more equitably among the species.

Table 10 represents the similarity analysis results between the baits according to Bray-Curtis index. The result of Bray-Curtis cluster analysis of similarity between the chicken meat and the liver baits (19%) and the chicken meat and the fish baits (36%) appears to be low, indicating that these baits do not share a lot of similar species. Nevertheless, similarity between the liver and the fish baits was higher (51%).

Table 9: Alpha diversity indices of fly assemblages associated with different bait types during early autumn in the Mediterranean region of Algeria.

Index	Chicken meat	Chicken liver	Fish
Species richness S	3	2	6
Abundance N	27	36	105
Dominance D	0.358	0.625	0.252
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Shannon Diversity Index H	1.061	0.5623	1.549
Equitability J (Pielou's Evenness)	0.963	0.877	0.784

Table 10: Similarity matrix of fly assemblages associated with different bait types during early autumn in the Mediterranean region of Algeria according to Bray-Curtis Similarity Index.

Bait type	Chicken meat	Chicken liver	Fish
Chicken meat	1	0.19	0.36
Chicken liver	0.19	1	0.51
Fish	0.36	0.51	1

4 Early fly colonizers in different geographical regions during spring

4.1 Fly composition and abundance

Liver bait was selected to study the early fly species abundance in different biogeographical regions of Algeria during spring due to its attractivity of the common species found on bodies. Fish was not considered as suitable bait since it emits more odours affecting the classical pattern of insect colonization.

According to Fisher's exact test, fly species composition differed significantly between the biogeographical regions ($p < 0.001$). Overall, the biodiversity of species in the Mediterranean zone was the highest compared to the semi-arid and the desert regions (Table 11 and Figure 25).

Calliphora vomitoria was present only in the desert region representing 10% of the catches. Nevertheless, *Calliphora vicina* was abundant in the Mediterranean, semi-arid, and desert regions with 31.5%, 28.5%, and 56.2% respectively of the total number of the trapped flies. *Lucilia sericata* was the most abundant species in the Mediterranean (52.5%) and semi-arid (71.4%) zones, occurring as well as in the desert region (37.5%). The Sarcophagidae were present only in the Mediterranean region (15.7%).

Necrophagous species, mainly species of the family Calliphoridae, begin to lay eggs on carcasses as early as the beginning of the fresh stage (Gennard, 2012).

Calliphora vicina and *Lucilia sericata* are well represented in the three biogeographical regions. *L. sericata* and *C. vicina* has been recorded throughout Algeria in the humid, subhumid, semi-arid and Saharan bioclimatic zones (Taleb et al. 2018a).

It should be noted that *Calliphora vicina*, very common during the cold season, decreases its activity during summer while *Lucilia sericata*, rare in winter, appears in very large numbers in summer (Taleb et al. 2018a). These observations explain the presence of both *Lucilia sericata* and *Calliphora vicina* during our study since it was dominated by a temperate climate.

Ch. albiceps favours warm to hot temperatures and is present in the humid, subhumid and semi-arid climatic regions. *L. sericata*, *C. vicina* and *Ch. albiceps* are present in all habitat types (Taleb et al. 2018a). However, there is a lack of data on the distribution of *Ch. megacephala* in Algeria since it has been reported recently for the first time in Algeria in Blida and Tipaza (Taleb et al. 2018b).

Table 11: Abundance of the early fly species colonizing liver baits in different biographical regions of Algeria during spring.

Family	Species	Mediterranean		Semi-arid		Desert	
		N	%	N	%	N	%
Calliphoridae	<i>Calliphora vicina</i>	18	31.5	6	28.5	27	56.2
	<i>Calliphora vomitoria</i>	0	0	0	0	3	6.25
	<i>Lucilia sericata</i>	30	52.6	15	71.4	18	37.5
Sarcophagidae	<i>Sarcophaga</i> sp.	9	15.7	0	0	0	0
Total		57	100	21	100	48	100

N: Absolute abundance; %, Relative abundance.

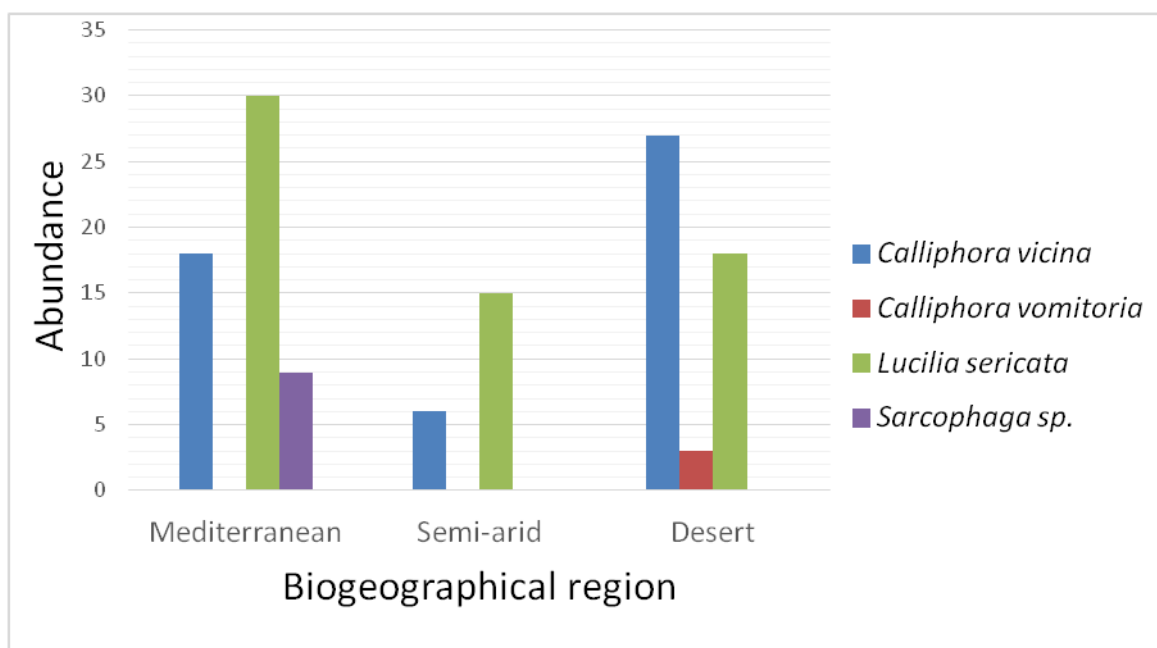


Figure 23: Bar graphs of the distribution of the early fly species colonizing liver baits in different biogeographical regions of Algeria during spring.

4.2 Biodiversity results

Diversity indices of the early fly species are depicted in Table 12. Alpha diversity metrics reveal that the Mediterranean and desert zones not only have the highest diversity, but also species are distributed more equitably.

The result of Bray-Curtis analysis (Table 13) shows average similarities between all the biogeographic regions, being the highest between the Mediterranean and desert zones (68.57%) followed by the desert and the semi-arid zones (60.86%), and the Mediterranean and the semi-arid zones (53.85%).

Table 12: Alpha diversity indices of the early fly species colonizing liver baits in different biogeographical regions of Algeria during spring.

Index	Mediterranean	Semi-arid	Desert
Species richness S	4	4	3
Abundance N	63	57	39
Dominance D	0.338	0.368	0.538
Simpson's Index of Diversity $1-D$	0.662	0.632	0.461
Shannon Diversity Index H	1.213	1.163	0.790
Equitability J (Pielou's Evenness)	0.841	0.8	0.735

Table 13: Similarity matrix of the early fly species colonizing liver baits in different biogeographical regions of Algeria during spring according to Bray-Curtis similarity index.

Region	Mediterranean	Semi-arid	Desert
Mediterranean	1	0.5384	0.6857
Semi-arid	0.5385	1	0.6086
Desert	0.6857	0.6087	1

5 Morphology and biology of the early fly species colonizing baited traps

5.1 *Calliphora vicina* Robineau-Desvoidy, 1830

C. vicina is a large fly, which size range from 10 to 14 mm long (Figure 26A). The head is black in colour, with the lower part of the bucca or “cheeks” appearing orange to yellow. The thorax appears black with dark longitudinal stripes on the dorsal surface between the bases of the wings (Figure 26B). The abdomen is a noticeable metallic blue, patterned with silver. Overall the body appears very bristly with yellowish basicosta and haired dark bristles (Figure 26C) (Szpila, 2012 ; Akbarzadeh et al., 2015 ; Lutz et al., 2017).

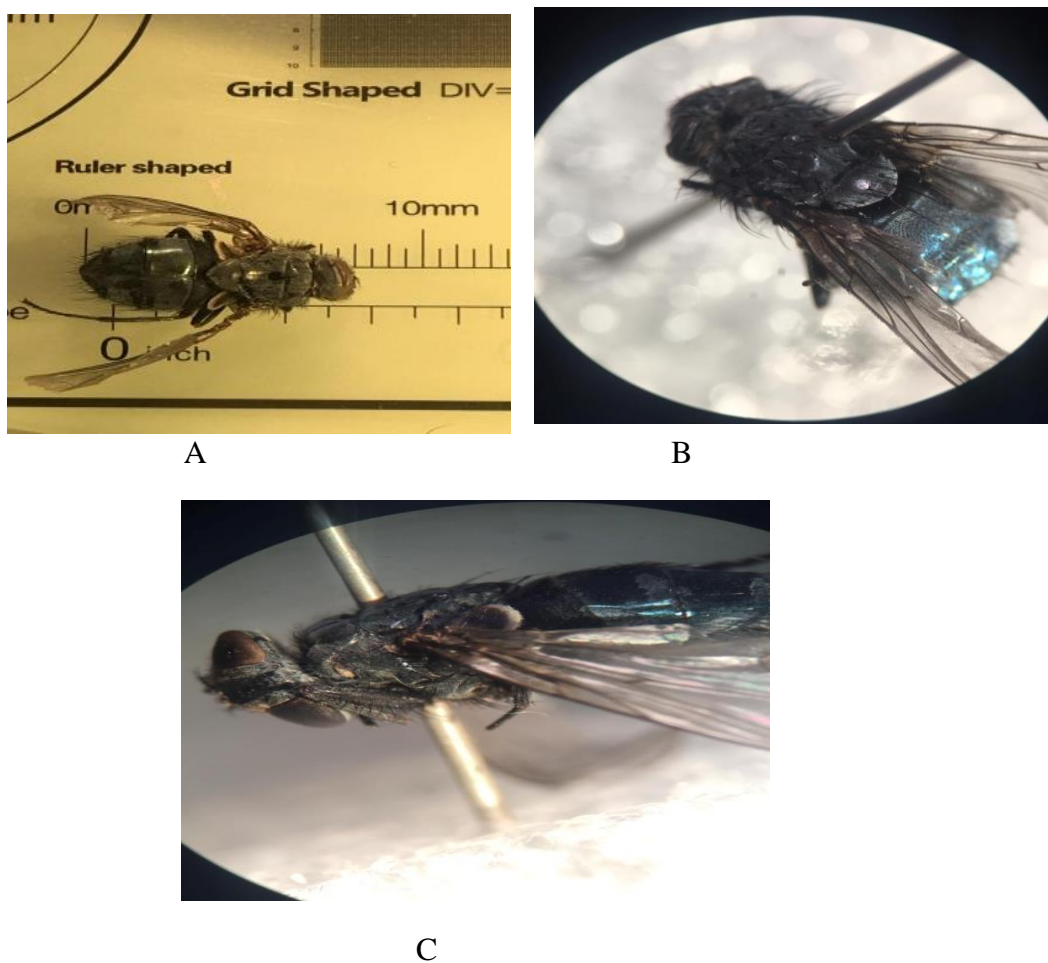
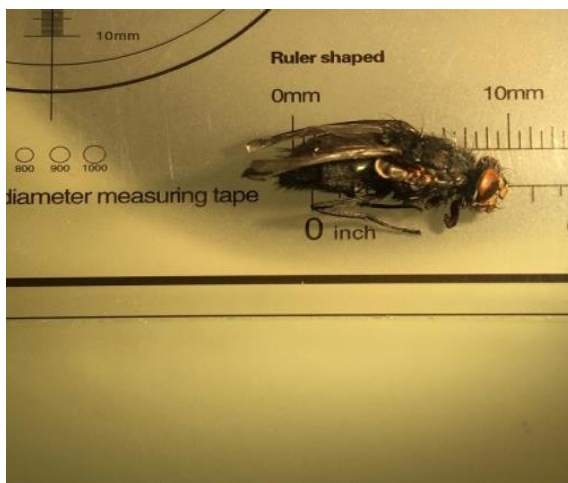


Figure 24: *Calliphora vicina* Robineau-Desvoidy, 1830. A and B: Dorsal view. C: Lateral view.

5.2 *Calliphora vomitoria* (Linnaeus, 1758)

C. vomitoria size ranges from 7 to 13 mm in length (Figure 27A). The thorax is dark blue to black, with a light gray dusty coating. Darker blue longitudinal stripes also may be visible on the dorsum or upper surface of the thorax. The abdomen is metallic blue, but appears to have a light coating of silver-gray powder as well. This pollen-like coating masks much of the metallic nature of the colour, but a glint of metallic sheen is still visible when specimens are examined closely. It is distinguished by the presence of orange hair in the lower part of genal dilation (Figure 27B) (Szpila, 2012 ; Akbarzadeh et al., 2015 ; Lutz et al., 2017).



A

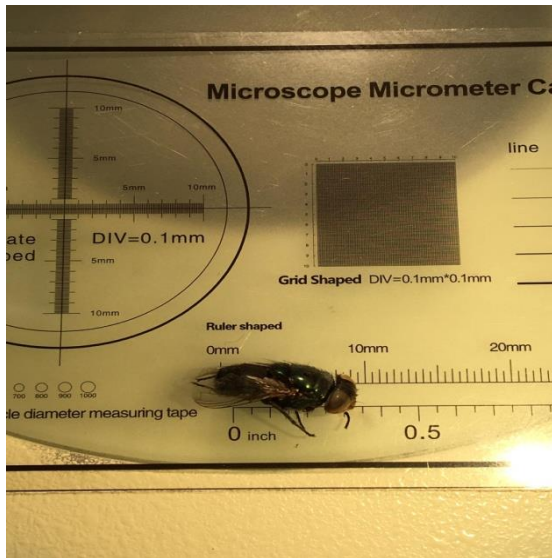


B

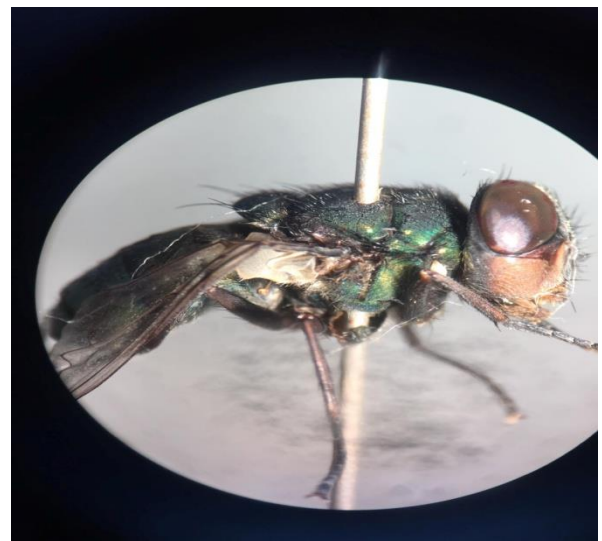
Figure 25: *Calliphora vomitoria* (Linnaeus, 1758). **A:** Dorsal view. **B:** Lateral view.

5.3 *Chrysomya albiceps* (Wiedemann, 1819)

Unlike *Lucilia* species, *Chrysomya* species have a hairy stem vein, distinguishing the subfamily *Chrysomyiinae* from *Luciliinae*. *Ch. albiceps* is easily identified by a range of morphological characters. *C. albiceps* size is about 10 mm (Figure 28A). It can be distinguished by the bright metallic-green adults with whitish-yellowish pollinosity on the face (Figure 28B), white anterior spiracles and dark posterior edges of the abdominal tergites (Figure 28B) (Szpila, 2012)



A



B

Figure 26: *Chrysomya albiceps* (Wiedemann, 1819). A: Dorsal view. B: Lateral view

5.4 *Chrysomya megacephala* (Fabricius, 1774)

C. megacephala size is about 10 mm (Figure 29A), the adults have short stout bodies, the eyes are unusually large and a very prominent shade of red (Figure 29B), making this flies easily recognizable in the field. It is known by an anterior thoracic spiracle brown and a brownish lower calypter (Figure 29B) (Szpila, 2012).



A

B

Figure 27: *Chrysomya megacephala* (Fabricius, 1774) A: Dorsal view. B: Lateral view

5.5 *Luclia sericata* (Meigen, 1826)

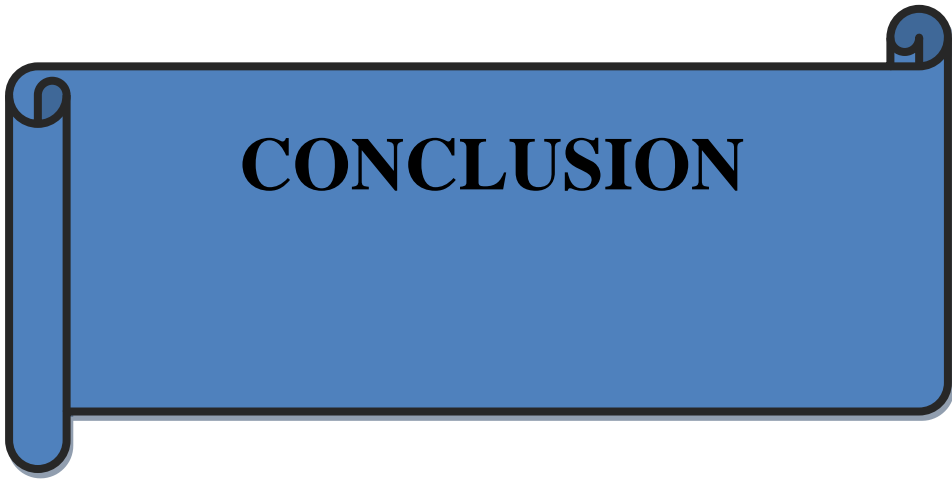
Adult *L. sericata* are 6 to 9 mm in length (Figure 30A). This fly is a brilliant metallic blue-green, yellow-green, green, or golden bronze (Figure 30B). The thorax has three prominent transverse grooves on its dorsal surface and the front femora are black or deep blue and bright yellow basicosta, a useful character in identification (Figure 30B) (Szpila, 2012).



A

B

Figure 28: *Luclia sericata* (Meigen, 1826) A: Lateral view. B: General view.



CONCLUSION

Conclusion

The study of distribution of necrophagous Diptera in Algeria is crucial because of their forensic and medical, and veterinary relevance. The establishment of a national catalogue of forensically and medically important Diptera is essential to fill the gap in applied forensic entomology research in our country,

Due to the lack of data on the primary necrophagous flies, the current studies investigated the effectiveness of several baits in trapping necrophagous Diptera and determined the species composition and relative abundance in urban habitats in different biogeographical regions in Algeria during warm seasons.

The most frequent species belongs to family Calliphoridae, subfamilies *Calliphorinae*, *Luciliinae*, and *Chrysomyiinae*. The main forensically important Algerian blow fly species, namely *Calliphora vicina*, *Chrysomya albiceps*, and *Lucilia sericata* were widely represented among the sampled populations.

The biodiversity of the species in the Mediterranean zone was the highest compared to the semi-arid and the desert regions. *C. vicina* and *L. sericata* were present in all the regions. During early autumn and spring. *L. sericata* was the most abundant species while *C. vicina* was less frequent. *Ch. albiceps* and *Ch. megacephala* occurred only on fish and chicken meat baits.

As perspectives, it would be interesting to:

- Conduct further studies in different habitats and provinces;
- identify the immature stages;
- Establish an identification key of the Algerian necrophagous fly fauna.

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Appendix

Table 14: general data of the captured flies during the studies.

Location	Date (season)	families	species	abandace	Bait	T°
Ghardia	-29/03/2021(spring).	<i>Calliphoridae</i>	<i>Caliphora vicina</i> <i>Caliphora vomitoria</i> <i>Lucilia sericata</i>	27 01 18	liver	20
Tiaret	-31/03/2021(spring)	<i>Calliphoridae</i>	<i>Caliphora vicina</i> <i>Lucilia sericata</i>	06 15	liver	19 24
Tipaza: Cherchell	-10/04/2021(spring) -11/04/2021(spring)	<i>Calliphoridae</i>	<i>Caliphora vicina</i> <i>Lucilia sericata</i>	18 02	liver fish	19 17
Tipaza: Cherchell	-20/10/2020(autumn)	<i>Calliphoridae</i> <i>Sarcophagidea</i>	<i>Caliphora vicina</i> <i>Lucilia sericata</i> <i>Sarcophaga sp.</i>	03 03 03	fish	22
Bousmal	-20/10/2020(autumn)	<i>Calliphoridae</i> <i>Sarcophagidae</i>	<i>Caliphora vicina</i> <i>Chrysomya albiceps</i> <i>Sarcophaga sp.</i>	01 01 01	Fish	
Damous	-20/10/2020(autumn)	<i>Calliphoridae</i>	<i>Chrysomya albiceps</i>	03	chicken	
Fouka	-21/10/2020(autumn)	<i>Challiphoridae</i> <i>Finiidae</i>	<i>Chysomya megacephala</i> <i>Fania canicularis</i> <i>Fania sp</i>	04 02 01	chicken	

Blida :	-20/10/2020(autumn)	<i>Calliphoridae</i>	<i>Lucilia sericata</i>	02	Fish	24
Blida		<i>Sarcophagidae</i>	<i>Sarcophaga liopygia</i>	02		
Affrone	-18/10/2020(autumn)	<i>Calliphoridae</i>	<i>Chrysomya albiceps</i> <i>Chrysomya megacephala</i> <i>Lucilia sericata</i>	01 04 01	fish	
		<i>Sarcophagidae</i>	<i>Sarcophaga sp</i>	01		
Tipaza: Fouka	22/05/2021 (spring)	<i>Calliphoridae</i>	<i>Lucilia sericata</i>	05	liver	18
		<i>Sarcophagidae</i>	<i>Sarcophaga sp</i>	02		
Blida: Blida:	29/05/2021 (spring)	<i>Calliphorodae</i>	<i>Lucilia sericata</i>	03	liver	22
		<i>Sarcophagidae</i>	<i>Sarcophaga sp</i>	01		
Mouzaia:	20/05/2021(spring)	<i>Sarcophagidae</i>	<i>Sarcophaga sp</i>	02	chicken	
Bouinan:	17/05/2021(spring)	<i>Calliphoridae</i>	<i>Lucilia sericata</i>	07	fish	
		<i>Sarcophagidae</i>	<i>Sarcophaga sp</i>	05		
Blida:	10/05/2021 (spring)	<i>Calliphoridae</i>	<i>Lucilia sericata</i>	01	liver	