

Maggots, those Tiny Wriggling Creatures, Play a Fascinating Role in Forensic Investigations

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Abstract - Today, insects and arthropods have become beneficial to forensic science, they can help the entomologist reconstruct and estimate the postmortem interval (PMI), by studying their characteristics, life cycle, behavior, and habitat. The purpose is to study what maggots found on corpses can tell us in forensic science, they were able to determine the location of wounds, including evaluate the period after death. This lesson collected the maggots from mouth and reared them until adults for identifying and looking them under a micro-CT scanner, aspects of these postmortem changes were revealed to be near the truth, and all data provided was useful in administering justice to avoid misinterpretations and evaluation errors. This article can be considered a consultative tool for forensic pathologists and crime scene investigators during a crime scene investigation and the autopsy.

Keywords - Arthropods, Maggots, Postmortem, Forensic Investigations, Forensic Entomology.

I. INTRODUCTION

Forensic Entomology was first used in 1235 in China by Sung Tz'u, who was a "Death Investigator" and he wrote a book in the Medico-Legal Text Book titled "Hsi Yüan Chi Lu" in Chinese. English as "The Washing Away of Wrongs". This is the first book written for investigative purposes (Dorothy, 2007). In the book, Mr.Sung also educated about the blow fly that likes to swarm on the open organs of the corpse, such as the eyes, ears, nose, mouth, anus and vagina, especially wounds. In the 19th and early 20th centuries, forensic entomology began to be used in more investigations. Until in 1996, the American Board of Forensic Entomology was established, which is a certification board for forensic entomologists (ABFE, 1996) in Thailand.

There is the first report on forensic entomology by a professor from Chiang Mai University. The body was found near the police station in Lampang province. On the corpse's leg, a honeycomb-like wound was found, with many maggots in the wound, which brought them to the medical team Department of Parasitology of Chiang Mai University studies found the maggots in stage 3. Its scientific name is *Chrysomya rufifacies* (Macquart). From this information, it can be used to estimate the time of death (Sukontason, Kabkaew L., K. Sukontason, P. Narongchai, S. Lertthamnongtham, S. Piangjai and J. K. Olson, 2001).

Worms or fly larvae call "maggots", growth found on corpses is of interest in forensic science. This is a field of study the relationship between insects and corpses call "forensic entomology". When examining the corpse pathologists and investigators need to know what type of maggots were found on the corpses. Knowing the type of insect can help to determine how long it has been growing. And how many days does it take to grow into an adult? This is information that can help indicate the time of death. The location of insects found on the corpse can indicate the location of wounds on the corpse's body too.

Flies are the most common worms found in Thailand. They are all the good decomposer and witness in crime scene because they are not lie. They can come to the body within 1-2 hours to lay eggs, 24 hours for the fly maggot to hatch from the egg. After that, the maggots will grow into stage 1-3 maggots. This is the stage where the maggots develop. It will have a body length of approximately 1 - 1.5 centimeters, which will take

approximately 7 days. The body will be milky white and will begin to crawl around the corpse, find a dry place to prepare for the pupa stage. Generally, it crawls about 1-2 meters away from the corpse. Normally, the pupa takes about 3-10 days to become an adult. The average adult lives for 28-30 days.

Working in the field of forensic entomology is in accordance with the Forensic Science Services Act B.E. 2016. The use of entomologists in crime scene investigations are normally and is becoming increasingly important as insects arrive at the scene quickly and are considered excellent first evidence and first witness (UiTM, 2014). Nowadays, the use of insect and animal evidence to determine the cause of death, time since death and can initially identify the location of the wound are accepted in modern forensics:

Broadly speaking, forensic entomology is the study of insects applied to any sort of legal issue; as a general formula, one could use insects + legal = forensic entomology. That means every time insects are involved in a situation that requires the intervention of the law, it could be a case where forensic entomology is applied (Gemmellaro Denise, 2017). It's basic meaning is "the science of collecting and analyzing insect evidence to aid in forensic investigations" (Amendt, J., C. S. Richards, C. P. Campobasso, R. Zehner and M. J. R. Hall, 2011).

Following this logic forensic entomology is easily divided into three branches: (Lord and Stevenson, 1986)

1. Medical Entomology (Medico-legal Entomology).
2. Urban and in house.
3. Store Product Pests (Lee, 1989).

A. Medical Entomology (Medico-Legal Entomology)

This section focuses on the criminal component of the legal system (Byrd J. H. and Tomberlin J. K, 2020) and deals with the necrophagous (or carrion) feeding insects that typically infest human remains. Insects are related to humans and cause disease by contact with secretions, touching a wound, or eating infected food. The insects or arthropods that are related and easily contaminated are maggots, flies, cockroaches, mosquitoes, mites, ticks, etc. They are vectors and carriers of diseases in humans such as dengue fever, malaria, elephantiasis, typhoid fever, etc. Animal diseases, or diseases transmitted from animals to humans, are called "zoonosis." This includes diseases from humans that are transmitted to animals as well. Some people clearly call it "reverse zoonosis" or "anthroponosis." (Zoonosis, 2015).

B. Urban and in House

This part aspect deals with the insects that affect man and his immediate environment. This area has both criminal and civil components as urban pests may feed on both the living and the dead. The damage caused by their mandibles as they feed can produce markings and wounds on the skin that may be misinterpreted as prior abuse. Urban pests are of great economic importance and the forensic entomologist may become involved in civil proceedings over monetary damages. Insects can also affect the interpretation of blood spatter pattern analysis. Roaches simply walking through pooled and splattered blood will produce tracking that may not be readily recognizable to the untrained observer. Specks of blood in unique and unusual areas (such as on ceilings) may mislead crime scene technicians unless they are aware of the appearance of blood contaminated roach tracks.

C. Store Product Pests

These insects are commonly found in foodstuffs and the forensic entomologist may serve as an expert witness during both criminal and civil proceedings involving food contamination (Byrd J. H. and Tomberlin J. K., 2020). Examples of this could be moths we find in a rice box stored in our pantry or beetle-infested cereals in a warehouse (Russell W. C., 1947). In situations like this, it is important to find the source of the infestation to assess some responsibility. Are the moths in our rice found there because we left the package open for too long? Or, is the box completely sealed, meaning the infestation occurred before that package got to our pantry, possibly in the supermarket or, even earlier, when the rice was being packaged? The entomologist, in this case, would be responsible for identifying the insects in question, determining their development stage, and based on the knowledge about the insects' feeding behavior, typical habitat, and biology give your opinion on when and how the infestation might have occurred (Gemmellaro Denise, 2017).

II. RESEARCH STUDY

A. Case Study

In this research, it is case Bang Pa-In Police Station, Ayutthaya Province, in the central of Thailand. The corpse looked deteriorated. He was a man with a thin build, reddish-skinned skin, and short black hairs. Found a lot of maggots around the mouth and nose in Figure 1. In general, flies lay eggs, taking about 1-2 days to hatch from maggots, depending on many factors such as temperature (Deonier C. C., 1940), light, humidity, etc. These factors are all interrelated is especially important in assessing the Post-Mortem Interval (PMI). (Aballay, Fernando H., M. Cecilia Dominguez, and Florencia Fernandez Campón, 2012).

III. METHODS

Take a sample from the corpse. These are creamy-white maggots, little spine on dorsal and no legs in Figure 2. In collecting it must be recorded as follows:

1. Details of the terrain: city, countryside, desert, forest, grassland, etc.
2. The place where the body was found: the ground condition is sandy soil, loamy soil, clay rock, hillside, plain, whether there is a road through it, etc. Type of plant found What kind of plant is it? Aquatic plants, endemic plants, etc. If you don't know (ask a botanist).
3. Date, time, and temperature.
4. Species of insects (arthropods) found.
5. Case number.
6. Recorder's name (Rowe, 2007).

Note: In taking notes on the collection of samples from the crime scene, photography is also an important part of helping researchers work more efficiently (Lee and W.D. Lord, 1994).

A. Specimen Collection Step

1. Preserved samples include specimens that were placed directly into 80% ethyl alcohol: maggots (label the details). (Adams Z. J. O. and Hall M. J. R., 2003)
2. Collected live specimens of maggots from the same areas as the preserved samples (label the details too).
3. Place approximately 50–75 maggots of representative sizes on a food medium (raw pork or beef) and wrap loosely in aluminum foil. Be sure that the container lid is ventilated with very small holes to avoid escaping maggots. Place the raw meat into a rearing container with ½ to 1" of vermiculite (pupae no add meet) (Saigusa, Kiyoshi, Masataka Takamiya, Masatoshi Matsumasa, and Yasuhiro Aoki, 2006).
4. Separate the live maggots into additional containers and record the details as mentioned above.
5. After collecting live maggots, feed them (in a temperature room) until they are adults to confirm the fly. It's useful for the entomologist to estimate, as close as possible to the truth, the _{min} PMI.

B. Steps to Make Slides

1. Take the insects (specimens) collected from the corpse and then bring them to the cutting laboratory and place it in the cassette. Bring the cassette to the automatic tissue processor for the preparation process, treated with a chemical solution so that the treated specimen is sufficiently hardened to be sectioned (Adams and Hall M. J. R. 2003).
2. Prepare the chemically specimen through an automatic tissue processor and embed it in a liquid wax medium contained in a mold of various sizes according to the size of the specimen using a tissue embedding machine. The hardening medium will support the specimen in such a way that it can be easily cut into thin tissues 3-5 microns in size by following (Making Permanent Microscope Slides of Insects) and also recording the number of blocks in the embedding record. Insert the cartridge with the tissue embedding center.
3. Take the embedded specimen into the paraffin block and cut it into thin tissue (paraffin section) with a rotary microtome. Normally, we will cut the thin flat tissue, 3-5 microns in size, with the tissue plate embedded on a glass slide. by following (Nelson Riley C. and Karin Gastreich, 2001).
4. Put the glass slides with paraffin section into the hot air oven by adjusting the temperature into two ranges, namely 90-110 °C for 15-20 minutes and 60-65 °C for 30-60 minutes to help. The adhesion of the

paraffin section to the slide surface prevents the section from falling off easily during staining, by following (Slide Mounting Techniques for very small to microscopic animals) and recorded in a record of slide incubator drying (Nelson Riley C. and Karin Gastreich, 2001).

5. Glass slides containing paraffin sections were stained by using conventional staining methods (Hematoxylin & Eosin staining) using an Automatic slide stainer following (Prepared Microscope Slides: Insects Specimen)
6. Take a glass slide with paraffin section at tissue staining and cover the slide. By covering it on the tissue, using a mounting medium to help fix the cover glass firmly on the slide. To promote easy and convenient microscopic examination and a good way to preserve specimens.
7. Once the biopsy slides are completed, they are analyzed under a microscope.

C. Study Maggots under Microscope

Principles of operation of an x-ray imaging machine with a small computer (X-Ray Computerized Tomography Scan), using techniques to photograph the sample material with x-ray radiation without destroying the sample and relying on computer processing to create three-dimensional images (3D) (Keklikoglou K. et al., 2019). Each maggot was preserved in 80% ethanol, dehydrated in ethanol, transferred into hexamethyldisilazane for several hours, and air dried for 24 hours. No stain was used. After that was scanned with a SkyScan 1172 microtomograph. To scan the whole maggot, the following were used: voltage (~80 kV) and current (~50–100 μ A) were customized for each scan based on maggot size and density to obtain maximum signal-to-noise ratio and optimal contrast. The reconstruction of the raw image dataset and “cleaning” were performed using the Bruker-Skyscan free software to reconstruct and process the images, permitting not only reconstruction but also virtual slices used to achieve the final volume rendering reconstructions. A detailed description of the procedure was published (Alba-Tercedor, 2014).

D. Raring Maggots to Adults

Maggots to become adults makes it easier to classify types. The process of raising in the laboratory is controlled at room temperature, humidity, and light. The optimum temperature is 30°C–35°C (± 3 °C), and the humidity is in the range of 70%–75%. The appropriate light period must not be less than 2,230 lux. In the laboratory, maggots are grown to adults successfully. They were classified according to taxonomy and systematic principles under a microscope (Natural History Museum in London).

IV. RESULTS

A. Maggot

1. Studied morphology under the microscope of maggots. It is creamy white, spine on dorsal, no legs, the shape some slender some robust, and it looks like a grain of rice. Body length is about 1.6 cm–2.3 cm. Classified as an Order Diptera (di = two, ptera = wings) refers to a group of insects with two wings, which are the flies.
2. The general body shape has a pointed head and an obtuse tail. The head has a mouth hook, and the tail has a pair of posterior spiracles (Jason H. Byrd and Jeffery K. Tomberlin, 2020).
3. Microscopic analysis of second- and third-stage maggots. Inside the posterior spiracle, two and three spiracular slits are found, respectively (Figure 3, Figure 4), and each piece is an oval fragment.
4. When examining by sectioning, to identify which family of maggots belongs to the family, specimens must be processed for at least 1 day for each specimen. A complete slide will be provided to study the life cycle, habitat, and behavior of each maggot species identified for the purpose of reconstruct and estimating the time of death (post-mortem interval = PMI) as close as possible (minimum post-mortem interval = $\min$ PMI)

The presence of 2nd and 3rd maggots, blowflies, Family Calliphoridae, Order Diptera, have a complete metamorphosis (Davies L. and Ratcliffe G. G., 1994). The slower or faster life cycle of the blowfly depends on temperature, humidity, sunlight and the flies' food (Deonier, 1940).

Blowflies prefer to lay their eggs on carcasses or on wounds, especially rotten wounds or on carcasses. Founding maggots in the body's mouth and nose, in addition to evaluate the period after death alongside

decomposition, it is also useful in helping to pinpoint the location of wounds once you arrive at the scene and sometimes it can also be said whether the body was moved or not after death.



Figure 1. Maggots on Face Especially Mouth and Nose



Figure 2. Maggots with a spine, no legs, shaped oval like a rice

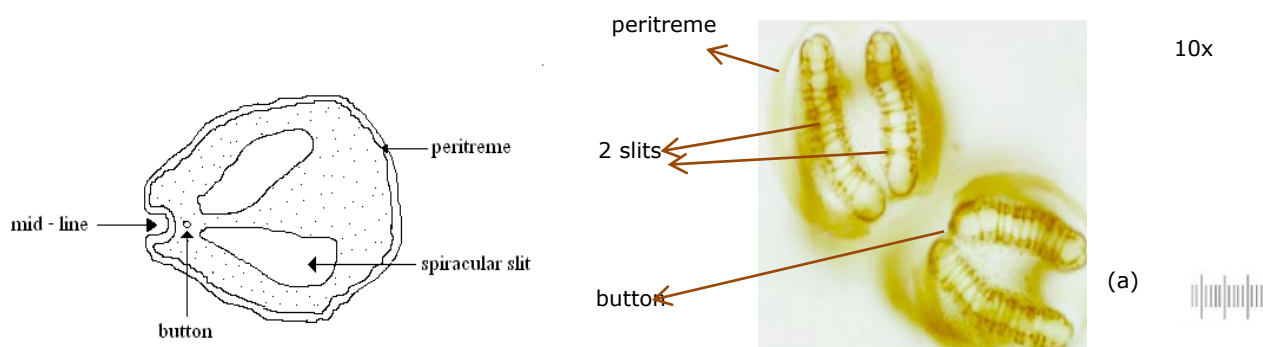


Figure 3. A Tip of Spiracle, Maggot 2nd Stage

(a) This Scale is Ocular Micrometer Scale 10 Times Magnification under Micro-CT Scanning.

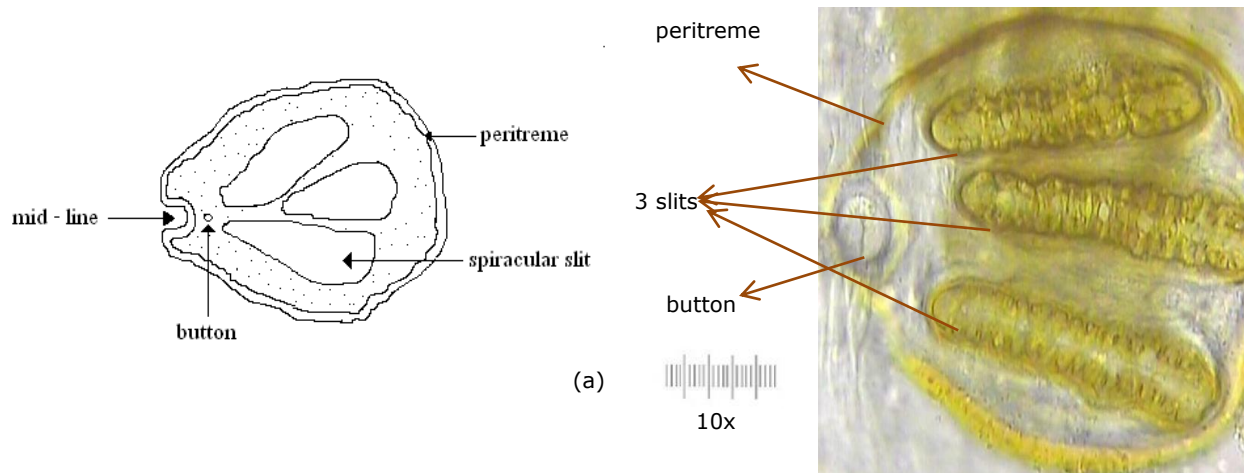


Figure 4. A Tip of Spiracle, Maggot 3rd Stage

(a) This Scale is Ocular Micrometer Scale 10 Times Magnification under Micro-CT Scanning.

V. DISCUSSION AND CONCLUSION

By studying the insect population and the developing larval stages, entomologists can estimate the postmortem index, any change in position of the corpse as well as the cause of death. When examining insects found on a corpse, particularly around wounds, (in this case a mouth and nose) it provides valuable information for forensic investigations. Let's delve into this fascinating topic: blowflies (*Calliphoridae*): these insects are often the first to arrive at a corpse, sometimes within minutes after death depending on multiple factors such as climate and individual ways a body begins breaking down. The conclusion of this case is that this corpse has been dead for at least 2-3 days from observing the external condition of the corpse and the decomposition process of the remains. Many maggots were found in the mouth and nose, making it known that this corpse had wounds inside the mouth. Which police said was caused by using a gun to shoot himself in the mouth to commit suicide. From raising maggots collected from corpses, made a slide and looked at it under a microscope. It was found to be a blowfly, Family *Calliphoridae*. The maggots were in stages 2 and 3 by studied the spiracle of maggots (Figure 3, 4). Flies swarming into wounds might seem unpleasant, but their behavior actually serves a purpose. Let's explore why flies are attracted to wounds and what it can tell us:

1. Feeding Behavior

- Flies, especially blowflies, are attracted to wounds because they feed on organic matter, including dead tissue and bodily fluids.
- When a wound is open, it provides an ideal feeding ground for flies. They land on the wound to consume any decaying tissue or exudate present.

2. Myiasis

- The phenomenon of flies infesting living animals (including humans) with their larvae (maggots) is called myiasis.
- Flies lay eggs near wounds, and when these eggs hatch, the maggots feed on the surrounding tissue. This process can be beneficial in certain situations.
- In some cases, medical professionals intentionally use maggots to clean wounds and promote healing. This practice, known as maggot therapy, has been used for centuries.

3. Healing and Infection Prevention

- Maggots have enzymes that break down dead tissue, helping to debride wounds.
- By removing necrotic tissue, maggots create a cleaner environment, which can aid in wound healing.
- Additionally, the movement of maggots stimulates blood flow and prevents bacterial growth, reducing the risk of infection.

While the sight of flies swarming into wounds may be unsettling, their behavior can serve a purpose in wound care. However, it's essential to differentiate between beneficial maggots and harmful pathogens carried by flies. If you encounter this situation, seeking professional medical advice is crucial. In Thailand the first type of insect that typically arrives at a dead body is usually a blowfly (*Calliphoridae*). These flies are attracted by body fluids and gases emitted during decomposition. Within 24 hours after death, blowflies lay their eggs on the corpse. In summary, blowflies are drawn to corpses due to the Volatile Organic Compounds (VOCs) released during decomposition, and their presence and life cycle stages can help forensic experts estimate the post-mortem interval (time since death). These insects play a crucial role in forensic entomology, aiding in investigations related to time of death and other relevant details in the justice process.

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