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IDENTIFICATION OF NECROPHAGOUS FLIES IN KERANDANGAN NATURE PARK, LOMBOK

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Abstrak

Lalat nekrofag merupakan serangga pemakan bangkai selama proses dekomposisi material organik hewan. Keanekaragaman jenis dan kelimpahannya dipengaruhi oleh faktor lingkungan dan posisi geografis. Penelitian ini bertujuan untuk mengidentifikasi keanekaragaman dan kelimpahan lalat nekrofag di Taman Wisata Alam Kerandangan, Lombok. Pada penelitian ini, kami menggunakan bangkai tikus sebagai makanan dari larva nekrofag. Hasil penelitian ini, didapatkan empat famili Calliphoridae, Sarcophagidae, Muscidae, and Piophilidae, dan 12 spesies yang terdiri dari Chrysomya megacephala, Chrysomya rufifacies, Chrysomya bezziana, Calliphora spp., *Lucilia* spp., *Hemipyrellia linguriens* (Diptera: Calliphoridae)*, Hydrotaea ignava, Antherigona orientalis* (Diptera: Muscidae)*, Protopiophila* sp. (Diptera: Piophilidae); *Sarcophaga bravicornis, Sarcophaga cf australis, dan Sarcophaga cf lincta* (Diptera: Sarcophagidae)*. Lalat nekrofag yang paling melimpah adalah Chrysomya megacephala dan Chrysomya rufifacies*.

***Kata Kunci**: Entomologi, Forensik, Identifikasi, Lombok.*

Abstract

Necrophagous flies are flesh-eating insects during the decomposition process of animal organic substances. Their diversity and abundance are influenced by environmental factors and biogeographical positions. This study aimed to identification the diversity and abundance of necrophagous flies in Kerandangan Nature Park, Lombok. In this study, we used rat carcasses as food for necrophagous larvae. The results of this study obtained four Family flies, there is Calliphoridae, Sarcophagidae, Muscidae, and Piophilidae, and 12 species of necrophagous flies, they are *Chrysomya megacephala, Chrysomya rufifacies, Chrysomya bezziana, Calliphora* sp., *Lucilia* sp., *Hemipyrellia linguriens* (Diptera: Calliphoridae)*, Hydrotaea ignava, Antherigona orientalis* (Diptera: Muscidae)*, Protopiophila* sp. (Diptera: Piophilidae); *Sarcophaga bravicornis, Sarcophaga* cf *australis*, and *Sarcophaga* cf *lincta* (Diptera: Sarcophagidae). The most dominant necrophagous insects present are *Chrysomya megacephala* and *Chrysomya rufifacies*.

***Keywords**: Entomology, Forensic, Identification, Lombok.*

1. INTRODUCTION

The forensic science field has many mysteries within it that require attention to detail and foresight to solve every case. Each section of forensic science employs a specific method to provide information related to the case and substance found at the crime scene or on the victim's body (Trasia, 2022). However, when the carcasses were found, it usually in not fresh condition, and it frequently undergoes decomposition until skeletonization. This decomposition process could eliminate evidence on the victim's body due to tissue damage caused by bacteria, fungi, and other factors. Necrophagous insects are one of decomposers of decaying organic substances on carcasses (Franceshetti *et al*., 2021).

Necrophagous flies are usually found in the bodies of deceased animal carrion and human corpses. The presence of necrophagous flies on carcasses occurs after several hours of death until skeletonization. The succession of each necrophagous fly during the decomposition period is important evidence for estimating physical state, body temperature, and carcass changes.

In criminal investigations, necrophagous flies could be exceedingly important. Entomological evidence can be used as evidence in criminal proceedings, primarily to determine how somebody died.

This study discusses forensic entomology and the types of flies that could be found on carrion. Blow flies, flesh flies, and other types of flies exist in carrion. Each fly has specific characteristics and could be observed on different steps of decomposition. Some flies, for example, will arrive at the early body breakdown process, while others will arrive at the end of decomposition. Investigators could estimate how long a victim has been deceased by examining the species of flies and their life cycle phases.

LITERATURE REVIEW

2.1. Necrophagous Insect

Necrophagous insects are insects that colonize organic material from corpses and carcasses since the first day of death (Iancu et al., 2016; Vilte et al., 2019). These invertebrates are usually as a dominant factor in the decomposition process (Benbow et al., 2013; Essarras *et al*., 2021). Necrophagous insects interact with the odor produced due to tissue damage resulting from putrescine and cadaverine. This results in the interaction of chemical substances to attract the presence of necrophagous insects on corpses and animal carcasses (Rivers and Gregory, 2014).

The use of necrophagous insects in forensic entomology has been applied for centuries. Insect-based evidence obtained at a crime scene can be used to estimate the minimum postmortem interval, identify whether a carcass/corpse has been carried, and help ascertain the cause and manner of death (Hartmann *et al*., 2021).

The first case report using insects as evidence in a criminal case came from China (13th century), for the first time insects and other arthropods were studied by Tźu in the book Hsi yűan chi lu ("The Washing Away of Wrongs"). The book discusses murder by stabbing the victim in a rice field area. Investigators explained that flies appeared on invisible blood spots (Rivers and Gregory, 2014). Insects as forensic indicators were documented in Germany on April 6, 1881, during the excavation of mass graves carried out by Dr. Reinhardt. This discovery showed that there were insects that were breeding in the cracks of corpses and taxonomic identification was carried out, resulting in the remains of Phoridae fly pupae and beetles in graves that were more than 15 years old (Benecke, 2001).

In the world of forensic entomology, insects from the Order Diptera are the dominant species that are often involved in the world of forensics (Meena et al., 2020). The fly species that are frequently present are Calliphoridae, Muscidae, Fannidae, and Sarcophagidae (Arnaldos et al., 2001; Battán-Horenstein and, Raquel, 2018; Battán- Horenstein et al., 2020). Calliphoridae and other types of flies carry out life cycle stagesthat are used to be studied by forensic entomologists. The colonization of necrophagous flies on organic material from corpses and animal carcasses has a dynamic effect on the environmental and biographical conditions of the location where the source is found (Vilte et al., 2019).

The first fly that appeared was a female fly. Female flies will look for a placeto oviposition with signals in the form of chemicals. The central location signal for flies to oviposition is associated with decaying animal and human tissue and the odor emitted during the decomposition process (Brundage and Byrd, 2016). Chemical compounds released by decomposing bodies give signals to flies. The fly nervous system consists of three parts, namely dendrites, cell bodies, and axons. One neuron and another do not toucheach other but are close to each other. The gaps between the dendrites of neurons and other axons are called synapses and signals are passed from one cell to another by a groupof chemical messengers called neurotransmitters (Rivers and Gregory, 2014).

2.2. Postmortem Interval

The high level of criminal cases caused the fast and simple methods of investigation, it could be done by associating the necrophagous insect development with the decomposition level. Insects could be

used as general evidence to estimated time of the victim's death (Meena et al., 2020; Dawson *et al*., 2021). Estimated time since death also known as Post-mortem Interval (PMI) is one of the main data to investigate. Forensic entomology is mainly used to calculate postmortem minimum intervals (PMImin) by estimating the time of insect colonization (Brundage and Byrd, 2016; Byrd and Lerah, 2020).

Furthermore, forensic entomology can help with issues such as determining the time of death, the location of the death, and the cause of death due to latent infection in the corpse or carcass. Sampling and tools that help provide information of value to forensic science (Ren et al., 2018). The development of necrophagous insects as evidence to calculated the PMI are influenced by biogeographic and environmental conditions such as temperature, humidity, and rainfall (Mahat et al., 2019).

3. RESEARCH METHOD

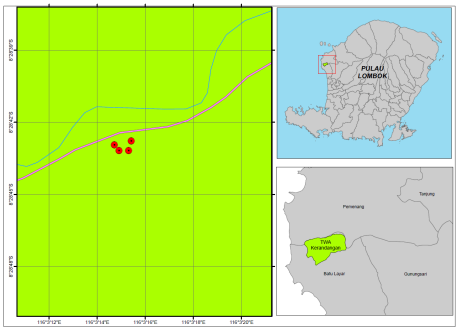

Figure 1. Location of Research

This research was carried out at the Kerandangan Nature Tourism Park (TWA), Western Lombok, West Nusa Tenggara. Flies have been collected from wistar rat (*Rattus* sp.) carcasses placed in the forest. The average body weight of rats is around 200-300 grams. Four groups of rats which each group containing six rats were placed in parallel iron cages ± 20 meters from each other (Federer, 1967). The iron cage functions to keep the carcass from the scavenger animals. Each mouse was euthanized using ketamine subcutaneously (Fig. 1).

Observations of the decomposition stages of the carcass and the life cycle stages of flies on the carcass were made twice a day. The adult flies were sampled every day to identify the fly species that visited the carcass.

The adult fly samples that have been taken are placed in a killing jar containing cotton that has been moistened with isopropanol as a dry preservation method. Larvae and pupae were subjected to two treatments, the half part were preserved and the other were rearing. Rearing was conducted in the Biology Laboratorium, Mathematics and Natural Science Faculty, Mataram University. Fly identification is carried out by referring to Animal Health (2020); Couri (2010); and Meiklejohn (2012).

4. RESULTS AND DISCUSSION

The results of this research identified 12 species of necrophagous flies consisting into four families, Calliphoridae Muscidae; Piophilidae, and Sarcophagidae.

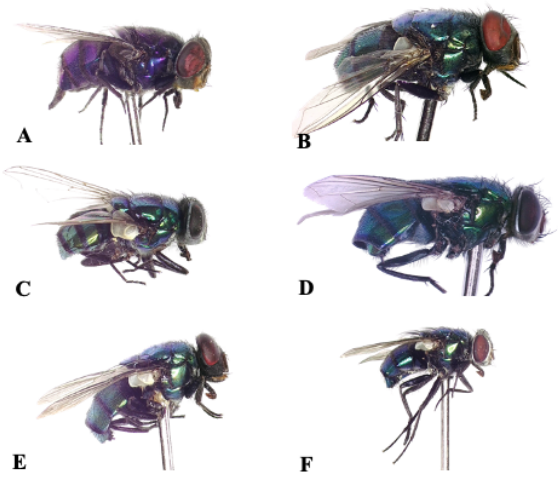

Figure 2. Family Calliphoridae A. *Chrysomya megacephala*; **B.** *Chrysomya bezziana;* **C.** *Chrysomya rufifacies*; **D.** *Lucilia* spp; **E.** *Calliphoridae;* **F.** *Hemipyrellia linguriens*

Figure 3. Family Muscidae. A. *Hydrotaea ignava*; **B.** *Antherigona orientalis*

Figura 4. Family Piophilidae. *Protopiophila* sp

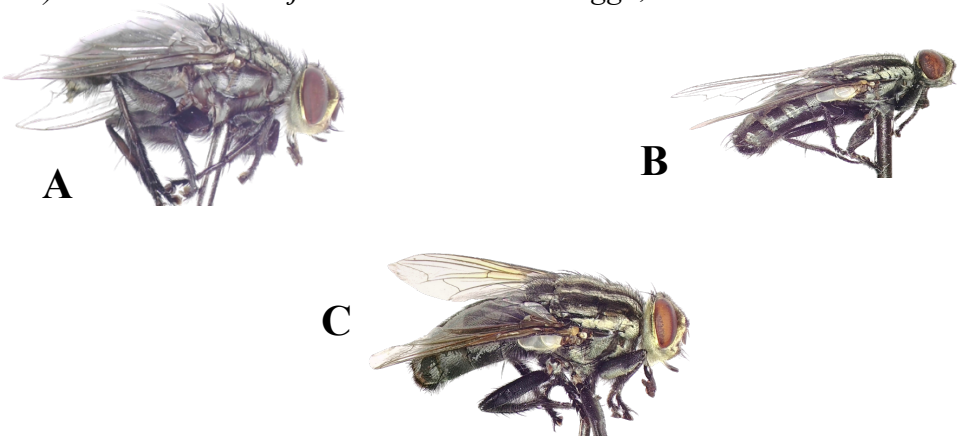

Figure 5. Family Sarcophagidae. A. *Sarcophaga brevicornis;* **B.** *Sarcophaga* cf *australis*; **C.** *Sarcophaga* cf *lincta*

The first observation was conducted in the afternoon of the first day. The decomposition process could be recognized into three stages. They are autolysis, putrefication, and skeletonization. The autolysis stage is the stage where enzymes digest body cells, putrefaction is the process of destroying fat and muscle tissue by Clostridium and Bacteroides bacteria whereas skeletonization is the final stage of decay. At this point, the body consists of hair and bones. Decay is best defined in terms of the decay of specific body parts, such as leg and foot bones, skull, and ribs (Gennard, 2012).

The fly activities around the carrions and the cage have been observed since the first day. Female flies will be present firstly on the carrion body (Byrd and Gregory, 2014). Female flies are more attracted to fresh carrions, while adult male flies are more attracted to carrions that are entering the active decomposition stage (Liu *et al.,*2016). Fly activities on carrions are predation and oviposition of eggs. Eggs have been deposited on nostrils, ears, mouth, genital, and body parts that are not directly exposed to heat (Amendt *et al.,*2010). Flies lay eggs on parts of the body that are not directly exposed, to protect the eggs from drying out due to exposure to direct sunlight (Byrd and Gregory, 2014).

During the fresh stage, several species of flies were present on carrions laid after 6 hours of death, and some flies oviposited on the bodies of the carrions. The flies present were *Chrysomya megacephala*; *Chrysomya rufifacies*; *Chrysomya bezziana*; *Lucilia* spp; *Calliphor*a spp; *Hymepirellia lingurriens*; *Lucilia* spp; *Protopiophila* sp.; *Calliphora* spp (Family Calliphoridae); *Anterigona orientalis* (Family Muscidae); *Sarcophaga* cf *lincta*; and, *Sarcophaga brevicornis* (Family Sarcophagidae).

In the second day, the decomposition of carrions entered the swelling stage (The Bloated Stage). This phase is characterized by the swelling of the stomach due to trapped gas in the body (Dawson *et al.,* 2020). This gas is caused by the process of nutrient metabolism by anaerobic bacteria and an increase in internal body temperature (Gennard, 2012). In this day, the fly cycle stage has entered the larvae phase, larvae were found carrying out predation inside the body and on the underside of the carrions' body. At this stage, the presence of adult flies are increased. The flies are attracted to the odor emitted from the carcass body, as a result of the activity of anaerobic bacteria. The flies that were present on the second day were *Chrysomya megacephala*; *Chrysomya rufifacies; Chrysomya bezziana; Hymepyrellia lingurriens; Calliphora* spp. (Family Calliphoridae); *Anterigona orientalis* (Family Muscidae); *Sarcophaga* cf *lincta*; *Sarcophaga brevicornis*; and, *Sarcophaga* cf *australis* (Family Sarcophagidae). At the beginning of decay (fresh stage) adult Calliphoridae flies are the dominant fly species present on carrions and

oviposition on carrions in several parts of body not exposed to direct sunlight.

On the third day, the carrions decomposition stage entered the active decomposition stage. The active decomposition stage is characterized by the skin and hair separating from the body. Apart from that, the gas in the body comes out, and the body experiences compression (Byrd and James, 2000). Due to internal pressure caused by the formation of gas, at this stage fluid escapes into open parts of body and produces ammonia (Amendt *et al.,*2010). In this phase, the number and activities of larvae and adult of flies of flies are increased. In the next stage occured fermentation of carcass which has produced butenoic acid and caseinic acid. Thereafter there is an initial decomposition period involving ammonia fermentation from the body, which attracts other insect populations (Goff, 1993). At this stage, larval predation increases. Most of the larvae are in the abdominal and genital tissues. Interspecific competition can be found (Ahmad and Baharudin, 2018). The flies present on the third day were, *Chrysomya megacephala; Chrysomya rufifacies; Chrysomya bezziana; Lucilia* spp; *Calliphora* spp; *Hymepirellia lingurriens*; *Lucilia* spp; Protopiophila sp.; *Calliphora* spp (Family Calliphoridae); and, *Sarcophaga* cf *lincta* (Family Sarcophagidae). Apart from that, there are differences in the presence of flies from the Piophilidae family, in Ramos-Pastrana (2018) research, there were no Piophilidae family flies present.

On the fourth day, the decomposition of carrions entered the post-decomposition stage in four groups. In post-decomposition stage remains skin, hair, and bones. Body tissue experiences drying (Wardani and Arif, 2019). In this stage, we found two morphological types of flies larvae, they are smooth and hairy larvae. The hairy larvae species identified as *Chrysomya rufifacies*. Meanwhile, the smooth larvae are thought to be larvae from the Calliphoridae and Muscidae families based on measurements of the length of the preserved larvae. At this stage, it was also found that some larvae migrated into the ground or buried themselves, to prepare for the pupa stage which was found in four groups. The adult fly that emerged on the fourth day was *Chrysomya megacephala; Chrysomya rufifacies; Chrysomya bezziana; Lucilia* spp. (Family Calliphoridae); *Protopiophila* sp.; *Hydrotaea ignava* (Family Muscidae); and no fly species from the *Family* Sarcophagida*e* were found.

On the fifth day, the decomposition stage in the four groups was the post-decomposition stage. Apart from that, in the life cycle stages of flies in carrions, some larvae migrate into the soil and other still predate on the body of carrions. The dominance of larvae in this phase are hairy flies larvae which are refered to *Chrysomya rufifacies* larvae. The soil was excavated to a depth of 10 cm and found pupae and several larvae migrating. The number of adult flies present on the carrions decreased, the flies present were *Chrysomya rufifacies; Chrysomya megacephala; Calliphora* spp.; *Lucilia* spp. (Family Calliphoridae); and, *Sarcophaga* cf *australis* (Family Sarcophagidae). Research conducted by Vasconcelos *et al.,* (2013), used pig carrions (*Sus scrofa* L.) weighing 15 kg. In the post-decomposition stage, the larvae that have migrated turn into pupae. In addition, the species *Lucilia* spp and *Calliphora* spp are flies that colonize carrions in the post-decomposition stage.

On the sixth day, the decomposition stage enters the final stage, namely skeletonization. In the skeletonization stage, only hair and bones remain. Apart from that, the association of flies on the bodies of carrions decreased compared to the previous day (Gennard, 2012). There was only adult fly activity on the bodies of the carrions and pupae were found under the bodies of the carrions and in the soil to a depth of 10 cm around the cage. The flies present on the carrions are *Chrysomya rufifacies*; *Lucilia* spp; and *Calliphora* spp (Family Calliphoridae). In addition, the post-decomposition and skeletonization stages, adult flies and *Chrysomya rufifacies* larvae were the dominant species.

This study found the dominant larvae flies in carrions and rearing are *Chrysomya megacephala* and *Chrysomya rufifacies*. on carrions (Goff, 1993). The results of this study are similar to research conducted by Adrus and, Nor (2018), using Malaysian carrions of plantain squirrels (*Callosciurus notattus*), chickens (*Gallus gallus*), and toads (*Duttaphrynus melanositctus*). The dominant flies present in each carrion were *Chrysomya megacephala* and *Chrysomya rufifacies* from the fresh stage to skeletonization. In this study, we recorded the larva of *Chrysomya rufifacies* larvae were preyed the other larvae *Chrysomya rufifacies* larvae have a larger size than the other larvae (Goodbrod, and Madison, 1990; Dawson *et al*., 2022; Chin *et al*., 2009).

The other two species Calliphoridae (*Lucilia* sp and *Calliphora* sp,) are present in carrions from the first day of decomposition into the skeletonization stage. These two species are among the dominant adult species present in carrions. As the family has the highest number of species, this also explains the sense of smell which detects very low odors and is not detectable by humans. Calliphoridae fly species are also attracted to freshly dead carcasses, so this also shows the importance of Calliphoridae flies as the dominant species present on carrions and corpses (Koffi *et al.,*2018).

This study is similar to Ramos-Pastrana *et al.,* (2018), using three pig carrions (*Sus scrofa domestica*) weighing around 9 kg. Carrions are placed in rural areas in Colombia. The results show that the active decay stage is dominated by the Calliphoridae, Sarcophagidae, and Muscidae families.

Anterigona orientalis and *Hydrotaea ignava* (Family Muscidae), are flies that present on carrions from the fresh stage to the post-decomposition stage. The results of this research have similar with Wangko *et al.,* (1993) which was carried out in Winangun, Manado using domestic pigs. Muscidae family flies were present on first days to eight day, apart from that there were larvae found on to 14 days in pig carrions.

Propiophila sp (Family Piophilidae) was the fly with the lowest number. This species present at the active decomposition stage to post-decomposition. The results of this study have similarities with Carvalho *et al.,* (2000), using pig carrions and human corpses placed in the urban forest, in Campinas, Brazil. They reported the presence of Piophilidae in their Piophilidae could be found from the active to the post-decomposition stages with different numbers and types of species.

The result in this study is similar with Grisales *et al.,* (2010) using pig carrions that were placed in the forest of Pereira City, Colombia. This research has similarities with Silva *et al.,* (2023), using pig carrions (*Sus scrofa* L.) placed in the Inhamum Municipal Envormental Protection Area in July - August 2010 and March - April 2011. Ramos-Pastrana *et al.,* (2018), using three pig carrions (*Sus scrofa domestica*) weighing around 9 kg. Carrions are placed in rural areas in Colombia. The results show that the active decay stage is dominated by the Calliphoridae, Sarcophagidae, and Muscidae families.

Other species in the Calliphoridae family, namely *Lucilia* sp and *Calliphora* sp, are present in carrions from the beginning of the decomposition process to the skeletonization stage. These two species are among the dominant species present in carrions. As the family has the highest number of species, this also explains the sense of smell which detects very low odors and is not detectable by humans. Calliphoridae fly species are also attracted to freshly dead carcasses, so this also shows the importance of Calliphoridae flies as the dominant species present on carrions and corpses (Koffi *et al.,*2018). This research has similarities with Silva *et al.,* (2023), using pig carrions (*Sus scrofa* L.) placed in the Inhamum Municipal Envormental Protection Area in July - August 2010 and March - April 2011.

5. CONCLUSIONS AND SUGGESTIONS

The conclusion of this study, the flies present at the carcass of wistar rat in Kerandangan Nature Tourism Park were *Chrysomya megacephala, Chrysomya rufifacies, Chrysomya bezziana, Calliphora* spp., *Lucilia* spp., *Hemipyrellia linguriens* (Diptera: Calliphoridae)*, Hydrotaea ignava, Antherigona orientalis* (Diptera: Muscidae)*, Protopiophila* sp. (Diptera: Piophilidae); *Sarcophaga bravicornis, Sarcophaga* cf *australis*, and *Sarcophaga* cf *lincta* (Diptera: Sarcophagidae). The dominant species is *Chrysomya rufifacies* and *Chrysomya megacephala* (Calliphoridae).

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