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Species diversity of medically important necrophagous flies in Southwest Iran

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Abstract. *Akbari M, Rafinejad J, Fazeli-Dinan M, Aivazi AA, Jalilian A, Sheikhi S, Akbarzadeh K. 2023. Species diversity of medically important necrophagous flies in Southwest Iran. Biodiversitas 24: 1467-1472.* Species diversity of medically important necrophagous flies in Southwest Iran. Biodiversitas 24: 1467-1472. The insects are the most successful group in the phylum Arthropoda. Flies are among the most diverse group of insects and organisms. Flies can affect human health in many ways, such as the transmission of a wide range of pathogens and myiasis. Species diversity is the middle level of the hierarchical system of biodiversity. Flies, like other insects, are sensitive to biotic and abiotic factors, and their biodiversity will change under different climatic conditions. This study was performed in Ilam province, in southwest Iran. Two methods were used for collecting the flies, including bottle traps and insect nets. Fly collections were done monthly at each collection site for a year, and to identify the trapped flies, reliable identification keys were used. Calculation of fly species diversity indices was done based on the Shannon-Wiener index, species dominance based on the Simpson dominance index, species richness employing the Margalef's index, and evenness was performed based on the Evenness index. Different aspects of species diversity were calculated using PAST V.3 software. The dominance and abundance of necrophagous flies were higher in the south of the province. The highest evenness was related to the north of the province. A comparison of the diversity of necrophagous flies also indicated that the north of the province has a high species diversity. A significant and positive relationship between fly frequency and the temperature was seen. There was also a significant and negative relationship between the frequency of flies and humidity. The results of this study completed one of the research puzzles on the biodiversity of Iranian flies. The abundance and diversity of necrophagous flies were high in the Ilam province, especially in spring.

Keywords: Biodiversity, Diptera, forensic entomology, necrophage

INTRODUCTION

The Arthropoda is the largest animal phylum, and insects are the most successful group in this phylum. Diptera (Di: two, pteron: wing) are among the most diverse group of insects and organisms distributed worldwide, with about 153,000 known species in approximately 180 families (Zhi-Qiang 2013), so about 1 in every 10 animals described is a Diptera includes all true flies.

Diptera probably has more considerable health and economic effects on humans than any other order of insects in many ways (Badii 2020). Flies are one of the most ecologically and species-rich diverse insects and are classified under Cyclorrhapha or Muscomorpha suborder. They are also called filth flies and live in a wide range of habitats except for Antarctica (Sanei-Dehkordi et al. 2020). Because of the association with detritus and human wastes, many flies (especially house flies) are involved in the transmission of a wide range of pathogens such as viruses, bacteria, fungi, helminth eggs, and protozoan, especially in the hot seasons. Flies transmit pathogens mechanically from a contaminated substrate (contaminative mechanical transmission) or an infected host (direct mechanical transmission) through their legs, body hair, and mouthparts or feces and vomit on food to a susceptible host (Khamesipour et al. 2018, Yin et al. 2022).

Some flies also are blood feeders (Hematophagous), for example, the tsetse fly, which transmits sleeping sickness (African Trypanosomiasis) biologically to humans. Other biting flies, such as stable flies (*Stomoxys calcitrans*), horseflies, Hippoboscidae, and some others, play a role in the transmission of livestock diseases, including trypanosomes, as well as the transmission of Haemoproteus to birds (Mozaffari et al. 2020).

In addition to their role in the transmission of various pathogenic agents, larvae of flies can cause myiasis (Akbarzadeh et al. 2012). Human myiasis may be benign and asymptomatic, or it may have moderate to dangerous symptoms and even death (Hazratian et al. 2020). Because necrophagous flies are usually the first insects to arrive at vertebrate carrion and may lay eggs in it, they are critically important organisms for criminal investigation. The use of necrophagous insects in forensic entomology provides invaluable information, such as an estimate of the minimum Post-Mortem Interval (PMImin) index (Jafari et al. 2019).

Humans have been aware of the concept and importance of biodiversity for centuries. Plato repeatedly mentioned the principle of abundance and believed that we would have a better world if we had a high diversity (Keesing and Ostfeld 2021). Biodiversity means diversity among all organisms from all sources, including terrestrial, oceanic, and ecological collections that include these resources and includes diversity within species, between species, and ecosystems (Hamilton 2005). Species diversity is the middle level of the hierarchical system of biodiversity (Hamilton 2005). It examines the diversity of species in specific areas and points out the differences between taxonomic groups and geographical areas (Roswell et al. 2021). Species diversity is one of the characteristics of a biological society and has a large part in biodiversity studies. Sampling, calculation of indices, and statistical analysis are the three main parts of biodiversity surveys (Khoobdel et al. 2020). Ecologists and biologists measure the biological diversity of a given region or site to study the ecological and evolutionary processes and to analyze changes in diversity, similarity, and dominance of species on a time scale (Magurran and McGill 2011). Flies, like other insects, are sensitive to biotic and abiotic factors, and their biodiversity will change under different climatic conditions. Knowledge of the biodiversity and ecology of these various types of flies is important from an epidemiological point of view (Davari et al. 2018).

There have not been many studies on the biodiversity of necrophagous flies in Iran. Most of the cases have been done in a limited time and place, and even in a period from the previous decades to the last decade, no comprehensive study has been done on flies (Mozaffari et al. 2020). Ilam province has three different climates. Due to the diversity of its habitats, it is one of the most important animal husbandry areas in Iran (Akbari et al. 2020). Also, the placement of Ilam province on Iran's border with Iraq and having a border crossing brings many Iranian and foreign travelers to this area. Flies are important in terms of medicine, veterinary and forensic entomology. There is little information available on the biodiversity of important flies in terms of forensic medicine (necrophagous flies) in Iran. It seems necessary to conduct basic studies in different ecological conditions. This research was the first study of these insects in Ilam province to provide basic information for future studies. In this study, our objective was to gain knowledge on the species diversity of necrophagous flies and to compare it in different climates throughout the Ilam province.

MATERIALS AND METHODS

Study sites

Ilam province lies in central Zagros Mountain, southwest Iran. Forty-five sites in eight counties across Ilam province were surveyed for medically important necrophagous flies over one year (October 2019-September 2020; Figure 1). Sites were categorized into three regions (north, central, and south) based on the climate. The north region consisted of sites in (33_ 344790 to 33-848894 N, and 46-274941 to 46-766784 E), and it was primarily made up of oak forests, having a cold climate and long winters. The central region consisted of sites in (32-760639 to 33-363349N and 46_ 747637 to 47-669131 E), owning a temperate to warm climate with hot summers and mild winters. Lastly, the south region consisted of sites in (32_ 417832 to 33-374358 N and 46_ 264227 to 47-818933 E) with plains of the west and southwest of the province along with a dry hot climate.

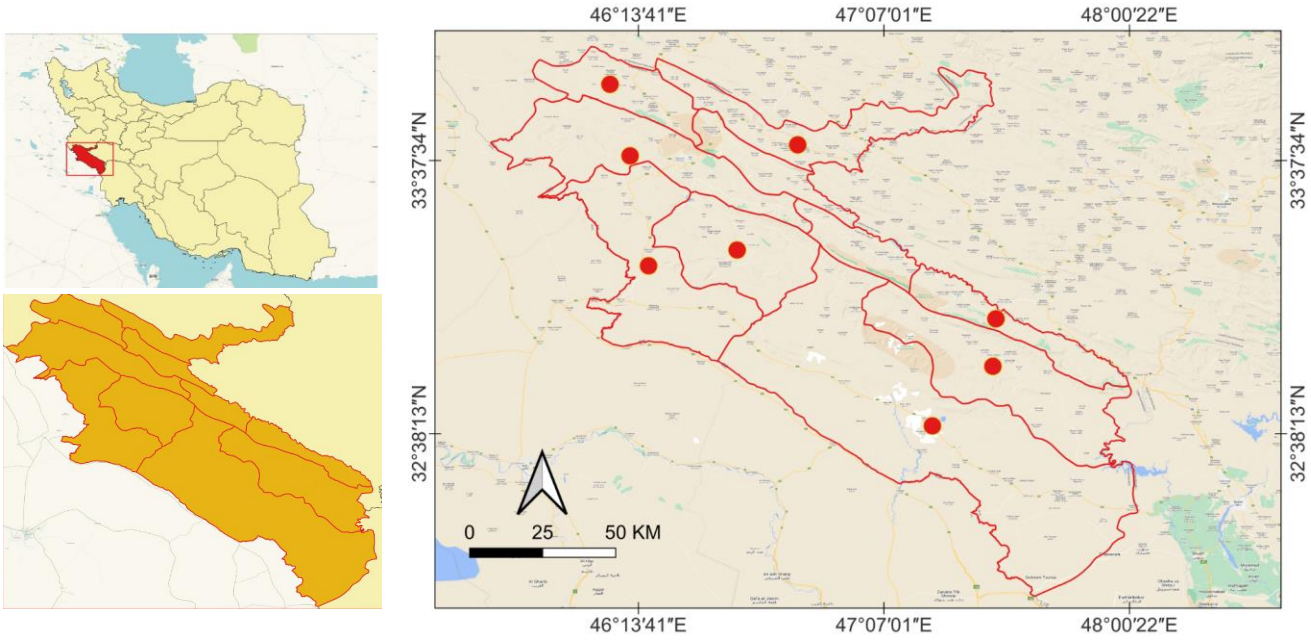

Figure 1. The geographical location and sampling stations in the counties of Ilam province, Iran, 2020

Sampling

The purpose of the study was to trap adult flies. To do so, two methods were used; bottle traps were made from plastic water bottles, also insect nets were used in sampling sites after direct observation for half an hour. To attract the flies, some baits such as liver, horse feces, or meat were used. Flies enter the trap to get to the bait but cannot find the way out. For other non-target insects, the traps should be installed at the height of about two meters above the ground. Fly collections were done monthly at each collection site for a year. The traps were collected 2 or 3 days later, and trapped flies were killed using chloroform and were mounted using entomological pins. Then the sample information, including the time and place of trapping, was attached, and all the flies were sent to the laboratory for identification.

Identification

To identify the trapped flies, reliable identification keys were used (Couri 2010; Akbarzadeh et al. 2015). The identification of Sarcophigidae flies was made only with the use of male flies (Richet et al. 2011).

Data analysis

Calculation of fly species diversity indices was done based on Shannon-Wiener index H' = -Σpi×lnpi, species dominance based on Simpson dominance index D $=\sum_{i=1}^{S} P_i^2$, species richness employing the Margalefs index ($D_{Mg} = \frac{S-1}{ln_N}$), and evenness, was performed based on the evenness index (Exp (H)/S).

In the above equations; N is equal to the total number of individuals, and S is the total number of species in the sample. Also, $P_i = \frac{n_i}{N}$ where Pi is equal to the ratio of individuals in a species, and ni is the number of individuals in each species.

Hill number indices were used to estimate species diversity. For this purpose, the Hill number was calculated based on Shannon entropy (Hill 1=Exp (H´)) and the inverse Simpson index (Hill 2=1/ (D)).

Different aspects of species diversity were calculated using PAST statistical software. To estimate the reliability limits and to compare the biodiversity parameters of the caught flies on temporal and spatial scales, the bootstrap method with 10,000 samples was used. Pearson's correlation test was used to analyze the relationship between the frequency of flies and temperature and relative humidity variables with the help of PAST V.3 software. Excel software was also used to draw the graphs.

RESULTS AND DISCUSSION

Important indices of species diversity were calculated to measure species richness, evenness, dominance, and diversity for necrophagous flies in different months in Ilam province (Table 1). The highest abundance of necrophagous flies was in September, while the lowest frequency was in February. Species diversity indices (Shannon-Wiener and Hill 1 and 2) showed species diversity reached its peak in March. The slope of the species diversity of necrophagous flies in Ilam province does not have the same behavior due to having two completely different climates, for example, in March in the south of the province, which has a warm climate, the spring season begins, but in the north of the province, cold weather prevails. The abundance and species richness in this province due to completely different conditions, as well as hot winds and unpredictable dust, were also very variable. In another study in Abadan, the highest biodiversity of flies was based on Shannon-Wiener Index same as our study in spring. The highest diversity of dominant species based on the Simpson Index was in autumn, but in our study was in spring. This difference in the results may be because the studied climates in Ilam province are different from Abadan in Khuzestan province.

In addition, these parameters were also calculated in different climates of Ilam province (Table 2). The results of this study showed that the species richness in the three climatic zones was the same during one year of sampling. This finding could be due to the proximity of these three regions to each other and the sampling of places that are a border between the three climates. The highest abundance of necrophagous flies was in a warm climate, while the lowest frequency was in a cold climate. Flies' community diversity and abundance vary between tropical and temperate regions (Zahra et al. 2018). Species diversity indices (Shannon-H and Hill 1 and 2) was high in cold climate compared to other climates.

A comparison of evenness also showed that the highest evenness was related to the north of the province, which has a cold temperature compared to other sites (Figure 2). Species evenness refers to how close in numbers each species is in an environment.

Table 1. Monthly results of necrophagous flies species diversity indices, Ilam province, 2019-2020

Indices	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar
Taxa_S	23	24	23	21	22	24	24	23	22	23	21	24
Individuals	1365	1633	1556	1622	1797	1856	1398	1250	809	469	406	812
Hill 1	9.87	10.9	10.1	8.01	7.37	8.40	8.31	8.44	9	11.11	10.6	11.6
Hill 2	6.98	8.22	7.61	6.36	5.83	6.23	5.09	5.26	5.03	6.87	7.04	8.37
Dominance_D	0.14	0.12	0.13	0.16	0.17	0.16	0.2	0.19	0.2	0.145	0.14	0.12
Simpson_1-D	0.86	0.88	0.87	0.84	0.83	0.84	0.80	0.81	0.80	0.85	0.86	0.88
Shannon-Wiener	2.29	2.39	2.31	2.08	2	2.13	2.12	2.13	2.2	2.40	2.36	2.45
Evenness_e^H/S	0.43	0.45	0.44	0.38	0.33	0.35	0.35	0.37	0.41	0.48	0.50	0.48

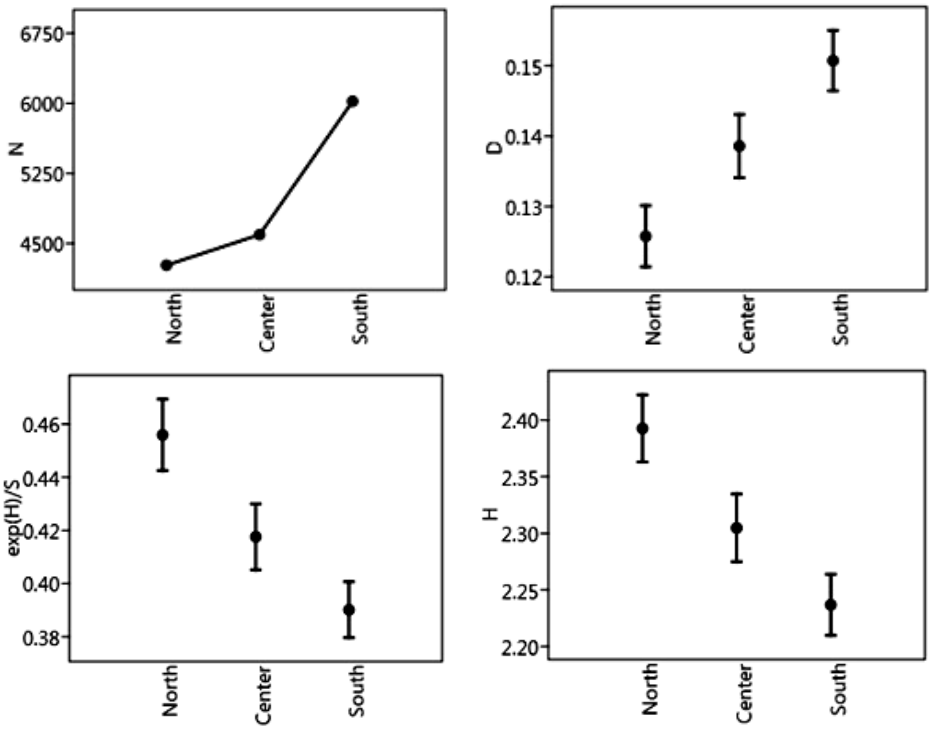

Figure 2. Comparison of flies' species diversity indices, Number (N), Dominance (D), Evenness (exp(H)/S), and Shannon (H) indices for necrophagous flies captured in traps placed in Ilam province from 2019-2020

Table 2. Results of necrophagous flies species diversity indices in different studied climates, Ilam Province, 2019-2020

Indices	Cold climate	Temperate climate	Warm climate
Taxa_S	24	24	24
Individuals	4266	4593	6021
Hill 1	10.95	10.02	9.37
Hill 2	7.95	7.22	6.64
Dominance_D	0.13	0.14	0.15
Simpson_1-D	0.87	0.86	0.85
Shannon_H	2.39	2.31	2.24
Evenness_e^H/S	0.46	0.42	0.39

The results of the Pearson's correlation test between abundance with temperature and humidity changes showed that there was a significant and positive relationship between flies' abundance and temperature ($P < 0.0001$; rs: 0.91) (Figure 3). As the temperature increases, the abundance of flies increases, but it will not always be possible, and with overheating, the abundance of flies decreases. In a warm climate or arid climates with hot and dry summers and rare rainfall, the flies' population typically peaks in spring and autumn.

There was also a significant and negative relationship between the abundance of flies and relative humidity ($P < 0.0001$; rs: -0.92) (Figure 4). Therefore, it is expected that with decreasing relative humidity, the population of fly species will gradually increase, which is as same as the study by Jafari et al. (2019). In Qeshm Island, the abundance of species decreases with increasing temperature and humidity, which is because the temperature and humidity in the Persian Gulf islands are much higher than in Ilam cities (Khoobdel et al. 2015). Between 50 and 80% of Relative Humidity (RH) and between 18 and 28°C is ideal for insects, including flies, but each species has slightly different needs (Tougeron et al. 2020). In this study, the highest abundance was between 60 and 70% relative humidity.

Flies have a global distribution and are established everywhere in tropical and temperate climates throughout the world. The only area free of flies is Antarctica (Akbarzadeh et al. 2018). Some flies are classified as synanthropic, which can be mechanical vectors for some contagious diseases in humans and many animals (Stoffolano Jr. 2022). Also, larvae of some species are the cause of myiasis in human and livestock animals. Necrophagous flies have a significant role in their ecosystems, where they are in a highly competitive situation with each other and struggling with their localities' geographical conditions.

Despite the obvious importance of the diseases caused by flies, such as myiasis, little data has been published about their agents in Iran (Akbari et al. 2021). Most studies have been performed in limited space and time, and even in a period until recent decades, no comprehensive studies have been performed on flies. Also, human myiasis is not officially defined as a notifiable disease in Iranian health reporting systems.

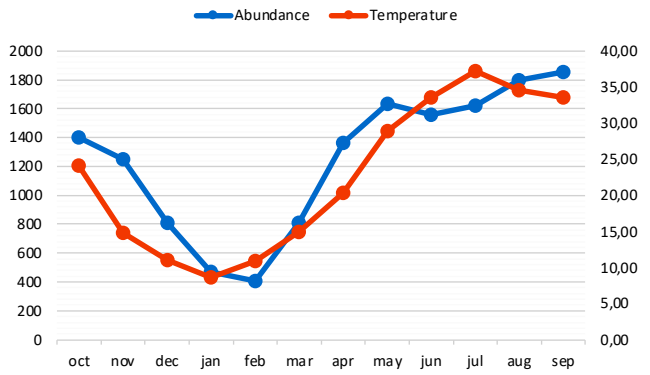

Figure 3. Population fluctuations of necrophagous flies with temperature changes in Ilam province, 2019-2020

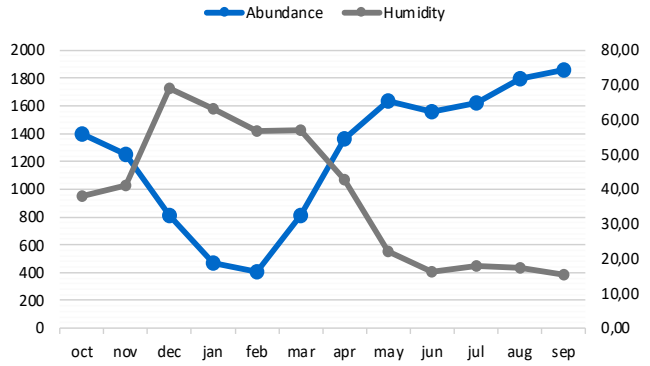

Figure 4. Population fluctuations of necrophagous flies with relative humidity changes in Ilam province, 2019-2020

Insects are very sensitive to environmental conditions. The species richness and abundance of flies in different regions are related to many reasons, such as availability of resources, humidity, optimum temperature, etc. The comparison of the species diversity (species richness and abundance) of insects in different climates may not be without drawbacks, but this issue proves how much the climate affects the composition, distribution, abundance, and dominance of insects (Courtney et al. 2017).

Biodiversity indices can show the structure of target taxa in their community. Species diversity, or at least species richness, can be a useful tool for the ecologist, but one must be clear as to the particular definition and the measure being used. Species diversity is one of the more frequently measured quantities in ecology, yet how to measure it is complex and sometimes contentious. The past decade has seen great advances in comparing and unifying various diversity metrics and also in developing ways to standardize samples before measuring diversity (Bohn and Huth 2017).

Despite valid critiques, species diversity-species richness, the Shannon index, and the Simpson index are still widely used in ecology (Roswell et al. 2021). Developing a robust diversity metric has been challenging because, unlike many variables measured by ecologists, the diversity of a community often cannot be estimated in an unbiased way based on a random sample from such a community (Skevington and Dang 2002). Over the past decade, ecologists have begun incorporating two important tools for estimating diversity: coverage and Hill diversity. Coverage is a method for equalizing samples that is, on theoretical grounds, preferable to other commonly used methods, such as equal-effort sampling or rarefying datasets to equal sample size (Chao et al. 2014).

Local trade and transit of various agricultural products and livestock can facilitate the distribution of necrophagous flies. Human movement and pastoralism can affect fly species' biodiversity. There is no reliable data on the total number of nomadic pastoralists in Ilam province. They have various paths on their traveling, which mostly depend on their prediction of pastures. In addition to the high mobility of adult flies, the nomadic behavior of shepherds in the province may facilitate the dispersal of the flies along the round trip of their hosts. This condition might be resonated with the moving of the herds by trucks which prepare immediate transportation of the flies. This issue can be one of the most important reasons for the similarity in biodiversity indices in a large-scale study in this province.

In conclusion, the results of this study completed one of the research puzzles on the biodiversity of Iranian flies. The abundance and diversity of necrophagous flies were high in the Ilam province. Throughout the year, especially in spring, the weather conditions have been suitable for the growth and activity of these insects.

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