TRAKYA FAUNASINDA DİPTERA FAMİLYALARININ TAYİNİ: ADLİ ENTOMOLOJİ İÇİN MUSCİNA STABULANS'IN İLK KAYITI

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ÖZET

Amaç:

Bu çalışma kapsamında daha önce adli entomoloji açısından değerlendirilmemiş olan Kırklareli ili Lüleburgaz ilçesinde adli entomolojik açıdan önemi olan Diptera türlerinin belirlenmesi hedeflenmiştir

Yöntemler:

Bu amaçla 1 Nisan 2010 tarihinde başlatılan çalışma, 28 Eylül 2010 tarihinde sonlanmıştır. Çalışma alanında günlük sıcaklık ve nem miktarları ölçülmüş, Diptera türlerinin bıraktığı yumurtalar, gelişen larvalar, pupalar ve yumurta bırakmak üzere gelen erişkin dipteralar toplanmıştır. Örnekler laboratuvar ortamında geliştirilip incelenmiş ve adli entomoloji açısından en önemli üç familya olarak kabul edilen Calliphoridae, Muscidae ve Sarcophagidae ailelerine ait bireylerin bölgenin Adli Entomoloji faunasının içerisinde yer aldığı belirlenmiştir. Daha sonra tür tayinleri yapılan örneklerin 25ºC ve %60 nem miktarı altındaki gelişim süreleri belirlenmiştir. Çalışmamızda belirlenen üç familyadan; Calliphora vicina, Lucilia sericata, Muscina stabulans, Musca domestica, Wolhfahritia magnifica olmak üzere toplamda beş tür tespit edilmiştir.

Bulgular:

Adli entomolojide önemli bir veri olan ADH (Türlerin toplanma süresi) ile türlerin cesede geliş sıraları ve zamanları belirlenmiştir. Tayin edilen bireyler ekolojik ve biyolojik yönden araştırılmış, sıcaklık ve nemin gelişimlerine etkisi incelenmiştir. L. sericata larvaları sabit sıcaklık ve nem miktarı altında beslenme davranışları yönünden incelenmiş ve tek bir L. sericata larvasının tüketebileceği besin miktarı belirlenmiştir. Kırklareli iline bağlı Lüleburgaz ilçesi, çalışmamızla birlikte adli entomolojik olarak ilk kez gözlenmiş olup, adli entomolojide önemli olan Diptera faunasının cesede geliş sıraları belirlenmeye çalışılmıştır.

Sonuç:

Çalışmamızın ülkemizin adli entomolojisin de kullanılan Diptera faunası kayıtlarına katkıda bulunması ve benzeri çalışmalara yardımcı olması hedeflenmiştir.

Anahtar Kelimeler: adli entomoloji, Diptera, Calliphoridae, Sarcophagidae, Muscidae

IDENTIFICATION OF DIPTERA FAMILIES IN FAUNA OF THRACE: FIRST RECORD OF MUSCINA STABULANS FOR FORENSIC ENTOMOLOGY

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ABSTRACT

Objective:

In this study, we aimed to identify diptera species of relevance in forensic entomology in Luleburgaz town located in Kırklareli province, not evaluated before in terms of forensic entomology.

Methods:

The study was carried out between April 1, 2010 and September 28, 2010. Temperature and humidity were measured daily; eggs left by diptera species, developing maggots, pupas and the adults that came to leave eggs to the trial device were all collected. Samples grown under laboratory conditions were investigated. Individuals belonging to the most important three families relevant in forensic entomology, Calliphoridae, Muscidae and Sarcophagidae, were in the region's forensic entomology fauna. Afterwards, we investigated the duration of development of the identified samples under 25°C temperature and 60% humidity. Of the three families observed in our study, Calliphora vicina, Lucilia sericata, Muscina stabulans and Musca domestica, Wolhfahritia magnifica five species in total were identified.

Results:

Species arrival sequences to corpse and the arrival times were determined by using ADH (Accumulated Degree Hour), a method important in forensic science. The identified individuals were ecologically and biologically analyzed; their effects on temperature and humidity were observed. Identified maggots of L. sericata were analyzed with respect to their nutritional behaviors and constant temperature and humidity in laboratory conditions and the amount of nutrient that the one L. Sericata maggot can consume was determined.

Conclusion:

In our study, it is aimed to assist similar studies and contribute records of Diptera fauna which is used in our country's forensic entomology.

Key words: *forensic entomology, Diptera, Calliphoridae, Sarcophagidae, Muscidae*

INTRODUCTION:

Forensic medicine which plays a major role in justice is an important branch of science co-operating with many specialist areas. The branch of science which reveals data about the time and place of death of a person by examining a series of insects which come to the corpse on a regular basis within a few minutes after the death and lay eggs, which is to be exploited because it can provide important information according to the nature and conditions of the case in all circumstances and in each case is called forensic entomology or medicocriminal entomology (1,2).

The science of forensic entomology can be described as the use of biology, ecology and behaviors of insects examining them in many ways in forensic cases to determine the place of death and post mortem interval (PMI) in forensic investigations (3). In case of a suspicious death, the determination of the place of death and PMI correctly is very important for justice to be done. For a corpse found in the first 24 hours after the death, the determination of PMI can be said with the accurate measurement of body temperature. PMI can also be determined in the first 72 hours by measuring the amount of potassium in the eye fluid with biochemical methods (4). But, in the case of corruption has begun in the first 36 hours and even in the first 24 hours in hot weather, the sensitivity in determining PMI decreases. Even in the cases where weather conditions are appropriate, the margin of error may rise from hours to days in the determination of PMI. In this case, forensic sciences benefit from Forensic Entomology. Forensic Entomology is the most precise method used to determine the PMI in the period from the first day until the first two months (5,6).

In order to benefit from Forensic Entomology in the most accurate way, insects should be known in detail (7). Insects that constitute the most dominant group among the world's known animal groups emerge everywhere in the nature in various events. Biologists name today as the "Insect Age". Insects are unrivaled in terms of the number and diversity in the animal kingdom. The number of the species is thought to exceed three million after naming those who haven't been identified yet, especially the ones located in the tropics. Insects are the only flying invertebrates, along with this feature; the featured structure of their bodies, external skeletons composed of chitin layer, breeding, feeding and respiratory systems enabled them to be today's most dominant animal group (8).

Insects are directly related to human life carry out the most important step in corruption of the corpses. In the Forensic Entomology, members Diptera (two-winged), Coleoptera (Beetles), Lepidoptera (Butterflies), Hymenoptera, Isopoda (Armadillos), Nematoda (Roundworms), Protura (Sensor-legged), Mites, Collembola, Opiliones and Acarina are exploited. But the most important and the first team is Diptera team (9,10).

Dipteras; is a team including more than 150.000 species connected to a total of 158 known families. It is stated in "A Manual of Forensic Entomology" which is accepted a classic work for forensic entomology that 23 families of Dipteras can be associated with forensic entomology (11). In a study conducted in Brazil, it was stated that 22 families can be associated with human corpses, and only seven of these (Calliphoridae, Muscidae, Fanniidae, Phoridae, Piophilidae, Sarcophagidae and Stratiomyidae) have forensic entomological importance. In a recent study conducted in Brazil, the Drosophilidae family was included to the seven families which are important for forensic entomology and it was stated that eight families are assessable (12). Dipteras that come to the corpse within the first few minutes start the decomposition by leaving their eggs or larvae to the appropriate and protected areas of the corpses. The most common diptera species in Forensic Entomology are the species belonging to Calliphoridae, Sarcophagidae and Muscidae families. As a result of collecting and evaluating egg, larva, pupa and imago of the members of other Diptera team that make a progress process depending on the temperature and succession in a way that can be associated with the corpse, PMI can be determined, an answer can be found to the question whether the corpse was carried from the place of death or not and as a result of various biochemical analysis applied to the larvae, the determination of the toxic substances caused the death can be done. Forensic entomology may not only lead the forensic sciences in cases of murder, but also in cases of negligence and abuse. Insects should be examined in detail ecologically and biologically and the forensic entomology fauna should be determined to determine the succession for each geographical region in order forensic entomology to help criminal incidents (7).

The purpose of the study is to determine the insect fauna including Luleburgaz district of Kirklareli province which is important in terms of forensic entomology and to determine PMI from the forms of evolution of the insects belonging to the Diptera team.

Figure 1: Picture of flies leaving their eggs in outdoor study

MATERIAL AND METHOD:

Our study was conducted in two phases as field and laboratory study to cover the spring and summer seasons.

Field Study: The field phase was conducted for experimental group of the determination study of the diptera species used in forensic entomology in Luleburgaz district, Kirklareli province, 41°24'32.41"N latitude and 27°21'53.77"E longitude, for the control group 41°24'32.32"N latitude and 27°21'55.56"E longitude. There is approximately 120 m distance between the two stations. The continental climate is observed in the region.

Two 3-liter plastic-transparent container and funnel in the field phase of the study, and petri dishes, falcon tubes, forceps and alcohol are used during the stages from collecting the samples to the laboratory stage. The study was initiated on April 1, 2010 at 10:00 am 360 g calf muscle tissue that has just died for sure was left in 2 mechanisms in the region as experimental group and control group. There is approximately 120 m distance between the two stations. Our aim is to identify necrophagia species by comparing the species that come to the two mechanisms and to distinguish the species that come by chance and leave without laying eggs (Figure 1). Mature flies were able to get inside with the help of funnels located on the entrance of the mechanisms and they stayed inside after they laid eggs. Mature insects stayed in the mechanism were kept after putting them in petri dishes with the help of a forceps. Those which should be examined dead of the samples taken from the larvae after the development of the eggs were put in 70% alcohol and kept in falcon tubes, and those that should be examined alive were put in petri dishes in order to keep them alive and grow them. The study took 155 days in total.

Laboratory study: Laboratory phase of the samples within the scope of the study was carried out in Systematic 1 Laboratory at Istanbul University, Faculty of Science, Department of Biology. In the laboratory phase of the experiments carried out within the scope of the study, petri dishes, falcon tubes, forceps, two 60x60 sized wire meshes surrounded by veil, wood chips, precision balance, 160x200 sized magnification binocular microscope, camera, alcohol and KOH (potassium hydroxide) were used. Extech brand, cabled, continuous measurement

termo&hygrometer was used for the measurement of humidity in the laboratory. In addition, open containers filled with water were placed in the laboratory in order to make the humidity as much as possible. The opening of the chamber was opened or closed sometimes in order to make the humidity stable (Figure 2).

The experimental setup established in the laboratory was divided into two groups consisting of 60x60 sized cages. The petri dishes in which samples brought from the land on the day of April 17, 2009 were put in the first cage together with pieces of calf muscle tissue left in order the larvae to feed. The wood chips placed on the bottom and sides of the petri dishes enabled an appropriate field for the samples that completed their feeding and enter into the 3rd instar larva stage to enter pup stage. After a species-specific while, the larvae started to enter into the pup stage and the pups were taken from the first cage, put into clean petri dishes and were placed in the second cage in order the mature flies to come out. Mature flies that come out of the pup stage were taken from the petri dishes that are closed and then some of them were taken pictures and examined, some of those that are identified as from different species were kept under stable humidity and temperature conditions to lay eggs. Thus, development time for each species under 25 C and 60% humidity was determined. An experimental group was formed on September 30, 2009 and the second step of the laboratory study was initiated. A study carried out with a total of 15 groups, 10 of them is the subject group and 5 of them is the control group, in this study carried out with the species that are identified as Lucilia sericata was initiated. Only 15g calf muscle tissue and no larvae were left in the first 5 petri dishes in order to calculate the amount of evaporation for the control group under 25 C temperature and 50% humidity. Five young instars that have just come out of egg were put over the 15g calf muscle tissue placed in the other 10 petri dishes and the experiment was initiated. Amount of food the larvae of the samples belonging to L. sericata species in the period until they enter the pup stage in laboratory conditions consumed was determined with this study (Figure 3). The amount of meat given and the amount of evaporation observed in control groups, the amount of meat left after the larvae enter the pup stage and the weights of pups were evaluated by measuring them with precision balance.

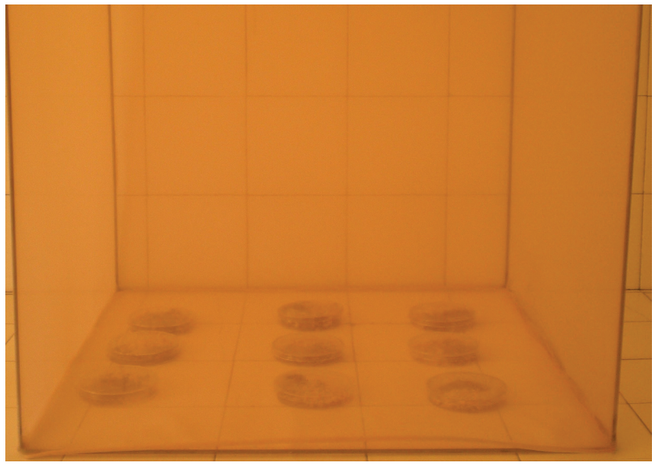

Figure 2: Cage system in laboratory study

RESULTS:

During the field study, 134 mature individuals and 110 larvae were examined. In the laboratory study, the stages of development and feeding of 50 larvae were observed. During the period of field study carried out in Luleburgaz district of Kirklareli province, 3 families, 2 subfamilies, 5 genus and 5 species belonging to Diptera team have been identified. Mature individuals of 5 different species which are Muscina stabulans and Musca domestica Linnaeus 1758 from Muscidae family (Fallén 1817), Lucilia sericata (Meigen 1826) and Calliphora vicina (Robineau-Desvoidy 1830) from Calliphoridae family, Wohlfahrtia magnifica (Schinner 1862) from Sarcophagidae family were examined under a microscope and

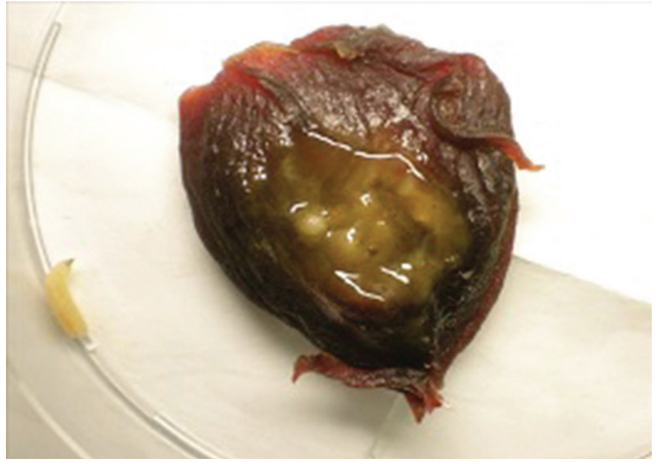

Figure 3: System used in detecting consumed amounts of larvas

after waiting the larvae of these species in 10% KOH solution for 10-12 hours to examine them, their species determinations were made and photographed. The determinations of the species were made according to Greenberg and Kunich (2002), Cutter (2002) (13,14).

During the study period, the temperature and humidity findings in Luleburgaz district of Kirklareli province have been followed from the website of State Meteorology Works.

DISCUSSION:

Typically, it was the fly species belonging to the Calliphoridae family that first appeared around the corpse. The gravitating time of the Calliphoridae family onto the body was different. A study, took place in England, proved that C. vicina reached the corpse within a couple of minutes (15). Firstly, in our study, C. vicina and L. sericta were brought into our testing apparatus. The most dominant group among the species discovered during the study, was L. sericata. Amongst the other studies of forensic entomology carried out in Turkey, in the study of Sabanoglu (2007), C. vicina and L. sericata were observed and was evaluated in terms of the forensic entomology (16). Two out of eight species discovered in the study of Coban (2009) in Edirne, were C. vicina and L. sericata (17). Alongside the species that are common between Edirne and Kirklareli, the diversity of the species observed distinctly, was thought to be stemmed from the difference in the time frame the two studies took place, the different climatic factors and the geographical differences. The pubescence period of the eggs of C. vicina under 50% humidity at 25°C was determined as 15-19 (360-456 hours) days (13). Greenberg and Povolny (1971) stated that C. vicina was observed in the winter months in subtropical zones and in spring and autumn in temperate zones (18). In our study, C. vicina appeared in April, May and September 2009. By the evaluation through ADH method, it was determined that the larvae hatched after 33 hours under 60% humidity at 25°C, approximately 152 hours later it developed into pupa, the residence time inside the pupa lasted 180 hours, and their evolution was completed in approximately 365 hours (Table 1). The maximum temperature and the amount of humidity in the months when C. vicina was observed, was measured as 65% humidity at 31.9°C in May 28, 2009 and 88% humidity at 29°C in September 6, 2009 in our study zone.

Tantawi et al. (1996) stated that, the period of getting mature individuals out of the eggs under 50% humidity at 23°C was 15 days (260) hours (19). In our study, it was determined that the larvae of L. sericata hatched after 21 hours under 60% humidity at 25°C, 123 hours later the larvae developed into pupa and the hatching time of the mature individual was 146 hours, and their evolution was completed in 290 hours (Table 1). The time difference between 360 hours of evolution period Tantawi et al.(1996) stated, and 290 hours of evolution period in our study was thought to be stemmed from

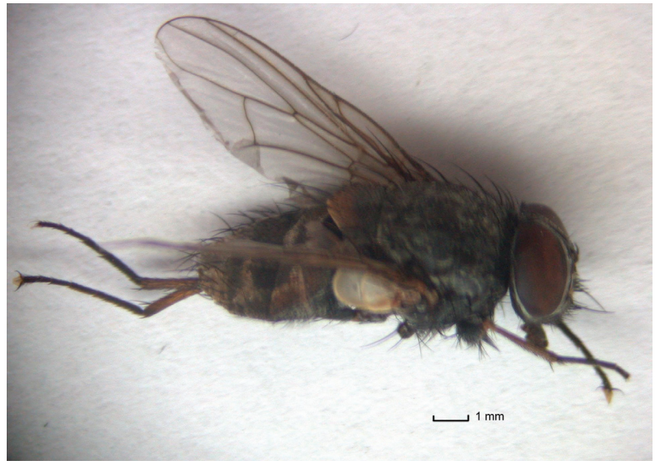

Figure 4: Muscina stabulans

the difference that the two studies were carried out under 50% humidity at 23°C and 60% humidity at 25°C. It was observed that, L. sericata appeared on the testing apparatus during the period of study, to lay eggs within each month except for the first days of April. Despite C. vicina and L. sericata are cosmopolite species, it is well-known that C. vicina is observed mostly in urban zones. C. vicina was thought to be appeared, as our study had been carried out in a green zone at the center of the district. According to the sources we were able to reach 21 species relevant to the Calliphoridae family as reported before for our country (20).

The less observed species in our study was specified as M.domestica. In a study carried out in Samsun (AKDEMIR) it was stated that, for M. domestica, it required 16 days for pubescence under 45% humidity at 24°C (21). In our study, it was observed that the larvae hatched after 43 hours under 60% humidity at 25°C, 117 hours later the larvae developed into pupa, the residence time inside the pupa lasted 165 hours and their evolution was completed in 325 hours (Table 1). In Samsun, Akdemir (2005) stated 384 hours period for 45% humidity at 24°C (21). In our study, the evolution period was determined as 325 hours fewer than 60% humidity at 25°C. The time difference was thought to be stemmed from the amount of humidity where the two studies were carried out and the pubescence period descended as the amount of humidity increased. In this case, in the environments reserving high humidity such as forestry, lake bed and lakeshore, it was considered to pay attention to the pubescence period of the flies on the corpse. Known as a house fly, M. domestica's being one of our samples, made us think that it was because of the houses around the zone where our study was carried out. It also made us consider that the corpses on which eggs or larvae of M. domestica found, might not have necessarily died at home.

The other species of the same family, M. stabulans (Figure 4), is a species that could be observed mostly in rural areas, and also in the event of stable, farm or similar building areas' structuring in the urban zones of occupation. It is one of the most common individual of this family along with M. domestica. The flies belonging to M. stabulans were especially related with corpses around Iberian Peninsula (22). However, M. stabulans, a species commonly observed in the summer months, was not seen in the thesis studies and publications that we were able to reach aiming at determining the Forensic Entomological fauna in Turkey, in our study, this species was observed in June, July, August and September 2009. In Brazil, a study carried out for M. stabulans stated that it required 40 days (960 hours) at 16°C, 20 days (480 hours) at 20°C, 12 days (288 hours) at 26°C and 15 days (360 hours) at 31°C to get mature individuals out of the eggs (23). In our study, we observed that the larvae hatched within 25 hours fewer than 60% humidity at 25°C, the larvae developed into pupa at the end of 120 hours and the pupation lasted approximately 160 hours. The total time lasted to get mature individuals out of the eggs was determined 305 hours

Table 1: Growth periods at constant moisture 60% and temperature 25°C

Species	Ovulation Period/ Hour	Larval Period/ Hour	Pupal Period/ Hour	Total Developmental Period/Hour
L. sericata	21	123	146	290
M. stabulans	25	120	160	305
M.domestica	43	117	165	325
C. vicina	33	152	180	365
W. magnifica	44	150	216	411

(Table 1). M. stabulans and M. domestica were observed in our study zone in June, July August and September 2009.

Mature W. magnifica, one of the species of Sarcophagidae family, are located in the meadows or in similar zones. W. magnifica, rarely seen in the environments like stable or house, is the second dominant species after L. sericata, observed in our study. In a study carried out in America it was determined that the evolution of W. magnifica was completed in a period between 80 days (1920 hours) and 100 days (2400 hours) (24). In our study, it was observed that the larvae hatched after 44 hours under 60% humidity at 25°C, 150 hours later the larvae developed into pupa, the time required for the mature individual to get out of the eggs was 216 hours and the total time to complete their evolution lasted 411 hours (Table 1). The difference between evolution periods was thought to be stemmed from the difference between the temperature and the humidity levels.

In the study carried out in laboratory phase, for calculating the amount of aliment that one of the individuals of young larvae belonging to the species L. sericata had consumed along the period lasted until pupation, it was observed that the samples of the species L. sericata developed into pupa under 50% moisture at 25°C in 168 hours. Considering the values of evaporation amounts inside the control groups, the average was determined as 9.73g. As a result of evaluating the remaining amount of meat, average consumed meat amount was measured as 1.193g, and it was determined that a single larva consumed 0.238g (Table 2,3). Aliment and after the evaluation of pupa weights, average pupa weight of L. sericata was specified as 0.067g.

The evolution period of the species under constant temperature and humidity amount, that had been discovered in our study was observed, pursuant to the results it was determined that the species completed its evolution sharpest was L. sericata under 60% humidity at 25°C in 290 hours, M. stabulans followed it in 305 hours, M. domestica in 365 hours and W. magnifica in 411 hours that completed its evolution in longest period of time. These values proved that, in case of finding larvae of W. magnifica or pupas on a corpse in a zone the study was carried out, this person remained death much longer than another corpse from which the larvae of M. domestica or L. sericata were picked for determining the time of death.

During the period our study, for only first five days of April 2009 the egg sample was not taken, it was observed that one or two flies appeared rarely on the testing apparatus had not laid eggs, and the reason was thought to be the minimum 5.5°C temperature and 30% humidity level in Luleburgaz region along the five days of April 2009.

In the course of our study, the most significant factor affected

Table 2: Rest amount of meat at evaporation and after evaporation in Control groups

Control Groups	Evaporation Amount/g	Rest Amount of Meat/g
C1	9,30	5,70
C2	9,28	5,72
C3	9,27	5,73
C4	9,25	5,75
C5	9,25	5, 75

the evolution of the species belonging to the dipteral family, was the temperature and it was observed that, like low temperatures and low amount of humidity, the temperatures over 30°C also affected the evolution and delayed the evolution period of the species. It was observed that, the evolution picked up speed at the highest temperature and humidity environments in the optimum temperature ranges; hatching time for larvae and the time required for larvae to molt, was descended. Therefore it was required to analyze the evolution period of all the species of diptera used for the forensic entomology in Turkey at different temperatures and amount of humidity, and to upload the results to the database related to the forensic entomology and forensics. These values are of significant importance getting proximate results with the reality in investigations relevant to the forensic cases. In the study we carried out, the differences between the evolution periods of the dipteral was thought to be depended on the temperature and the amount of humidity in our working environment.

Table 3: Rest amount and consumed amount of meat in Experimantal groups / evaporation and rest amount of meat in Control groups

Experimental Groups	Rest Amount of Meat/g	Consumed Amount of Meat/g	Average Evaporation Amount/g
E1	4,73	1	9,27
E2	3,73	2	9,27
E3	4,88	0,85	9,27
E4	4,93	0,8	9,27
E5	4,73	1	9,27
E6	4,23	1,5	9,27
E7	4,25	1,48	9,27
E8	3,83	1,9	9,27
E9	4,33	1,4	9,27
E10	4,49	1,25	9,27

Several studies carried out in our country, proved that the species L. sericata, C. vicina, M. stabulans and W. magnifica we discovered in this study, also caused myiasis in Turkey. It was stated that, in a study carried out at Nigde and Ankara University, called as Myiasis Flies Continuing Evolution on The Remainings of Cattles, the Calliphora vicina and the Muscina stabulans; in a study carried out Kirikkale and Selcuk University, called as Gingival Myiasis Case Arose from Wohlfahrtia magnifica of A Lamb, the Wohlfahritia magnifica; in a study carried out at Gaziantep, Kocatepe and Ankara University, called as Geniral Myiasis Case of A Cat and in a study carried out at Adnan Menderes University, called as Occular in A Cat Arose From the Larvae of Lucilla sericata (Diptera: Calliphoridae) and Traumatic Myiasis Case of A Dog, the species Lucilia sericata caused myiasis (25-28). It was observed that, most of the species discovered in our study matched with the myiasis case. This proves that most of the species of diptera used for the forensic entomology, has also caused myiasis.

There are two significant factors in terms of the importance of the team diptera in the forensic entomology and to discover these species correctly. First one is that, the determination keys are sufficient, comprehensive, and explanatory, the second one is the interest and sensitivity of the taxonomists in this subject. Studies, relevant to the subject should be conducted, and in these studies, it is required to specify the features of the area, climatic ecological data, faunas and succession thoroughly. This is the only possible way to make use of the insects correctly in the forensic entomology. Today, a more practical and different systematic of Diptera is tried to be set up for the team Diptera, still regarded as complicated and congested (12).

In consequence, in the study carried out in Luleburgaz district of Kirklareli three most common species of the team Diptera, Calliphoridae, Muscidae ve Sargophagidae; C. vicina, L. sericata, M. domestica, M. stabulans and W. Magnifica regarded as significant in terms of the forensic entomology, were discovered. The species M. stabulans, significant in terms of the forensic entomology and had not been detected inside the studies carried out in Turkey, was discovered in the area we conducted our study. It was determined that, this species is a new input among the species dipteral used for the forensic entomology. Thus, M. stabulans will enter our forensic entomology database as a new input. With the species discovered, the succession of the forensic entomology fauna of the Luleburgaz region was specified.

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