Application of different insects in forensic field

Siqi Chen *

Beijing No.4 High School, Beijing, China

* Corresponding Author Email: siqichen@ldy.edu.rs

Abstract. Forensic entomology is a burgeoning applied science. It advocates the use of analysis of morphological characteristics, developmental stages and species of insect samples, in order to calculate PMImin, and conjecture the cause of death and crime environment. Due to the absence of an accepted standard and the peculiarities of each case, forensic entomology still has not reached its maximum forensic application value. To achieve more extensive use in the forensic field, the effectiveness and unique superiority of entomological evidence need to be shown. This research primarily focuses on how entomology knowledge applies in court. The existing researches related to forensic entomology have been summarized and analyzed in the following. Factors like bias in evidence collection hinder the development of forensic entomology. However, entomological analysis's potential of being the most efficient PMImin calculation method could not be ignored. To better show how entomology could apply in crime investigation, two Diptera families with forensic significance have been analyzed in detail. The first family is Sarcophagi Dae, which has its special oviposition behavior and specialty of colonizing indoor corpses. The research on Calliphoridae, a species with significant carcass colonization ability, is also essential for the forensic entomology field. On account of the importance of accurate species identification, the global distribution, habitat preference and spawning habits of each family. To improve acceptance of insect evidence in forensic application, the popularization of entomological knowledge needs to be improved, and the absent standard in evidence collection and case report writing also needs to be made up.

Keywords: Forensic entomology, Molecular identification, Sarcophagidae, Calliphoridae.

1. Introduction

With the rapid development of technology, science became a helpful tool to solve crimes including civil offences and formal offences. However, crimes nowadays have also advanced in sophistication, along with modern offenders depending on scientific concepts to either "hide" or even perpetrate crimes [1]. To deal with these more complicated civil and criminal matters, forensic science, an area of study that conducts experimentation and applies proven facts and principles to deal with challenges related to the law [1], was invented. Generally speaking, forensic science is an intersection of social science, life science and medicine [1]. Similarly, forensic entomology, just as its name suggests, is an interdisciplinary that entomology and forensic science combines and influences each other.

Forensic entomology is a relatively new research field. The field of forensic entomology has only been around for 140 years, since the first forensic entomology scientific paper was published in 1883. Moreover, forensic entomology's importance for forensic casework was not acknowledged until the early 1900s [2]. In the past 20 years, forensic entomology has made great progress and substantive breakthroughs, and acquired certification from two of the top forensic institutions in Europe, the entomological laboratories of the Belgian Institute National de Criminalistique et Criminologie and the French Institut de Recherche Criminelle de la Gendarmerie Nationale [3].

In the class Insecta, insects from orders like Coleoptera, Diptera, Hymenoptera have a habit of performing frequent life activities on dead bodies corpses. Therefore, Diptera, Coleoptera, Hymenoptera, and other necrophilic insect groups are the most frequently involved in forensic entomology-related study and practice [4]. Physiological development rates, ecological succession, molecular species identification, and biochemical profiling of these necrophilic insect groups are essential perspectives for forensic entomological research and practice [5]. Main task of forensic entomology is to analyze necrophilic insects and use analyzing results to infer the time of death, place of death and cause of death to assist crime investigation. In addition, other common application of forensic entomological analysis includes investigation of sexual offenders like rapist by examining the stomach and tissue composition of the larvae and pupae of the necrophilic insects to determine the source of their food by genotyping human DNA [4].

Forensic science requires the development of reliable standards. A unified standard can provide guidance for practice and improve the efficiency of work and exchange. Forensic entomology lacks precisely such a standard due to the relatively small number of past cases and the uniqueness of each case. For many in the forensic area, insects remain exotic as a result of this circumstance [5]. However, the value of entomological evidence in the investigation of criminal incidents and its advantages over other types of evidence cannot be ignored.

This research will summarize the results and methods of several forensic entomological studies below, in order to reflect the necessity and application value of entomological evidence in forensic analysis. The studies are mainly about the previous criminal investigation cases that applied entomological knowledge, the major challenges of forensic entomology development, how species with significant corpse-colonization ability be used in forensic entomology.

2. The performance of insects in forensic entomology

Lena Lutz et al. summarize case reports from the Institute of Legal Medicine Frankfurt, Germany, from 2001 to 2019, emphasize the significance of insects in providing legal investigations' evidence, and reveal the potential roadblocks that still stand in the way of a sufficient application [3]. Even forensic entomological analysis may be among the most effective methods for identifying a PMImin with the least amount of sample work, but their significant advantage still has not been identified by the majority of the legal field. Hence, the popularization of entomological knowledge among criminal investigators is much needed. The importance of cooperation of recovery and examination of the corpse from multiple discipline perspectives should also be realized.

They first raise the most significant factor in limiting progress of forensic entomology--the bias in collected evidence caused by the particularity (e.g. person responsible for evidence collection, sampling location) of each case. The non-trained or less experienced staff could only look for fly maggots, which are much easier to spot and identify than species like beetles, which eventually also influenced the statistical result of most frequently using evidential insects. However, they further assert that the evaluation of insect evidence in the forensic sciences is unaffected by this potential bias for Diptera due to the exclusive focus on integrated analysis of morphological characteristics of a particular insect species with the characteristics of the same insect species found at the body discovery site. In the following, they analyze the characteristics of carcasses colonized by most common evidential insect families in the field of forensic entomology, and their seasonality of oviposition and preferred environment respectively.

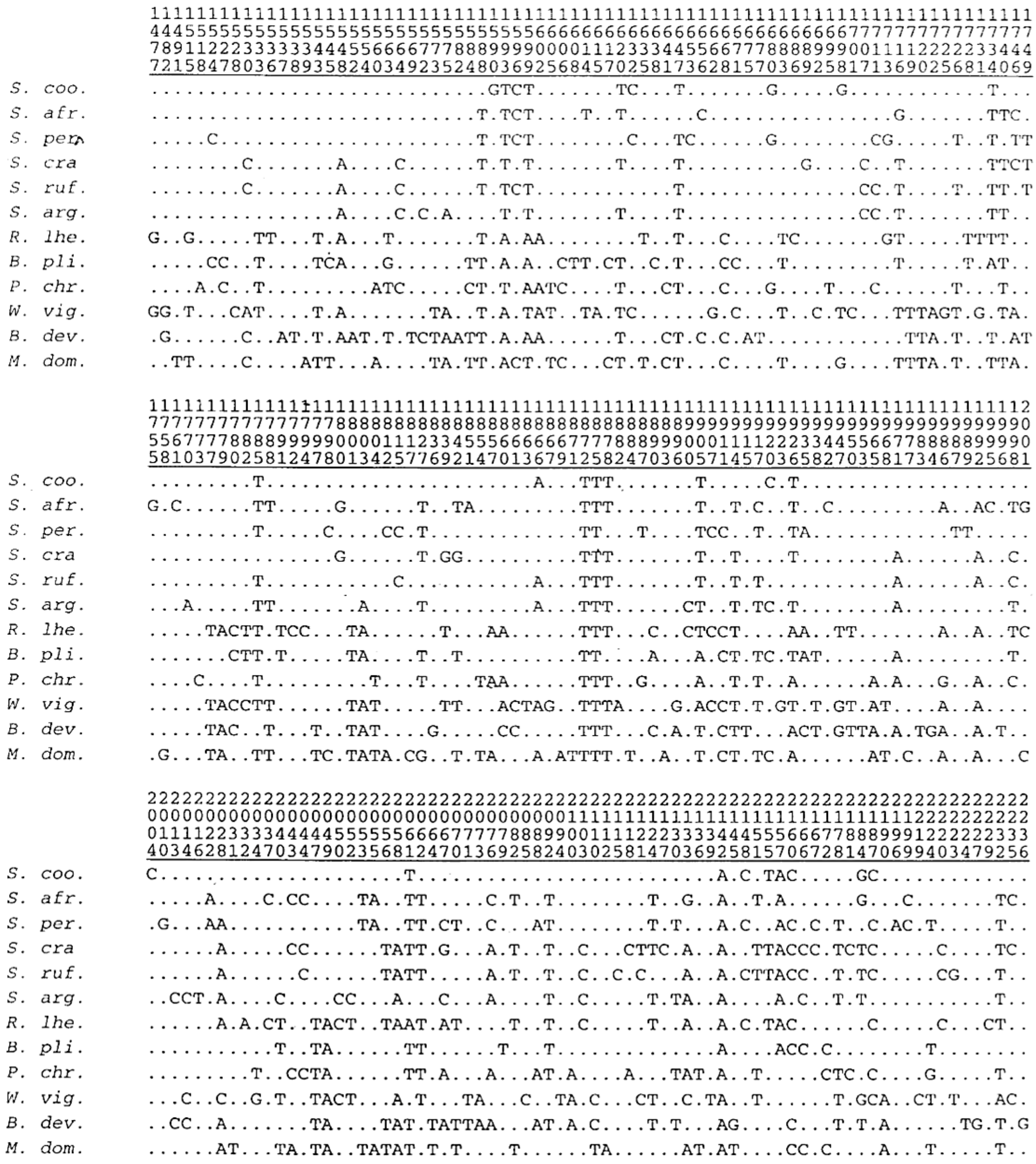
In the part of providing cases for the different PMI groups, the examples greatly exemplify how insects found on corpse could be used as evidence to estimate PMI based on their characteristics of different developmental stage, favored environmental temperature and other entomological evidence. However, in the summary section of "seasonal oviposition activity" of different insects' groups, the species sequence of related figures is not consistent with the narrative order of the text captions, which just create some difficulties in reading and connecting the images and texts [3].

Zanthé Kotzé et al. point out the biggest challenges for forensic entomology more extensive application in legal system are the deficiency in forensic entomological standards and the majority lack of cognition of the application of arthropod evidence [2]. Therefore, to help students new to the field, investigators, and legal counsel for clarity in a court of law, this page primarily discusses the criteria, terminology, limitations and applications of forensic entomology in criminal.

They firstly list several accepted standards and restrictions in forensic entomology: (1) The necessity of appropriate estimation of the exposing site of the bodies and insects. (2) The error of insects' developmental datasets introduced by difference between relative stable laboratory environment and more fluctuant field environment. (3) The influence of Myiasis on determination of

PMImin. (4) The probability that the entomological evidence originates from another organism's corpse. (5) When counting PMImin based on insects' spawning time, investigator always would not take nighttime hours into consideration, because most Diptera often do not oviposit at night. (6) The counting of PMImin needs to begin when the insects can approach the bodies freely. (7) Insects' succession patterns are only predictable to a certain extent.

They then introduce several frequently-used forensic entomological terms and the essence of clarifying their meanings. The following lists the most widely used keys, including pictographic, molecular identification, and various dichotomous keys. The sections required for the forensic entomology report are also recommended by the author. To help non-professionals better understand the contents of the case reports, they conclude the importance of proper use of scientific terminology and individuality of each case. Above all, this article is very all-sided and detailed guideline for constructing a forensic entomology case report [2].



```
           111111111111111111111111111111111111111111111111111111111111111111111111
           444555555555555555555555555555556666666666666666666666666667777777777777
           789112223333334445566667778889999000011123333445566777888899900111122222233444
           721584780367893582403492352480369256845702581736281570369258171369025681406 9
S. coo.    ...........................GTCT......TC..T.......G....G............T...
S. afr.    ..........................T.TCT...T..T......C................G.......TTC.
S. per.    .....C.....................T.TCT........C..TC.....G........CG.....T..T.TT
S. cra     ........C.......A....C......T.T.T......T....T..........G....C..T.......TTCT
S. ruf.     ........C.......A....C......T.TCT...........T...............CC.T....T..TT.T
S. arg.    ................A....C.C.A....T.T......T....T...............CC.T.......TT..
R. lhe.    G..G.....TT...T.A...T.......T.A.AA........T..T...C....TC.......GT.....TTTT..
B. pli.    .....CC..T....TCA...G......TT.A.A..CTT.CT..C.T...CC...T.........T.....T.AT..
P. chr.    ....A.C..T.........ATC.....CT.T.AATC....T...CT...C...G....T...C......T...T..
W. vig.    GG.T...CAT....T.A.......TA..T.A.TAT..TA.TC......G.C...T..C.TC...TTTAGT.G.TA.
B. dev.    .G......C..AT.T.AAT.T.TCTAATT.A.AA......T...CT.C.C.AT...........TTA.T..T.AT
M. dom.    ..TT....C....ATT...A....TA.TT.ACT.TC...CT.T.CT...C....T....G....TTTA.T..TTA.

           111111111111111111111111111111111111111111111111111111111111111111111112
           777777777777777788888888888888888888888888899999999999999999999999999990
           556777888899999000011123345556666667777888999000111122223344556677888889999 0
           581037902581247801342577692147013679125824703605714570365827035817346792568 1
S. coo.    .........T......................A...TTT.......T.....C.T...................
S. afr.    G.C......TT.....G......T..TA.........TTT.......T..T.C..T..C.........A..AC.TG
S. per.    .........T.....C....CC.T.............TT...T....TCC..T..TA............TT.....
S. cra     ................G......T.GG..........TTT.......T..T....T........A......A..C.
S. ruf.    .........T...........C...........A...TTT.......T..T.T...........A......A..C.
S. arg.    ...A.....TT.......A....T.........A...TTT......CT..T.TC.T........A.........T.
R. lhe.    .....TACTT.TCC...TA......T...AA......TTT...C..CTCCT....AA..TT.......A..A..TC
B. pli.    .......CTT.T.....TA....T..T..........TT.....A...A.CT.TC.TAT......A.........T.
P. chr.    ....C....T..........T...T....TAA......TTT..G....A..T.T..A......A.A...G..A..C.
W. vig.    .....TACCTT......TAT.....TT...ACTAG..TTTA....G.ACCT.T.GT.T.GT.AT....A..A....
B. dev.    .....TAC..T...T..TAT....G.....CC.....TTT...C.A.T.CTT...ACT.GTTA.A.TGA..A.T..
M. dom.    .G...TA..TT...TC.TATA.CG..T.TA...A.ATTTT.T..A..T.CT.TC.A......AT.C..A..A...C

           222222222222222222222222222222222222222222222222222222222222222222222222
           000000000000000000000000000000000011111111111111111111111111111112222222222
           011112233333444445555556666777778889900111122223333444555666778889991222222333
           403462812470347902356812470136925824030258147036925815706728147069940347925 6
S. coo.    C...................T...........................A.C.TAC.....GC............
S. afr.    .....A....C.CC....TA..TT.....C.T..T........T..G..A..T.A......G...C.......TC.
S. per.    .G...AA...........TA..TT.CT..C...AT........T.T...A.C..AC.C.T..C.AC.T.....T..
S. cra     ......A.....CC......TATT.G...A.T..T..C...CTTC.A..A..TTACCC.TCTC.....C....TC.
S. ruf.    ......A......C......TATT.....A.T..T..C..C.C...A..A.CTTACC..T.TC.....CG...T..
S. arg.    ..CCT.A....C....CC...A...C...A....T..C.....T.TA..A.....A.C..T.T...........T..
R. lhe.    ......A.A.CT..TACT..TAAT.AT....T..T..C.....T..A..A.C.TAC......C......C...CT..
B. pli.    ...........T..TA......TT......T...T..............A....ACC.C.........T........
P. chr.    .........T..CCTA......TT.A...A...AT.A....A...TAT.A..T.....CTC.C....G.....T..
W. vig.    ...C..C..G.T..TACT...A.T...TA...C..TA.C...CT..C.TA..T......T.GCA..CT.T...AC.
B. dev.    ..CC..A.......TA....TAT.TATTAA...AT.A.C....T.T...AG....C...T.T.A......TG.T.G
M. dom.    ......AT...TA.TA..TATAT.T.T....T......TA......AT.AT....CC.C....A...T.....T..
```

Figure 1. Nitrogen bases sequence of mitochondrial DNA in different Sarcophagidae species [6].

Ren et al. have identified and explained the distinct features of flesh flies that have made it easier for investigators to perform in PMImin estimate, like they deposit maggots directly on a corpse instead of eggs and their relatively large size [7]. They firstly give an overall review of distribution of flesh flies species based on different geographic features and climates of different regions from a global perspective. Due to the significant effects of geographical location on when different Sarcophagidae species arrive, scholars should pay more attention to constructing databases for each bio geoclimatic zone. Common flesh fly’s species also have the particularity of colonizing indoor cadavers and related cases have been listed in the following section. Furthermore, the case in which Sarcophagidae appears at higher elevations is also analyzed. The potential value of studying various drugs' influence on PMImin determination with the endotoxicology assistance is also clarified. DNA sequence identification techniques' role in Sarcophagidae species' forensic application is also crucial. Errors in Sarcophagidae species identification could induce a deviation in PMI estimation of more than one day. As shown in Fig. 1, an mtDNA sequence database allows for the identification of most Sarcophagidae species feeding on corpse [6]. In addition, accounts of traumatic myiasis brought on by Sarcophagidae species, and flesh flies' developmental patterns under different temperatures are also compiled. This article provides a comprehensive summary for Sarcophagidae species and their distribution, which could be a milestone of flesh flies application in forensic entomology [7].

Accurate species identification of closely related Sarcophagidae species remains a challenge, because external morphological identification and molar identification are all under development and facing multiple barriers. However, identification through measuring wings could be a useful substitute technique, which might be especially effective in Sarcophagidae family because of wing measurement's equal efficacy in determining gender. Szpila et al. develop a reliable wing measurement tool to aid in identifying forensically important Sarcophagidae species [8]. They mainly used European flesh flies as material, which were collected from thirteen European and Middle Eastern countries using entomological nets on flowers or slightly decomposed chicken liver. Wing measurement method developed for Muscidae and Calliphoridae has been referred They also included data from specimens of the Muscidae and Calliphoridae families in research in order to more effectively compare these three families (Sarcophagidae, Muscidae, and Calliphoridae) from the standpoint of the wings. The result shows wing measurement could successfully distinguish different families, but few Sarcophagidae genus have been misidentified as other family's genus. Additionally, only a few species' sexes could be identified by wing measurement. In a nutshell, Szpila et al.'s study proved that wing shape is an effective potential identification standard. In the future, sample size and identification procedure could further improve to lift success rate of genus [8].

Accurate species identification plays a key role in the application of Sarcophagidae in forensic entomology due to their significantly high colonization capacity for corpses and their special oviposition patterns. Therefore, Fuentes-López et al. compared the specimens taken from baited traps in Alicante with the sarcophagids taken from human carcasses from forensic cases in the same vicinity [9]. They used absolute ethanol to preserve the DNA sequence of sarcophagids. Pig liver and pig blood are used to set traps in order to attract sarcophagids. After cox1 mitochondrial gene extraction, PCR amplification, DNA sequencing and barcode analysis using ABGD, GMYC and BIN species delimitation methods of each specimen, they identified 8 species, which contain 6 Sarcophagidae species, 1 Calliphora species, 1 Wohlfahrtia species). The two species that are most frequently collected are Sarcophaga tibialis and Sarcophaga argyrostoma. In addition, they also study phylogeographic relationships among populations of the most widespread species in the area using the PopArt program. It was evident that the Alcoy S. argyrostoma samples were isolated. Above all, this article proves DNA-based and taxonomic identification approach is an effective method identifying Sarcophagidae species and estimating minPMI [9].

Badenhorst et al. introduce the taxonomy, morphological identification, molecular identification, phylogenetic relationships, biogeographical distribution, and forensic significance of Chrysomya [10]. Three primary morphological forms with distinct ecologies are found in Chrysomya: a "normal" form (Chrysomya pacifica Kurahashi), a "derived" form and a "feral derived" form. These forms diverge with each other due to habitat difference, and differentiate based on male morphology. However, some Chrysomya species have not been clearly distinguished in some former researches, so investigator need to verify taxonomy of older studies when using conclusion of these researches.

For different developmental stages of Chrysomya, different traits would be essential in different Chrysomya species identification. In addition, molecular identification plays an effective role only if the right marker molecules have been chosen. In most cases, mitochondrial DNA could be the first choice based on their superior marker's rate of evolution and numbers of copies. For species with close relationships, to prevent the possibility of confusion, phylogenetic analysis is required. The biogeographical distribution of Chrysomya megacephala has also been delineated in the following. The Chrysomya megacephala shows a preference for tropical and temperate climates and a tendency to colonize Europe and Canada in the future. Chrysomya megacephala do not show significant Habitat preferences, but female C. megacephala tends to lay eggs at night under warm conditions through walking. Under different temperatures, C. Megacephala's development rates would also be different. Richard and Villet's thermal accumulation models illustrate how temperature affects the course of C. megacephala development. Five C. Megacephala developmental milestones were timed and compared by Richard and Villet at nine different constant temperatures [11].

Chrysomya megacephala spread fast throughout the world due to their exceptional longevity and mean egg mass. Diseases including diarrhea and parasite infestations, which are related to urban forensic entomology and public health, are spread by Chrysomya megacephala. In addition, Chrysomya megacephala's behavior and diet also make them significant in the field of stored-product forensic entomology, environmental forensic entomology and the most important--Medicolegal forensic entomology. They are considered significant in minPMI estimate since they are typically the first and most dominant colonizers of corpses [10].

Lucilia sericata is one of the most represented forensic species, which is usually predominantly identified in samples. Case reports related to this species usually emphasize a large variability in development time. The PMImin estimation process frequently uses the accumulated degree days (ADD) approach, whose quality depends on the mathematical lower thermal threshold. Cervantès et al. studied how low the temperature would influence Lucilia Sericata's development rates [12]. The Lucilia sericata samples are mainly from natural capture using fish as bait in Rosny sous Bois, and specimens from fishing tackle shop could be a substitution when natural samples are not available. The researchers observe Lucilia sericata's development under different temperatures based on three reference points: first appearance of a third-stage larva, pupal stage and adult emergence. The researchers used the data in the table for linear fitting and extrapolated the lower mathematical development threshold through the x-intercept approach. Then, they used the ADD method to calculate the dates of oviposition for each group of Lucilia sericata under different temperatures, and compared the data obtained from ADD method and the data gathered from rearing experiments at the same temperatures. The result shows that the developmental stages' duration decrease with the increasing temperature. Additionally, there is also a clear tendency to decrease accuracy of the ADD method with decreasing temperature. This research reveals the limitations of ADD method in PMImin calculation and the effect of low environment temperature on Lucilia sericata development [12].

To assist entomologists, predict and quantify the effect of sub-optimal evidence, the consequence of using delayed and incorrect collection of forensic entomological evidence needs to be clarified. Archer et al. mainly investigated the effects of storage temperature on the growth and physiological development of unfed live maggot samples of varying ages. Two species with forensic significance (heat-adapted Ch. rufifacies and cold- adapted C. vicina) have been used as experimental subjects. They set up three size classes and gave each group four different temperature treatments. For comparison, they also set up a baseline group for each size class with the same four temperature treatments. For C. vicina, there was a significant variation in absolute length change across treatments, indicating size, and for Ch. rufifacies, there was an interaction between size, time, and temperature. The majority of the second instar larvae in Calliphora vicina maggots showed very little mortality. From the second to the third instar, Chrysomya rufifacies exhibited high mortality and little moulting. It took 48 hours for only "large" Ch. rufifacies maggots housed at room temperature to pupate. It is not advised to postpone preserving live, unfed maggot samples because these data demonstrate that development can continue in those samples even after they are collected [13].

3. Conclusion

In a nutshell, this research analyzes and summarizes 8 forensic entomology articles' purposes of research, research methods and research results respectively. This research explains the importance and former application of entomological evidence in the forensic field. Challenges and shortcomings of forensic entomology have also been identified and analyzed, which would be a potential research direction in the future. This research mainly focusses on two diptera families with forensic entomological significance-Sarcophagidae and Calliphoridae. For Sarcophagidae, the unique forensic advantages and distribution of flesh flies, and factors that may influence the calculation of PMImin by Sarcophagidae species have been identified. Based on the necessity of accurate species identification for application of entomological evidence, two identification methods-Wing shape measurements and DNA-based identification-have been shown to be promising and more efficient. For Calliphoridae, it is about identification (using morphological and molecular method), distribution and behavior of a particular Calliphoridae genus with forensic significance-Chrysomya megacephala. To achieve better use of entomological evidence, the influence of other external factors like low temperature environment and delayed collection of entomological evidence on Calliphoridae species have also been discussed.

References

[1] Rivers D B, Dahlem G A. The science of forensic entomology. John Wiley & Sons, 2022, 1 - 5.

[2] Kotzé Z, Aimar S, Amendt J, et al. The forensic entomology case report—A global perspective. Insects, 2021, 12 (4): 283.

[3] Lutz L, Zehner R, Verhoff M A, et al. It is all about the insects: a retrospective on 20 years of forensic entomology highlights the importance of insects in legal investigations. International Journal of Legal Medicine, 2021, 135: 2637 - 2651.

[4] Ren L P, Shang Y J, Guo Y D. Progress and Application of Entomological Evidence in Forensic Science. Fa yi xue za zhi, 2021, 37 (3): 295 - 294.

[5] Amendt J. Forensic entomology. Forensic sciences research, 2018, 3 (1): 1 - 1.

[6] Wells J D, Pape T, Sperling F A H. DNA-based identification and molecular systematics of forensically important Sarcophagidae (Diptera). Journal of Forensic Sciences, 2001, 46 (5): 1098 - 1102.

[7] Ren L, Shang Y, Chen W, et al. A brief review of forensically important flesh flies (Diptera: Sarcophagidae). Forensic sciences research, 2018, 3 (1): 16 - 26.

[8] Szpila K, Johnston N P, Akbarzadeh K, et al. Wing measurements are a possible tool for the identification of European forensically important Sarcophagidae. Forensic Science International, 2022, 340: 111451.

[9] Fuentes-Lopez A, Sala A P, Romera E, et al. DNA-based and taxonomic identification of forensically important Sarcophagidae (Diptera) in southeastern Spain. Science & Justice, 2021, 61 (2): 150 - 159.

[10] Badenhorst R, Villet M H. The uses of Chrysomya megacephala (Fabricius, 1794) (Diptera: Calliphoridae) in forensic entomology. Forensic sciences research, 2018, 3 (1): 2 - 15.

[11] Richards C S, Villet M H. Data quality in thermal summation development models for forensically important blowflies. Medical and veterinary entomology, 2009, 23 (3): 269 - 276.

[12] Cervantès L, Dourel L, Gaudry E, et al. Effect of low temperature in the development cycle of Lucilia sericata (Meigen) (Diptera, Calliphoridae): implications for the minimum postmortem interval estimation. Forensic sciences research, 2018, 3 (1): 52 - 59.

[13] Archer M S, Jones S D, Wallman J F. Delayed reception of live blowfly (Calliphora vicina and Chrysomya rufifacies) larval samples: implications for minimum postmortem interval estimates. Forensic sciences research, 2018, 3(1): 27-39.