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Forensic use of *Chrysomya albiceps* (Wiedemann, 1819): the first cases indicating postmortem interval for human corpses in Republic of Macedonia

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Abstract
The Republic of Macedonia is a country on the Balkan Peninsula without any history of forensic entomology use even when insects are the only available indicators of time since death. Here, we report forensic entomology cases in which the necrophagous blow fly *Chrysomia albiceps* was used for the first time to estimate the time between death and the discovery of the corpse or the postmortem interval (PMI). This is the first record of this species in Macedonia.

Keywords: Forensic entomology, *Chrysomya albiceps*, postmortem interval, case reports, Republic of Macedonia

1. Introduction
Forensic entomology, as part of forensic science, represents the use of insects in medico-legal context [1]. Forensic entomologists use their knowledge of insects and their life cycles and behaviors to give clues about a crime [2]. The decomposing corpse, as a temporary resource of nutrients, is exploited by a wide diversity of necrophagous insects. Among them, blowflies (Calliphoridae) are the first insects to arrive and lay eggs at carrion, attracted by the odor produced in the early stages of decomposition [3, 4]. The oviposition starts a biological clock whereby the subsequent determination of the age of the developing blow fly larvae is the basis for estimating the time between death and the discovery of the corpse or the postmortem interval (PMI) [5]. The rate of development of an insect is mainly governed by temperature [6]. Hence, knowing the stage of development of the oldest immature insects on the body, and the average environmental temperature of the crime scene while the body was in situ, it is possible to calculate an accurate PMI [7].

The calliphorid species, *Chrysomya albiceps* (Wiedemann, 1819) is one of the most studied blow flies because of its importance in human and animal health, as well as being an important forensic entomology indicator frequently occurring on human bodies [8, 9, 10, 11]. Several studies noted that the females are carrion breeders which oviposit slightly later than other blow flies [12, 13, 14]. The first instar larvae of *Ch. albiceps* feed on the decomposing carcass, while the second and third instars are predaceous on other necrophagous Diptera larvae. Due to this aggressive behavior of *Ch. albiceps* larvae Faria *et al.* [14] found that the abundance of body breeding fauna may significantly decrease. In this sense, Halide *et al.* [15] suggest that the knowledge of the life cycle and feeding behaviors of the species is useful for more precise estimation of postmortem interval and contributes to the avoidance of errors during a forensic investigation. Considering the morphological features, adults and larvae of *Ch. albiceps* are easily distinguished from other carrion-associated blow fly species in Europe. The adults have the stem-vein haired above, anterior thoracic spiracle yellow, whitish posterior thoracic spiracle, genae with lower half blackish and upper half pale brown covered with dense white hairs, 5 to 7 proepisternal setae, black transverse marginal abdominal bands on abdominal segments III and IV very narrow, up to about a quarter on AIII and usually not more than about sixth in AIV and dense white hairs on the ventral surface of the abdomen [16, 17]. The larvae of *Ch. albiceps*, commonly named "hairy maggots" (Fig. 1 and Fig. 2) are easily recognized by the presence of numerous prominent fleshy protrusions along their body [18].

Almost identical ultra-morphological characteristics are present in immature stages of *Ch. rufifacies* (Macquart), the other hairy maggot blow fly. However, this species differs from *Ch. albiceps* in having elongated tubercles and slender spines assembling only at the tips [19]. Apart from that, *Ch. rufifacies* was not yet reported in Europe [20].

Ch. albiceps originating from the Old World tropics represents an alien species recorded in many parts of Europe [8, 16, 21]. Although Grassberger *et al.* [8] suggested a wide distribution on the Balkan Peninsula, its presence in the Republic of Macedonia has not yet been confirmed.

There is no published data regarding the application of entomological evidence in estimating PMI in the country. In this paper, we describe forensic entomology cases in Republic of Macedonia in which the necrophagus blow fly *Ch. albiceps* was used for the first time to estimate the postmortem interval (PMI). This is the first record of this species in the country.

Fig 1: Third instar larvae of *Chrysomya albiceps*

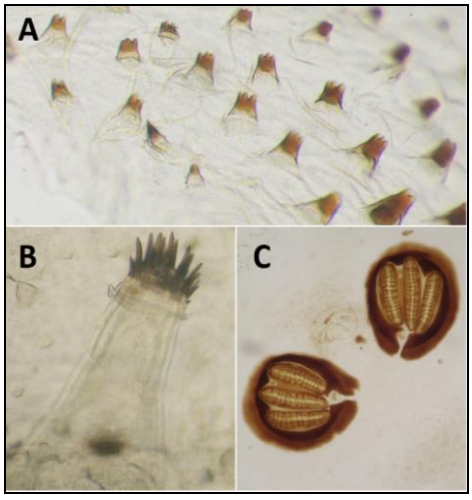

Fig 2: Third instar of *Chrysomya albiceps*: A – thoracic segment III, spines; B – fleshy proturbance; C – posterior spiracles

2. Materials and Methods

During July 2013, three criminal investigations involving entomological evidence were conducted. The cadavers were removed from the death scene and, autopsies were performed by forensic pathologists at the Institute of Forensic Medicine, Criminology and Medical Deontology in Skopje. The forensic entomologist was allowed to collect samples and document with photographs the decay stage of the cadavers. For a collection of entomological evidence, the standards and guidelines by Amendt *et al.* [4] were followed. The blow fly specimens were transported to the Laboratory for Invertebrates and Animal Ecology, at the Faculty of Natural Sciences and Mathematics, Skopje for further identification. Unfortunately, none of the specimens arrived alive.

To facilitate the identification of the blow fly larvae and to visualize the morphological details of the cephalopharyngeal skeleton and the integument, the clearing technique proposed by Sukontason *et al.* [22] was applied. Photographs were taken using Olympus SZX9 and LEICA WILD M3C Stereomicroscope with an integrated high-resolution digital camera. Morphological identification of the immature and adult blow fly specimens was done using the identification keys provided by de Carvalho and de Mello-Patiu [23] and Szpila [24]. Blow fly specimens are deposited in the Macedonian National Collection of Invertebrates at the Faculty of Natural Sciences and Mathematics, Skopje.

The developmental stages were identified and the length of individuals of most advanced stages was measured under a binocular microscope in 0.1 mm units using a vernier caliper. For estimating the PMI the age assessment of the oldest individuals that have developed on the body was determined using superimposed isomegalen and isomorphen diagrams prepared by Richards *et al.* [9]. The diagrams illustrate morphologically (length and stage) changes during development of the fly depending on the time and temperature.

3. Results and discussion

Case study 1

On July 8, 2013 the dead body of a 57-year old female murder victim was discovered in her ground floor apartment in the district of Aerodrom, Skopje (41.98°N, 21.46°E). The apartment door and windows were closed leaving only the balcony door slightly opened. According to the nearest weather station, the mean ambient temperature on the crime scene was 22.5 °C. The corpse was in an advanced stage of decay with the head completely skeletonized. Medical evidence suggested that the diagnosed substantial perimortem head trauma was the cause of death. Examination during autopsy showed severe maggot infestation, particularly in the head region. The majority of the larvae were large sized (10-15 mm) feeding third-instars of *Chrysomya albiceps* (Fig. 3A). In addition, the head and the body were widely colonized with other calliphorid larvae at different stages of development. Empty pupal cases were not found at the autopsy.

Plotting the length of the most mature individuals (third-instar larvae) of *Ch. albiceps*, and the temperature of the crime scene on the superimposed isomegalen and isomorphen diagrams by Richards *et al.* [9], we estimate the time interval between death and recovery of the body to be 11 or 12 days. Therefore, the oviposition probably took place between the afternoon of 29th and morning of 30th of June 2013. Police investigation proved the time of death to be the evening of 29th of June after the confession of the victim's son, who turned out to be the murderer.

Case study 2

On July 15, 2013 the body of a 30-year old male was found on the Veles hospital outdoor grounds (41°42'51.1"N 21°46'32.1"E). The cadaver was fully dressed, lying on his side, facing the grass and had no indication of any injury. Meteorological data from the nearest weather station showed that the mean ambient temperature was 29 °C. The autopsy

revealed that the body was in early stages of decomposition with high densities of larvae, especially on the face and neck as shown in Fig. 3B. The larvae and the adult specimens collected by the forensic entomologist were identified as individuals of *Ch. albiceps*. The age determination of blow fly larvae showed their transition from second to third instar (5-12 mm).

The development curves established by Richards *et al.* [9] show that at 29 °C, *Ch. albiceps* reaches 3rd instars in 2.2 days. This data indicates that the death most probably occurred July 13th 2013, which was in accordance with results from the police investigation. Namely, an autopsy confirmed that death occurred due to drug overdose two days before the discovery.

Case Study 3

In the last case presented by the authors, on the evening of 22th of July 2013, the corpse of elderly women was discovered stuck in a crack between a retaining wall and the rocks from the slope in an area called Zajcev Rid. The location (42°00'56.1"N, 21°23'55.9"E) is in the transition zone between the urban and rural area of Skopje. The woman was declared missing since 7th of July and had memory problems due to her old age. The weather data showed that the mean ambient site temperature was 25 °C.

The autopsy was performed on 24th of July. The head and arms were completely skeletonized, dorsal part of the body was dry and mummified and heavy maggot infestation was evident on the chest and abdomen. Because of more advanced postmortem changes in soft tissues, pathologists couldn't determine accurate time since death. Examination performed by forensic entomologist confirmed that *Ch. albiceps* third instar larvae (10-14 mm) were the most numerous on the human corpse (Fig. 3C), although they were not the oldest. In this case, the individual in the most advanced stage of development was a single dark brown *Ch. albiceps* pupa (Fig. 3D) that was used to estimate the time since death.

The age assessment following the method by Richards *et al.* [9] indicated that the third instar larvae were no more than 8 days old. Furthermore, the isomorphen and isomegalen diagrams suggest that at 25 °C, the pupal stage extends from the 8 to the 13 days from hatching. Therefore, the most probable day of oviposition for *Ch. albiceps* was between 12th and 17th of July.

Concerning this case, it is important to stress out that *Ch. albiceps* was the only insect evidence found on the deceased body in advanced stages of decomposition, which could be a result of the aggressive feeding behavior of second and third instar larvae. This predatory species may clear the corpse of all earlier arrivers [14], and therefore the only rough estimation of the post mortem interval (PMI) was made. In this case, the lack of other entomological evidence provides no information about the events that took place between the 12th and 7th of July (the day the victim went missing), so more accurate PMI estimation was not possible.

This study confirms the previous findings of many authors [8, 11, 25, 26] about the ability of *Chrysomya albiceps* in colonizing cadavers found indoors and outdoors in urban as well as rural areas. In this sense, the cases described in this paper point out the great value of even one single species in solving forensic cases in various types of habitats. Moreover, the occurrence of *Ch. albiceps* in the Republic of Macedonia in the warmer period of the year is in accordance with Grassberger *et al.* [8], Szpila *et al.* [16], Kosmann *et al.* [25] and Vanin *et al.* [26]. The authors suggest that, to complete the development this species requires temperature higher than 15 °C, which could be useful in determining the season of death. The daily average temperature in the country during the winter does not exceed 15 °C, thus the findings of the pupal cases of *Ch. albiceps* on human remains could indicate longer period since the time of death.

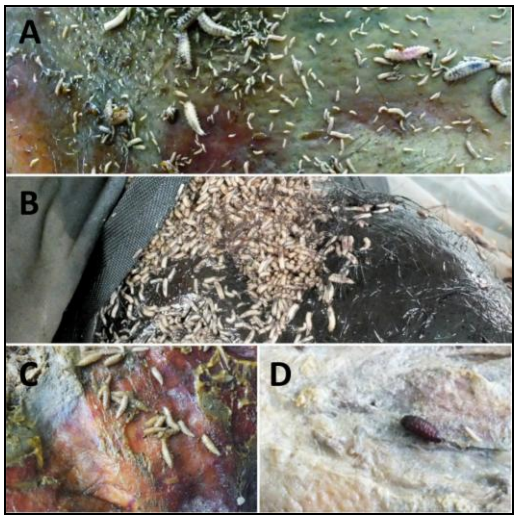

Fig 3: Body breeding fauna on human cadavers: A – Diptera larvae in different stages of development (Case 1); B – high densities of larvae (Case 2); C – Third instars of *Chrysomya albiceps* (Case 3); D - *Chrysomya albiceps* pupae (Case 3).

4. Conclusion

In this paper the necrophagus blow fly *Ch. albiceps* was used for the first time in Republic of Macedonia to estimate the time between death and the discovery of the corpse (PMI). For appropriate estimation of PMI, the authors recommend close cooperation between forensic entomologists and forensic pathologist from the visual observations of the cadaver in the scene, through the collection of insects and temperature data up to the final report with the interpretation of entomological and other biological evidence. Finally, we hope that the results presented in this study will initiate further research on the application of entomological evidence for forensic purposes in the Republic of Macedonia.

5. References

1. Byrd JH, Castner JL. editors. Forensic entomology: the utility of arthropods in legal investigations. Edn 2, CRC press, Boca Raton. 2009, 127-177.
2. Sharma R, Garg RK, Gaur JR. Various methods for the estimation of the post mortem interval from Calliphoridae: A review. Egyptian Journal of Forensic Sciences. 2015; 5(1):1-2.
3. Smith KGV. An introduction to the immature stages of British files. Diptera larvae with notes on eggs, puparia and pupae. Handbooks for the Identitication of British Insects. Royal Entomological Society of London, London. 1989; 10:11-13.
4. Amendt J, Campobasso CP, Gaudry E, Reiter C, LeBlanc HN, Hall MJ. Best practice in forensic entomology—standards and guidelines. International journal of legal medicine. 2007; 121(2):90-104.
5. Catts EP. Problems in estimating the postmortem interval in death investigations. Journal of Agricultural Entomology. 1992; 9(4):245-255.
6. Amendt J, Krettek R, Zehner R. Forensic entomology. Naturwissenschaften. 2004; 91(2):51-65.
7. Amendt J, Richards CS, Campobasso CP, Zehner R, Hall

MJ. Forensic entomology: applications and limitations. Forensic science, medicine, and pathology. 2011; 7(4):379-392.
8. Grassberger M, Friedrich E, Reiter C. The blow fly *Chrysomya albiceps* (Wiedemann) (Diptera: Calliphoridae) as a new forensic indicator in Central Europe. International journal of legal medicine. 2003; 117(2):75-81.
9. Richards CS, Paterson ID, Villet MH. Estimating the age of immature *Chrysomya albiceps* (Diptera: Calliphoridae), correcting for temperature and geographical latitude. International journal of legal medicine. 2008; 122(4):271.
10. Biavati GM, de Assis Santana FH, Pujol-Luz JR. A checklist of Calliphoridae blowflies (Insecta, Diptera) associated with a pig carrion in central Brazil. Journal of Forensic Sciences. 2010; 55(6):1603-1606.
11. Vairo KP, Corrêa RC, Lecheta MC, Caneparo MF, Mise KM, Preti D *et al.* Forensic use of a subtropical blowfly: the first case indicating minimum postmortem interval (mPMI) in southern Brazil and first record of *Sarconesia chlorogaster* from a human corpse. Journal of forensic sciences. 2015; 60(s1):3-5.
12. Braack L, Retief PF. Dispersal, density and habitat preference of the blow-flies *Chrysomyia albiceps* (Wd.) and *Chrysomyia marginalis* (Wd.) (Diptera: Calliphoridae). The Onderstepoort journal of veterinary research. 1986; 53(1):13-18.
13. Faria LD, Godoy WA. Prey choice by facultative predator larvae of *Chrysomya albiceps* (Diptera: Calliphoridae). Memórias do Instituto Oswaldo Cruz. 2001; 96(6):875-878.
14. Faria LD, Trinca LA, Godoy WA. Cannibalistic behavior and functional response in *Chrysomya albiceps* (Diptera: Calliphoridae). Journal of Insect behavior. 2004; 17(2):251-261.
15. Halide NA, Açikgöz A, Isbasar T. Predator behavior of *Chrysomya albiceps* (Fabricius) (Diptera: Calliphoridae) on human corpses. Türkiye Parazitolojii Dergisi. 2011; 35(2):105.
16. **S**zpila K, Matuszewski S, Bajerlein D, Konwerski S. *Chrysomya albiceps* (Wiedemenn, 1819), a forensically important blowfy (Diptera: Calliphoridae) new for the Polish fauna. Polish Journal of Entomology. 2008; 77(4):351-355.
17. Grella MD, Savino AG, Paulo DF, Mendes FM, Azeredo-Espin AM, Queiroz MM *et al.* Phenotypic polymorphism of *Chrysomya albiceps* (Wiedemann) (Diptera: Calliphoridae) may lead to species misidentification. Acta tropica. 2015; 141:60-72.
18. Baumgartner DL, Greenberg B. The genus *Chrysomya* (Diptera: Calliphoridae) in the new world. Journal of Medical Entomology. 1984; 21(1):105-113.
19. Sukontason KL, Sukontason K, Lertthamnongtham S, Kuntalue B, Thijuk N, Vogtsberger RC *et al.* Surface ultrastructure of *Chrysomya rufifacies* (Macquart) larvae (Diptera: Calliphoridae). Journal of medical entomology. 2003; 40(3):259-267.
20. Sanford MR, Whitworth TL, Phatak DR. Human wound colonization by Lucilia eximia and *Chrysomya rufifacies* (Diptera: Calliphoridae): Myiasis, perimortem, or postmortem colonization?. Journal of medical entomology. 2014; 51(3):716-719
21. Verves YU. Records of *Chrysomya albiceps* in the Ukraine. Medical and veterinary entomology. 2004; 18(3):308-310.
22. Sukontason K, Methanitikorn R, Sukontason KL, Piangjai S, Olson JK. Clearing technique to examine the cephalopharyngeal skeletons of blow fly larvae. Journal of vector ecology. 2004; 29:192-195.
23. Carvalho CJ, Mello-Patiu CA. Key to the adults of the most common forensic species of Diptera in South America. Revista Brasileira de Entomologia. 2008; 52(3):390-406.
24. Szpila K. Key for the identification of third instars of European blowflies (Diptera: Calliphoridae) of forensic importance. Current concepts in forensic entomology. Springer Netherlands, Dordecht. 2009, 43-56.
25. Kosmann C, Macedo MP, Barbosa TA, Pujol-Luz JR. *Chrysomya albiceps* (Wiedemann) and *Hemilucilia segmentaria* (Fabricius) (Diptera, Calliphoridae) used to estimate the postmortem interval in a forensic case in Minas Gerais, Brazil. Revista Brasileira de Entomologia. 2011; 55(4):621-623.
26. Vanin S, Caenazzo L, Arseni A, Cecchetto G, Cattaneo C, Turchetto M. Records of *Chrysomya albiceps* in Northern Italy: an ecological and forensic perspective. Memorias do Instituto Oswaldo Cruz. 2009; 104(4):555-557.