European Association for Forensic Entomology

13th meeting

Abstract Book

EAFE

European Association for Forensic Entomology

Budapest, Hungary
25 - 28 May 2016

XIII. International Meeting of
the European Assotiation for Forensic Entomology
HUNGARY, 25 – 28. May 2016.

Budapest

2016

Dear EAFE members and guest,

It’s my pleasure to welcome you all on the XIII. Conference of the European Association for Forensic Entomology.

In this year Budapest host this conference and I hope so that you would gain better knowledge of the Hungarian history and cultural heritage.

The wide variety of the present scientific conference program represent that the Entomological Society is so open-minded the relationships beyond the Europe borders, and the spirit which can be only created by the scientific work. Without borders, with continuous development of the investigation we serve the criminal matters solving.

This scientific cooperation manifested in the work of organizers, because the important partners of the judicial action is the universities, museums, research institutes. In this profession of faith, our partner is Hungarian Society of Parasitologists and Hungarian Natural History Museum.

I hope that all of you came here to know more about forensic entomology and how to use our acquired knowled gein the forensic cases but also to strengthen contacts and friendships. We registered almost 75 participants from 24 countries.

On behalf of organizers, I wish you all nice and effective days, and I believe that this conference will be fruitful, we will gain experiences from each other and we will have creative discussions about theory and practice.

Gábor Csorba, PhD Csaba Bozó, Dipl. Ing. Prof. Róbert Farkas, DVM. PhD, DSC

Dear EAFE members, colleagues and friends,

It is with pleasure and pride that we invite you to read and digest this abstract book, which compiles all the scientific work that will be presented during the 13th meeting of the European Association for Forensic Entomology.

The annual meetings are a window on forensic entomology. A window opened on a vast and varied landscape: from classic taxonomy to the latest developments in chemistry, from collecting evidence on crime scenes and rearing maggots to state-of-the–art instrumental analysis. More than ever forensic entomology is a very diverse area, where curiosity and creativity converges towards a better comprehension of phenomenons and ultimately, in helping justice.

Enjoy your stay In Budapest, and may this meeting be fruitful in every aspect.

Luc Bourguignon

President of the EAFE

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GENERAL INFORMATION

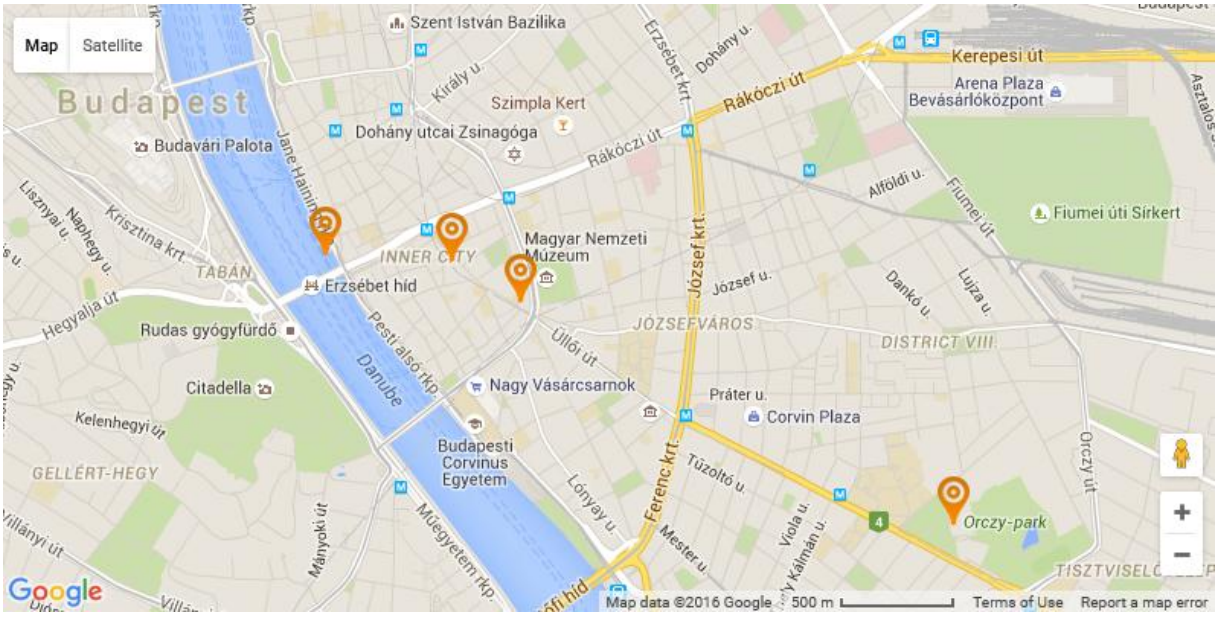
Date

25-28 May, 2016 Wednesday-Saturday

Venues and Map

Welcome reception (25 May, 2016): MészárSteak Kitchen, 1053 Budapest, Kecskeméti utca 14., Sissi room EAFE Meeting Budapest (26-27 May, 2016): Hotel Mercure Budapest Korona, 1053 Budapest, Kecskeméti u. 14., 1st floor Optional gala dinner (27 May, 2016): Zsófia Rendezvényhajó, 1052 Budapest, Petőfi tér, port nr. 9.)

Workshop (28 May, 2016): Hungarian Natural History Museum, 1083 Budapest, Ludovika tér 2-6.)



Public transportation

You can travel in the vehicles only with pre-purchased passes or tickets that you validate when starting your trip. Tickets are valid on the entire length of bus, tram, trolley bus and metro lines, the Millennium Underground and the cogwheel railway, but are only valid within the administrative boundary of Budapest on suburban railway lines called HÉV.

Prices in 2016 are as follows Single ticket HUF 350 (approx. 1,12 EURO) One-day travel card HUF 1 650 (approx. 5,3 EURO) Discount coupon book (10 pcs. of single tickets) HUF 3 000 (approx. 9,6 EURO) Metro section ticket (up to three stops on metro lines) HUF 300 (approx. 0,96 EURO) Tickets can be purchased at metro stations, at newsstands and at ticket vending machines.

Budapest Card

"The whole city in your pocket." is the motto of the Budapest Card. The card offers its holder several free and discounted services. **Prices in 2016** HUF 4 900 for 24 hours (approx. 15,8 EURO) HUF 7 900 for 48 hours (approx. 25,4 EURO) HUF 9 900 for 72 hours (approx. 31,8 EURO) Each card is valid for one adult and one child up to 14 years of age.

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Shops

Shops are open from 10:00 till 18:00 from Mondays to Fridays, but in the shopping malls they are open from 10:00 till 21:00 between Mondays and Thursdays, and from 10:00 till 22:00 from Fridays to Saturdays. Please note that shops are closed on Sundays.

Recommended taxi companies

To avoid any inconveniences please use one of the following taxi companies:

City Taxi: +36-1-2-111-111 **6x6 Taxi: +36-1-6-666-666**

Scientific program

	25th May 2016
17.30-19.30	Registration
18.00-21.00	Welcome reception at Meszarsteak

	26th May 2016	
8.30-12.40	Registration	
9.15-9.30	Welcome Speech **R., Farkas**	
9.30	**Plenary**	
9.30-9.50	**L., Rozsa**	New insights into the possible role of the human microbiome in the death and decomposition of human bodies
9.50	Oral communications	Section I chairman: **J. Amendt**
9.50-10.10	**A. E. Whittington**	Entomofauna of buried remains: motter's 1898 „Fauna of the grave" revisited
10.10-10.30	**L. M. Weidner, M. A. Monzon, G. C. Hamilton**	Effects of habitat on initial insect activity on piglet carcasses in diurnal and nocturnal conditions
10.30-10.50	**D. Martin-Vega, T. J. Simonsen, M. Wicklein, M. J. R. Hall**	Pictures of change: Micro-Computed Tomographic visualisation of blowfly intrapuparial development for minimum Post-Mortem Interval estimations
10.50-11.10	**Sasha Voss, Paola A. Magni, Christian Nansen, Ian Dadour**	Hyperspectral imaging: a new technique for aging blow fly pupae
11.10-11.30	Coffee break	
11.30	Oral communications	Section II chairman: **L., Papp**
11.30-11.50	**M., Földvári**	Variation in fly developmental time and its effect on PMI estimates
11.50-12.10	**V. Bernhardt, C. Schomerus, J. Amendt**	Of Pigs and Man – Growht rates of *Calliphora vicina* (Diptera: Calliphoridae) on human and porcine tissue
12.10-12.30	**A. Grzywacz, D. Wyborska, M. Piwczyński**	DNA barcoding allows identification of European Fannidae (Diptera) of forensic importance
12.30-12.50	**B. K. Zajac, J. Amendt, R. Zehner, B. Bettin,**	Application of Maldi-TOF MS for the identification and characterization of *Lucilia sericata* and *Calliphora vicina* larvae

	A. Karger	
12.50-13.10	**T. A. de Wolf,** **F. P. Drijfhout**	Metabolites as internal age markers in *Calliphora vicina*
13.10-14.10	Lunch Time and Poster Section	
14.10	Oral communications	Section III chairman: **K. Brown**
14.10-14.30	**S. Vanin,** **P. Harrop**	Cronobiological studies on body search and emergence of first colonizer flies in outdoor cases
14.30-14.50	**M.D. Gemmellaro,** **C. M. Sollami,** **C. Bucolo,** **E. Musumeci,** **L. M. Weidner**	First survey insects of forensic importance in Sicily and it's volcanic caves
14.50-15.10	Coffee break	
15.10	Oral communications	
15.10-15.40	**M. Heyns**	Forensic entomology at the University of Cape Town, South Africa
15.40-16.00	**M., Angyal,** **Cs., Bozó,** **A., Kricskovics,** **Gy., Árvay,** **E., Rácz,** **Z., Porpáczy,** **K., Sipos,** **Zs., Ujvári**	A pilot study as a forensic ecology experiment
16.00-16.20	**A. Williams**	A head by a nose: using decomposition chemistry to improve canine victim remains detection
16.20-16.30	Conclusion of the first day	
18.00-…..	[social event]	
	27th May 2016	
8.30-12.30	Registration	
9.30	Oral communications	Section IV chairman: **L. Bourguignon**
9.30-09.50	**C. Aubernon,** **V. Hédouin,** **D. Charabidzé**	Thermoregulation in Calliphorids larvae
9.50-10.10	**I. Hofer,** **A. Hart,** **D. Martin-Vega,**	Optimising crime scene temperature collection for forensic entomology casework

	M. J. R. Hall	
10.10-10.30	**S. E. Shin, J. Park, S. J. Jeon, K. S Ko, S. H. Park**	Effect of temperature on development of *Lucilia sericata* (Meigen) and insects attracted to human cadavers in Korea
10.30-10.50	**K. Brown, K. Jetten, H. Ody**	Forensic flies: is climate change affecting dipteran succession for time scene death estimation?
10.50-11.10	Coffee break	
11.10	Oral communications	Section V chairman: **M. J. R. Hall**
11.10-11.30	**L. Lutz, J. Amendt, G. Moreau**	Decomposition and insect colonization on conceal pig carcasses
11.30-11.50	**S. Sya, N. Degallier, R. Garrouste, A. N. & D. Azar**	Scavenger insects association to human decomposition process, four case reports from Lebanon
11.50-12.10	**G. Moreau, J.-P. Michaud**	But how probable is probable? A succession-based, probabilistic method for PIA estimation
12.10-12.30	**Sz. Matuszewski**	Estimation of Post-Mortem Interval based on presence and absence of subsequent developmental stages of insects
12.30-12.50	**K. Frątczak, Sz. Matuszewski**	The effect of multiple, *in vivo* measurements on the development of *Creophillus maxillosus* (Coleoptera: Staphylinidae)
12.50-13.50	Lunch Time and Poster Section	
13.50	Oral communications	Section VI chairman: **S. Vanin**
13.50-14.10	**T. C. Moretti, A. X. Linhares**	Missing: necrophagous social wasps – where did they go?
14.10-14.30	**M. Taleb, T. Moussa, B. Djedouani, G. Tail, F. Z. Kara, H. N. Açiköz**	Current situation of forensic entomology in Algeria
14.30-14.50	**Lavinia Iancu**	Necrophagous insects identified during several forensic entomology experiments in Romania, since 2009 to date
14.50-15.10	**E. Leonhardt**	The Hirox instruments

15.10-15.30	**L. Gombas**	3D advanced forensic solutions from Leica Geosystems
15.30-15.50	Conclusion of the second day	
15.50-16.10	Coffee break	
16.10-16.30	EAFE meeting	
19.00-22.00	Social dinner	

	28th May 2016
9.30-13.00	Workshop with **Z. Soltesz**: „future 3D" - identification keys of fly familie sand exercise of Sargophagidae genitalia preparation support by Hirox
13.00-…	„Time to Say Goodbye."[1]

[1]https://www.youtube.com/watch?v=LWQbuJ24Wzg

ORAL PRESENTATIONS

NEW INSIGHTS INTO THE POSSIBLE ROLE OF THE HUMAN MICROBIOME IN THE DEATH AND DECOMPOSITION OF HUMAN

Lajos Rózsa

MTA-ELTE-MTM Ecology Research Group, Pázmány s. 1/C, H-1117 Budapest, Hungary

The human microbiome consists of a 1-2 kg mass of taxonomically diverse microbes, mostly bacteria and fungi. Throughout the human life, they provide mutualistic services to ensure the host's long and healthy life. Apparently, this strategy is adaptive from their point of view because they benefit from a long-lasting period of transmission possibilities, even if low levels of host exploitation permits only a low intensity of transmission per unit time. The alternative, apparently less beneficial strategy would be a short-term intensive exploitation of the host body, i.e. a short period of severe disease and intensive transmission. The aim of my talk is to summarize recently published hypotheses (Rózsa et al. 2015; Krezalek et al. 2016) that modify this view. Presuming that (i) members of the mutualistic microbiome are capable to obtain information about the host survival chances (info on host age and health) and also (ii) that they are capable for quick phenotypic switches in levels of pathogenicity yields in a surprising prediction. Provided that these prerequisites are fulfilled, we expect that whenever host survival chances greatly decline, the microbiome exerts a sudden and coordinated increase of pathogenicity so as to increase host exploitation and thus to maximize immediate transmission chances. The microbial decomposition of human (and animal) bodies are mostly dominated by the formerly mutualistic members of the microbiome. Therefore, it seem likely that the maximization of this final short-term transmission effort may well include transmission from the human corpses through soil and water so as to establish new mutualistic relationships in new human hosts. Widespread burial or cremation habits does not prohibit this process in general because the human microbiome is not at all specific to humans. Calliphorid flies likely play an important role in this process.

Rózsa L, Apari P, Müller V 2015. The Microbiome Mutiny Hypothesis: can our microbiome turn against us when we are old or seriously ill? Biology Direct 10: 3.

Krezalek MA, DeFazio j, Zaborina O, Zaborin A, Alverdy JC 2016. The shift of an intestinal "microbiome" to a "pathobiome"governs the course and outcome of sepsis following surgical injury. Schock – in press

ENTOMOFAUNA OF BURIED REMAINS: MOTTER'S 1898 "FAUNA OF THE GRAVE" REVISITED

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A metadata analysis was conducted on data that were presented in a past paper that appears to have gone largely unnoticed save for occasional comment of an uncritical nature. Data were derived from 123 graves of 150 disinterments that included invertebrate grave fauna, which Dr Murray Galt Motter carried out in 1898 form the City of Washington cemeteries. Approximately 75 taxa (including some undetermined to species) were assessed in Motter's tables, from which the most informative group of thirteen species were extracted (representing the top 80.2% relative abundance). The remaining long tail of "singleton's", were mostly adventive species and incidental records that provide little context. The data analysis demonstrated clear patterns in faunal structure for the following parameters: month of interment, duration of interment, presence or absence of Adipocere, depth of grave, soil type and moisture content. These results confirm the prevalence of a small number of species restricted to particular conditions, which are discussed with reference to known ecological or behavior preferences for these species as well as a comparison with more modern research recorded in scientific literature.

Adipocere generally excludes insects and those species prevalent in graves where it is absent sometimes occur in much lower abundance (sig. $p < 1\%$). Lepismatidae and *Eleusis pallida* LeConte 1863 (Coleoptera, Staphylinidae, Osoriinae) demonstrated a preference for 1.52m grave depth, sandy-clay soil, wet to submerged grave conditions and burial in crypts (all sig. $p < 1\%$). As is to be expected faunas are higher from interments conducted in summer months (April to June) with a secondary peak of activity in autumn months (August – October). The occurrence of the two most abundant taxa (Lepismatidae and *Eleusis pallida*) at a duration of interment of 8 years and Lepismatidae peaked a second time at 11 years. Gamasid mites (*Uropoda depressa* Banks in Motter 1898) peak at 5 years duration of interment and the Staphylinid *Actobius umbripennis* LeConte 1863 peaked twice at 5 and 8 years. Most other species fluctuated and demonstrated several or non-significant peaks.

The prevalence of Lepismatidae, *Eleusis pallida*, *U. depressa* and *Actobius umbripennis* together with the less abundant members of this guild, suggest an association with fungal hypha, which may be the main food source for these species, rather than direct products of decomposition. Even so, the presence or absence of this guild helps inform forensic instances of burial taphonomy.

Reference:

Motter, M. G. (1898)."A contribution to the study of the fauna of the grave. A study of on hundred and fifty disinterments, with some additional experimental observations."*Journal of the New York Entomological Society* **6** (4): 201-231.

EFFECTS OF HABITAT ON INITIAL INSECT ACTIVITY ON PIGLET CARCASSES IN DIURNAL AND NOCTURNAL CONDITIONS

Lauren M. Weidner[1*], Michael A. Monzon[2], George C. Hamilton[2]

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There has been in an increase in the use of insects to determine a minimum post-mortem interval (m-PMI) due to their early arrival to remains. Blow flies (Diptera: Calliphoridae) arrive to remains often within minutes to hours after exposure in diurnal conditions, while what little is known about their arrival in nocturnal settings is controversial and inconclusive. Other insects, such as coleopterans and hymenopterans can also arrive to a carcass during early colonization and affect blow fly development, although the frequency and extent of these interactions is also uncertain. This study analyzed the initial six hours after a piglet carcass was placed out in two environments (rural and urban) in diurnal and nocturnal conditions with both continuous video recording and hourly observations. Four piglets were placed out every two weeks (24 in total) over the summer of 2014. Initial blow fly arrivals to the carcasses were recorded under diurnal conditions, and a significant difference was found between environments, with a mean (± SE) arrival time of 1:02 min. ± 21sec in the rural environment and 2:40 min. ± 59 sec in the urban environment. We recorded the orders associated with the carcasses in each environment (time of day and location), and we observed increased diversity at night. These recordings also included a novel interaction with yellow jackets (Hymenoptera: Vespidae), which to our knowledge has not been described in the literature before. This experiment provides baseline data on early insect colonization in various environments in New Jersey, and the implications for determining m-PMI will be discussed.

PICTURES OF CHANGE: MICRO-COMPUTED TOMOGRAPHIC VISUALISATION OF BLOW FLY INTRAPUPARIAL DEVELOPMENT FOR MINIMUM POST-MORTEM INTERVAL ESTIMATIONS

Daniel Martín-Vega[1], Thomas J. Simonsen[2], Martina Wicklein[3], Martin J.R. Hall[1]

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[2]Naturhistorisk Museum Aarhus, DK-8000 Aarhus C, Denmark
[3]Department of Neuroscience, Physiology and Pharmacology, University College London, WC1E 6BT London, UK

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Forensic entomology, defined as the analysis of insect evidence to aid in legal and criminal investigations, has its main application in the estimation of minimum post-mortem intervals (PMI_{min}). Most estimates depend on the use of developmental data from Calliphoridae (blowflies), which are generally the first colonisers of cadavers and, therefore, ideal forensic indicators. As the development rates of the immature stages of blowflies are strongly influenced by environmental temperature, the PMI_{min} can be accurately estimated taking into account crime scene temperature information and available developmental data for the pertinent species. For blowfly larvae, this can be achieved by modelling quantitative measures of age (e.g. body length) in relation to time; however, estimates based on the puparial stage are severely limited due to the lack of a reliable aging method. This is particularly critical as the puparial stage accounts for more than half of the developmental duration of the blowfly life cycle. The aim of the current study is to develop a reliable method for aging blowfly puparia for forensic purposes using micro-CT scanning, providing a greater temporal resolution than that currently available.

Specimens of two blow fly species, *Calliphora vicina* Robineau-Desvoidy and *Lucilia sericata* (Meigen), were reared at three different constant temperatures (15º, 20º and 24ºC) and collected at either 10% (*C. vicina*) or 20% (*L. sericata*) time intervals of the whole intra-puparial development. The specimens were killed in hot water, stained in 0.5M iodine and scanned using a Nikon Metrology HMX ST 225 CT-scanner. The resulting projections were reconstructed, rendered and visualized, and the tomographic data quantified.

The chronology of the development remained fairly constant in the two target species and in the three experimental temperature set-ups. Age-related morphological changes in key structures were determined and virtual three-dimensional reconstructions allowed for volume measurement of different organ systems, yielding a quantitative measure of age. Some organ systems, such as the alimentary canal, the central section of the brain or the indirect flight muscles are particularly informative with respect to the qualitative and quantitative changes in their morphology throughout development. The advantages and disadvantages of this innovative method for age determination and the interdisciplinary benefits of the current results to other areas of research will be discussed.

HYPERSPECTRAL IMAGING: A NEW TECHNIQUE FOR AGING BLOWFLY PUPAE

Sasha Voss[1], Paola A. Magni[2], Christian Nansen[3], Ian Dadour[4]

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[3]Department of Entomology and Nematology, UC Davis, USA
[4]Program in Forensic Anthropology, Dept. of Anatomy &Neurobiology,
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Blowflies (Diptera: Calliphoridae) are the predominant taxa used to indicate time since death (minPMI) as they are among the first insects colonising remains after death. The developmental duration of blowflies is driven by temperature and specimen age is determined using reference data detailing temperature-dependent developmental timeframes for specific life stages. Problematically, where the duration between stages is lengthy, for instance between pupal formation and adult fly eclosion, error can be introduced to the minPMI estimate. At present optimal estimation of age between life stages, particularly between pupal formation and adult fly eclosion generally involves destructive and/or invasive techniques. Ultimately non-destructive and non-invasive techniques are needed that allow for the specimen to remain intact and be used for later re-analysis.

Such invasive techniques include morphological examination of developmental changes using a conventional light microscope, histological staining, scanning electron microscopy (SEM), micro-computed and optical coherence tomography and gene expression analysis. However, these techniques are labour intensive, require expensive equipment and involve a high degree of specialist expertise to interpret results.

This research employed for the first time *hyperspectral imaging*(HSI) to age blowflypupae. HSI consists of the acquisition of imaging data in which each pixel is associated with a detailed reflectance profile. Under controlled experimental conditions it is assumed that different target objects, such as blowfly pupae of different age or species, will reflect light differently based on their difference in physical structure and biochemical composition of the cuticle.

This work developed a predictive model for determining pupal age for two blowfly species, *Calliphora dubia* Maquart and *Chrysomya rufifacies* Maquart at two temperatures (24°C and 30°C), correlated with the morphological changes occurring during pupal metamorphosis.

HIS is a promising technology in forensic entomology being non-destructive, non-invasive, suitable for both live and preserved specimens, portable (field or laboratory based), rapid and cheap.

VARIATION IN FLY DEVELOPMENTAL TIME AND ITS EFFECT ON PMI ESTIMATES

Mihály Földvári

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Immature stages of flies are paramount in establishing the post mortem interval (PMI) in forensic practice. There are numerous ways to sample dipterous larvae on a dead body, and the prediction will depend greatly on the properties of the collected individuals. Not only has the prediction an error, but the flies also have different developmental times due to several factors.

My focus is on differences in developmental time that may be caused by individual life history traits (decisions?) or genetic tendencies. All environmental effects come on top of these and create the final variation of a pool of fly larvae that can be found at one time on a dead body.

I will present data of a calliphorid species (Lucilia ampullacea), where 300 eggs had been produced within an hour and developmental time varied subsequently to a great extent: when the first flies emerged there were still first instar larvae in the food provided (pig liver).

My second experimental data are from the non-forensic Diptera species, the Asian stalk-eyed fly (Teleopsis dalmanni): egg lays ranging in offspring age of 3 to 4 days ended up creating a difference of 15 to 32 days range in adult fly emergence. In both cases temperature and humidity had been constant, and flies were kept in 12:12h day-night cycle.

The estimated PMI must be based on a wide range of collected flies (not simply the oldest/ largest/ widest individual), because it can be one extremity of a potentially bell shaped (Gaussian) distribution of developmental stages. Therefore it will bias the PMI in an unpredictable way. One possible solution can be to use large sample sizes and their body measurement means. This makes sure that the error of developmental stage estimation is smaller and the estimates will be more comparable.

These individual differences may have genetic, epigenetic background, or they may be simple consequences of independent life history decisions. Therefore studying their context with the help of well-designed experiments is vital. Nevertheless, even if the explanations are unclear the PMI predictions can be improved by addressing developmental variance with large, randomised sampling.

OF PIGS AND MAN - GROWTH RATES OF CALLIPHORA VICINA (DIPTERA: CALLIPHORIDAE) ON HUMAN AND PORCINE TISSUE

V. Bernhardt[1], C. Schomerus[2], J. Amendt[1]

[1]Institute of Legal Medicine, Goethe-University, Frankfurt/Main, Germany
[2]Institute of Anatomy II, Goethe-University, Frankfurt/Main, Germany

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Over the last decades, development studies on larval growth rates and development times of Diptera were performed by using non-human tissues (e.g. ground meat or chopped liver of beef/pork) as a nutrition medium. Such development data are used as a reference for the determination of the age of juvenile necrophagous insects sampled on a human body, using their age to estimate a minimum postmortem interval (PMI_{min}). However, some might ask whether these data reflect the growth on human tissue appropriately and this could lead to critical situations at court.
We studied and compared the larval growth rates and development times of *Calliphora vicina* (Diptera: Calliphoridae) on human muscle tissues of five body donors and several standard rearing pork tissues like e.g. pork loin. All human and porcine tissue studies were replicated three times at 25 °C. One replicate enclosed 300 larvae.
First results show that the larval growth rates within human and within one type of porcine tissue is consistent and predictable, but we noted a significant ($p \leq 0.05$) slower development on pork loin compared to human tissue. Time until first pupation differed up to 48 hours and until the emergence of the first fly up to 24 hours.
These preliminary results highlight the fact that best practice in forensic entomology so far neglected the question about the most suitable tissue for developmental studies. While a pig cadaver, depending on its size, seems to be an appropriate surrogate for a human body when performing insect succession experiments, pork tissue might not be the most suitable tissue for producing sound development data of necrophagous Diptera. Several studies indicated differences in growth and development of some fly taxa in relation to the organs and tissues (e.g. lung, liver, kidney etc.) they were feeding on within a body. There is even evidence that blowfly larvae grow faster on pig than on cow for example (Clark et al., 2006), i.e. show a food species specific difference regarding their development. We are discussing pro and contra of using pork tissue in forensic entomology as well as possible answers of this problem.

Clark, K., Evans, L. & Wall, R., 2006. Growth rates of the blowfly, *Lucilia sericata*, on different body tissues. Forensic Science International, 156(2-3), pp.145–9.

DNA BARCODING ALLOWS IDENTIFICATION OF EUROPEAN FANNIIDAE (DIPTERA) OF FORENSIC IMPORTANCE

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Fanniidae are one of the dipteran families attracted to and breeding in decomposing animal carrion and dead human bodies. Despite the fact that carrion has been revealed attractive for more than 50 species worldwide, many authors refer only to very few species. The most commonly recognizedspecies are *F. canicularis*, *F. leucosticta, F. manicata*, *F. pusio* and *F. scalaris*. Students often do not attempt to identify fanniidsbecause their identification may be considered difficult, particularly for non-experts. Thus, we investigated usefulness of molecular methods in species identification for European carrion visiting Fanniidae, as an alternative for morphology based identification.

For the study we have selected two sets of taxa. The first set included species regularly visiting and breeding in carrion (*Fannia aequilineata*, *F. canicularis*, *F. coracina*, *F. incisurata*, *F. latipalpis*, *F. leucosticta*, *F. manicata*, *F. monilis*, *F. pusio* and *F. scalaris*). Additional set included species of possible forensic importance or accidently visiting carrion. We sequenced barcode region (658 bp) of cytochrome oxidase subunit I (COI) gene for 63 specimens representing 25 species.The proportion of correctly identified specimens was estimatedusing best match, best close match and all species barcodes criteria in SpeciesIdentifier v1.8.

We observed low or even lack of intraspecific haplotype diversity. For several species specimens from different countries shared the same species specific haplotype. The highest intraspecific distance 2.4% we observed in *F. canicularis* and for the great majority of species the distance did not exceed 0.5%. The lowest interspecific distance (*F. aequilineata* vs. *F. latipalpis*) was found at level 4.4%. The proportion of correctly identified specimens was very high. The only species misidentified were those without conspecific barcodes in the database.

Low intra- and relatively high interspecific diversity of COI barcode region allows for precise species identification of Fanniidae. Our analysis revealed that molecular taxonomy may be successfully applied for taxonomical purposes in Fanniidae, matching unidentified females and immature stages to already known males.

APPLICATION OF MALDI-TOF MS FOR THE IDENTIFICATION AND CHARACTERIZATION OF *LUCILIA SERICATA* AND *CALLIPHORA VICINA* LARVAE

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Protein profiling by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) is rapidly evolving as a tool for the identification and characterization of insects and other arthropods for various applications over a wide range of research areas. We present here the application of this technique to two forensically important blow fly species, Calliphora vicina and Lucilia sericata (Diptera: Calliphoridae). Both taxa belong to the typical first colonizers of human corpses and the reliable identification and age estimation of the juvenile stages is a mandatory task for narrowing down the minimum time since death in crime investigations. Usually, species identification and age estimation of forensically relevant fly species are conducted using either traditional morphological and morphometric methods that often require experience and practical knowledge or by rather expansive and elaborate molecular methods. Here, we present an application that may be capable to solve both issues, species identification and age estimation of the same specimen, simultaneously.

Specimens of both fly taxa were reared under the same conditions at a constant temperature of 20°C. Oviposition took place only for a window of time of two hours to ensure that all larval replicates have the same age. Daily samples of larvae were performed every 24 hours. Proof-of-concept studies were performed for both species until day seven of larval development.

A reference database was constructed using commercial software (MALDI Biotyper) that allowed the separation of both taxa irrespective of the age of the samples. Evaluation of spectra revealed striking temporal changes of the spectral pattern during larval development, reflecting the development of protein composition of the larvae. A MALDI-TOF MS -based age estimation with exact to the day precision was possible during the earlier phase (days 1-3), while deviations of +/- one day were observed in the later phase of development (days 4-7). This was probably due to the observed increase of the variability of spectra with time. Thus, the results show that MALDI-TOF MS is not only a promising tool for the identification but also for the age determination of forensically relevant blow fly larvae.

METABOLITES AS INTERNAL AGE MARKERS IN CALLIPHORA VICINA

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Determining the age of any entomological evidence collected at the crime scene is crucial in all post-mortem interval estimations. Several methods exist to calculate the age of larvae collected at the crime scene. These include larval length, larval instar, ADH calculations as well as the more recent developed methods such as using cuticular hydrocarbons and DNA techniques. The analysis of hydrocarbons to determine age is based on surface compounds commonly found on the cuticle of the larvae. In this new preliminary study the focus is on internal small molecules; known as metabolites and how these can be used as age markers.
Organic and aqueous larval extracts were obtained by extracting larvae in a two-step process. The larvae were put in 2mL centrifuge tubes and weighed on an analytic balance. 500 µL aqueous solvent (methanol/water, 1:1) was added for samples< 50 mg and 1 mL solvent for samples> 50 mg. The larvae were cut into small pieces with scissors and homogenised. Samples were then sonicated for 10 minutes and centrifuged for 15 minutes. The supernatant was removed and retained as the aqueous extract. The remaining pellets were broken up by a pair of clean tweezers and the solvent for the organic extraction (dichloromethane/methanol, 3:1) was added to the samples using similar volumes as for the aqueous extracts. Larvae were extracted on a daily basis with 10 replicates for each day. Eggs and pupa were also extracted. The extracts were analysis on an Agilent LC-Q-TOF-MS with ESI using a C18 column. All extracts were injected with a volume of 5 µl while the column temperature was controlled at 35 °C. The solvents used as mobile phase were Milli Q water with 0.1% of formic acid and methanol, at a flowrate of 0.4 ml/min using gradient elution (95% aqueous to 100% organic).
Analysis of the data was performed using MassHunter ProFinder Software to extract relevant features (‘accurate mass’ and ‘retention time’ combinations) from each sample followed by MassHunterMass Profiler Professional Software to perform multivariate analysis on the obtained dataset. Results from this preliminary study indicate that using the complete profile of metabolites, larvae from different days can be distinguished in the PCA plot. This is the first study to age blowfly larvae using metabolites and in the presentation the use of this new method will be discussed, alongside any challenges using this method.

CHRONOBIOLOGICAL STUDIES ON BODY SEARCH AND EMERGENCE OF FIRST COLONIZER FLIES IN OUTDOOR CASES

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Circadian clocks have evolved to synchronize physiology, metabolism and behaviour to the 24-h geophysical cycles of the Earth. The understanding of the circadian clock mechanism is a crucial element of forensic entomology because it is able to control routines such as locomotor activities, locating of food sources, feeding, mating, ovipositing and emergence times.

The study of the insect colonisation of carrions and human cadavers allows for the estimation of the post mortem interval (PMI). However, it is thought by some scientists that flies are not active during the night time period and therefore are not able to reach a body and so to oviposit during this time or in general in dark conditions. Determining nocturnal activity in forensically important flies is of fundamental importance in order to make a more precise PMI estimation.

In previous works the circadian clock behavior of *Megaselia scalaris* (Diptera: Phoridae) was described in detail using the Trikinetics technology used previously in *Drosophila* studies, which allows for factual data rather than observational data as reported in many articles. The activity rhythms of *M. scalaris* were monitored using light/dark (LD 12:12) photoperiods at 20 °C. Using a new monitor for bigger flies the locomotor activity and the emergence time of bottles flies [*Protophormia terranovae*, *Calliphora vomitoria* and *Lucilia sericata* (Diptera: Calliphoridae)], belonging to the first colonization wave, have been investigated at different temperatures and photoperiods.

In contrast to the *M. scalaris* experiments, that demonstrated that this fly is both diurnal and nocturnal, all the experiments carried out with the bottle flies indicated a clear diurnal activity (diurnal vs nocturnal: p=0.00) with a residual activity in the dark phase (evening activity) for *Calliphora vomitoria*. No statistical difference was observed for either sex in all the species.

Pupa emergence experiments run in LD 12:12 photoperiod determined that bottle flies emerge during the light phase (>90%).

Our experiments have demonstrated that *P.terranovae, C. vomitoria* and *L. sericata* are diurnal in their locomotor activity and in their emergence. These results allow a better understanding of the observational and occasional data reported till now about the diurnal/nocturnal activity of the bottle flies of forensic interest. Focusing on the first body colonizers, this work has a direct impact on the minimum PMI estimation using an entomological approach.

FIRST SURVEY INSECTS OF FORENSIC IMPORTANCE IN SICILY AND ITS VOLCANIC CAVES

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In Sicily (Italy), in the area surrounding Mt. Etna, we conducted a survey to assess the composition of entomofauna of potential forensic interest on terrestrial and in volcanic subterranean environments; here we will present the results observed during the summer months. For this study we placed traps in 4 different volcanic caves, one on each site of the volcano, and on 3 terrestrial sites throughout the island. Insects were collected using Rescue! Pop! Fly Traps, which were baited with 20g of bovine liver.

The traps were deployed for 7 days at each site and then they were replaced with a clean trap and a new bait; the adult insects collected were cleaned with water and preserved in 75% ethanol. In order to monitor temperature and light, we used Hobo Pendant Temperature/Light Data Loggers, which we placed about 5-10 cm from each trap and which remained in that location forwhole duration of the sampling. Our survey continued for 6 weeks during summer of 2015.

The results we obtained were quite different from trap to trap; while some traps were full of insects, others contained only few, demonstrating the difference in abundance and biodiversity Thefamily that was most abundant in terrestrial environment was Calliphoridae, while in caves the family that was most abundant was Phoridae. *Calliphora vicina*Robineau-Desvoidy, a traditional winter species active in cold climates, was the most common Calliphoridae that we collected in the caves; this is probably due to lack or reduced light and temperature of subterranean environments.

FORENSIC ENTOMOLOGY AT THE UNIVERSITY OF CAPE TOWN, SOUTH AFRICA

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No forensic entomological research has been conducted at University of Cape Town or in the Western Cape, prior to the author joining the university in 2012. Forensic entomologicalresearch on the local blow fly populations of the Western Cape is now in its infancy.

One aim of our entomological research is to identify the blowfly species that are active in land based environments in the Western Cape and to determine baseline development rates for these species, which will aid in more accurate PMI (Post Mortem Interval) determinations. Blow flies of *Chrysomya spp.* and *Lucilia spp.* have been identified. The research focus is now on establishing the effect of factors such as climatic conditions, particularly temperature and humidity, maggot masses and xenobiotics on the development of the blow flies and thus the ability to estimate the PMI.

One study investigated the influence of Ritalin (methylphenidate hydrochloride / MPH) on the development of *C. chloropyga.*MPH was detected from treated larvae preserved in ethanol and by freezing. Furthermore, MPH could be detected from samples containing as few as three larvae, after 3.5 days incubation at ~30 °C. This may be indicative of an improved preservation and stability of MPH in insect specimens, especially given the labile nature of MPH. The influence of MPH on the duration of developmental stages was evident in an expedited larval stage of up to 17 hours, and a prolonged pupal stage of up to 16 hours.

Another study was to analyse the effect of amitriptyline on the development and growth rate of *C. chloropyga*and *L. sericata.*Results indicate that Amitriptyline delays pupation in blowfly larvae by at least 26 hours and the emergence of imago by at least 72 hours.

We also investigated flyspecks or artefacts caused by fly activity on a crime scene.Clear differences between flyspecks and legitimate bloodstains were noted and unique characteristics of fly artefacts to differentiate it from true blood spatters were distinguished.

Further research should be conducted into areas such as standardised methodology for entomological investigations, bioaccumulation, insect metabolism of drugs, and quantitative analyses of insect evidence.

A PILOT STUDY AS A FORENSIC ECOLOGY EXPERIMENT

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A number of changes can be found by the local inquiry of violent crimes when we studied the relationship between dead body (bodies) and the conditions of environment. These kind of processes (e.g. physical-chemical, physiological, and other biological ones) have static and dynamic features. For the present pilot study, we used to carry out the detection of the exhibits on model animals – in that case on four pigs (*Sus scrofa*) – towards PMI. The investigation of the time of death set forward by the study of indicated model work in accordance with the successful forensic entomological steps on carcass bodies (Centeno et al. 2002, Smith 1987).

The main purpose of our experiment was the introduction of a complex method, as new possibility in the Hungarian criminal practice. We would like to prove and model those algorithms that must be followed during on-site examination. Consequently, we have been created a document in accordance with the domestic law requirements. During the in situ experiment, we recorded several dipteral specimen, the bacterious and acarious populations, which represent the biotopes of nearly rural environment. At the same time, we investigated the concerning environmental conditions, succession of the insects, the appearing acarion fauna, and changes of nitrogen content and bacterial spectrum of the soil, by the exomorphological alteration of carcass as well.

The role of forensic entomology is excessively important in case resolving because the forensic entomology exhibit is stronger together with other exhibit. This scientific cooperation was great because it was an initial step, a partial success and hopefully we could develop it in our research.

AHEAD BY A NOSE: USING DECOMPOSITION CHEMISTRY TO IMPROVE CANINE VICTIM REMAINS DETECTION

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Specially trained victim remains detection (VRD) dogs are regularly used by police forces to detect human remains or bodily fluids. There are over 20 units that train VRD dogs, and within these units, the training methods used to introduce the dogs to the distinct smells of human decomposition vary wildly, meaning that operational success is sometimes the result of trial and error, rather than a scientifically-governed testing regime. VRD dog training is currently unregulated and unstandardized, and the testimony of dog handlers has been questioned in court, and the evidence based on VRD dogs' performance subjected to intense scrutiny.

This presentation will describe cutting-edge research currently being undertaken by the Forensic Anthropology Research Group at the University of Huddersfield that attempts to identify and catalogue the volatile organic compounds given off by a decomposing cadaver as a function of time, and to correlate this with the performance of specially trained dogs. Over 400 individual volatile organic compounds (VOCs) have been identified from the human decomposition process, but, at present, no-one knows which of these chemicals elicit a trained response ("alert" or "indication") from a VRD dog. We have, for the first time, undertaken odour experiments on VRD dogs from different forces to determine which components of the decomposition profile they respond to. The intention is to use this information to initiate scientifically rigorous training regimes for VRD dogs based on laboratory research, in an attempt to improve VRD dog training and operational performance. It may also be possible to inform the design and manufacture of more accurate 'pseudoscents' than currently exist.

THERMOREGULATION IN CALLIPHORIDS LARVAE

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It is well established that the maggots' development depends on local temperature. This temperature is primordial for minimum Post Mortem Interval (PMI) estimation. But, because of micro climate, and larval mass effect, estimating the temperature felt by larvae during their development on a corpse is difficult. In a recent study, we evidenced for 3 Calliphoridae species a specific temperature preferentially selected by larvae (Aubernon et al., 2016). We demonstrated that in a heterogeneous thermal environment, larvae of *Lucilia sericata* stayed at 33.3±1.52°C, *Calliphora vomitoria* at 29.61±1.63°C and *Calliphora vicina at* 22.58±1.55°C. According to results, we focused our research on the behavior of larvae according temperature changes and presence of other species.

We focused a first batch of experiment on the larval ability to react to local (food) temperature changes. We placed 80 *Lucilia sericata* larvae in a 40cm long setup with a hot spots located at each extremity. These hot spots were set at 25°C or 34°C with 20°C ambient temperature. Heating of the hot spots was switched on at different time (8h, 16 h or 24h). We observed that larvae first moved to the closest spot, resulting in 2 different aggregates, and then moved to the warmest spot. If this spot was then switched off, larvae were able to move to the other (and formerly cooler) spot. Last, we noticed that the reverse (switching the coolest spot to became the hottest) did not elicit such a clear result: larvae did not always move from 25°C spot they were located to the new 34°C spot.

In a second experiment, placing 40 *L. sericata* and 40 *C. vicina* larvae together on a thermal gradient, we studied the ability oflarvae having different preferential temperature to create heterospecific groups. We observed that behavioral balance between aggregation and temperature selection produced contrasted results. In some experiments, *L. sericata* larvae gathered with *C. vicina* around 22°C (*C. vicina*'s species specific temperature). But experiments with two monospecific groups, each one located on its species specific temperature, were also observed. In these cases, numerous larvae were observed located between the two groups.

These results highlight the thermal regulation abilities of blowflies' necrophagous larvae. Accordingly, average temperature felt by larvae during their development on a corpse may differ from any local or ambient temperatures values. These results may have consequences on temperature values considered for the PMI estimation in forensic entomology. Other experiments on development according to temperature and thermoregulation behavior are currently performed to understand the larval behavior and optimize the PMI estimation methods.

OPTIMISING CRIME SCENE TEMPERATURE COLLECTION FOR FORENSIC ENTOMOLOGY CASEWORK

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In suspicious death investigations, the value of minimum post-mortem interval ($_{min}$PMI) estimations from insect evidence using temperature modelling is indisputable. In order to investigate the reliability of the collected temperature data used for modelling $_{min}$PMI, it is necessary to study the effects of location and duration of placement of data loggers on the accuracy and precision of measurements.

Digital data logging devices are the most commonly used temperature measuring devices in forensic entomology. Their placement in three chosen locations, two outdoors and one indoors, had measurable effects when compared with actual body temperature measurements, some more significant than others depending on season, exposure to the environment and logger location. Overall, the study demonstrated the complexity of the question of optimal logger placements and the potential impact of inaccurate temperature data on $_{min}$PMI estimations, showing the importance of further research in this area and development of a standard protocol.

The minimum duration of data logger placement needed for a minimum degree of accuracy when retrospectively estimating crime scene temperatures from nearby weather station data, varies between rural and urban areas. For both urban and rural areas, however, an optimal data fit was generally reached after 4–10 consecutive days within a radius of up to 30km to the 'crime scene'. With increased distance and differing altitudes, a lower overall data fit was observed, although a diminishing return from additional data was also reached after 10 consecutive days. These results demonstrate the need for caution regarding distances and climate differences when using weather station data for retrospective regression analyses for estimating temperatures at crime scenes. This study provides initial recommendations for data logger placement and duration, and a baseline for further research into producing standard guidelines for increasing the accuracy of $_{min}$PMI estimations and, ultimately, higher reliability of forensic entomology evidence in court.

EFFECT OF TEMPERATURE ON DEVELOPMENT OF *LUCILIA SERICATA* (MEIGEN) AND INSECTS ATTRACTED TO HUMAN CADAVERS IN KOREA

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Information about forensically important insects attracted to human cadavers is scarce in Korea. In the last 5 years, we collected insects from medicolegal autopsies in northeastern Seoul in Korea and analyzed the temperature-dependent development of blow fly *Lucilia sericata* (Meigen), the most frequent indoor species for estimation of a minimum postmortem interval (PMI-min).

Adult beetles were identified to the species level by morphological characters. After the half of collected maggots were identified to the family level by the shape of the posterior spiracles, DNA barcoding using nucleotide sequences of mitochondrial cytochrome c oxidase subunit I gene (COI) was performed for species identification. Remained maggots were selectively reared for setting up a stock of *Lucilia sericata* (Meigen). Rearing for temperature-development data of *Lucilia sericata* (Meigen) duplicated repeatedly 5 times at 5 constant temperatures, 20, 24, 28, 32, and 35℃ (total 25 experiments). Under 70% relative humidity and 16(day): 8(night) photoperiod, a fragment of fresh pork liver was placed as rearing media. Four individual maggots were sampled at each 12 hours interval and soaked in boiling water for 30 seconds before measuring the body lengths and confirming the developmental stages.

As results, 3 orders (Diptera, Coleoptera, and Hymenoptera), 15 families, 48 species and 3,114 individuals were confirmed in 81 medicolegal autopsies. *Lucilia sericata* (Meigen) is the most frequent indoor species (53.1 %). So, researches on the development of this species in constant temperatures are preferentially carried out for applying to crime scene. Total 1,974 individuals were sampled and the average body lengths were 1.22 ± 0.1 (eggs), 2.55 ± 0.62 (1^{st} larvae), 6.39 ± 1.59 (2^{nd} larvae), 14.12(12.50-15.09) (3^{rd} larvae), 12.56 ± 1.58 (post-feeding larvae), and 7.78 ± 0.52 mm (pupae) (± standard deviation). Total development periods were 20.60 ± 1.53, 14.78 ± 0.61, 11.70 ± 0.84, 10.90 ± 0.55, and 10.70 ± 0.45 days at 20, 24, 28, 32, and 35℃, respectively. The optimum temperature was 23.85℃ (± 1.9℃: 95% prediction band). The minimum development threshold was 8.92℃ and the overall thermal constant was 240.99 ± 24.33 (± S. D.) accumulated degree days (ADD) above the threshold.

This study provides a snapshot of the general insects attracted to human cadavers in South Korea. This rearing data of *Lucilia sericata* (Meigen) has presented variance of measured results in spite of the same growth pattern in different zoogeographic regions. Therefore, this result indicates that consideration of geographical variances as well as rearing methods must be preceded for precise estimation of PMI-min.

FORENSIC FLIES: IS CLIMATE CHANGE AFFECTING DIPTERAN SUCCESSION FOR TIME SINCE DEATH ESTIMATION?

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Despite numerous carrion succession studies, only six have been published in the UK and none have been published in the past five years. An indication that climate change is affecting species distributions, in the USA, Europe and through personal observation, demonstrates increasing need for documenting the changing colonisation and succession patterns. The aim was to conduct such work to aid post-mortem interval estimation.

Liver-baited traps and rabbit carcasses were placed outside every month from April 2015. Hourly temperature and humidity measurements were taken using a datalogger. Observations and collections of forensically relevant, primary colonising Diptera were made daily, the latter using sticky traps placed near the rabbit and using a vial to catch flies on the rabbit itself.

The rate of decomposition increased with warmer weather as expected. In spring and summer, oviposition was observed by at least day 4, with larvae reaching 3rd instar by at least day 9. Skeletal remains were observed within the month of placement. Between January and March, there was very limited decomposition, with the rabbit appearing unchanged, and <100 larvae within the abdomen in early 3rd instar.

Previous observations indicated the primary Lucilia to frequent cadavers was *Lucilia sericata* (Meigen). This is now being outnumbered by *L. illustris* (Meigen) and *L. caesar* (Linnaeus), particularly in the spring months. In keeping with previous observations,*L.sericata*was the only Lucilinae to oviposit on cadavers. Despite this, *Calliphora vicina* (Robineau-Desvoidy) is the dominant primary coloniser of both carrion and liver-baited traps throughout the year. Some specimens showed combined *Lucilia* spp. characteristics (particularly acrostichal bristles) and variations from previous identification literature; these will be explored.

DECOMPOSITION AND INSECT COLONIZATION ON CONCEALED PIG CARCASSES

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Most forensic studies have examined decomposition and insect colonization of animal carcasses that are readily accessible to insects, but in homicides, cadavers are often concealed to a certain extent. However, decomposition and colonization by insects may differ if carcass accessibility for insects is constrained. In this comparative study conducted in Germany and Canada from May to September 2015, we tested the hypothesis that the permeability and structure of the material used to conceal a decomposing resource affect to different extents the colonization, survival, and interspecific interactions of insects attracted to the resource. For that purpose, insect colonization and carcass decay was documented at specific time intervals (10, 25, 45, 70, 100 days) on 30 domestic pig carcasses (15 in Canada, 15 in Germany), placed in trashcans, drums, and suitcases. Five pigs were allocated to each treatment in each country. The occurrence patterns of insect species around the concealed carcasses in Canada and Germany were documented throughout the study. Each type of container constituted a different level of insect exclusion. During the 100 days of the experiment, more than 50 000 insect specimens belonging to over 60 taxa were recorded. Results indicated that trashcans, drums, and suitcases had different impacts on insect colonization, and that the different levels of insect exclusion associated with these concealment units modified the decomposition process in a similar way on both continents, leading to the fastest decomposition in suitcases and almost no degradation but mummification in the bins. In addition, the results indicated that trashcans, drums, and suitcases altered the patterns of insect arrival and departure from concealed carcasses, the structure of insect communities, as well as the interspecific relationships between the insects of forensic importance, especially for Silphidae, Staphylinidae and Calliphoridae. Referred to this we observede. g. competition between the dominant predators *Creophilus maxillosus*, *Necrodes surinamensis* and *Necrophila americana* resulting in avoidance of each other by colonizing different types of containers. All in all these results support our general hypothesis and offer helpful information to assess the post mortem interval of concealed bodies.

SCAVENGER INSECTS ASSOCIATION TO HUMAN DECOMPOSITION PROCESS, FOUR CASE REPORTS FROM LEBANON

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Currently, entomology is not widely used in forensic investigations in Lebanon due to the dearth of insect succession studies and on the entomofaunal knowledge. Though after the assaults that took place in the North eastern area of the country during 2014-2015, forensic entomology becomes officially implied in the forensic investigations. Here we report the occurrences of insect taxa among both Diptera and Coleoptera that were collected during autopsy of 4 human corpses in the Central Military Hospital Badaro Baabda District – Lebanon. This preliminary data is considered as bedrock for a forensically relevant insect database for the country.

Case report 1:Postfeeding larvae of the flesh fly *Wohlfahrtia magnifica* and one *Chrysomya albiceps* adult were discovered during the decay stage of decomposition, together with two beetles, male and female *Saprinus magnoguttatus*.

Case report 2: Calliphoridae pupae, adults of *Lucilia sericata*, *Creophilus maxillosus* and *Carpophilus* sp. were sampled during the advanced stage of decomposition.

Case report 3: larvae stage 3 of *Calliphora vicina*, adults of *Dermestes frischii, Sphaeridium scarabaeoides and Dinothenarus (Dinothenarus) flavocephalus were collected during the advanced stage of decomposition.*

Case report 4: Calliphoridae pupae, *Nitidula flavomaculata* and Scarabaeidae adults were sampled from a mummified corpse.

Each corpse is unique in its decomposition process and the taxonomic composition of insects attracted to it is affected by several abiotic and biotic variables which should be clarified. Within the 4 cases mentioned above, only the case that involved necrophagous flesh flies maggots and necrophilous histerids was in the early stage of decomposition, the death was recent and the minimum postmortem interval $(PMI)_{min}$ was estimated within a margin error of few days, however in the rest of the cases the time of death was estimated within a greater margin error. Rigorous research will be the most important factor that support forensic entomology field in Lebanon and convince the legal community on the reliability of insect evidence and on the precision in calculating the PMI.

BUT HOW PROBABLE IS PROBABLE?A SUCCESSION-BASED, PROBABILISTIC METHOD FOR PIA ESTIMATION

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Entomological evidence can usually be classified as being of deterministic or probabilistic nature. A good example of the latter is the heterotrophic succession of insects on carcasses, which is somehow predictable but nonetheless variable from one carcass to the next. As such, any prediction about species occurrence during heterotrophic succession requires the estimation of probabilities. Although the successional patterns of taxa collected on corpses could be used to develop probabilistic estimates of the period of insect activity (PIA), few approaches of PIA estimation have been proposed in the forensic entomology literature since the first computer algorithm written in 1992 by Schoenly, Goff, and Early. However, the development of faster computers and the implementation of iterative statistical techniques now provide fast and stable numerical methods for the computation of PIA estimates and their prediction intervals, something that is critically lacking in forensic sciences. These iterative methods can also be adjusted to account for the biotic and/or abiotic conditions at the scene that may influence insect occurrence, as long as the database used to produce the PIA estimate allows for inference.

In this talk, we will examine a succession-based, probabilistic method for PIA estimation developed using data from New Brunswick, Canada. Fresh pig carcasses, used here as human surrogates, were exposed recurrently throughout the whole annual period when carrion-related insects are active in this area. The entomofauna associated with these carcasses was sampled daily. Abiotic parameters were recorded on-site using a weather station.

A total of 131necrophageous and predacious insect species representing 2 orders, 18 families and 75 genera were recovered from carcasses. The model accounted well for the occurrence of some adult species along the degree-day/decomposition gradient and readily identified the effect of some environmental variables (e.g., rain).However, only some of the species/genera that form the complex of carrion-related insects exhibited predictable occurrence patterns in relation to time since death on a degree-day scale. The method used to produce a succession-based probabilistic estimate by combining occurrence/absence data of several species will also be presented and discussed.

ESTIMATION OF POSTMORTEM INTERVAL BASED ON PRESENCE AND ABSENCE OF SUBSEQUENT DEVELOPMENTAL STAGES OF INSECTS

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Postmortem interval (PMI) may be estimated from the succession of insects on cadavers. The succession-based methods may be divided into the presence and the absence-presence methods. In the former, the PMI estimate is based on the presence of two insect taxa, in the latter, on the absence of an early arriving taxon and the presence of a long residing taxon. These approaches meet however serious limitations. Large dependence on the context of a case seems to be the most important. Insect assemblages on carrion are largely affected by season, habitat, type of cadaver exposition and cadaver mass. Analysis of these limitations leads to a conclusion that the most important weakness of the succession-based methods is their partial reliance on insect disappearance (departure) times.

Here, I propose a novel succession-based method for PMI estimation, which is based on appearance time of subsequent developmental stages of a definitive species. This is a presence-absence estimation. Lower PMI is delineated by the pre-appearance interval (PAI) of the developmental stage which was collected on a cadaver, whereas upper PMI is defined by the PAI of the stage which is next in the life cycle but yet absent on the cadaver.

The method may be divided into several steps. First, corpse fauna is collected, identified and classified. Second, the definitive species is chosen. This is the species which was collected as immature stage and which is the most successionally advanced of all the species present (i.e. it colonizes cadavers later than all the other species collected). In the third step, PAI of the collected developmental stage is estimated. In the fourth step, PAI of the subsequent, yet absent stage is estimated. In both steps PAI may be estimated using temperature methods or average seasonal or monthly PAI may be used. For example if 2nd instar larvae of *N. littoralis* were collected, and *N. littoralis* was chosen as a definitive species, PAI for its 2nd larval stage will define lower PMI, and PAI of the next, yet absent 3rd larval stage will define upper PMI. In the last step the confidence interval for the PMI estimate is approximated. Because current method involves several sources of error (insect sampling error, temperature approximation error, PAI estimation error etc.), the best way to provide meaningful interval estimate is to use the previously specified error rates of the estimation procedure.

The method was exemplified for selected carrion species colonizing cadavers in rural habitats of Central Europe, namely *Lucilia caesar* (Calliphoridae), *Thanatophilus sinuatus*, *Necrodes littoralis* (Silphidae) and *Stearibia nigriceps* (Piophilidae). It was validated using results of a large-scale pig carcass experiments. Validation tests revealed surprisingly high accuracy of PMI estimates (for most configurations of stages relative error of estimation ranged between 0.1 and 0.3). Prospects and limitations of the method will be discussed. Moreover, the current presence-absence approach for PMI estimation may be similarly effective in the case of non-entomological methods for PMI estimation.

THE EFFECT OF MULTIPLE, *IN VIVO* MEASUREMENTS ON THE DEVELOMPENT OF *CREOPHILUS MAXILLOSUS* (COLEOPTERA: STAPHYLINIDAE)

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While creating development models for necrophilous beetles, *in vivo* measurements of age indicators are usually made. Individual insects are measured many times from hatching until eclosion. Multiple measurements of the same individual may however affect development, and eventually the quality of the model. Accordingly, here we test the hypothesis that repeated, *in vivo* measurements affect the development of a beetle species *Creophilus maxillosus*, a common predator for maggots on large carcasses.

Experiment was carried out at four constant temperatures: 25, 27.5, 30 and 32.5 °C. Growth rate of 40 individuals was studied at each temperature. Twenty individuals were measured (length and mass at larval stages and mass at pupal stage) at intervals representing 10% of the stage duration (at each temperature about 40-50 measurements per individual), whereas 20 individuals were inspected only in terms of the development stage. Insects were kept individually in small containers and fed *ad libitum* with larval blowflies. Measured individuals were taken out of the incubator for about 5 minutes to the room where the temperature was about 25 °C and were measured there.

Consistent, significant but rather small differences in the rate of development between measured and non-measured insects were observed at each temperature. Non-measured individuals passed more quickly through the stages. These differences accumulated with the progress of the life cycle, being the largest at eclosion. The differences were similar at each temperature regime, consequently they cannot be accounted for temperature variations occurring during measurements, but are rather the effect of stress resulting from repeated measurements. Mortality was the same for measured and non-measured insects.

In conclusion, we have demonstrated that multiple, *in vivo* measurements affect the duration of pre-adult development in *C. maxillosus*. However, the effect is not very large and only a slight correction of the development model will be necessary.

MISSING:NECROPHAGOUS SOCIAL WASPS — WHERE DID THEY GO?

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This is more a story than a typical congress presentation. Still, we believe it is worthwhile to alert our fellow researchers. Although vertebrate carrion plays an important role in the nourishment of some social wasps, especially *Agelaia* Lepeletier and *Angiopolybia* Araujo (Silveira et al. 2005), few studies on insects associated with vertebrate carcasses have dealt with these insects. We aim to elucidate the possible forensic importance of the epiponine wasp *Agelaia pallipes* (Hymenoptera, Vespidae, Polistinae). We are currently examining the behavioral dynamics and the relationships between this wasp and other insects regularly found on decomposing vertebrate carrion in Neotropical environments. Yet, this wasp, abundantly collected only about 10 years agoin rural and forest sites in São Paulo State (southeastern Brazil) (Moretti et al. 2011), is now difficult to find. One possible explanation for its absence at carrion baits is a reduction in its populations, possibly due to the exceptional dry season in São Paulo State in 2015. To determine whether species or families are declining is often difficult, especially with Vespinae, which are often prone to under-recording. Examination of published reports on the possible decline of social wasp populations and interviews with residents in both tropical and temperate zones revealed that the reduction of populations of some social wasp species is likely to be widespread. In addition to Brazil, social wasps are declining, *inter alia*, in northwestern Costa Rica, French Guiana, Cameroon and Great Britain. We provide some examples and possible causes for this phenomenon. The decline in the recorded presence of wasps requires further investigation, also from the forensic standpoint, having in mind that these insects, when utilizing vertebrate carrion, function as both predators and necrophages.

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CURRENT SITUATION OF FORENSIC ENTOMOLOGY IN ALGERIA

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Forensic entomology has grown immensely as a discipline in the past thirty year. In Algeria, forensic entomology was introduced by the National Institute for Criminalistics and Criminology of the National Gendarmerie (NICC). However, all the work that has been done so far in this growing field in Algeria has been unknown at both the national and international levels. In this context, the aim of this presentation is to describe the state of forensic entomology in Algeria.

The Laboratory of Entomology of the NICC is the only one of its kind in Algeria. It started its activities in 2010, consisting of two specialists. Currently, the laboratory is performing different tasks such as the expert work required by investigators to estimate the post-mortem interval using insects. To assure the quality of the entomological evidence, crime scene personnel are trained by the laboratory of Entomology of the NICC. Recently, undergraduate and graduate students have been studying carrion ecology and insect activity in different geographic locations of Algeria using rabbits, dogs and wild boar carcasses. The Laboratory of Entomology of the NICC has been involved in some of these research projects. Entomotoxicology experiments have also been conducted at this laboratory.

Official bodies have been adopting more and more the use of entomological evidence in criminal investigations in Algeria, which is commendable. It is important, therefore, that steps are taken to fill in the gaps in knowledge necessary for entomological evidence to have a useful future in criminal investigations in Algeria.

Keywords: forensic entomology, PMI, criminal investigations, Algeria.

NECROPGAHOUS INSECTS IDENTIFIED DURING SEVERAL FORENSIC ENTOMOLOGY EXPERIMENTS IN ROMANIA, SINCE 2009 TO DATE

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At present, forensic entomology is not used for PMI estimation in Romania, neither is included among the other forensic expertises. Starting with 2009, several experiments were performed in order to investigate the necrophagous entomofauna and to emphasize the importance of introducing forensic entomology in this European country.In Europe, this expertise is recognized and used in many countries (e. g.:United Kingdom, France, Italy).
Consequently, this paper summarizes some of the forensic entomology experimental research performed in Romania since 2009. Five experimental models will be presented. The experiments were performed in Bucharest (Romania), in different years and seasons, by using different carcasses and exposure techniques. During all experiments the meteorological data were recorded continuously. The necrophagous insect species were identified (both adults and larvae) and their time presence and families' succession were noted, also the decomposition stages were indicated during each experiment.
The first experimental model was developed in an indoor environment, between 4July and 26August 2009. Pieces of swine meat were used as bait. Calliphoridae, Sarcophagidae and Histeridae species were identified and the developmental cycle of each species was monitored.
The second experimental investigation involved fox carcasses, and lasted between 9 March 2011 and 9 May 2011. The experiment was set outdoor, in the urban area of Bucharest, in a park-type environment. Necrophagous insect species were identified as belonging to Calliphoridae, Muscidae, Scathophagidae, Silphidae, Staphylinidae, Histeridae and Dermestidae.
During the third experiment, badgers carcasses and three exposure ways were used: outdoor, buried, and wrapped in plastic materials and placed outdoor. The experimental location was the same as described above and the experimentation time frame was 21 March 2011-24 October 2011. Calliphoridae, Muscidae,Fanniidae, Scathophagidae, Silphidae, Staphylinidae, Histeridae, Scarabeidae, Carabidae and Dermestidae species were identified, except for the buried carcass, where no insect species could be observed or sampled.
Between 20 April 2011 and 10 May 2011 the fourth experimental model was set in the same outdoor location, and used jackal skulls as bait. In this case, only Calliphoridae and histerid beetles were identified.
The fifth experiment was divided into cold (November 2012-May 2013) and warm season (10 July-10 October 2013). Six swine carcasses were mounted outdoor, in the same park-environmental setting. From both experiments Calliphoridae, Muscidae, Fanniidae, Anthomyiidae, Sepsidae, Piophilidae, Silphidae, Cleridae, Histeridae, Staphylinidae, and Dermestidae species were identified and their succession specified. Two Sepsidae species (*Themira nigricornis* (Meigen, 1826) and *Meroplius fukuharai* (Iwasa, 1984) were identified as new records for the Romanian territory and the south-eastern part of Europe.
All the experiments presented the succession of insects during decomposition, in different seasons and under different exposure ways, with the intention of continuing the experimental researchby emphasizing the importance of entomology as a forensic method in Romania.

POSTER ABSTRACTS

HOW THE ALTITUDE AFFECTS THE CALLIPHORIDAE COMMUNITY ALONG A CORPSE DECOMPOSITION

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Studying the sarcosaprophagous community is really important for forensic practice since it can provide useful information in different aspects concerning death. Then, studying and understanding the wide variety of species related to corpses and their succession is a useful and necessary tool in forensic studies. Hence the need of performing experiences of colonization and succession of such fauna on animal carcasses models (Prado e Castro, 2005; Al-Mesbah et al., 2012).

It is well known that such community is affected by different environmental variables enhancing the need of studying this community in different microclimatic environments, even if the different sites are close to each other (Anderson, 2001, among others).

As it concerns the community, the most important group to be considered for forensic purposes is the necrophagous, mainly Diptera, some of which are known to be the first arthropods to access the corpse. Among them Calliphoridae is the most important family, being the best indicator of postmortem interval and usually the first to reach the corpses. The Calliphoridae community was studied at three different altitudes (400 m, 900 m and 1500 m) of Sierra Espuña, a mountainous area in Murcia province (SE Spain), using a modified Schoenly trap baited with a 5 Kg piglet (*Sus scrofa* Linnaeus, 1758). Samples were taken daily for the first two weeks from corpse exposition. The experiment was replicated at each altitude for the four seasons of the year, between autumn 2006 and summer 2007. The succession took different times depending on the altitude. While no significant differences in the faunal composition were found, some differences concerning the decomposition rates and the Calliphoridae community behaviour have been detected.

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FIRST DATA ON LIFE-CYCLE LENGTH OF *HYDROTAEA CAPENSIS* (DIPTERA: MUSCIDAE) BRED IN LABORATORY CONDITIONS

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Family Muscidae is an important group belonging to the sarcosaprophagous fauna due to its wide distribution and its synanthropic habits. Their females usually lay eggs on the natural openings of a corpse, wounds or clothes moisted by cadaveric fluids. *Hydrotaea capensis* (Wiedemann, 1818) is a cosmopolitan hemi or eusynanthropic species (Gregor et al., 2002) often found in the Mediterranean area. It can belong to the sarcosaprophagous fauna; some data point to its frequent presence in forensic cases (Lefevbre & Pasquerault, 2004). Despite such potential applied interest, its biology is still poorly understood. Lefevbre & Pasquerault (2004) tried to study its life cycle characteristics at different temperatures but did not achieve any conclusive data.

This contribution provides the first conclusive data on the life cycle of this species bred under laboratory conditions (controlled relative humidity: 50-60%, photoperiod: 12:12 and four different temperatures: 18, 20, 25 and 30°C). Four replicates for each thermal regime were considered. As could be expected, the whole cycle was longer as the temperature was lower. At 18ºC, larvae III failed to pupate, died and dried without this could be due to the breeding conditions. The fastest development occurred at 30ºC, followed by that at 25ºC. Both were quite similar, showing a rapid growth followed by a sharp reduction in size before the pupation started, so fitting the usual pattern of other Diptera of forensic interest (Grassberger & Reiter, 2002). Duration of the whole preimaginal development is provided for both most favorable thermal regimes, as well as data on larval stages duration for the other two temperatures.

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THE IMPACT OF PARACETAMOL ON THE GROWTH RATE OF TWO SPECIES OF DIPTERA CALLIPHORIDAE (*CALLIPHORA VICINA AND LUCILIA SERICATA*)

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This project consisted in studying the impact of paracetamol on the growth rate of *Lucilia sericata* (Meigen, 1826) (Diptera, Calliphoridae) and *Calliphora vicina* (Robineau-Desvoidy, 1830) (Diptera, Calliphoridae).
Males and females of the two species were collected at the National Institute of Criminalistics and Criminology's campus in Bouchaoui, Algeria (36° 44' 0" N, 2° 55' 0" E). The foodstuff was prepared; four rabbits (albinos) of the same sex (all males) and approximately of the same weight were injected through the marginal ear veins with different paracetamol doses (dose 01: 60 mg/Kg, dose 02: 100 mg/kg, and dose 03: 200 mg/kg) every four hours during the day time (at 08:00, 12:00, and 16:00). A fourth rabbit was dedicated to serve as a control (00 mg/kg). Three days later, the rabbits were sacrificed 20 minutes after their last injection. Meanwhile, the insects were stimulated for oviposition. For each species four rearing containers were prepared; one to be used for the control samples and three for the three selected doses. Finally about 150 to 200 eggs of each species were transferred into a rearing container, provided with 200g of the rabbit meat, and then put into a rearing chamber at 24°C and 70% relative humidity. In order to assess the impact of the drug on the growth rate of the target species, two parameters were assessed: length of the maggots in different periods (each 24 hours for *L. sericata* and 48h for *C. vicina*), and duration of life cycle. For this, a random sample of 10% of the population was taken, killed in hot water (about 80°C), and measured. The appearance of the first pupae (3 to 4) were considered as the end of the sampling processes and the appearance of the first adults (more than 3) was considered as the end of the life cycle.
The results of the length measurement, for both species, showed that the higher the drug's dose in the substrate was, the bigger the maggots were compared to the controls. This was clearly observed during the first 72 hours of the maggots' life. However the life cycle length of both species was not affected except for *L. sericata* reared on the substrate containing dose 01 and dose 02; which extended the life cycle by approximately 17 hours.
These results showed that paracetamol may affect the development of *Lucilia sericata* and *Calliphora vicina*, which is crucial to take into consideration to estimate the PMI correctly. Further work is strongly recommended in order to assess more parameters and doses.

Keywords: *Lucilia sericata*, *Calliphora vicina*, development, paracetamol, entomotoxicology

CASES FROM CENTRAL ITALY: INDOORS VS OUTDOORS

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In a previous work (Bugelli et al., 2015) the authors analyzed and compared indoors and outdoors cases from Central Italy in order to have a better understanding of the body colonization process and of the factor affecting it.
Three new cases are here presented, the results confirm and strongly support the previous general results. In addition, the presence of *Megaseliascalaris* in indoors cases is confirmed.
Case 1 -At the end of April the body of a49 years old man was found inside his car parked in a wood. The body, in a supine position, was wearinga sweater, shirt, jeans and the shoes. The victim was reported alive 6 days before the body discovery and the cause of deathwas indicated as a suicide.
Larvae belonging only to a species, *Lucilia sericata* (Diptera sericata) were collected from the body.
Case 2 - At the end of July the body of a 77 years old man was found lying on the bed in a supine position wearing only underpants. The body was found in an advanced decay and no sign of injury was reported with the autopsy. The victim was reportedalive few days before the body recovery.
Larvae from two species were collected from the body and the crime scene: *Megaseliascalaris* (Diptera, Phoridae) and *Sarcophaga* sp. (Diptera, Sarcophagidae)
Case 3 - At the end of July the body of a84 years old woman was found lying on the floor in a supine position. The body was in an advanced decay and no sign of injury was present. The victim was seen alive 6 days before the body discovery.
Three species were collected from the body: *Chysomyaalbiceps* larvae (III), *Lucilia sericata* (III) (Diptera, Calliphoridae) and *Sarcophaga* sp. (Diptera, Sarcophagidae).

HOW SHOULD LIVING ENTOMOLOGICAL SAMPLES BE STORED?

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Sampling, killing and transferring/storing of insect evidenceis a very important task in forensic entomology because different methods can influence survival and growth rates of the living samples or bias the morphological examination of the dead specimens. The majority of "Best Practices" and "Guidelines" in forensic entomology recommend that fly larvaeshould kept under controlled or at least known conditions, most suitable at 2-6°C. They suggest in addition that larvae should be stored in vials with an air-permeable lidand that these vials should be equipped with coarse sawdust or paper for taking e.g. excretion liquids. Living samples should be then brought to an expert within 24 hours. While keeping this window of time seems to be a realistic approach, cooling the samples or catering them during storage seems to be aserious problem for some crime scene technicians or forensic pathologists. Neglecting guidelines or best practice recommendations might lead to a weakening of the entomological evidence at court or even its exclusion. However, it is not always clear whether and which of the recommendations based on experiences, opinions or scientific evidence.
What happens if the cold chain is not maintained, or if there was no air-condition or no supply of coarse sawdust?
We stored different numbers and stages of larvae (L1-3 in numbers of 50 and 100) of the blow fly *Lucilia sericata*in 100 ml plastic cups at room temperature (~20°C) or in the refrigerator (5°C) for 16 hours without air, supply of food and sawdust. After that they were kept at 2-6°C in a Styrofoam box for 8 hours, simulating a transport situation. A second treatment was the storage of the larvae for a period of about 72 hours at 5°C, simulating storage of an insect infested body in the morgue before performing the sampling and the autopsy.
Mortality was much more related to the amount of larvae in the vials than to the storage temperature. Surviving larvae were reared until adult eclosion. Results showed that low temperature episodes cease development in almost all immature stages and that development was much better calculable for specimens which were kept at cool temperatures than at room temperature. However, an influence on the rate of development after returning the specimens to higher temperatures could be detected. The degree of this effect depended on the stage in which the cold episode took place: The younger the specimen the longer was the total rate of development. Consequences for the PMI estimation are discussed.

DNA BARCODING IN FORENSIC ENTOMOLOGY – A NGS APPROACH FOR FAST AND RELIABLE IDENTIFICATION OF SPECIES COMPOSITIONS ON CARCASSES

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Introduction:
Forensic entomology aims to use insects to determine the postmortem interval of a carcass. A major problem however is the rapid, exact, and reliable identification of the insect species. Eggs and early larval stages of many insects share similar features, making it difficult to distinguish between species based on morphology. Often, collected larvae need to be reared under constant conditions to the imago, which offers better differential features. This time consuming method is not beneficial when a crime case must be solved quickly. A promising alternative is species identification using molecular markers. The Bavarian State Collection of Zoology (SNSB-ZSM) uses DNA barcoding to obtain genetic fingerprints of all animals, fungi, and plants found in Germany. Approximately 17,000 animal species were already successfully barcoded and more than 100,000 barcode sequences were provided for the international Barcode of Life Database (BOLD).In this study, we created a DNA barcode reference library for arthropod species playing an important role in forensic entomology and we applied NGS techniques in order to obtain simultaneous species identifications of bulk samples.

Material and Methods:
We investigated insects extracted from 30 carcasses stored in 50mL Falcon tubes, provided by the local morgue. For reference library construction, we sorted out visually distinct morphospecies and different developmental stages of all sample containers; tissue samples were removed from each specimen and transferred into 96 well plates for subsequent DNA extraction. Sequence data and trace files were then uploaded to BOLD. In a second step, we tested the potential of bulk extractions and subsequent species identification using NGS and the application of the barcode reference data in one single analysis step.

Results& Discussion:
The analysis of the NGS data comparison with the BOLD database revealed31 different BINs of insect species,of which only 13 could be identified by references of the forensic insect library which we created on BOLD. The whole procedure took a total of 30 working hours from DNA extraction to species identification for the whole bulk sample, which clearly demonstrates thatNGS is especially promising, as it saves time and costs in comparison to convential methods. This study illustrates that DNA Barcoding might be a promising addition for forensic entomologists, because it facilitates accurate and fast species identification of all developmental stages even when specialized taxonomists are lacking.

CLASSIFICATION OF LARVAL SILPHINAE (COLEOPTERA: SILPHIDAE) ACCORDING TO INSTAR

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Larvae of beetles may be classified according to instar based on measurements of quantitative morphological features. For this purpose statistical classifiers of the species level were created and a very high accuracy of classification was achieved. Here we test the concept that subfamily level classifier may be similarly successful in the classification task. For this purpose, larvae of four closely related species of carrion beetles i.e. *Necrodes littoralis*, *Oiceoptoma thoracicum*, *Thanatophilus sinuatus* and *Thanatophilus rugosus* (Silphidae: Silphinae) were studied.

Larvae of *T. sinuatus* and *T. rugosus* were reared in laboratory conditions. Larvae of *O. thoracicum* were collected on pig carcasses during 2011 field studies. For *Necrodes littoralis* we decided to use measurement data from our previous study. Specimens just after ecdysis and fully sclerotized were sampled. All larvae were killed and kept in 70% ethanol. Subfamily level classifier was created using 60 training larvae (20 per instar) of each species. Classifier was tested with training larvae and 30 test larvae (10 per instar) of each species. Distance between dorsal stemmata, width of the mesonotum and width of the pronotum were measured. Measurements were taken from digital photographs made with Leica M165C stereomicroscope. Classifiers were created and validated using a linear discriminant analysis (LDA). LDA generates classification functions which are used to calculate instar-specific classification values from measurements of the classified specimen. The largest value indicates larval instar to which the specimen belongs to.

Distance between dorsal stemmata and width of the pronotum were incorporated into the model. Validation with test larvae gave perfect results in the case of *N. littoralis* and *O. thoracicum*. Few misclassifications were however observed in the case of *T. sinuatus* and *T. rugosus*. Accordingly, a separate genus level classifier was created for larvae of *Thanatophilus*.

Due to the high classification accuracy of the subfamily level classifier we recommend to use it for all larvae of *N. littoralis* and *O. thoracicum*, as well as for the fully sclerotized larvae of *Thanatophilus*. As for the *Thanatophilus* larvae just after ecdysis, it is suggested to use the genus level classifier.

ULTRAMORPHOLOGY OF PREIMAGINAL STAGES OF *HYDROTAEA CAPENSIS* (DIPTERA: MUSCIDAE)

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Despite its interest for forensic purposes, family Muscidae has not been deeply studied concerning its preimaginal stages although, recently, some papers (i.e. Grzywacz et al., 2013, 2014) have paid attention to the comparative study of such stages of several species. Concerning *Hydrotaea capensis* (Wiedemann, 1818), a species of forensic interest (i.e. Lefevbre & Pasquerault 2004, Arnaldos et al. 2014) few data are available on its immature stages.

A laboratory colony of *H. capensis* was founded with larvae and puparia from a forensic case in Murcia. Once the adults emerged it was confirmed that they belonged to *H. capensis*. Specimens of all preimaginal stages were obtained and studied on SEM using the methodology adopted in Paños-Nicolás *et al.* (2015). Larvae were also studied by light microscopy to observe their internal structures. This contribution provides the first images of all preimaginal stage of *H. capensis*, as well as those of the cephalopharyngeal skeleton of the three larval stages. For a comparison of larval stages, the different body regions have been studied separately. As it concerns the puparium, the only equivalent structures in relation to larval stages that were studied are the creeping welts and the anal division.

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APPLICATION OF THE HIGH RESOLUTION MELTING (HRM) ANALYSIS ON THE IDENTIFICATION OF FORENSICALLY IMPORTANT FLY SPECIES (*BRACHYCERA*)

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When estimating the minimum postmortem interval (PMI_{min}) by age determination of juvenile insects, especially necrophagous flies collected from corpses or crime scenes, species determination plays an important role. Since the development duration is temperature dependent on the one hand but species specific on the other hand, knowledge about the species is essential.

So far, species identification is accomplished by using morphological keys or by sequencing DNA-fragments with species specific sequence variants, whereby both methods are characterized by several disadvantages as the need of extensive expertise or a high amount of labour and costs.

A cost-effective and rapid method already used as a diagnostic tool in various disciplines that constitutes an alternative opportunity for species differentiation is the High Resolution Melting (HRM) Analysis. Here, the specific melting temperature of a particular DNA-fragment is determined. The melting temperature of a DNA-double strand depends on fragment length and base composition and therefore theoretically varies between genetically diverging species even if there is only one single nucleotide polymorphism.

Here we present melt curve analyses of 33 different species from 7 forensically relevant fly families: *Calliphoridae, Fanniidae, Piophilidae, Phoridae, Muscidae, Sarcophagidae* and *Drosophilidae*. Primers for two mitochondrial (*cytochrome-c-oxidase subunit 1* gene) and one nuclear marker (*28S ribosomal RNA* gene) have been designed, to generate amplicons, whose melting temperatures could be assigned to certain species. Furthermore, the respective sequences have been aligned and a score based on the number and location of existing sequence variations has been calculated. This score was compared to the melting temperatures of the respective species.

Moreover all collected data were analyzed via variance analysis (ANOVA) and tested for significance.

IDENTIFICATION OF CARRION VISITING MUSCIDAE (DIPTERA) BY MEANS OF WING MEASUREMENTS

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The presence of Muscidae on decomposed bodies has been recorded in numerous case reports and carrion experiments. Carrion attract numerous species and genera of houseflies, both regular elements of carrion insect assemblages and accidental visitors. Thus it is recommended that identification keys of adult Muscidae associated with carrion should cover wide range of species. Adult Muscidae identification is based mostly on chaetotaxy and wing venation. Their identification may be considered difficult, particularly by non-experts. Thus, we have investigated possibility of semiautomated muscid identification by means of wing measurements as an alternative for classic morphology and DNA-based approaches.

For the study we have used 583specimens representing 11 genera and 21 species of Muscidae. Most genera, except *Hydrotaea* and *Muscina*, were represented by single species. Both wings have been detached from the body and their images were obtained using USB camera with resolution of nine megapixels. On the wing images 15 landmarks have been determined using computer mouse in IdentiFly software. Coordinates of the landmarks were analyzed using methods of geometric morphometrics. Identification of the species was based on Canonical Variate Analysis. Identification error was assessed using leave-one-out cross-validation.

Most genera were identified without error. Misidentification between genera occurred only in two cases. Single specimen of *Phaonia* was identified as *Mydaea* and single specimen of *Helina* as *Phaonia*. Also identification of species within *Muscina* was relatively high and only single specimen of *M. prolapsa* has been misidentified as *M. stabulans*. Correct identification rate of species within *Hydrotaea* was lower and ranged from 93 to 100%.

Our results revealed relatively high success in both genus and species identification of carrion visiting Muscidae. Automated identification by means of wings measurements can be used by non-experts and does not require sophisticated equipment. The method can be an alternative to more difficult and more time-consuming identification based on taxonomic keys.

DURATION OF THE POST-FEEDING AND PUPAL STAGE OF THE BLOW FLY *CALLIPHORA VICINA* AS A FUNCTION OF THE PUPARIATION SUBSTRATE

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Growth rates of blow flies are major tools used to estimate the PMImin in forensic entomology casework and considerable effort has gone into generating developmental data for a number of temperature regimes and species of forensic importance. Under laboratory conditions several types of pupariation substrate are offered to post-feeding larvae e.g. sawdust or sand. However, real case scenarios are highly variable regarding their possible pupariation substrates and this might influence the time spend in dispersal and finally the pupariation process itself. Hence, the present study examines the influence of seven kinds of pupariation substrates on the durations of the post-feeding stage and the pupal stage of the forensically relevant blow fly *Calliphora vicina*. Larvae were reared on pork liver at 20 °C until post feeding stage and then placed in boxes (10 per box) with different pupariation substrates, including a thick woollen carpet, a unstructured smooth carpet, soil, sawdust, two kinds of sand (playground sand and sand for bird breeding) and no substrate. Additionally, by offering several choices between two kinds of substrates a study of post-feeding larvae preferences was carried out. To determine the effect of natural disturbance by other larvae on the developmental timings, either 1, 2 or 5 larvae were placed into plastic tubes. All experiments were performed at 12 °C without light. The times to pupariation and adult eclosion were noted. The disturbance-experiment was repeated four times and the substrate-experiment three times. ANOVA-tests were performed to analyse the significance of differences. To examine the preference behaviour of post-feeding larvae, boxes with different choices of two substrates were offered. After approximately two weeks, pupae in each substrate were counted. Control boxes were prepared, where both sides contained the same substrate. T-tests were performed to analyse the choices.
Results showed significant differences of pupariation timings on different kinds of substrates in two of three experimental runs, whereas adult eclosion timings were only significantly different in one of three replicates. The longest post-feeding and pupal stage durations were observed on the soil, the woollen carpet and on no substrate. The developmental timings based on the disturbance factor due to different numbers of larvae showed significant differences, with an increasing number of larvae within a limited space leading to an increase in the post-feeding duration. Choice-experiments showed that soil was preferred the most, whereas sand was avoided.

ALTERNATIVE USES FOR BLOWFLY LARVAE IN THE DETECTION OF SEMEN FOR POST MORTEM SEXUAL ASSAULT CASES

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Profiling offenders of sexual assault is an accepted practice in forensic investigation, however the persistence of semen following the onset of decomposition is largely unknown. Consequently, the success rates of offender identification in rape-homicide cases are uncertain. Whilst there are established protocols into the collection of evidence for the living, the lack of existing research into post-mortem cases indicates these guidelines cannot be applied, paving the opportunity for further research.

Blowflies are primary colonisers of cadavers, most commonly laying eggs in open and accessible orifices.The impact feeding larvae have on post-mortem semen persistence within the vagina is undetermined. The feeding behaviour of insects is destructive and unspecific, facilitating the decomposition process. Alongside this, cadaver exposure to environmental and chemical factors may be detrimental. With the high level of consumption undertaken by larvae masses, tied with low levels of biological material present at a crime scene, it is viable that the larvae crop may present a protected environment for semen, allowing for prolonged, successful evidence recovery.

The aim of this research is to explore the potential for larvae crop content analysis as tool in rape-homicide investigations, determining the impact insect activity has on post-mortem semen persistence. Variables will be observed and alternative evidence protocols will be discussed.

Semen will be deposited within piglet carcasses and placed outdoors. Behavioural observations will be made daily, and larvae feeding in deposition sites will be collected. Larva crops will be dissected and DNA profiled for the presence of a male specific gene. Alongside this, current swabbing protocols will be followed for comparison purposes. Finally, the degree in which external factors impact these success rates will be explored.

Techniques and methodology investigated throughout the pilot study will be presented, alongside support and justification from current literature, considering crop content analysis, decomposition and the potential for this research in future investigations.

STAY FROSTY! THE USE OF ICE-SPRAY FOR SAMPLING AND KILLING BLOW FLY LARVAE

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In forensic entomology, the correct sampling and storing of insects as evidence material is of great importance. Several guidelines and best practice papers recommend the killing of the fly larvae by means of hot water and the subsequent storing in > 70% ethanol. As a golden rule, this procedure usually guarantees an appropriate quality of the material for morphological and molecular analysis, but hot water may not be available at crime scenes. Moreover, killing the larvae with hot water requires the subsequent placing of the specimens in ethanol. This procedure is not always performed at crime scenes and instead the larvae are stored alive for a too long period, which may often impede the successful analysis of entomological evidence. Several procedures are recommended to simplify the sampling method, e.g. killing and storing the specimens in ethanol, or just sampling the specimens and keeping them alive at low temperature until analysis.

In the present study, we tested the killing of larvae of the two forensically important blow fly species, *Calliphora vicina* and *Lucilia sericata*, by using a medical and an industrial ice-spray. Followed by the subsequent storage of these specimens in 96% ethanol, we examined the influence of this method of killing on the state of preservation.

Larvae were reared on pork ground meat at 25°C until the larval stages L2 and L3 were reached. Then, groups of 40 larvae each were treated as follows: a) killed by placing them in hot water (HW), followed by transfer and storage in 96% ethanol; b) killed and stored in 96% ethanol (ETH); c) and d) sprayed with a medical or industrial ice-spray for about 4 seconds, then transfer and storage in 96% ethanol; e) and f) likewise sprayed with these ice-sprays for about 8 seconds, followed by transfer and storage in 96% ethanol. With both types of ice-spray all specimens were killed within 4 seconds. However, larvae killed by this method tend to be reduced to a smaller size than those of the control groups HW and ETH, but this difference was less evident when applying the ice-spray for about 8 seconds. Depending on the fly species and its larval stage, this difference was significant, but often disappeared after one week of storage in 96% ethanol. L2 larvae showed fewer changes in their body size than L3 specimens. In additional experiments we found that the type of vial used for the killing of the larvae may have influenced the results. The potential application of this new sampling method in forensic entomology will be discussed.

STUDY OF INSECT FAUNAL SUCCESSION ON A DECOMPOSING PIGLET CARCASS (SUS SCROFA L.) PLACED IN A MAN-MADE FRESHWATER POND IN PUNJAB, INDIA

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Forensic entomology deals with the use of the insects and their arthropod relatives that inhabit decomposing remains to aid legal investigations and is used mainly to estimate Post mortem interval based on the developmental rates and the successional ecology of specific insects that feed on a carcass. Forensic entomology has focused on terrestrial saprophagous insects, and a very few studies emphasizing on biota colonizing corpses in aquatic environments. Present entomological study was conducted in a man-made freshwater pond in a countryside area of District, Ludhiana, Punjab (India), from 25thFebruary to28th March 2015 (Winter season) by using piglet (*Sus scrofa*) carcassasas research model. A 3.7 months old piglet (16 kg), was thrown into a man-made fresh water pond. Observation was made until the complete decomposition of carcass. It comprises of two visits per day i.e. morning and evening. Climatological data and water analysis was recorded. It took thirty-three days for complete decomposition of carcass. Total five stages of decomposition were observed according to early observation made by Byrd and Castner (2001). During submerge fresh stage, the piglet carcass submerged to the bottom of the pond. The carcass reappeared and floated on the surface of water after seventh day of experiment. Ants were the first insect appeared on exposed carcass and there activity is reported on carcass. The flies of family calliphoridae i.e.*Chrysomyamegacephala* and *Chrysomyarufifacies* started to oviposit eggs on the ninth day. Flies oviposit their eggs at the mouthparts and neck region. Abdomen turned light blue and purple showing mark of livor mortis. Bunches of blow fly eggs were seen on the exposed body surface on the tenth day (floating decay), along with first and second instars of *C. megacephala* crawling on the piglet's skin, mouthparts and neck region. On the elevanth day, adult blow flies, *C. megacephala*, *C. rufifacies*, *Lucilia sericata*, *Lucilia cuprina,* Sarcophagid flies, muscid flies i.e. *Musca domestica*, *M. sorbens* and Hister sp. of family histeridae from order coleopterawere observed on the carcass. Numerous first and second instars of flies were observed on the body surface with *C. megacephala* larvae being the predominant one. Bloated deterioration stage began on thirteenth day exposing ribcage, intestine and leg bones.Beetles of family Histeridae, carabidae, scarabaeidae, Staphylinidae and cleridae were dominating in this stage. Carcass was partially sinking with three maggot masses were observed on neck and abdomen on seventeenth and eighteenth day. On day eighteenth maggot masses were still visible on the exposed parts of the body. Ontwentyeighth day one third of carcass sunk with larvae and pupae were floating on the surface of water. Adult flies of family sarcophagidae and muscidae including histerid beetles were still sighted on the carcass during this stage. A total of 1580 specimens belonging to order diptera (calliphoridae, sarcophagidae, muscidae), coleoptera (histeridae, scarabaeidae, carabidae, Staphylinidae and cleridae) and hymenoptera (formicidae) were collected. The carcass along with the maggots sunk on thirty second day leaving behind an oily layer on water surface.Present study was conducted to prepare a baseline data ofinsect faunal succession in case of aquatic environment which ishelpful in estimating the time since submergence (TSS) of body.

A MOLECULAR, MORPHOLOGICAL AND PHYSIOLOGICAL COMPARISON OF ENGLISH AND GERMAN *CALLIPHORA VICINA* (DIPTERA: CALLIPHORIDAE)

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The blow fly *Calliphora vicina* is a common species distributed throughout Europe and can play an important role as forensic evidence in crime investigations. Published development data act as references for the growth rate and enable age estimation of the immature stages, leading to the establishment of a minimum post-mortem interval. As this published reference data originate from different geographic populations, it is important to determine whether or not populations of the same species show different rates of growth related to their geographical origin.

Therefore, the aim of this study was to compare developmental rates of *C. vicina* from Germany and England under different temperature regimes, and to look for potential molecular and morphological variation between the two populations.

For the developmental study, colonies of German and English *C. vicina* were cultured and kept at the Institute of Legal Medicine in Frankfurt, Germany. Three different temperature regimes were applied in the laboratory, two constant (16°C & 25°C) and one variable (15-19°C, Room temperature = RT). At 7 time points (ADH: 600, 850, 1200, 1450, 1800, 2050, 2400)10 larvae (2 per container) were selected randomly and measured; additionally, the times of the post feeding stage, pupariation and emergence of the adult flies were recorded. For the morphometric and molecular study, 60females and 51 males from five different populations (4 Germany, 1 England) were sampled. Right wings were prepared on glass slides, photographed, digitized and measured based on 19 landmarks following Hall *et al.* (2014). DNA was isolated from three legs per selected specimen (n = 28) using 5%chelex. A 784 bp long fragment of the mitochondrial cytochrome b was sequenced and these sequences were aligned and phylogenetically analyzed.

For German flies, development from egg to adult took 20 days at 25°C, 37 days at 16°C and 34 days at RT, whereas English flies took 23 days, 34 days, and 36 days at the same temperatures, respectively. Wing shape variation using canonical variates analysis, discriminant function analysis, and cross-validation test showed that there was no overlap between the two geographic populations. *P*-value of two groups were highly significant differences at $P<0.0001$. Moreover, a cytochrome b analysis was able to distinguish between 2 of the analysed colonies, the laboratory colonies of Germany and England.

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METHODS FOR THE INVESTIGATION OF POST-FEEDING LARVAL DISPERSAL IN UK BLOWFLIES: PRELIMINARY RESULTS

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In order to determine the minimum time since death in forensic investigations, it is essential to collect the oldest insect specimens associated with the body. Often these are not found on the body itself, but have left to find a suitable pupariation site, and therefore may be some distance from the body on which they were feeding. A literature review revealed that much information is either contradictory or missing concerning the post-feeding larval dispersal behaviour of blowflies. This has highlighted a need for a more complete study looking at the many factors that may affect larval dispersal. Therefore, based on published experimental studies, multiple methods were explored to determine the most appropriate to study these factors, with varying results. The best shape and size of the arena in which to hold dispersing larvae was investigated, and it was determined that a long (6m), narrow arena was best for determining distances travelled by larvae and that a large (2.5m diameter) circular arena was most appropriate for looking at distances travelled in conjunction with the directional preferences exhibited by the larvae. Various sampling techniques were tested and it was determined that manually sorting through the substrate for the specimens, although time-consuming, resulted in the greatest pupal recovery. After testing different software packages, ©Tracker Software Products was determined as the most suitable as it was able to track multiple larvae simultaneously, was the most simple to use and was free. To refine the results of this study a SYNTECH ServoSphere was successfully tested for recording the speed and direction of individual dispersing larvae. These preliminary studies have established which methods are most precise, reproducible and representative of the natural environment, and therefore which should be employed for a full-scale study.

USE OF CARNIVORE CARRION RESOURCES BY ARTHROPOD AND VERTEBRATE SCAVENGER COMMUNITIES IN WILD HABITATS OF SOUTHEAST SPAIN

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Understanding carrion decomposition and the factors affecting its variability is crucial for strengthening and improving the accuracy and reliability of forensic investigations of death; this is particularly important in forensic entomology casework as basic knowledge of carrion ecology can minimize the error in minimum post-mortem interval estimations (Tomberlin *et al.*, 2011). However, there remains much to be done on the study of arthropod communities and carrion decomposition from an ecological perspective. For instance, it is known that vertebrate scavengers accelerate carrion decomposition rates (Barton *et al.*, 2013), but studies on carrion insect succession typically exclude this factor by avoiding the access of vertebrates to the experimental carcasses. Moreover, while scavenging studies of vertebrate carrion have focused on herbivore and omnivore carcasses, carnivore ones have been largely neglected. Here we present the first preliminary results from a study aimed at yielding new insights into the partitioning of carnivore carrion resources amongst the arthropod and vertebrate scavenger communities in wild habitats of southeast Spain.

Data were collected from 20 red fox (*Vulpes vulpes* L.) carcasses, placed at two areas in the Region of Murcia (SE Spain) for two months (from mid-winter to early spring 2016), with an inter-carcass distance of >1km within areas. The carcasses were attached to trees by a wire, allowing access to both insect and vertebrate scavengers. Insects were collected manually whereas vertebrate scavenger activity was monitored by automatic cameras activated by movement and placed close to each carcass. Red fox carcasses were visited by several avian and mammalian scavengers, but only one carcass was partially consumed (by golden eagle *Aquila chrysaetos* L.). The avoidance of red fox carrion by mammals allowed wide exploitation of this resource by necrophagous insects. Diptera, Coleoptera and Hymenoptera were the three insect orders driving the decomposition process, with blow flies (Calliphoridae) being the first colonisers in every carcass and Silphidae and Staphylinidae beetles becoming particularly abundant in later succession stages. The composition of the carrion insect community in the framework of a partitioned resource and in comparison with other types of carcasses used in forensic entomology studies will be discussed.

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ANATOMY OF A BUBBLE: UNDERSTANDING EARLY PUPAL METAMORPHOSIS OF BLOW FLIES AND ITS SIGNIFICANCE IN FORENSIC ENTOMOLOGY STUDIES

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A correct understanding of insect biology is pivotal for strengthening forensic entomology research. In blow flies, the processes taking place during the puparial stage are of special interest as this period lasts more than 60% of the total immature development; hence, it can be a crucial time-span for estimating a minimum post-mortem interval. Although thepuparial stage is usually referred to as the "pupal stage", the actual pupa only occurs during a relatively short period of time, i.e. approximately between the first 7.5–30% of the total puparial period, after which the pharate adult is the stage which continues developing inside the puparium until its emergence. The pupa is, however, the stage which shows the most dramatic changes during metamorphosis, from an apparent head-less and leg-less larva to a winged pharate adult fly. The current study aims to enhance our understanding of this fascinating process through the visualisation of the key morphological changes using X-ray images and micro-CT scanning reconstructions of pupae of the blow fly *Calliphora vicina* Robineau-Desvoidy.

White prepupae (i.e. irreversibly contracted post-feeding larvae) reared under a constant temperature of 24°C ± 0.8^0C were considered as the time zero for this study. At 0, 3, 4, 6, 13, 18, 24 and 28 hours, 9–10 puparia were placed in a Nikon Metrology HMX ST 225 micro-CT scanner and imaged with an X-ray beam of 110kv and 203µA, passed through a 0.1 mm aluminium filter. Two 28 hours-old puparia were imaged every minute during 3.5 hours, in order to register the major morphological changes occurring at that time. Additionally, 5 puparia were collected at six-hour intervals during the first 48 hours, killed in hot water, stained in 0.5 M iodine and scanned using the same micro-CT system (exposure: 500 ms; voltage: 110 kV; current: 100 µA). Slice stacks in the three principal planes (cross, horizontal and sagittal) were rendered for each specimen and segmented for 3D visualisations.

The pupal stage is delimited by the larval-pupal and pupal-adult apolyses. The most significant event is the development of a gas bubble connected to the tracheal system which grows increasingly in volume and plays an essential role in the transformation of the amorphous cryptocephalic pupa into the headed phanerocephalic pupa. This massive morphological change takes place during approximately 1.5 hours, i.e. less than 1% of the total puparial stage. The significance of these changes for forensic entomology, including the fixation and preservation of pupal samples, will be discussed.

ESTIMATIONOF POSTMORTEM INTERVAL BASED ON PUPARIA OF *PHORMIA REGINA* (MEIGEN) AND LARVAE OF *NECRODES LITTORALIS* (L.) – A CASE REPORT FROM POLAND

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On 16thof July 2015, corpse of an adult man in advanced decomposition was found in the rubble located in an open area of the suburb of Śrem (Poland). The postmortem interval (PMI) established by the forensic pathologist was from 3 to 6 weeks. Entomological evidence was collected by the assistant prosecutor and included insects present on the corpse and in soil samples from below the corpse. Various species and stages were identified. Puparia of *Phormia regina* from the soil samples were the most developmentally advanced specimens of blowflies. Moreover, third instar larvae of *Necrodes littoralis* were collected directly from the corpse. Pre-adult development time of *P. regina*was estimated using thermal summation method and accumulated-degree days (ADD) reported by Marchenko (2001). To approximate PMI from larval *N. littoralis*, the pre-appearance interval (PAI) and the development interval were estimated. PAI was calculated using methods and models reported by Matuszewski & Szafałowicz (2013) and Matuszewski & Mądra (2016).The age of third instar larvae was estimated using thermal summation method and ADD after Dekeirsschieter (2012). All calculations used original and corrected (1˚C and 2˚C) average daily temperatures from the nearby weather station. The PMI estimates were:

- 36-38 days using *P. regina* and 37-40days using *N. littoralis* (for the uncorrected temperatures),
- 31-34 days using both *P. regina* and *N. littoralis* (temperatures corrected by +1˚C),
- 24-27 days using *P. regina* and 28-29 days using *N. littoralis* (temperatures corrected by +2˚C).

It was concluded that death occurred between 7 and 23 of June 2015, and most probably between 13 and 23 of June 2015.This was the first report when PMI was approximated by the age estimates combined with the PAI estimates. Moreover, the case demonstrates an urgent need for the more robust developmental model of *N. littoralis*, as it proved to be highly useful for the estimation of PMI.

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THE HISTORY OF MEDICOLEGAL ENTOMOLOGY IN BRAZIL

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With its enormous territory and wide variation in abiotic and biotic factors, which allow for a large variety of carrion-associated entomofauna, Brazil has great potential for studies in medicolegal entomology. In addition, high crime rates in some Brazilian metropoles account for many corpses and a consequent demand for forensic-entomology tools. This report on the development of medicolegal entomology in Brazil was inspired by two major sources: first, the comprehensive paper by JRPL and colleagues (2008), which markedthe 100th anniversary of the first medicolegal studies in this country. Second, the chapterby TCM and WACG (2015)in the book"Forensic Entomology: International Dimensions and Frontiers",edited by JK Tomberlin and ME Benbow. In this chapter, the authors provided a general overview of the history, accomplishments, and challenges of forensic entomology in South America, especially in Brazil. The foundationof medicolegal entomology in this countrywas the study byOscar Freirein 1908,14 years after the publication of "La faune des cadavres" by Mégnin.Freire presented to the Medical Society of Bahia (northeastern Brazil)the first Brazilian collection of carrion-associated insects, as well as the results of his studies on necrophagous entomofauna found on the remains ofhumansand small vertebrates.Following the study by Freire, forensic entomology research continued actively in Brazil until the 1940s. These studies then suffered a 40-year drought (1940-1980), broken by Monteiro-Filho and Penereiro (1987) who described insect succession on carcasses of small rodentsin a second-growthwoodland in the state of São Paulo. Since then, research on medicolegal entomology in the country has continued. Here, we present an overview of the first published reports and main milestones in this field in Brazil.

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APPLICATION OF WING MORPHOMETRIC AND TERMINAL RESTRICTION FRAGMENT LENGTH POLYMORPHISM (T-RFLP) ANALYSES FOR SPECIES IDENTIFICATION OF FORENSICALLY IMPORTANT FLIES

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Correct species identification is an initial step in forensic entomology e.g. for accurate post-mortem interval estimation as each species has its own developmental rate. Several identification techniques based on morphological and molecular characters. However, the use of classical morphology requires expert knowledge for correct identification, especially immature stages are quite difficult to identify on species level because of a lack of comprehensive keys for all life stages. In this study, we applied the use of wing morphometric as well as terminal restriction fragment length polymorphism (T-RFLP) techniques to facilitate species identification of forensically important flies for non-experts in entomology.

In wing morphometric analysis, we used 19 landmarks (Hall *et al.* 2014) for separation of 12 blow fly species. Canonical variates analysis was used for species discrimination. Discriminant function analysis and cross-validation test was used to assign the specimen to the correct species. Moreover, wing shape dimorphism in males and females of each species was analyzed.

In T-RFLP analysis, a 452 bp of the mitochondrial COI gene was amplified using two differently fluorescence-labeled forward and reverse primers. The amplicon was digested with a combination of five restriction enzymes BpmI, BsaBI, BstEII-HF, PstI-HF, and RsaI. The digested products were mixed with a DNA size standard and visualized by capillary electrophoresis using an automated sequencer. The two generated fragment profiles (blue and green peak) from each specimen were analyzed.

Both wing morphometric and T-RFLP analyses can be used for species identification. The advantages and drawbacks of each technique for applying in species identification are discussed.

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THE STUDY AND APPLICATION OF UNDERWATER DECOMPOSITION FROM AN ENTOMOLOGICAL PERSPECTIVE FOR THE PURPOSE OF POST-MORTEM INTERVAL ESTIMATION

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Although the decomposition of human remains in water is known to differ from that on land, very little research has been conducted into how these differences extend to forensic entomology. To date, no research covering insect succession on remains decomposing in aquatic habitats, which is vital knowledge for accurately estimating the minimum post-mortem interval, has been undertaken in the United Kingdom. A pilot study is underway in Portsmouth, South East England, to compare insect succession on rabbit carcasses allowed to decompose naturally in small plastic tanks of fresh stream water and of sea water. Preliminary data suggest that colonisation of remains in fresh water occurs earlier than in salt water, where the carcass has been visited by known early colonisers of remains but no oviposition has occurred.

During the decomposition process, soil and water samples will be taken which can then be analysed to identify any changes in the microfauna as decomposition occurs, and the skeletal remains of the carcass from the non-aquatic woodland study will be monitored for approximately one year for beetle gnawing. Species colonisation patterns for each habitat will be discussed.

Following this initial testing phase, further field studies will be conducted in which piglet carcasses will be allowed to decompose naturally on land (in a small local wooded area), in fresh water (in a pond located in the same wooded area) and in sea water (suspended from a raft in Langstone Harbour).

MOLECULAR IDENTIFICATION OF FORENSICALLY IMPORTANT CALLIPHORIDAE AND SARCOPHAGIDAE SPECIES USING ITS2 NUCLEOTIDE SEQUENCES

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Necrophagous flies are useful evidence for estimating the postmortem interval (PMI). To estimate PMI in forensic entomology, the identification of fly species is essential because growth rates vary with the species. The molecular identification has been suggested as an alternative strategy to identify species due to the limited number of expert taxonomists. Previous studies showed that the mitochondrial cytochrome c oxidase subunit I (COI) marker could be used to identify fly species. However, in some cases, these maternally inherited markers cause confusion. Therefore, nuclear DNA markers such as the internal transcribed spacer2 (ITS2) are also required.

The 11 Calliphoridae and 5 Sarcophagidae fly species collected between 2005 and 2014 in Korea. The specimens were firstly identified using a morphological approach, and then processed to perform a molecular identification. The specimens were utilized for PCR amplification and nucleotide sequencing of ITS2 locus. Phylogenetic trees and pairwise distance matrices were generated from obtained nucleotide sequences.

According to this study, the 11 Calliphoridae and 5 Sarcophagidae fly species could be distinguished using ITS2 nucleotide sequences. The interspecific distances were all above 0.019 except for that between the sibling species. The sibling species such as *Lucilia illustris* and *Lucilia caesar* were also distinguished even the very low level of interspecific diversity (0.003). The sequence distances of ITS2 locus in intraspecies were 0.000 for 13 fly species and 0.001 for 3 fly species. The phylogenetic tree of Calliphoridae fly species showed no species- or subfamily-level paraphyly. Also, The phylogenetic tree of Sarcophagidae fly species demonstrated no species-level paraphyly.

In conclusion, the ITS2 marker was possible to successfully distinguish the 11 Calliphoridae and 5 Sarcophagidae fly species collected in Korea. This is the first study that widely analyzed nucleotide sequences of the ITS2 locus from Calliphoridae and Sarcophagidae fly species collected in Korea.

CALLIPHORA VICINA DEVELOPMENT UNDER WIN 55,212-2 CANNABIONOID INFLUENCE

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The blue bottle fly *Calliphora vicina* (Robineau-Desvoidy, 1830) is one of the most frequent species in the Basque Country (North of Spain) and an early colonizer of human remains. It has been reared under laboratory conditions and isomegalen- together with isomorphen-diagrams have been established between 5 and 30ºC. Thanks to the collaboration of researchers from the School of Medicine at the University of the Basque Country (UPV/EHU), a research under controlled conditions has been done to study the potential influence of cannabinoids in combination with an *anesthetic in the development of the maggots*. Maggots were reared in rat carcasses that received an overdose of WIN 55,212-2 (synthetic cannabinoid) and *Chloral hydrate (anesthetic)*. A control was euthanized with CO2 to prevent any chemical influence in blowfly development. Three parameters were evaluated, development time, maggot size during development, and adult size at the end of it. We observed assortment in maggot size at the beginning of the development (D1-D4) in carcasses with drug; maggots increased their size after D5, and it did not affect to the final size of the adults. With this research we confirm that drug consume can influence insects development and may introduce an error in the estimation of the postmortem interval (PMI) based on the age of the maggots. Therefore, drugs should not be underestimated when estimating PMI based on the period of insect activity (PIA).

***Key words**: Calliphora vicina*, Calliphoridae, cannabinoid WIN 55,212-2, *Chloral hydrate*, entomotoxicology, *post-mortem* interval estimation.

IDENTIFICATION OF SOME FORENSICALLY IMPORTANT BEETLES (COLEOPTERA: SILPHIDAE) BASED ON COI GENE IN INDIA

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Forensic entomology is the application of insect biology in criminal investigations. Two main orders of insects which are considered important from evidential point of view are Diptera and Coleoptera. But most of the studies are based on flies while a little data is available regarding importance of beetles in forensic entomology. However, generations of entomologists have realized that not only the flies, but also the beetles found on corpses tell a story. Beetles are tends to be associated with the later stages of decomposition process, which is very important in terms of dry bones of the body. They are generally found on corpse when it is more decomposed and their investigation has enabled scientists to determine the time of death of corpse, PMI (Post mortem interval).The diversity of beetles found on a body changes over time and can therefore provide evidence of ecological succession. But the most crucial step in using insects as evidence in criminal investigations is the accurate identification of that particular insect which is found on the dead body. Morphological identification of beetles poses a great problem as keys are not available for each and every family of Coleoptera and most of the forensic experts does not have entomological background. In this scenario, different molecular markers can be used as the great tools for identification.

Several nuclear and mitochondrial genes can be used for identification purposes. In present study, cytochrome oxidase subunits one (COI) sequence on mitochondrial DNA was studied for molecular identification of forensic significant beetles. Seven beetles species belonging to superfamily Staphylinoidae and family Silphidae were collected from two locations in India. Collected specimens belonging to 2 subfamilies and 4 genus were *Necrophila* (*Calosilpha*) *ioptera* (Kollar & Redtenbacher, 1848), *Necrophila* (*Deutosilpha*) *rufithorax* (Wiedemann, 1832), *Necrophila* (*Calosilpha*) *cyaniventris* (Motschulsky, 1870), *Necrodes littoralis* (Linnaeus, 1758), *Necrodes nigricornis* (Harold, 1875), *Nicrophorus nepalensis* (Hope, 1931) and *Thanatophilus minutus* (Kraatz, 1876). CTAB method was used for the DNA extraction from beetles and obtained sequences were used for phylogenetic analysis of these beetles.

FORENSIC FLIES OF THE HUNGARIAN NATURAL HISTORY MUSEUM

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Forensic entomology –study of insects for medico-legal purposes – is an important ecosystem service. There are many ways insects can be used to help solving a crime but safe identification is one of the most important criteria. Well-identified comparative materials preserved in museum collections are essential for forensic studies.

Though the Hungarian Natural History Museum collection survived the World War II, in 1956 the Zoological Department was hit by a soviet fire bomb. The fire destroyed 75% of the whole Diptera collection (250,000 fly specimens). At present the HNHM has one of the largest Diptera collection in Central Europe, housing more than one million specimens. The Palaearctic Region is the best represented in the collection. It is richin Diptera from Mongolia, Afghanistan and Korea. The Oriental Region (Vietnam, Nepal, Malaysia, Thailand, Indonesia and Taiwan) and some of the Africa countries are also well represented. 75% of the specimens identified to species level, but almost every specimen is determined at least to family level.

Two-thirds of the collection is from the Carpathian Basin (so far more than 6,500 have been proved from Hungary alone), representing almost all the important forensic species from this region. They belong to the following families: Phoridae, Sepsidae, Heleomyzidae, Piophilidae (incl. Thyreophoridae), Milichiidae, Sphaeroceridae, Scatophagidae, Anthomyiidae, Fanniidae, Muscidae, Calliphoridae and Sarcophagidae. Majority of the specimens are pinned but there are also an imago (20,000 ind.) and larvae (19,000 ind.) collections preserved in ethanol. The digital inventory of the collection is availableat generic while the Carpathian Basin collection registered at species level. Database of slide preparations is also available.

Current research activities by the staff and associated researchers are taxonomy and systematics of Sphaeroceridae, Agromyzidae, Heleomyzidae, Drosophilidae, Chamaemyiidae, Milichiidae, Cryptochetidae, Carnidae, Hybotidae, Empididae, Pipunculidae; Dipteran pollination; Diptera communities of small, ephemeral sources; blood sucking vector flies and forensic flies.

Since the mid-1980s three significant reference works were edited by former curators the 13 volumes of Catalogue of Palaearctic Diptera, the 4 volumes of the Contributions to a Manual of Palaearctic Diptera (including a forensic dipterology chapter by F. Introna and C. P. Campobasso) and the Checklist of the Diptera of Hungary.

CHECKLIST AND RECORDS OF THE MOST COMMON SARCOPHAGOUS DIPTERA FROM NORTH ALGERIA

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Knowledge of the carrion-breeding species present in a location during a particular time is important and necessary to apply forensic techniques. Algeria is situated in North Africa, bordered by the Mediterranean Sea in the north. Data regarding the occurrence of sarcophagous insects are not available in Algeria, therefore, the aim of this work is to identify and inventory the most important sarcophagous Diptera in northern Algeria to improve fundamental knowledge regarding the distribution of these species.
The specimens were collected between 2011 and 2015 on human corpses, animal carcasses and baited traps and were morphologically identified.

Identified specimens belong mostly to the Calliphoridae family followed by the Sarcophagidae and the Muscidae families. The Calliphoridae are represented mainly by *Lucilia sericata* (Meigen, 1826) and *Chrysomya albiceps* (Wiedemann, 1819) during the hot seasons and by *Calliphora vicina* (Robineau-Desvoidy, 1830) during the cold seasons. The recorded flesh flies are *Sarcophaga africa* (Wiedmann, 1824) and *Sarcophaga carnaria* (Linnaeus, 1758). Muscidae species are mainly *Muscina stabulans* (Fallén, 1817) and *Hydroteae (Ophyra) capensis* (Wiedemann, 1818).

Data from this study provide basic information on carrion insects' fauna in Algeria. They also form a basis for similar studies in different geographical and climatic regions of Algeria.

Keywords**:** Diptera, carrion, Calliphoriae, Sarcophagidae, Muscidae, North Algeria.

MEGASELIA SCALARIS (DIPTERA: PHORIDAE) ACTIVITY AT DIFFERENT TEMPERATURES IN LONG AND SHORT PHOTOPERIODS

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Circadian clocks have evolved to synchronize physiology, metabolism, and behaviour to the 24-hour geophysical cycles of the Earth. The understanding of the circadian clock mechanism is a crucial element of forensic entomology because it is able to control routines such as locomotor activities, location of food sources, feeding, mating, ovipositing, and emergence times. Colonization of carrion and human cadavers by insects allows for the minimum Post Mortem Interval (mPMI) to be estimated.

The prevailing opinion is that flies that are the first colonizers of a cadaver are not active during the night-time and therefore do not oviposit during this time. Determining the prevalence – if any – of nocturnal activity in forensically important flies, is fundamental for an accurate estimation of the mPMI.

Previous studies demonstrated that the scuttle fly *Megaselia scalaris* (Diptera: Phoridae) has nocturnal activity during the night and in dark conditions under 12:12 LD photoperiod in controlled condition.

In this paper we present the effects of longer 16:8 and shorter 8:16 photoperiods on the activity of this fly at 15, 20 and 25°C. Independently to the experimental temperature we demonstrated that after being entrained in long and short photoperiods, flies recover a 24hr cycle if maintained in dark conditions, confirming the role of the circadian clock in the activity of this fly. Experiments showed that the total amount of activity depend on temperature and that the evening peak is more pronounced than the morning peak.

As in previous work, these experiments were also performed using Trikinetics technology, commonly used in *Drosophila* studies, which allows for factual data rather than observational data as reported in many articles.

EFFECTS OF ETHANOL ON THE DEVELOPMENT OF *MEGASELIA SCALARIS* (DIPTERA: PHORIDAE)

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In Forensic Entomology the estimation of the age of the insects is used for the estimation of the minimum post mortem interval (mPMI). Insect development rate is mainly temperature dependent despite of other parameters can affect the developments. Several studies demonstrated that drugs and other chemicals can affect the growth of larvae, feeding on the dead body, leading to incorrect mPMI estimations. The presence of high level of ethanol is associated with several deaths.

The aim of this study was to investigate the effect of ethanol on the development of *Megaselia scalaris*, a common species in indoor cases both in Europe and in the USA. This species is very important for mPMI estimation in indoor cases, as observed by the authors and reported in the specific literature.

Larvae of *M. scalaris* were reared on pork liver with four different concentrations of ethanol (0=control, 1ml, 2ml, 4ml on 32 gr of maggot food (commercial pet food, patè). Despite ethanol affect the developmental time (larval eclosion delayed, larval development accelerated) it does not affect the larval size (ANOVA, $p=0.432$) and the pupal size (ANOVA, $p=0.946$).

In conclusion this experiment demonstrated that on *M. scalaris* ethanol has an effect on the immature developmental time but not on the immature stage length with consequences on the mPMI estimation.

A COMBINED PROTOCOL FOR IDENTIFICATION OF MAGGOTS OF FORENSIC INTEREST

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In Forensic Entomology the estimation of the age of insects is used for the estimation of the minimum post mortem interval (mPMI). Insect development rate is mainly temperature dependent and species specific, so correct species identification is fundamental for any other consideration.

Species identification is usually performed by the classic morphological identification based on phenotipic features analyses. More recently the molecular approach based on sequencing and comparison of specific mitochondrial or nuclear regions has been developed and largely used, specially for immature stages of insects development.

In the majority of the cases, the molecular identification is based on a destructive approach of the morphological characters. For this reason, depending on the legal system, it requires a specific authorization of the authority in charge of the case, especially when only few larvae or insect fragments are available.

In this paper we demonstrate that a molecular identification can be performed several times on the same specimen (larva) without affecting the anatomical characters used for morphological identification. The suggested technique allows the preservation of the larval esoskeleton and of the not used tissue in the same vial under ethanol as preservative solution in order to be able to repeat both the molecular and morphological analyses.

The technique has been tested using larvae of different size from centimeters [*Calliphora vomitoria* and *Lucilia sericata* (Diptera: Calliphoridae)] to millimeters [*Musca domestica* (Diptera: Muscidae) and *Megaselia scalaris* (Diptera: Phoridae)]. The cuticle was removed from each larva, fixed by diaphanization and stored in ethanol: soft tissues were progressively halved till obtaining the smallest fragment which was $1/_{1024}$ for *C.vomitoria*, $1/_{512}$ for *L.sericata*, $1/_{128}$ for *M.domestica* and $1/_{64}$ for *M.scalaris*. DNA was successfully extracted from each larval fraction and the obtained amount was enough for the amplification and sequencing of a portion 650 bp long of COI gene. Molecular identification confirmed the previous morphological identification.

This protocol allows the repetition of both the morphological and molecular identification at least for immature stages of flies species belonging to Diptera order. Furthermore this protocol reduces the risk of losing the evidence and guarantees any further requested analysis.

EFFECT OF FUR ON THE MICROBIAL AND ENTOMOLOGICAL COMMUNITIES ON RABBIT CARCASSES: FINAL RESULTS

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Different factors, both intrinsic and extrinsic, have been reported affecting the decomposition of a carrion/body. These factors mainly interact with the speeds of the biological and chemical reaction happening after the death. The biological reactions are mainly due to the activity of microorganism and insects. Pigs (*Sus scrofa domesticus*) have been used as a model for human studies and the results obtained have been applied to other mammals without considering the effect that fur can have on the decomposition process and on the insect or microbial colonization. In order to investigate this point, rabbits (*Oryctolagus cuniculus)* with fur and without were used in two sets of experiments in Huddersfield in 2014 (summer) and in 2015 (spring).

Entomological data for the first experiment revealed the presence of Diptera Calliphoridae (*Lucilia sericata, Calliphora vicina, Protophormia terranovae*), Muscidae (*Ophyra* sp); Sphaeroceridae (*Leptocera caenosa, Coproica vagans, Coproica hirticula, Coproica hirtula*), and Piophilidae (*Allopiophila vulgaris*), Hymenoptera Pteromalidae (*Nasonia vitripennis*) and Coleoptera Cleridae (*Necrobia rufipes*) and Dermestidae (*Dermestes lardarius*). Differences in colonization time were observed only in spring: animals without fur were colonized two days before animals with fur. No significant differences were observed in summer experiment.

The microbial community was investigated using BIOLOG EcoPlate™ and by pyro-sequencing (data under analysis). The functional diversity of the bacterial community on all carcasses showed a big variability dependent on the stages of decomposition and the sampling region (skin, mouth, soil-carrion interface). The content of water seems to play the most important role in the bacterial community growth, whereas the presence or absence of fur does no affect the functional diversity (TWO WAY ANOVA: fur-no fur $p=7.121$; body regions $p=0.00$; interaction $p=0.952$). At the beginning of the sampling the bacterial community is very high in the mouth area, whereas the community in the interface soil-carrion is negligible. This community increased its diversity during the decomposition process through to the end of the experiment (4 months). The community on the exposed skin is a function of the drying process with a belt shape: limited diversity at the beginning and at the end of the decomposition process and a maximum during the active decomposition. At the phylum level four main phyla of bacteria were found among analyzed carrions. During Active Decay of the decomposition process Proteobacteria was the most abundant phylum (68.8%). followed by Bacteriodetes (33.17%). Firmicutes (13.89%) and Actinobacteria (3.49%). Over the decomposition. Proteobacteria decreased becoming the second most abundant phylum (24.47%) during the Advanced Dry stage. Bacteroidetes increase becoming the most abundant phylum (66.83%). Also Actinobacteria increased (18.38%) while the amount of Firmicutes didn't change significantly. The analysis at the family level was able to highlight differences at the temporal scale but as well between carrion with and without fur.

AN EXPERIMENTAL APPROACH TO CHARRED ENTOMOLOGICAL EVIDENCE IDENTIFICATION

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Despite the great importance of entomology in the determination of time of death, there is very little data in the literature concerning its application to the frequently encountered scenario of burnt bodies. In addition, a commonly asked question in court is whether it is possible to determine if a body has been burnt before or after decomposition. In order to answer this question some experiments were performed using puparia of *Calliphora vomitoria*, *Calliphora vicina*, *Lucilia sericata* (Diptera, Calliphoridae) and *Megaselia scalaris* (Diptera, Phoridae) in order to estimate their burn point.

A hot-stage microscope with a digital imaging system was used to record pictures and reflected light intensity profiles of puparia heated at 10 °C min^{-1} to 700 °C under static air. Under these conditions, the pictures (recorded at 25 °C intervals) showed that the puparia are completely destroyed between 475 and 550 °C. No significant differences were detected between species ($p=0.09$). Interestingly, the shape of the posterior region and the spiracles were not affected by the heating until immediately prior to complete combustion allowing potential identification of the samples.
The results to date can be considered as an useful tool when investigating burned remains.

IFLY: A MOBILE FORENSIC ENTOMOLOGY APPLICATION FOR CAPTURING SITE SPECIFIC DATA

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The use of mobile computing applications in the field of forensic entomology is an emerging area that revolutionizes the accuracy of capturing data in the field, allowing for reliable data gathering, scene visualization, archiving and computations in remote locations. Moving from paper record keeping to mobile computing poses challenges and opportunities in developing the type of the data collected, and expands our ability to understand spatial relationships between species in ways not previously easy to achieve. Additionally, this transition presents an opportunity for the integration of multiple user datasets for metadata analysis. We present iFly®: an iOS® application for the iPad®that provides a central input format that is based upon the body of the carrion being investigated. This applicationcreates a mobile platform for capturing data on-site, allowing for the integration of environmental data directly into the site database and allows for specimen tracking withease,a feature not previously obtainable from traditional techniques. Central to iFly® is the use of integrated visual keys for quick identification of mature specimens that automatically captures nodal decisions and properly attributessource for the dichotomous keys utilized. It also allows for the assessment of total body score for decomposing remains, and the automated call-down of weather data from national weather centers, with the ability to identify and specify the weather stations and data date ranges needed. We plan to implement this program beginning in the United States, and then continuing to other regions as resources and partnerships are available.

NON-INVASIVE MOLECULAR APPROACH FOR ADULT FLY IDENTIFICATION

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A correct species identification is the key point for all the fields dealing with studies that involve insects. For these purposes, alongside the common morphological identification, a molecular approach, based on the characterisation and analysis of specific mitochondrial or nuclear regions is becoming increasingly frequent in last years.Despite the good results that can be reached, the molecular characterization is frequently identified as an invasive technique that often leads to the destruction of the sample. A good preservation of the integrity of an entomological sample is fundamental although it may have different purposes based on the field of work. In this work we propose a non-invasive method that allows for DNA extraction withoutdamaging the sample. This technique was tested on abdomens of Sarcophagidae flies collected in different places and on different years (from 2004 to 2015). DNA was extracted using a QIAamp® DNA Mini kit (Qiagen). Alterations were made to the amount of proteinase K used (40ul) and the incubation time (24 hours) as both of these changes increased the total DNA yield. TheCytochrome c oxidase subunit I (COI) and the mitochondrial encoded NADH:ubiquinoneoxidoreductase core subunit 5 (ND5) target genes were then amplified using a PCR before being visualised on agarose gel. Samples that showed a clear band of the appropriate molecular weight were then purified and sequenced for species identification.DNA was successfully amplified using primers aimed at the ND5 gene but not for that of the COI.This could be related to the difference in base length of the amplifiedsequence, from 297 bp of the ND5 gene to 658 bp of the COI's. PCR performed with primers designed for a COI smaller inner region showed positive results.

This study examines a way to non-invasively identify species through their genomic DNA.From both a forensic interest and the perspective of a museum collection maintaining the physical characteristics of entomological samples is desirable as it allows for repeat analysis and preservation of the morphology.

Collaborative exercise 2015

	Sample 1				Sample 2				Sample 3				Methods used			
CODE	Calliphoridae	*Lucilia*	*caesar (*)*	Gender	Calliphoridae	*Phormia*	*regina*	Gender	Piophilidae	*Stearibia*	*nigriceps*	Gender	Morphology	Time taken (min)	DNA	Time taken (days)
14	x	x	*caesar/illustris*	-	x	x	x	-	Sepsidae	-	-	-	x	-	x	-
27	x	x	x	x	x	x	x	x	x	x	x	x	x	100	-	-
41	x	x	x	x	x	x	x	x	x	x	x	x	x	60	-	-
54	x	x	x	x	x	x	x	x	x	*Piophila*	x	x	x	25	-	-
68	x	x	x	x	x	x	x	x	x	x	x	x	x	480	-	-
82	x	x	x	x	x	x	x	x	x	x	x	x	x	30	-	-
95	x	x	x	x	x	x	x	x	x	x	x	x	x	30	-	-
109	x	x	*illustris*	x	x	x	x	x	Sepsidae	-	-	-	-	-	-	-
123	x	x	x	x	x	x	x	x	x	x	x	x	x	30	-	-
136	x	x	x	x	x	x	x	x	x	x	x	x	x	30	-	-
150	x	x	*caesar/illustris*	-	x	x	x	-	x	-	-	-	-	-	-	-
163	x	x	x	x	x	x	x	x	Muscidae	*Thricops*	*beckeri*	x	-	-	-	-
177	x	x	x	x	x	x	x	x	x	x	x	x	x	15	-	-
191	x	x	x	x	x	*Chrysomya*	*albiceps*	x	x	x	x	x	x	90	-	-
204	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
218	x	x	x	x	x	x	x	x	x	x	x	x	x	40	-	-
231	x	x	x	x	x	x	x	x	x	x	x	x	x	60	-	-
245	x	x	x	x	x	x	x	x	x	x	x	x	x	30	-	-
272	x	x	x	x	x	x	x	x	x	x	x	x	-	-	-	-
286	x	x	*caesar/illustris*	-	x	x	x	-	x	-	-	-	x		x	3d
300	x	x	x	x	x	x	x	x	x	x	x	x	x	30	-	-
313	x	x	x	x	x	x	x	x	x	x	x	x	x	10	-	-
327	x	*Chrysomya*	*rufifacies*	-	x	*Chrysomya*	*rufifacies*	-	Sarcophagidae	*Boettcherisca*	*karnyi*	-	-	-	-	-
340	x	x	x	x	x	x	x	x	x	x	x	x	x	720	-	-
354	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
368	x	x	*illustris*	x	x	x	x	x	x	x	x	x	x	-	x	-
381	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
395	x	x	x	x	x	x	x	x	x	x	x	x	-	-	-	-
408	x	x	x	x	x	x	x	x	x	x	x	x	x	25	-	-
422	x	x	*illustris*	x	x	x	x	-	Sepsidae	-	-	-	-	-	-	-
436	x	x	*illustris*	x	x	x	x	x	x	x	x	x	x	15	-	-
449	x	x	*ampullacea*	x	x	x	x	x	x	x	x	x	x	180	-	-
463	x	x	x	x	x	x	x	x	x	x	x	x	x	120	-	-

(*) The distinction between Lucilia species caesar and illustris is generally possible based on the morphology of the genitalia.
The answers "caesar" and "caesar/illustris" were both considered correct.

- = no answer
x = correct answer

„FUTURE 3D”

WORKSHOP SYLLABUS

Identification key to the important forensic flies family in Europe

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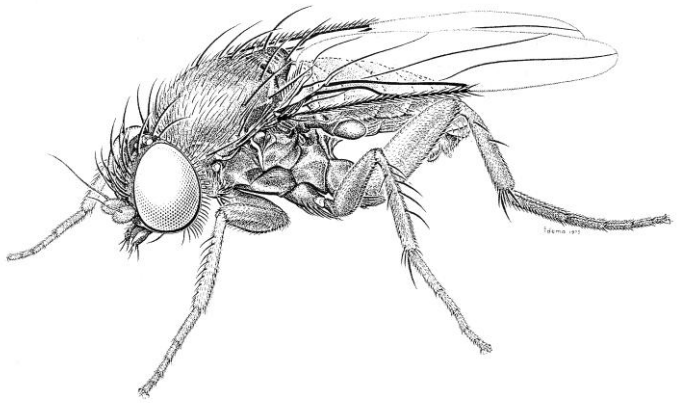
1 Antennae usually long, with scape and pedicel and at least 6 homonomous flagellomeres, usually longer than the head and thorax. Palpi usually with 3-5 segments **Diarchineura, Polyneura, Neoneura, Anisoneura (oldname, non monophyletic group: Nematocera)**

- Antennae shorter, scape and pedicel usually short, flagellomere sheteronomous: first flagellomere large, (sometimes annulated), second and other flagellomeres in the form of a stylusor arista; usually less than 6 flagellomeres. Palpi usually with 1 or 2 segments .....(**Brachycera) 2**

2 R veins strongly thickened, terminating along with costa at about middle of anterior margin. Other veins much weaker, usually rather parallel, running obliquely across wing. Antenna apparently two-segmented, globular or discoid with dorsal arista. Head and palpi usually with strong, serrate setae. Mostly hump-backed small flies with strong legs ..... **Phoridae**

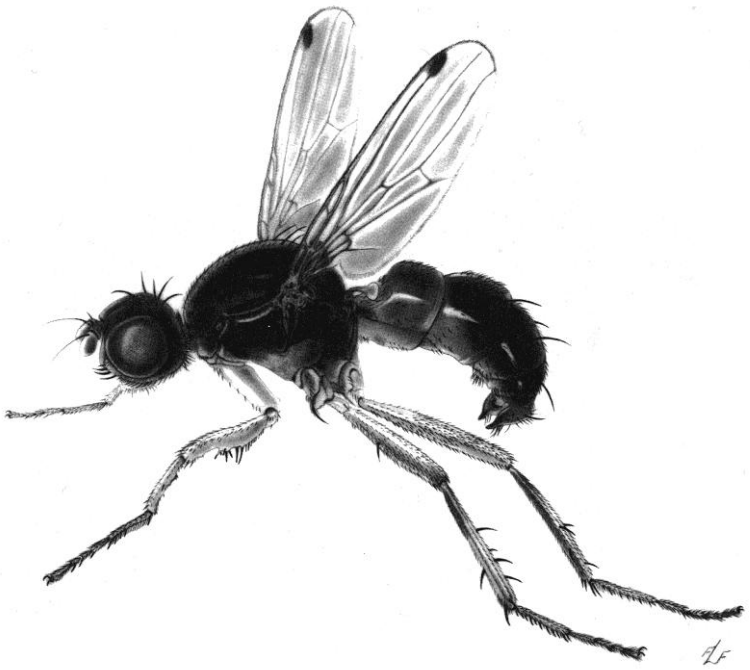


- Wing wenation more complex with at least one cross wein **3**

3 Greater ampulla present as a distinct bulbous swellings lightly below and in front of wing insertion. Pedicel dorsally always with a distinct linear seam. Lower calypter (thoracic squama) usually well-developed. Vein Sc more or less complete and separate from R1. In most genera and species one or more vibrissae as well as incurved lower fronto-orbital setae present ..... **(Calyptrata) 8**

- Greater ampulla not markedly developed. If better developed, pedicel without a dorsal linear seam (it is soin a majority of the families which run here), and/or lower calypter (thoracic squama) small or even vestigial. Combination of other characteristics different…… **(Acalyptrata) 4**

4 Costa without any break.One or more setae present at the posteroventral margin of the posterior spiracle. Small bare black flies, head rounded, arista bare, ocelli present ……. **Sepsidae**

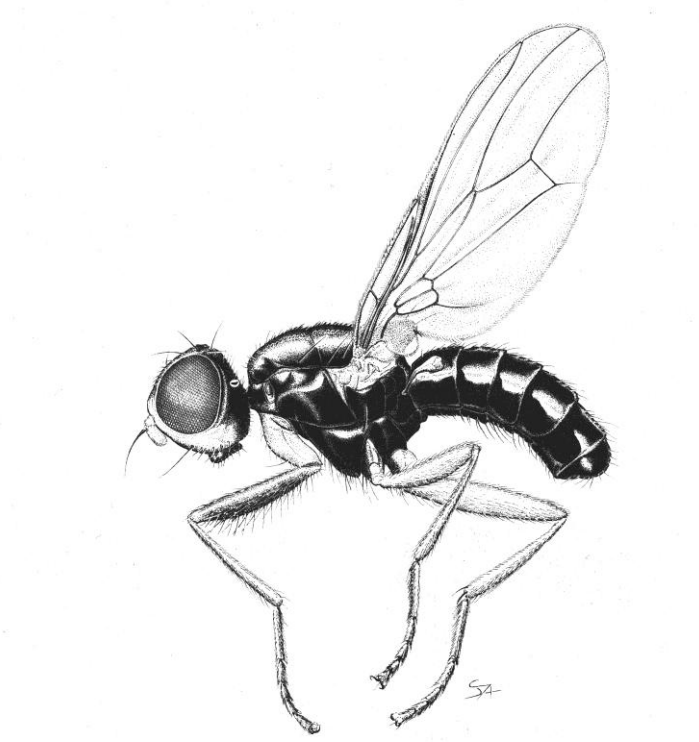


- Costa with at least a subcostal break. **5**

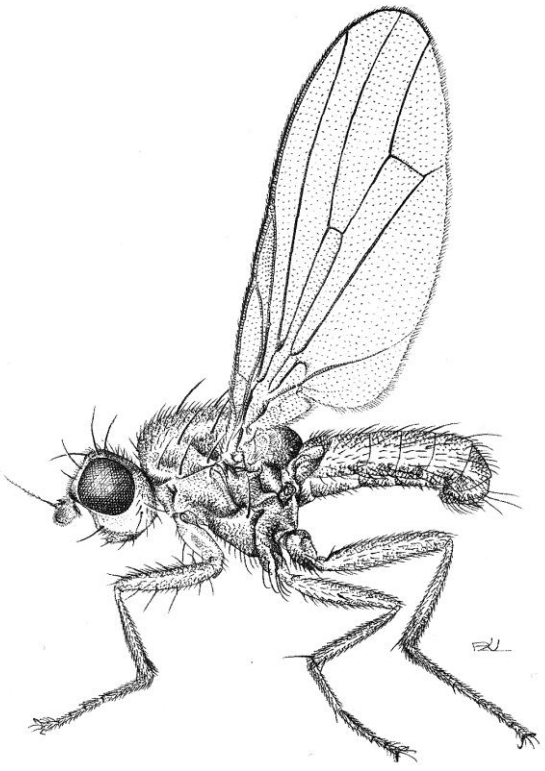
5 Subcostal vein complete, separate from vein R1 along it's whole length and ending in costa. **6**

- Sub costal vein in complete, either in distinct distally or fused with vein R1 distally (proximally to costa), or joined to R1by sclerotization of the intervening area. 7

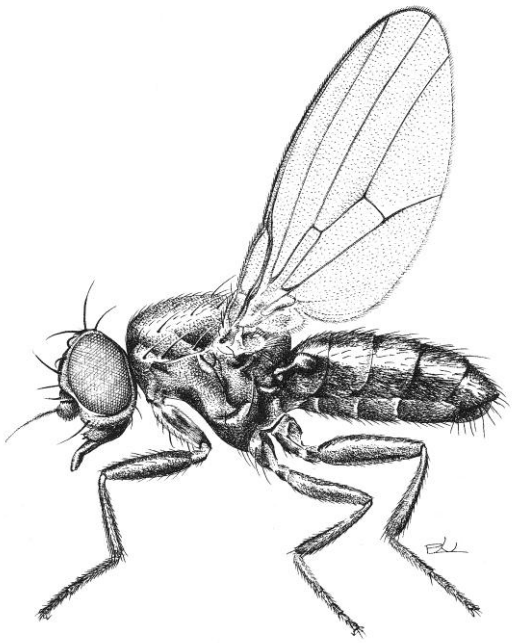
6 No tibiae with dorsal preapical seta. Postocellar setae divergent ..... **Piophilidae**



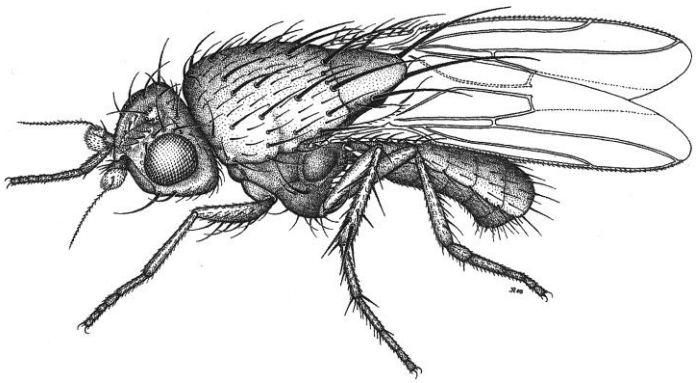
- Some orall tibiae with dorsal preapical seta. Postocellar setae convergent .. **Heleomyzidae**



7 Lower fronto-orbital setae medio-clinate. Hind basitarsus normal..... **Milichiidae**



- Fronto-orbital setae latero-clinate. Hind basitarsus shortened and broadened ……. ……**Sphaeroceridae**



8 Meron usually bare or with weak hairs or setulae. If seldom setose, vein M1 not distinctly bent forward distally **9**

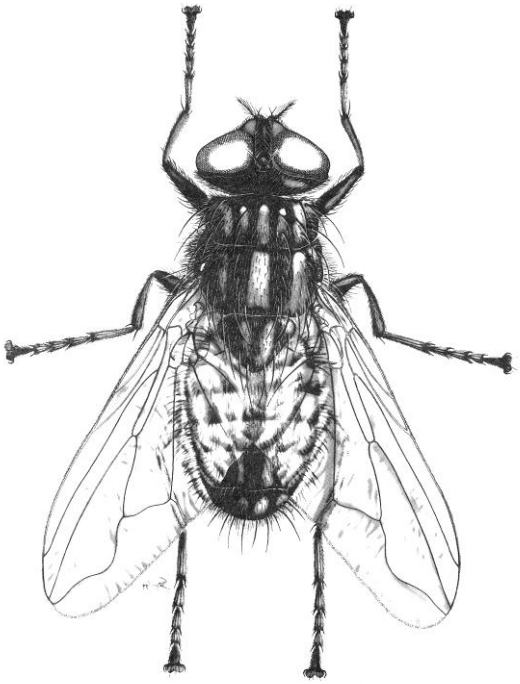
- Meron with a series of long setae arranged in a more or less vertical row. Vein M1 usually strongly bent forward distally..... **12**

9 Vein A1 not reaching wing margin..... **10**

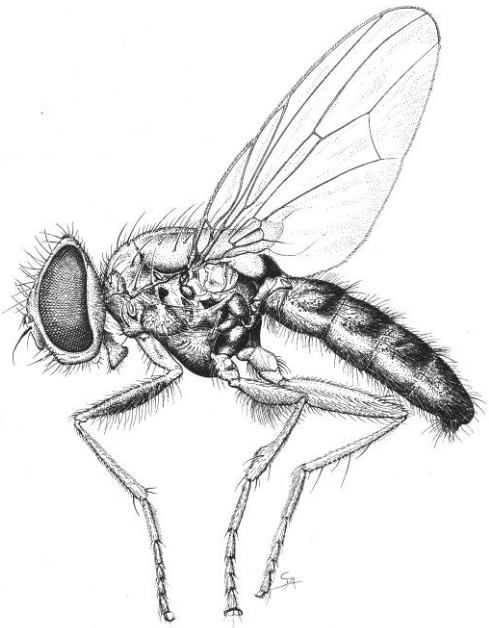
- Vein A1 reaching wing margin; sometimes only as a faint but still distinct crease-like .. **11**

10. Vein A2 not curved forward beyond apex of A1, it's imaginary extension running to wing margin and not meeting Al. Hind tibiain some genera with a strong submedian seta but this is never exactly in dorsal position. In question able cases, Sc diverging from R1 at a

point very near base of both veins; crossed interfrontals present in females of a few genera .....**Muscidae**

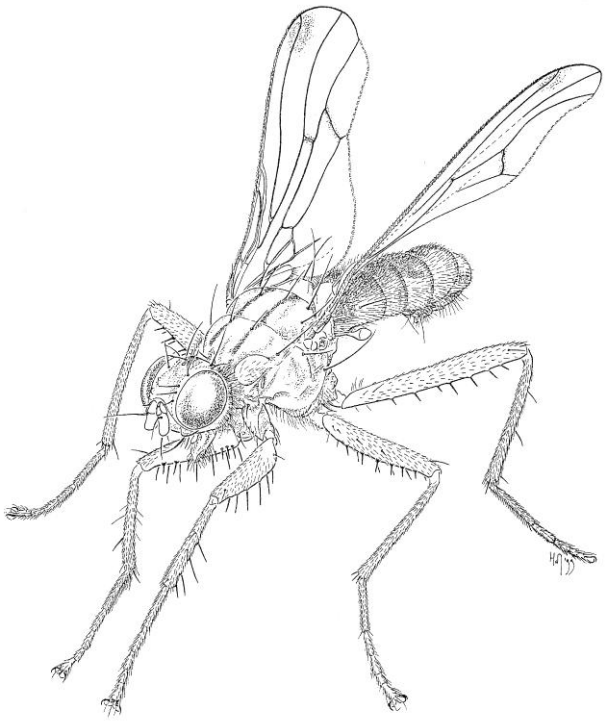


- Vein A2 curved forward beyond apex of A1, it's imaginary extension meeting A1 before wing margin. Hind tibia with a true dorsal submedian seta in line of the dorsal preapical seta. In cases of any bias, Sc diverging from R1 at a point very near base of both veins; crossed interfrontals always absent (females)……...**Fanniidae**

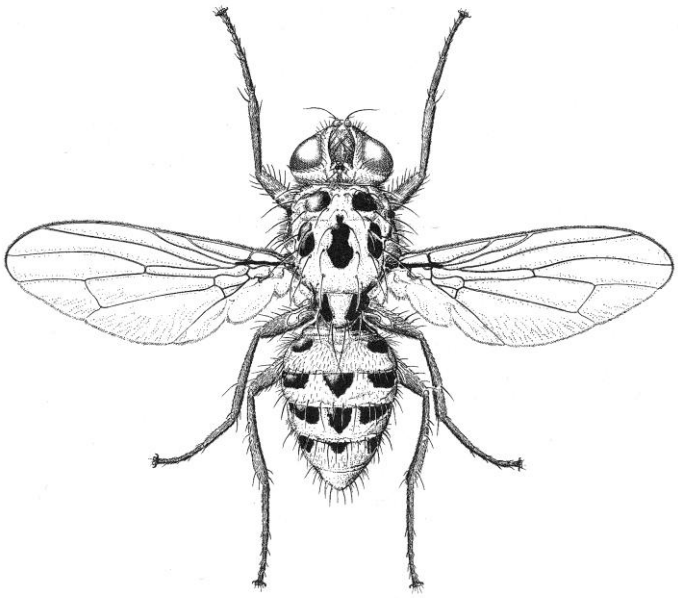


11. Scutellum bare on ventral surface. Occiput ventrally with fine pale, silky hairs. Transverse suture on scutum widely broken medially. Katepisternal setae not seldom

absent. Frons broad in both sexes, frontal vitta (interfrons) without a pair of crossed setae. Lower calypter (thoracic squama) linear, not at all convex and mostly smaller than upper calypter (alar squama) ..... **Scathophagidae**



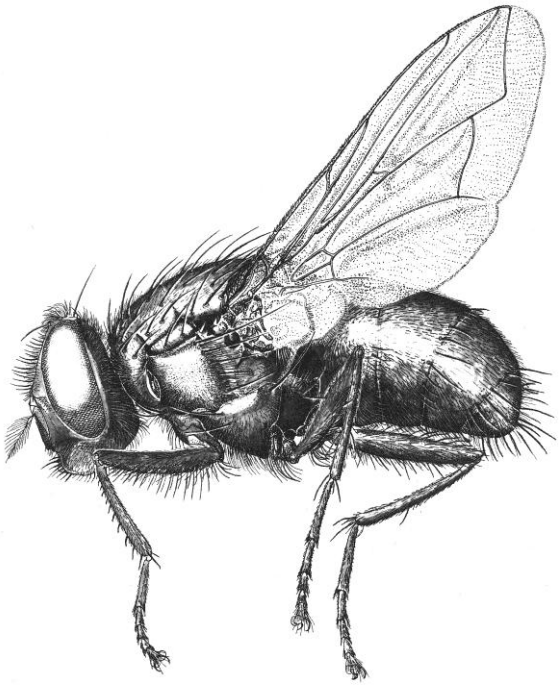
- Scutellum with fine pale hairs (in cases forming a tuft) beneath apex. Occiput ventrally without fine pale, silky hairs but with coarse black setae or hairs. Transverse suture on scutum not or narrowly broken medially. Katepisternal setae always present. Frons narrow in males (at least so in a majority of genera), frontal vitta (interfrons) of females mostly with a pair of crossed setae. Lower calypter (thoracic squama) convex, though may be narrower than, or subequal to upper calypter (alar squama) ..... **Anthomyiidae**



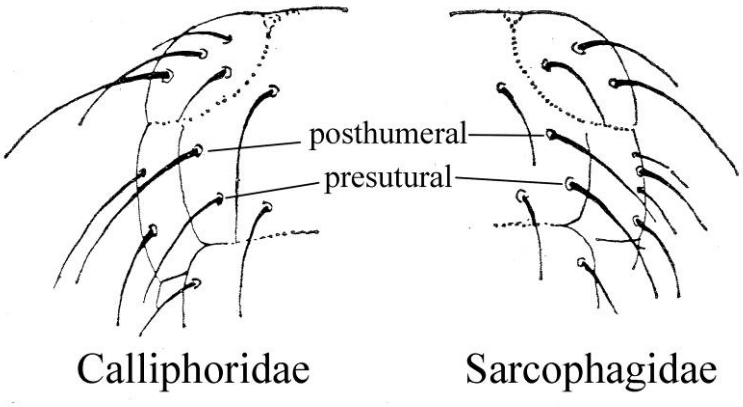
12 Postscutellum strongly developed as a rounded lobe beneath scutellum; anterior lappet of posterior spiracle without tuft of long hairs .....(Tachinidae)

- Postscutellum weakly or not at all developed, lappets of posterior spiracle absent, or anterior lappet with a tuft of long fine hairs toward dorsal margin .....(Be careful)**13**

13 External posthumeral seta distinctly lateral to presutural seta; notopleuron witht notopleural setae. Vein M1 usually angled at a point nearer to wing margin than to apex of cell dm. Abdominal sternites mostly with sensilla trichodea (alpha setae). Colour blue or green metallic (in some cases partly so), or not metallic but shiny; if microtomentose, usually with long fine pale hairs; scutum rarely with three longitudinal stripes, but colour never checkered……**Calliphoridae**

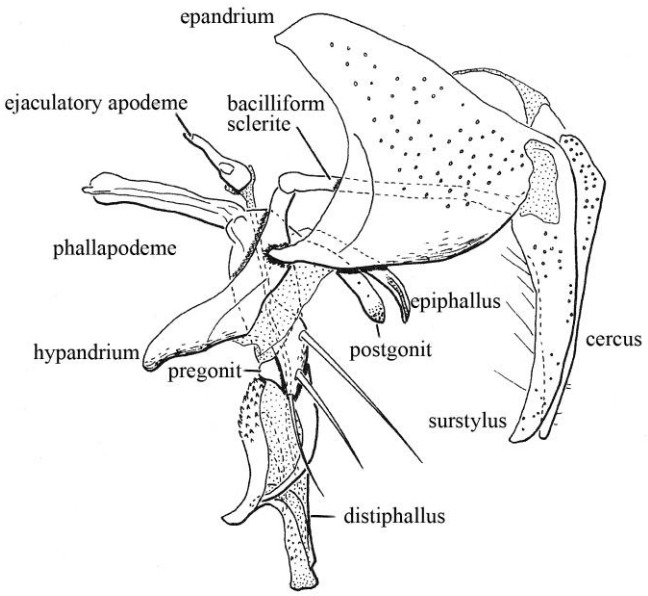


- External posthumeral seta absent, if present, situated medially and upper than presutural seta; notopleuron usually witht wo subprimary setae alternating with the two primary ones, or only two notopleurals. Vein M1 usually angledat a point nearer to apex of cell dm than to wing margin. Abdominal sternites without sensilla trichodea (alpha setae). Colour never metallic, abdomen usually checkered, striped, banded or spotted; scutum frequently with three longitudinal stripes .....**Sarcophagidae**



How to make permanent genitalia preparations

1. Soften abdomen with a drop of water, or in case of small specimens, keep them in a water-vapour chamber for some minutes
2. Cut off the abdomen with small scissors (over water)
3. Immerse the dissected part of the abdomen in some hot sodium-hydroxide (ca. 10% solution) for five to ten minutes; wash it in water
4. Incubate the minconcentrated lacticacid for five to ten minutes, there –after the genitalia are to be dehydrated in ethanol series
5. Preserve the preperatum in glycerol in a plastic microvial or in Euparalon a microscope slide



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