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Establishing Forensic Entomology in Albania - a Primer

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Abstract

Forensic entomology is the study of insects within a dead body, a subspecialty of forensic sciences whereby information is analysed to draw conclusions on legal matters. By calculating the age of immature insect stages feeding of human corpses, a possible evaluation of post-mortem intervals from the first day to several weeks might be available. The attractiveness of a decaying body differs for different necrophagous insects. Therefore, the appearance of this species and their relationship to the stages of decomposition, has been studied intensively on a wide variety of target organisms, humans included. The post-mortem interval (time of death) is a highly important forensic clue during investigation of crime scenes. It is of crucial importance to adopt internationally accepted standard and approaches, whenever this evidence is collected and evaluated for forensic purposes.

Keywords: forensic entomology; insects; post-mortem interval [PMI]; ecological process; decomposition.

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INTRODUCTION

Forensic entomology is a subset of forensic science whereby information and samples of insects and other arthropods are analysed to draw conclusions on legal matters, especially in forensic medicine (1). Years of research and application have turned it into one of the most important tools to establish the time since death in the later post-mortem interval when medical approaches no longer give accurate estimations (1, 2). The use of forensic entomology in the western hemisphere was only recognized at the beginning of the 20th century (3). It encompasses urban entomology, stored product entomology, veterinary entomology and last but not least medico-legal entomology (4). The emphasis in medico-legal entomology (and sometimes in veterinary too) is on estimating the minimum post-mortem interval (PMI_{min}), which is equivalent to the time since the first insect colonization of a body, by determining the age of juvenile insect stages developing on the decaying remains. In addition, successive patterns of insect arrival, persistence and departure from cadavers are important. Forensic entomology can contribute to the success of an investigation days, weeks, and sometimes even years post mortem, depending on the taxa found on the body and the relevant season.

Insects can also provide information when investigating the circumstances surrounding death, e.g. possible poisoning or post-mortem manipulation, as well as providing estimates of the cause and duration of neglect of people in need of care (1).

a. Insects and death

The decomposition of dead organic matter is one of the key ecological processes central to ecosystem functions (5, 6). Even though carrion or cadavers are unpredictable, patchy and ephemeral resources (7), a large group of specialized insect species is able to exploit this resource pulses for survival and reproduction (Table 1). Many of them can be used to a greater or lesser extent for PMI_{min} estimations in a forensic context.

All these species are attracted to decaying matter and some arriving at a corpse immediately after death, often within minutes (8). Still, the attractiveness of a decaying body differs for different necrophagous insects and that's why the appearance of this species and their relationship to the stages of decomposition, has been studied intensively on a wide variety of target organisms, like humans (9, 10, 11), dogs (12) or pigs (13, 14). The so called succession patterns on a decaying body can typically be described based on the decomposition stage the insects arrive and their overall sequence (Fig. 1).

The most important insects in forensic entomology are mainly species of the order of true flies (Diptera) and beetles (Coleoptera). Blow flies (Diptera: Calliphoridae) are usually the first necrophagous insects, colonizing carrion immediately or the first few hours after death.

Table 1 Selection of insects of forensic importance. Adapted from Amendt et al. (1)

Order/Family	Important genera
COLEOPTERA/BEETLES	
Cleridae (Checkered beetles)	*Necrobia*
Dermestidae (Larder beetles)	*Attagenus, Dermestes*
Geotrupidae (Dung beetles)	*Geotrupes*
Histeridae (Clown beetles)	*Hister, Saprinus*
Silphidae (Carrion beetles)	*Necrodes, Nicrophorus, Thanatophilus*
Staphylinidae (Rove beetles)	*Aleochara, Creophilus*
DIPTERA/FLIES	
Calliphoridae (Blow flies)	*Calliphora, Chrysomya, Lucilia, Phormia*
Fanniidae (Latrine flies)	*Fannia*
Heleomyzidae (Sun flies)	*Heleomyza, Neocleria*
Muscidae (House flies)	*Hydrotaea, Musca, Muscina, Ophyra*
Phoridae (Scuttle flies)	*Conicera, Megaselia*
Piophilidae (Skipper flies)	*Piophila, Stearibia*
Sarcophagidae (Flesh flies)	*Liopygia, Sarcophaga*
Sepsidae (Black scavenger flies)	*Nemopoda, Themira*
Sphaeroceridae (Small dung flies)	*Leptocera*
Stratiomyidae (Soldier flies)	*Hermetia, Sargus*
Trichoceridae (Winter gnats)	*Trichocera*
LEPIDOPTERA/BUTTERFLIES	
Tineidae (Clothes moths)	*Tineola*
HYMENOPTERA/(parasitic) WASPS	
Ichneumonidae (Ichneumon wasps)	*Alysia*
Pteromalidae (Fly wasps)	*Nasonia, Muscidifurax*

They therefore have the potential to provide a time estimate closest to the time of death and represent the most important insect group in forensic entomology. Adult blow flies are highly attracted to the odour produced during decomposition and are able to detect those even over large distances e.g. some flies are able to pick up the scent of death from 16 km away (15, 16). The interaction of olfactory stimuli, vision, colour and the presence of individuals of the same species play also an important role in the attraction of necrophagous insects (17, 18). Female blow flies in particular are attracted by the decomposing resources, as they use the carrion for the development of their offspring. Here, ammonia-rich compounds and hydrogen sulphide act as important stimulants for their oviposition as well as moisture content of the decomposing resource and pheromones (19). Most blow flies lay their eggs in body cavities (e.g. eyes, mouth, nose) or in wounds on the dead body, due to optimal conditions for the development of the flies' offspring. Temperature and humidity are thereby the major factors governing oviposition and the subsequent rates of development (20).

b. Myiasis

Myiasis is described as the infestation of living humans and vertebrates with fly larvae which feed at least for a period of time from dead or living tissue, liquid body substance or ingested food of the host (21).

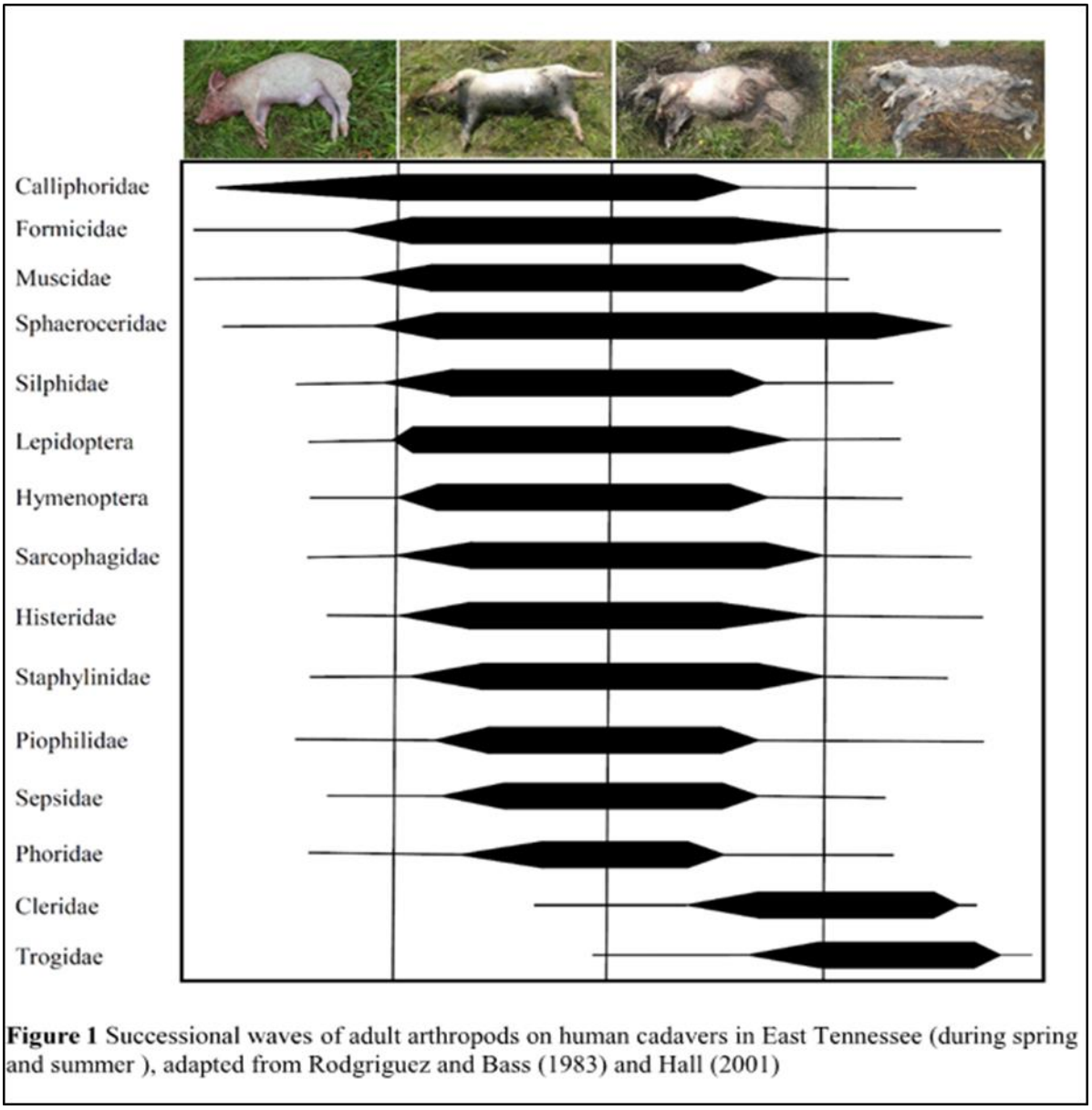

Figure 1 Successional waves of adult arthropods on human cadavers in East Tennessee (during spring and summer), adapted from Rodgriguez and Bass (1983) and Hall (2001)

In addition to obligatory parasitic species whose larvae penetrate the intact skin, there are also facultative parasitic species that use necrotic body areas, that produce comparable substances as described above, as well as fresh wounds for colonization. While this is still mainly a problem of the tropical regions, Europe could be more in the spotlight soon as climate change and globalization enables for the invasion of hitherto non-native species, but also for climatic conditions that promotes the establishment of these species especially of the blow fly genus *Chrysomya* (22). Furthermore, the autochthonous fauna might be impact by heat waves and high humidity. In our latitudes, representatives of the green and bronze shimmering genus *Lucilia* are particularly noteworthy. Such development is of veterinary

and human medicine relevance and forensic entomology expertise will help to understand timelines of infestation but also to combat or even prevent Myiasis. Last but not least, myiasis also has a forensic significance, since an infestation during the victim's lifetime could lead to an incorrect establishment of the PMI. Hints like a bandaging, an unusual place of infestation or an atypical fauna can help here.

Figure 2 Wohlfartiosis (Traumatic myiasis caused by the flesh fly *Wohlfartia magnifica* (Diptera: Sarcophagidae) of sheep eye; photo by Martin Hall, Natural History Museum London.

ENTOMOLOGICAL METHODS FOR PMI_{MIN} ESTIMATION

A PMI_{min} estimation based on entomological material can give evidences for a pinpoint period ranging from 1 day up to more than 1 month (20). It is important not to mistake that period with the time since death because there can be variable time gaps of less than an hour up to several days (or even longer) between death and first colonization by insects. As mentioned before, blow flies are the most important and also dominant taxa on a cadaver right after death. The typical life cycle of a blow fly consists of four sequential stages: egg, larva, pupa and adult. Their development times, and thus their age on the time of sampling, depends not only strongly on the ambient temperature but are also species specific. Therefore, an accurate PMI_{min} estimation is based on the knowledge or reconstruction of the ambient temperature at the scene of the crime. Moreover, the correct species identification is the first essential step when it comes to the entomological evidence itself, which should be done with identification keys for the necrophagous insect fauna of the geographic region where the body was found. There are also approaches to use molecular instead of morphological data for identifying the taxa of interest, e.g. by sequencing a 648-base pair region of the mitochondrial cytochrome-c oxidase subunit 1 (COI) gene. The latter became famous as the "DNA barcoding region" and the resulting DNA barcode libraries are a potential tool for identifying specimens without a significant taxonomic knowledge (23). But under no circumstances should one forget that the distinction between taxonomic units does not necessarily lead to a solid species-specific identification, and that these databases may still contain DNA sequences derived from voucher samples that have been morphologically misidentified. Hence, double-checked identifications (via DNA *and* morphology) are recommended if possible.

Knowing the species identity, the next step is the age determination of its immature stages (24, 25, 26). Age determination is made by relating the

stage of the oldest immature specimens sampled from the cadaver with the environmental conditions, to which they were exposed to. Then it is compared with known growth rate data, recorded from baseline rearing of Diptera, ideally from the same zoogeographical area, at known temperatures. The development of the insects of interest can be visualised in growth curves at constant temperatures, for example in isomegalen (27) and isomorphen diagrams (25), that illustrate morphological changes during fly development, e.g. length growth or moulting, in relation to temperature and time. By means of such diagrams the age can be determined by checking and measuring the immature stages.

A second widely used method for age estimation of carrion breeding insects is the calculation of accumulated degree hours (ADH) or days (ADD), which describe the summed thermal input a species accumulated during growth (28). It is based on the relation between the development rate and the ambient temperature expressed in a linear curve in the midrange of a sigmoidal curve above a lower development threshold (LDT). The LDT defines the temperature at which the development of a specific species stops. ADD and ADH are calculated as followed:

$$ADH = (ambient\ temperature\ [°C] - LDT\ [°C]) \times time\ [hours]$$

$$ADD = (ambient\ temperature\ [°C] - LDT\ [°C] \times time\ [days]$$

The required ADHs and ADDs of species of forensic importance to reach specific developmental stages have been determined in various experimental studies in different geographic regions (29, 30, 31, 32, 33), under constant (25, 26) and fluctuating temperature (34, 35) and under the influence of other biotic factors like intra (36, 37) - or interspecific competition (38). Despite such studies certain parameters might impact the accuracy of the data evaluation, e.g. precocious egg development (39, 40) circadian rhythms (41, 42), development plasticity (43, 44) or diapause (45, 46, 47, 48). They should be keep on mind on a case specific level when estimating the PMI_{min}.

During the late postmortem period, the estimate is based on the presence and absence of certain taxa on the body (49). The study of arthropod succession, i.e. the successive sequence of insect species over time and decay, enables scientists to associate each species or group to a more or less established decomposition stage. Knowing the chronology of insects colonising carrion in a certain area, an analysis of the fauna on a cadaver can be used to give a rough approximation of the PMI in the late postmortem period (50, 51). Forensically useful timetables indicating the presence and sometimes even the relative abundance of different insects at different times, but much more work needs to be done in this area on a local geographic level (52, 53).

a. Example

The application of the entomological method to determine the time of death consists essentially of two main procedures:

1. During the early postmortem period, the estimate is based on a direct age assessment of the oldest individuals that have developed on the body (minimum PMI) using ADD/ADH or isomegalen- and isomorphen-diagrams.

To age fly larvae you can kill and measure them.

- Measure the size of the larvae (length/weight), their stage of development, and check if they are feeding (full crop) or post-feeding (empty crop)
- Determine as accurate as possible the temperature to which the larvae were exposed during their development. This also requires temperature reconstruction of the crime scene
- Relate the size and feeding stage to their age using experimental references.

For details of isomegalen/isomorphen diagrams of typical species like *Calliphora vicina* (27), *Lucilia sericata* (25), *Chrysomya albiceps* (54) or *Protophormia terraenovae* (26) see the original studies.

A PRACTICAL GUIDE FOR COLLECTING AND REPORTING ENTOMOLOGICAL EVIDENCE

a. Sampling, labeling and preserving

For the collection of entomological evidence at a crime scene a special safety clothing, i.e. gloves, overalls, shoe covers or boots, is needed to avoid a contamination of the death scene. Furthermore, a list of following equipment is recommending:

- Tool box
- Protocol sheets to note when, where and what kind of entomological evidence was sampled (*A protocol sheet is added as supplementary data at the end of the paper*)
- Dark pencil or a pen with water-proof and ethanol proofed ink
- Labels for the vials
- Fine and medium forceps
- Spoons for collecting maggots from maggot masses on the cadaver
- Fine paintbrush for collecting eggs and small maggots
- Vials and storage boxes in different sizes
- Shovel for taking soil samples and searching for buried larvae/puparia
- Paper or plastic bags for litter and soil samples
- Tissue paper for handling eggs
- Thermometer for measuring the body and ambient temperature, as well as larvae masses
- Ethanol (75 – 95 %) for preserving dead specimens
- Camera/video for documentation of the death scene
- Cooler bag with re-usable ice packs for storing living insect samples
- A thermos flask with hot water for killing larvae*
- A sieve to drain the hot water*
- Insect net for sampling adult specimens

- 2 temperature Data logger for measuring the temperature at the death scene for at least 5 - 10 days

* if the living samples will be transferred soon (about 1-2 hours after the sampling started) to a laboratory, the killing with hot water (and sieving them afterwards) can be done in the laboratory

b. Methods for storing entomological evidence

Dead specimens should be stored in 70-95 % ethanol. Living specimens should be stored as followed:

Eggs: Place them in ventilated vials with moistened tissue paper on the bottom and transfer the samples to an expert for rearing within 24 h.

Larvae: The majority of specimens should be stored in vials that allow entry of air but preventing escape of the larvae. It is important to note on the vials as well as on the protocol sheet where the samples were taken from. The samples should be handed over to an expert for rearing within 24 hours, until then they should be stored under cold conditions. The remaining specimens should be killed with boiling water and stored in vials filled with 75 – 95 % ethanol. If hot water is not available kill the larvae as soon as the samples arrive in the laboratory. Never insert living larvae directly in ethanol because they will darken within a few days and then an identification is quite complicated.

Pupae: The samples should be transferred for rearing within 24 h, if this is not possible they should be stored under controlled condition (humidity, temperature) in a ventilated vial.

Adults: Living adults should be stored in vials and killed by freezing at – 20 °C for at least 1 h and stored in 75 – 95 % ethanol.

c. Where to collect entomological evidence

(A) On the corpse (at the death scene and during autopsy)

Caution should be exercised when sampling entomological evidence form a corpse using forceps or other tools to avoid postmortem artefacts on the corpse. This sites should be included for sampling:

1. The natural orifices and eyes (preferred oviposition sites)
2. Traumatic wounds (preferred oviposition sites)
3. At the interface between the body and the substrate and especially under the body
4. In the pleats of clothes and pockets, shoes, socks
5. If the cadaver is wrapped in material (carpet, blanket, bag etc.), search in the material
6. From the plastic body bag

(B) At the scene

Check the surrounding area of the body before its removal and afterwards.

Search the area in a radius of at least 2 m. Search beneath everything that provides protection and shade, i.e. in the field under e.g.

stones and between leaf litter, and e.g. under carpets, pillows and behind skirting boards at indoor scenes to ensure that you don't miss any entomological evidence like migrated and pupated specimens:

- *At an outdoor scene* take soil samples up to 2 m away from the body in a depth of 10 cm or even more for pupae or buried larvae. Also collect leaf litter.
- *At an indoor scene* check different rooms, because wandering larvae can disperse widely before pupariation.

d. Microclimate conditions and ecological features of the scene

1. Describe the condition of the corpse and of the death scene using the protocol sheet.
2. Collect temperatures: ambient, body, ground surface, soil and larval masses.
3. Request weather/temperature data from the nearest meteorological weather station for the time since the person was last seen until 5-10 days after the body was found. You will need this data for temperature reconstruction, which is essential for calculating the PMI_{min}
4. If possible, the temperature at the crime scene should be recorded hourly for 5-10 days with an electronic data logger. If this is not possible temperature maxima and minima should be obtained. For more details regarding temperature and its reconstructing see e.g. (55, 56, 57).

e. Documentation

1. Document the name of the instructing authority and principal contact and the time and type of approach.
2. Document date and time of sample collection.
3. Specify the whole sample clearly with A SINGLE code (number and/or name); this code will be your reference to the case in the future, and it has to be placed on any sample.
4. Label each vial you use and note the position of sampling (e.g. head, leg, carpet) on a protocol sheet.

PROCESSING ENTOMOLOGICAL EVIDENCE AT THE LABORATORY

It is strongly recommended to send the insect evidence to an expert in forensic entomology, who is familiar with local species and in processing the specimens at the laboratory.

(A) Rearing immature insects

Any living, immature specimen that is not going to be killed should be reared to the adult stage in a controlled (incubator) or at least monitored environment. For detailed description of rearing of forensically important insects see Byrd et al. (58). That means:

1. Rearing should be done in a (certified), calibrated incubator with constant temperatures.
2. If an incubator is not available, it is advisable at the very least to rear the juvenile stages at ambient temperature, to be

able to document the remaining part of the life cycle of the samples and to aid identification, because adults can be easier to identify than their immature stages. Assure a careful documentation of the temperature profile, e.g. by using a (certified) thermometer.

3. Monitor development. Time of pupariation and emergence of adult specimens should be noted.
4. One should keep the hatched flies as an option for further experiments with the population of the corpse. Feeding them with water and sugar the first days and then providing a source of blood (e.g. a piece of beef liver) for egg maturation should keep the stock running

(B) Soil and leaf-litter samples

1. Sieve and examine the samples visually. Search for immobile pupae, puparia or dead adults as well as for living larvae → if you find specimens, see above (rearing immature insects) for further instructions
2. When the samples are not checked within 24 h after collecting, they should be stored in a refrigerator, to avoid further development of immature stages and the growth of fungus.

(C) Preparation and identification of insects

An accurate species identification is an essential step for PMI_{min} estimations. Therefore, it is important to use identification keys representative of the geographical region and characteristic fauna. The procedure should be:

1. Label any identified or examined specimen, micro- scope slide, or other sample with the laboratory code for the individual case AND with the code of the specific vial.
2. If you are not able to examine and identify the insects, immature or adult, in an appropriate manner, keep them well-labelled in a vial filled with 70–95% ethanol for later molecular identification or for sending them to an expert.
3. If you are not confident with your species identification send the specimens to an expert for identification, and/or double check the identification by her/him.
4. Use specific keys for each development stage e.g. first, second, third instar larvae, pupae, puparium, adult specimens.

REPORTING INSECT EVIDENCE – DO'S AND DON'TS

Being a very specialised forensic discipline, the entomological report has to be very clear, transparent and focused. Unlike e.g. a molecular biological expertise, the entomological evaluation is not just evidence of fact, it is also evidence of (expert) opinion, and human beings, here: experts, might see different things in a different context. However, there should be a general template for an entomological report which can serve not only as a guide for the preparation of a report but also as a tool for ssessing its quality. The following issues should be taken into account: Table 2.

Table 2 Toward the production of an entomological report

Introduction and Case file	This part should not only provide and present the necessary background information of the case and time lines of the inquiry, but also tells the reader who was informing (and therefore maybe briefing…) you and what were the sources of your information.
Mission	Specify the inquiry and explain what you have been asked to do and by whom. Like 1. this is for reasons of transparency and for understanding later on the focus of your analysis.
Samples	The type, quality and amount of samples have to be described. Specify who sampled the specimens and when it was sampled. Depending on the amount and type of samples only a selection of samples is further investigated - explain the reason and criteria for selection. Describe the way of transport and storage and whether you received living or dead specimens. If you will rear any sampled specimens, give just a short note on how this will be done in your laboratory (temperature and diet for breeding). Also state which temperatures you take into account and explain whether own measurements were carried out at the place of discovery.
Forensic Entomology at a glance	The majority of readers will not be familiar with forensic entomology, so a brief introduction to the background of this discipline and the methods used is helpful.
Results	Indicate (Figure and/or table) which temperature(profile) you used for analysing the activity and growth or age of the later on described insects. Explain the source of your data and how far the weather station, if any, is from the place of discovery. If you reconstruct the temperature explain the quality of this reconstruction. After mentioning the morphological or molecular method and/or references for identifying the specimens, give the results of your entomological work in a certain order, e.g. on a taxonomic sequence or sorted by case specific relevance. Provide the name of the species, its taxonomic position, the developmental stages and numbers of specimens, and the age of the specimens (which indicate a certain date of oviposition). The latter step should base on a certain reference, i.e. published data for this species. Mention, if you have used your own reference data from your research work. This section does not yet deal with the final results, i.e. the combined evaluation of the findings.
Expert opinion	Recall the question and the schedule of the case and then the identity of the sampled specimens. Here, the taxonomic resolution and detail of information you give should be related to the importance of the taxa in the present case. It is not necessary to mention all taxa in detail again if their presence do not add any value to your examination. Mention one more time the age of the most important specie(s) and perhaps their arrival and presence times on a body and identify by using such times a PMI_{min}. Depending on the knowledge about the species of interest and the case data, you might be able to speculate about a delay in locating the body. However, try to answer and discuss only questions you were asked and avoid too much speculating. Don't be too accurate: Usually it is impossible to identify a window of time of e.g. 2 hours. Try to be accurate to the day and **always** include a minimum PMI, i.e. a window of time in which you can be sure that there is no smaller one.
Summary	This should be written in a bullet point style, with respect to your conclusion. The entomological evidence should be comprehensible just by reading this section.
List of references	All references you have used in your report for e.g. introducing into forensic entomology, identifying the insects or calculating their age, should be given in an own section.

ESTABLISHING FORENSIC ENTOMOLOGY IN ALBANIA

Forensic Entomology is an effective and low budget tool for estimating the PMI in a e.g. homicide investigation. However, there is a need for a regional baseline and reference for producing reliable data and results in case work. For achieving this we recommend the following landmarks.

A. Monitor and collect the necrophagous fauna for different habitats in Albania (some might prioritize the list e.g. according to the habitats with the most crime scenes) over different seasons and years; this can be done by just trapping them (see e.g. (59)) and/or by conducting succession experiments with a proxy like e.g. a pig carcass (see e.g. (60)). The latter would be helpful for identifying indicator species for different PMI's and different stages of decomposition.

B. Establish a list of "Necrophagous insects of Albania", accompanied by references of published or self made keys which enables an interested person to ID the most important taxa and their different stages in Albania; try to "barcode" as much as possible species and bank their sequences online.

C. Analyse "real cases" = insect infested human bodies (even without an official inquiry) for practising the sampling of insect evidence and the estimation of a PMI_{min} in a realistic context. Forensic Entomology is an applied science which relies on as much input from the real word as possible.

D. By doing in compliance with protocols *(please refer to the protocol sheet added as supplementary data at the end of the paper)*, a small number of fly species will quickly be highlighted by being particularly frequent and therefore relevant. Studying their growth rate in the laboratory and/or in the field will produce own Albanian data and validate the already existing, published data for that species, which were gathered in different geographic regions.

E. Train and inform police, medical examiners, judges, prosecutors, etc. and be present in the public (via social media, etc.): Knowing the method and its power will to its application.

F. Network yourself on a national and international level. Get in touch with national investigating authorities, universities and (police) academies and forensic pathologists. Become a member of the European Association for Forensic Entomology – they are open not just for long time professionals but also for beginners from different disciplines. Visiting conferences and presenting your cases and studies.

CONCLUSIONS

Forensic entomology is a subset of forensic science whereby information and samples of insects and other arthropods are analysed to

draw conclusions on legal matters, especially in forensic medicine. It can contribute to the estimation of the time since death days, weeks, and sometimes even years post mortem; and is an effective and low budget tool, that is used all over the world in a wide variety of geographical regions. A practical guide *(with a protocol sheet as attached to this paper below the references)* for collecting entomological evidence is helpful for field operators, along with recommendations how to report such evidence during expert work with a crime scene.

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Protocol sheet for the collection of entomological evidence (page 1/3)

Collected by: _____ date/time: _____Case Number: _____

SPECIFICATIONS OF THE BODY

Name: _____ Age: _____ Sex: m □ f □; Height: _____cm; Weight: _____ kg

POSITION: sitting □ ; horizontal □ (**V**entral, **D**orsal, or **L**ateral _____); hanging □; contact with the ground ? □ ; remarks: _____

CLOTHING: fully clothed □ ; partially clothed □ ; naked □ ; remarks: _____

ACCESSIBILTY: readily accessible □ ; hardly/not accessible □ ; body covered ? □ with: _____ _____; buried □ ; depth: _____ cm; remarks: _____

DECOMPOSITION: fresh □ ; early decomposition □ ; bloating □ ; advanced decomposition □ ; adipocire □ ; skeletonization □ ; mummification □ ; scavenging □ ;

WOUNDS* (which, how big, where): _____

SCENE OF DEATH

BUILDING: Garage/Storehous □ ; vehicle □ ; dwelling house □ ; floor: _____; on which surface ? _____; in which room ? _____; heated □ ; open window □ ; closed window □ ; remarks: _____

OUTDOOR: forest □ ; field □ ; pasture/grassland □ ; public park □ ; backyard □ ; shrubbery □; on grass/soil □ ; on sealed ground □ ; remarks: _____

MISCELLANEOUS (e.g. car): _____

TEMPERATURES

AMBIENT: 2 m above ground: _____ °C; 5 cm above ground: _____ °C

BODY SURFACE: _____ °C; **INTERFACE BODY/GROUND**: _____ °C

LARVAL MASS (LM): LM 1*: _____ °C; LM 2 *: _____ °C; LM 3*: _____ °C; **SOIL** (in a depth of 20 cm): _____ °C

! ! Record air temperatures at the scene of death for 5 – 10 days after discovery of the body ! !

DATALOGGER 1 (date and time of placement)**:** _____

DATALOGGER 2 (date and time of placement)**:** _____

POST MORTEM INTERVAL (PMI)

WHEN WAS THE BODY FOUND: _____;

LAST SEEN ALIVE: _____; **PMI** (days): _____;

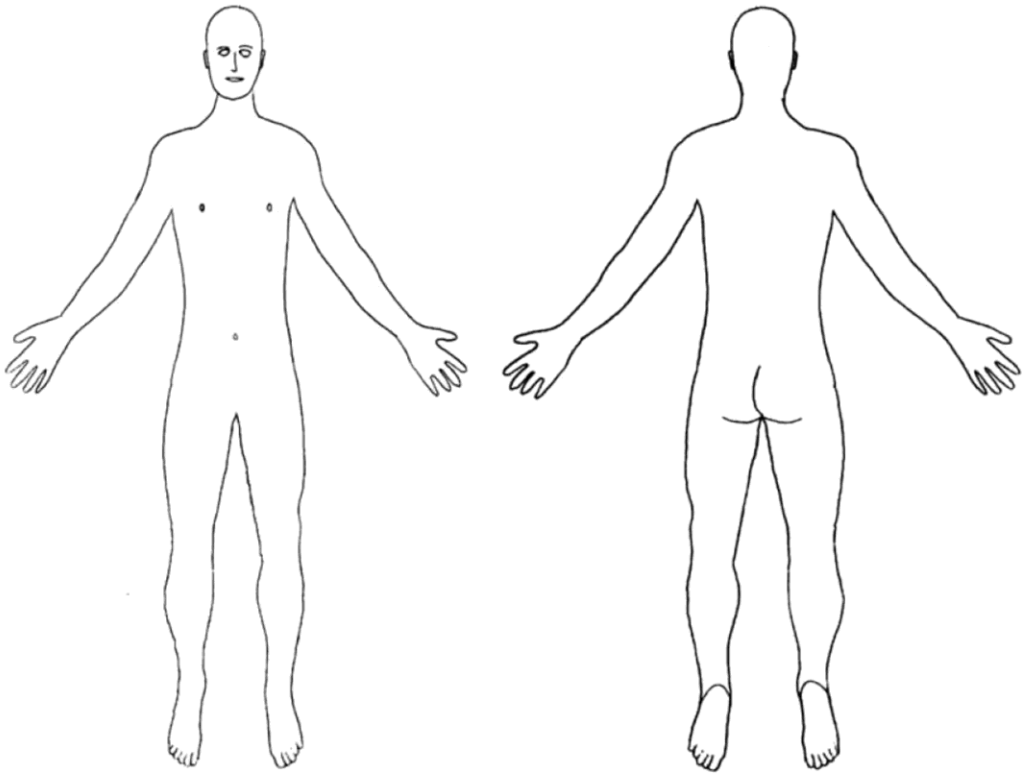
remarks: _____

Protocol sheet for the collection of entomological evidence (page 2/3)

Collected by: _____ date/time: _____Case Number: _____

Please use this sketch for marking

- Partial clothing (hatched) ▨
- Traces of scavenging (SC →)
- Wounds (W →)
- Larval masses (LM_1, LM_2, ….. →)
- Sample location (1, 2, 3, 4, …..)



INSECTS AT THE SCENE OF DEATH

ON THE BODY

FLIES (DIPTERA): eggs □ ; maggots □ ; prepupae □ ; pupae □ ; puparium □ ; adult flies □

BEETLES (COLEOPTERA): eggs □ ; larvae □ ; pupae □ ; exuviae □ ; adult beetles □

AT THE BODY

FLIES (DIPTERA): eggs □ ; maggots □ ; prepupae □ ; pupae □ ; puparium □ ; adult flies □

BEETLES (COLEOPTERA): eggs □ ; larvae □ ; pupae □ ; exuviae □ ; adult beetles □

other insects: _____

Protocol sheet for the collection of entomological evidence (page 3/3)

Collected by: _____ date/time: _____Case Number: _____

L = fly larvae, **PA** = Pupae, **PR** = Puparium, **AF** = adult fly, **BL** = beetle larvae, **AB** = adult beetles, **E** = exuviae; **pres.** = killing and preserving, **alive** = keep them alive for e.g. rearing; *please mark the positions on page 2 of this sheet

Sample N°	Approx. number	Type	Pres./alive	Location on body *
1		L □ PA □ PR □ AF □ BL □ AB □ E □		
2		L □ PA □ PR □ AF □ BL □ AB □ E □		
3		L □ PA □ PR □ AF □ BL □ AB □ E □		
4		L □ PA □ PR □ AF □ BL □ AB □ E □		
5		L □ PA □ PR □ AF □ BL □ AB □ E □		
6		L □ PA □ PR □ AF □ BL □ AB □ E □		
7		L □ PA □ PR □ AF □ BL □ AB □ E □		
8		L □ PA □ PR □ AF □ BL □ AB □ E □		
9		L □ PA □ PR □ AF □ BL □ AB □ E □		
10		L □ PA □ PR □ AF □ BL □ AB □ E □		
11		L □ PA □ PR □ AF □ BL □ AB □ E □		
12		L □ PA □ PR □ AF □ BL □ AB □ E □		
13		L □ PA □ PR □ AF □ BL □ AB □ E □		
14		L □ PA □ PR □ AF □ BL □ AB □ E □		
15		L □ PA □ PR □ AF □ BL □ AB □ E □		