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(Review Article)

Forensic entomology: A novel approach in crime investigation

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Abstract

Forensic entomology is an application of studies related to insects and other arthropods in legal matters. Depth knowledge and experience of the biodiversity, biology, ecology, and behavior of forensic insects found on a human corpse or at a crime site, can provide information about time since death, location of the crime, and in some cases cause of death. Although forensic insects are used in crime investigation for three to four decades, it aroused recently as a very popular and independent branch of forensic science in crime investigation. While observing and examining insects on a corpse, knowledge of regional insects' assemblages and their population dynamics play an important role in medico-legal entomology for an efficient criminal investigation.

Forensic entomology has three components, named as urban, stored products, and medico-legal. As different groups of insects are attracted to different stages of decay, forensic insects invade the corpse in a typical sequence. The type of insects' colonization found on corpses provides important information about the decomposition stage. Calliphoridae provides valuable evidences, particularly in determining time since death in criminal matters, where the time since death is prolonged and use of other conventional methods for estimation of time since death is limited. Two well-established ways used for estimation of post mortem interval are based on maggot's development and succession waves of entomofauna. The latest scientific and technological developments in the field, such as its combined approach with forensic toxicology, forensic palynology, DNA based molecular identification of insect species, and other various methods to estimate post mortem interval, are many emerging fields under this periphery and forensic entomology lately became very popular among criminal investigators and forensic entomologists.

Keywords: Medico-Legal Entomology; Succession Waves; Entomofauna; DNA Based Molecular Identification; Time since Death

1. Introduction

Insects are generally considered as noxious which badly affect humans' health, crops, livestock, and other household materials, however, they also provide valuable commercial products such as honey: also known as liquid gold or food of the gods, silk: declared as queen of all textile fibers, and lac: used as sealing wax, cosmetics and dyes. Moreover, approximately one third crop production is due to insect pollination. Insects' contribution in field of genetics is remarkably enormous and unforgettable. It also works as an environmental cleaner by acting as a scavenger during decomposition. Forensically important insects being scavengers, play a crucial role in crime investigations. When a corpse is found, either a human's or wildlife animal's, the typical questions of investigative science involve; time since death, the causes, and the place where actually the death was occurred. In most of the cases where decomposed and mutilated dead bodies are found, Forensic entomology acts as an indispensable evidential tool in investigation. Many

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scientists have used forensic entomology to estimate postmortem intervals in humans' death cases. As a result, forensic entomology is emerged as a very distinct and acknowledged branch of forensic science, and the admissibility of entomological evidences in courts is progressing across the globe [1]. Forensic Entomology is defined as the study of insects' fauna associated with dead bodies in criminal investigations for the court of law. Many forensic entomologists claimed that even after 72 hours, forensically important insects' evidences are often most reliable, accurate, and sometimes the only means for estimation of elapsed time since death [2,3,4,5]. Conventionally, estimation of time since death has been determined with the help of observing the external appearance of the corpses, considering other parameters such as temperature, cooling of the body, eye changes, skin color, muscle sagging, rigor mortis and post mortem lividity [6, 7]. If any of these characteristics are not observable due to corpses' decomposed and mutilated states, forensic entomology acts as a significant evidential tool and by the study of succession waves, post mortem interval (PMI) can be calculated [8]. Legal entomofauna analysis as a forensic tool has acquired more recognition because insects are the first organisms to arrive at a decomposing body or a crime site [9, 10] in a very unique sequence, i.e. succession wave [11, 12]. The predictable succession waves(which vary according to bio-geolocation, time of year or season and habitats) and their developmental and metamorphic larval stages are used in determining time since death and possible cause of death precisely[13, 14].The investigative agencies can be provided with very useful information to elucidate possible civil and criminal cases by a forensic entomologist as an expert witness. Insects are identified as well-established forensic markers since 13th century. The first documented instance in which tracheate hexapod were used as a crime detection tool during a criminal investigation in a murder case, occurred in China in 1235 as mentioned in Sung Tzu's medico-legal book, "The washing away of Wrongs: A training manual on investigating the death." During the case, it was recorded that the mass landing of different calliphoridae (blow flies) on a particular sickle caused a murderer confesses murdering a fellow Chinese farm worker with the same sickle [3]. A particular contribution in a legal case, which helped medico-legal entomology being recognized as a cogent forensic marker or tool for investigating criminal cases, was the murder of a newborn baby. During the investigation great medico-legal forensic entomologist Dr. Bergeret, who is considered as the first forensic entomologist, found a mummified body of an infant, encased in a chimney? Dr. Bergeret did an autopsy on the corpse and recovered some larvae of sarcophagidae (Flesh fly), particularly *Sarcophagacarnaria* and some moths. Beregeret made many pronounced estimations based on observed entomofauna and concluded that the infant body has been sealed up in 1848 and the moths had gained access in 1849 [15]. Megnin, 1894 published a book entitled "La faune des corpses" in which he gave eight theories for succession waves of freely exposed corpses and two theories for buried corpses. His theory was focused on wing venation, posterior spiracles, and the overall anatomy of the insects for identification. Megnin's work got vast recognition in forensic entomology [5, 11].

2. Types of forensic entomology

Entomology is derived from the ancient two Greek words 'entomon ' (insect) and 'logia' (study), which mean the systematic study of insects. Forensic entomology is the name given to any aspect of the study of insects and their arthropod counterparts that interacts with legal matters. Forensic (pertaining to law) entomology is the study of the insects associated with a human's or any protected animal's corpse to determine especially elapsed time since death and other relevant investigative queries. Lord and Stevenson divided forensic entomology into three major parts:

2.1. Urban entomology

Legal proceedings involving insects and related animals which affect man-made structures and other aspects of the human environment.

2.2. Stored products entomology

Proceedings involving insects infesting stored commodities such as cereals and other household products.

2.3. Medico-legal entomology

Sometimes termed as "forensic medical entomology", and in reality should be termed as "medico criminal entomology" (because it focuses on violent or heinous crimes), relates primarily to-

- Determine time since death (postmortem interval).
- Site /location/place of death (primary and secondary crime site).
- Manner/ mode of death [4].
- Cases of death especially with the help of forensic entomo-toxicology.
- Negligence of old age persons.
- Possible criminal act or misuse of insects [16].

Forensic entomology is inextricably linked with the broader scientific fields of medical entomology, forensic palynology, wildlife forensic entomology, taxonomy, and forensic pathology.

3. Decomposition of Human corpse and entomofauna association

After death of an organism, cells-tissues-organs start degrading and biocatalysts begin digesting the cells inside-out in a cyclic manner called autolysis, and finally the corpse starts to decompose. In gastrointestinal path, bacteria begin lysis of soft tissues; as a result internal organs are turned into liquid and produce gases such as carbon dioxide, methane, hydrogen sulphide, ammonia, sulfur dioxide, and hydrogen [17]. Forensically important insects are attracted by the volatile organic compounds known as apenumones, which are released by the decomposing corpse. It is a very significant parameter of estimating time since death because forensic insects' behaviors are mostly modified by the specific products released during subsequent decomposing stage [18].

Generally, four categories of forensic entomofauna can be found on the decomposing corpse [5]:

3.1. First category

Nacrophagous species, feeding on the corpse

3.2. Second category

Predators or parasites' species, feeding on the nacrophagous species

3.3. Third category

Omnivorous species, feeding on corpse and other arthropods (wasps, ants, and coleopterans)

3.4. Fourth category

Springtails and spiders use corpses as an extension of their sweet homes.

Five decomposing stages based on the external physical appearance of a human corpse and internal temperature suggested by Tullis and Goff [19] are as follows-

3.4.1. Fresh stage

This is the Initial decomposition stage. In this state cellular disintegration occurs, forensic entomofauna attendance found, there is no oviposition and it lasts for 24-48 hours.

3.4.2. Bloated stage

In this stage Putrefaction starts and gases accumulate in body. There is massive attendance of insects and oviposition is found. Duration of this stage is 2-7 days (seasons and habitats should also be considered).

3.4.3. Active decay stage/ decay stage

The corpse starts rotting in this stage. Due to release of gases, the walls of the abdomen get penetrated, which causes the carcass to deflate and bloat. Rise in Internal temperature, a pungent smell, drop-in corpse's body weight, conversion of third instar larvae into the pupate stage are some characteristics of this stage, and duration of the stage is 5-13 days.

3.4.4. Advanced decay stage/ post decay

This is a phase of larval development which feeds on the residual bony parts, cartilage, hard tissues like hair, and tiny proteins. Characteristics feature of this stage is a large quantity of wet, viscous material called decay by-products (BOD's) which works as a substrate site for insects' developmental stages activities, and duration of this stage is 10 to 23 days [17].

3.4.5. Dry remains stage/ Skeletonized stage

This is the final corpse's decomposition stage. There is shrinkage in the entomofauna population and BOD is dried up to this stage. Duration of this stage is 18-90 days [19].

4. Insect as a forensic indicator in crime investigation

Different questions which are raised in the process of crime investigations and medico-legal examinations of a site of crime or the dead body, such as time since death, cause of death, and the actual site of death are effectively answered by study and observation of Forensic entomofauna succession waves, and age, weight length, and growth of maggots. Forensic insects on a corpse in various habitats act as forensic detectives and are validated as cogent forensic indicators, because insects arrive at a decomposition corpse in a very particular sequence [9,11,12]. This predictable pattern of the life cycle of insects varies according to bio-geolocations, seasons and habitats. Insects' development and larval growth rates are used to help accurately determine the time since death and cause of death [13,14].

4.1. Major forensic entomofauna

4.1.1. Calliphoridae (Blow flies)

Known as the first visitor of crime scene

Blue bottle flies

Calliphora vomitoria, Calliphora livida, Calliphora hilli etc.

Green bottle flies

Chryosomya rufifacies, Chryosomya megacephala, Lucilia sericata, Lucilia cuprina, Lucilia illustris etc.

Black blow flies

Phormia regina

4.1.2. Sarcophagidae (Flesh flies)

Sarcophaga ruficornis, Sarcophaga pregrina, Sarcophaga albiceps, Sarcophaga africa, Sarcophaga caerulescens, Sarcophaga argyrostoma, Sarcophaga dux etc.

4.1.3. Muscidae (House flies)

Musca domestica, Musca sorbens, Stomoxys calcitrans etc.

4.1.4. Piophilidae (Cheese flies)

Piophila casei

4.1.5. Phoridae (Coffin flies)

Conicera tibialis, Megaselia scalaris etc.

4.1.6. Staphylinidae (Rove beetles)

Staphylinidae lameere, Bryocharis analis, Tanygnathu sterminalis etc.

4.1.7. Silphidae (Carrion beetles)

Nicrophorus vespilloides, Nicrophorus nepalensis etc.

4.1.8. Cleridae (Checkered beetles)

Necrobia rufipes, Korynete scaeruleus etc.

4.1.9. Dermestidae (Skin/ hide beetles)

Dermestes lardarius, Dermeste sater, Dermeste smaculatus etc.

5. Dipteran insect shows complete metamorphosis, i.e. they have at least four metamorphic life stages. A typical lifestyle of an insect goes through following stages

A typical life cycle of an insect is passed in the following stages:

5.1. Adult insect (flies) laid eggs

An adult female fly searches for a site/place to lay eggs. Natural orifices such as mouth, nostrils, anus, eyes, and open wounds are often considered as the perfect sites for oviposition in a corpse.

5.2. Hatching of Eggs

In most of the species, eggs are 1 to 2 mm long and it typically takes 1 to 2 days for hatching.

5.3. First instar larvae

After hatching of eggs, it is converted intofirst developmental stage called first instar larvae and also known as maggots. Maggots feed on the quasi-liquid bodily fluids, so different biocatalytics/enzymes are released by them to break down the protein surrounding them. In the process maggots start showing developments and after few days first moulting occurs, when they shed their first exoskeleton.

5.4. Second instar larvae

After first moulting, maggots convert into the second stage, called second instar larvae. As breaking down of the body tissues by the enzymes continues, they feed on the resulting decomposed materials and continue to grow in size, length and weight. When second instar larvae are mature enough, they molt exoskeleton for the second time.

5.5. Third instar larvae

After second moulting, larvae convert into third instar larvae. In this stage, maggots stop feeding (non-feeding stage) and they start surrounding themselves into a soft soil for pupation.

5.6. Prepupa

Mature larvae of insect called prepupas start to shrink in size and become slightly hardens.

5.7. Pupa

The last exoskeleton does not shred, it hardens and gradually turns from light brown to dark brown and finally black.

5.8. Empty puparium

After the emergence of adult insects empty puparia are left.

6. Identification of larval instars

Following morphological features are used to identify and differentiate between the larval instars.

- Total larval segments (11/12) could be differentiated (first or cephalic segments, which can be drawn into seconds, is called pseudocephalon).
- Differentiation between families is possible on the basis of unique arrangements of posterior spiracles of Calliphoridae, Sarcophagidae and Muscidae.
- Three major taxonomic identification features of larval stages are cephalophyaryngeal skeleton, anterior and posterior spiracles.
- Cephalophyaryngeal skeleton of second instar larvae is darkly pigmented and accessory sclerite below the mouth hook is not found, but in third instar larvae cephalophyaryngeal skeleton is heavily pigmented and accessory sclerite is found below the hook part.
- The anterior spiracles of second instar and third instar larvae have 8-9 papillae each which are arranged in a single line pattern which varies from species to species.
- The posterior spiracles of second instar larvae has two spiracular slits with no thickening of peritreme, it differentiates it from third instar which has three slits for each posterior spiracle.

- In first instar only last trunk segment mid- dorsally bears a pair of D- shaped posterior spiracles, which are the external opening of body tracheae for respiration.
- Second and third instar larvae have a pair of fan-shaped anterior spiracles, dorsally upon thorax, which helps in respiration.
- Second and third instar larvae are differentiated based on weight, length, color, etc,
- There are differences in the level of sclerotization of particular parts of cephaloskeleton.
- Level of sclerotization of posterior spiracular peritreme is different.
- The shape of posterior spiracular slits is highly differentiating.
- The general shape of anterior spiracles, pattern of arrangement of their lobes, distribution, and shape of spines/ warts on inner band area of segments are also important for differentiation.
- Observation under stereo zoom microscope (50X) and binocular microscope helps in identification and differentiation of different stages.

7. Evidential value of forensic entomology in the court of law

The judicial value of evidences obtained from forensic entomology can affect direction of crime investigation or legal proceedings in various ways. Oral and written reminiscence pertaining to forensic arthropods may be very useful as investigative weapon together a present or retrospective look at relevant circumstances. Sometimes, forensic entomofauna evidence may lead to very important other aspects, during investigation, which in turn may reveal the truth. Scientific information extracted from insect evidence may simply corroborate other actual and testimonial evidence. If entomological evidence is to have any impact on the outcome of litigation, it somehow much finds its way into the court proceedings. Forensic insect evidence is evaluated by session courts, High courts and Supreme Court of India and other countries under the general rubric of scientific expert testimony. Conclusively, this means that the entomological data must be analyzed by someone qualified to render an opinion regarding how such evidence fits the facts of the case at hand. Such expert testimony is governed by different state and central rules of evidence, or the codification thereof that exist in country jurisdictions.

7.1. Insects' collection, entomological preservation methods, simulation and rearing

The insect collection and their preservation methods are as follows:

7.1.1. Collections of adult flies

Collection of flying arthropods from the simulated sites and actual crime scenes can be done with the help of aerial netting.

7.1.2. Collections of eggs and larval stages

Collections of insects' eggs can be done with the help of a paintbrush (zero number) and the average sample size should be 50 to 100 eggs. Larval stages and pupae can be collected with soft nose forceps or with a disposable spoon.

7.1.3. Preservation

Collected specimens should be preserved in 70% ethanol.

7.1.4. Drying, pinning and taxonomic labeling

All collected specimens should be brought to the laboratory for drying, labeling, and identification. Specimens are first kept at room temperature and further in the desiccator box for drying, then pinned for taxonomic identification.

7.1.5. Microscopy

Microscopic images of all insect stages should be done with a high-resolution stereo zoom microscope or Scanning Electron Microscopy (SEM).

7.1.6. Environmental conditions

Climatological parameters like temperature, relative humidity, and rainfall, etc. of the geolocation of the crime site should also be recorded. These data can be easily obtained from the nearest Government Regional Meteorological Center.

7.1.7. Simulation and rearing

Carcasses should be placed at different geological habitats (sites) and seasons in a white tray of standard size or in case of a coffin, it should be placed in a wooden box (standard size). During the study, the tray should be covered with an iron case of standard size which should be protected with chicken wire and stones, so that the sites would not be disturbed by livestock or wild animals. For rearing, plastic pots and wide-mouth bottles covered by socks over the mouth of the container are used (Plate 4).

8. Recent advances in forensic entomology

8.1. Entomo-toxicology

Entomo-toxicology can be defined as the study of detection of poisons, toxins or drugs from various insects' developmental stages.The entomological evidences collected from crime sites can be used to determine the cause of death due to poisoning. Insects feeding on the corpse could be used in determining if any toxic substances or drugs were present in human tissues before death. Insects and insects' remains (empty pupal cases or dead insects) can be vital in gaining entomo-toxicological data in case of highly decomposed or mutilated dead bodies when important tissues/ organs have been destroyed. On the other hand, a forensic insect can be used to detect the presence of drugs of abuse. J C Beyer, 1980 concluded that using arthropods in a corpse or at crime site investigation can determine whether toxins were present in a body at the time of death [20]. Thus Forensic Entomo-toxicology was emerged as new sub branch of forensic entomology. J C Beyer, 1980 is known as father of entomo-toxicology for his pioneer significant contribution into the field. For the first time, the toxins, poisons or drugs from entomological evidences, such as maggots which feed on human corpse was detected by J C Beyer in a criminal case where a young girl's corpse with a history of suicidal attempts discovered fourteen days later after death. Due to advanced decomposition state of body, no organ's or tissue's remains were available for toxicological analysis. With the help of gas chromatography and thin layer chromatography techniques, *Cochliomyia macellaria* (Diptera: Calliphoridae) larvae was found and phenobarbital was detected. Furthermore, various scientific studies from western countries viz–in France, show the contribution of insects in toxin detection. Earlier in some cases drugs were not discovered during the analysis of body tissues after two months of death, but Kintz detected trizopam in maggots through liquid chromatography technique. Some studies suggested that maggot's tissues are more suitable than muscles tissues in toxins' detection [12] and even, in some cases, poisons can be detected from diapauses phase of insects. The use of pupal cases and larval skins allow scientists to detect toxins in a body, years after death. Corpse recovered in advanced stages of decay gives challenges in a different way, as the samples usually taken for toxicological examination viz viscera, vomit, blood, urine and others, were not available [21]. In such circumstances, the forensic insects (mainly flies and beetles) associated with the corpse can be sampled as a reliable alternate source for analysis of poisons, toxins, and various kinds of drug substances. During experimental studies, large number of toxic substances has been detected from insects' developmental stages that feed upon a corpse. Toxic substances like heroin, morphine, cocaine [22] triazolam, oxazepam, phenobarbital [23, 24], methamphetamine [25] lead, and arsenic [16] have been detected in maggots' tissues. Surprisingly, toxins can also be detected from insects' shed casting and their fecal matters, and its presence is detected and analysed by highly sophisticated techniques such as thin layer chromatography (TLC), high performance liquid chromatography(HPLC), Gas chromatography–mass spectroscopy (GC-MS), Liquid chromatography-mass spectroscopy (LC-MS) and Fourier-transform infrared spectroscopy (FTIR) [26, 27].

8.2. Wild life Forensic Entomology

The importance of forensic entomology is equally applicable on wildlife crime investigation cases like illegal hunting, poaching, trade which come under The Wildlife Protection Act 1972 (Since it provides information, such as time since death, cause of death, geo-location of crime site as in case of human death). In 2010, two national level sensational cases of tiger cubs' death in Ranthambhore and tiger's death in Sariska National Park (a tiger reserve in Rajasthan) were solved by collection and taxonomic identification of entomofauna from the dead body of cubs and tigers (specifically, species of *Chrysomya* commonly called as 'hairy maggot blowflies').After the study of life cycle of insects (considering factors affecting such as season, temperature, rainfall, relative humidity etc.), the development and growth rate of larvae and pupa helped in the estimation of postmortem intervals in both of the cases [28].

8.3. Instant insects' identification (DART-HRMS technology)

For the first time, the University of Albany has applied a technique called direct analysis in real-time with high-resolution mass spectrometry or DART-HRMS, for the examination of Calliphoridae fly's eggs. DART-HRMS, developed by Dr. Chip Cody of JEOL, is an ambient ionization mass spectrometry technique for the samples to be directly analyzed without any time-consuming sample preparation steps, and perhaps most importantly without destroying the sample.

The technique has demonstrated the possibility of almost instant differentiation between various insect species based on the amino acid profiles of the eggs (For instance alanine, isoleucine and proline were common but glutamine and tryptophan were only present in the eggs belonging to *P. regina*).

8.4. Molecular identification of insect species

The traditional methods used for morphological identification is outmoded and obsolete, moreover, practically impossible for some species and unreliable for immature life stages. These limitations lead scientists to evolve a molecular approach based on Polymerase Chain Reaction-Restriction fragment length polymorphism (PCR-RFLP) of the mt-DNA. Mitochondrial COI and COII genes are suitable molecular markers because, relatively, a high degree of genetic variations has been reported in this region. Due to difficulties associated with identification by phenotypic characteristics, molecular identifications are preferred techniques, for example, DNA barcoding and PCR-RFLP are preferred for accurate and quick identification. Techniques of molecular biology are being used to identify and differentiate insect's species which helps in estimation of PMI. PCR-RFLP analysis are also used for identification of closely related species of forensic importance from different life stages, and it is an easy, fast and low-cost technique for routine diagnostic purposes [5, 11, 29, 30, 31].

8.5. Human DNA identification from larval gut portion

Techniques for molecular identification of species of insects or detecting and distinguishing human DNA in insects' gut which feed on human's corpse have recently been developed to increase the efficacy of the field. Maggot's crops are suitable source to obtain DNA for the identification of both the insects and their gut contents. Human STR profile could be obtained from maggots feeding on decomposing tissues (if skeleton could not be found) in an advanced decaying stage when collecting samples from the corpseis not possible [32, 33].

9. Wide range of applications and multifarious approach of forensic entomology

- Estimation of time since death in humans (Post mortem interval).
- Biogeographical location (site or place of death).
- Movement of the dead body (displacement).
- Determination of primary and secondary crime scene
- Mode/ manner of death [4].
- Cause of death- Entomotoxicology.
- Possible criminal misuse of insects [16].
- Negligence of older persons at home.
- Negligence of newly borne babies in the hospitals.
- Traffic accidents with no immediately obvious cause.
- Entomotoxicology -detection of possible poison/drug through insect evidence.
- Human DNA identification through a larval gut portion.
- Wildlife forensic entomological examination and investigation.
- Trafficking of narcotic plants in border areas.

10. Future perspectives

The decay pattern of corpse and insects' succession waves should be studied thoroughly in different regional climatic zones, seasons, and habitats to construct a location-based regional database of forensic entomofauna. It needs specific studies of forensic insects with reference of temperature, relative humidity, and rainfall, etc. to a make maturation scale of different developmental stages of a particular insect species. There is a necessity of funding and financial assistance from different research and development agencies for actual crime scene data collection, simulation site data collection and to discover different molecular markers to identify insect species. It is very necessary for crime sites to be visited by forensic entomologist to practice forensic entomological exercises in routine criminal cases for more scientific and technological development.

11. Conclusion

During a criminal investigation process, police investigators, medico-legal experts, and forensic investigators gradually become more and more aware of the spectacular contribution made by the silent testimony of entomofauna. Entomofauna from crime sites or corpses were neglected and considered as nuisance in the investigation process.

However, presently, due to advancement in scientific research and technology, the study or examination of forensic insects' infestation and behavior is benefiting as a valid scientific tool. Forensic entomology has emerged as the main branch of forensic science with the passes of time in western and as well as some Asian countries. Its role in the justice delivery system has become more and more significant and relevant. Nowadays, forensic medico-legal entomologists are being hired in various law enforcement agencies like the FBI (USA), CBI (India) as expert witnesses and have employed forensic entomologists as an expert. Potential use of forensic entomofauna needs a vast and depth working knowledge and experience about the regional entomofauna which are vital as forensic indicators. Similarly, depth information about the life history, insect habits, habitats, immature developmental stages, biogeographical location, insect biodiversity, taxonomy and insect species identification is a must in solving different crime cases. A better application of various aspects of medico-legal entomology as a crime detection tool by the investigators and forensic entomologists can change the latitude of crime scene investigation.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors declared that they have no conflict of interest.

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