Use of Scanning Electron Microscopy (SEM) In Forensic Entomology

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Abstract- *Forensic entomology is an excellent branch of entomology dealing with the study of insects and other arthropods found in and around the corpse and use them for the estimation of post mortem interval (PMI). Fly larvae and fly eggs are used to aid in the determination of a PMI. In order for the data to be useful the larvae and eggs must be identified down to a species level to get an accurate estimate for the PMI. There are many techniques currently being developed to differentiate between the various species of forensically important insects and scanning electron microscopy is one of those.*

Keywords— *F*orensic entomology, PMI, SEM

I. INTRODUCTION

Forensic entomology is a science pertaining to the study of insects and other arthropods related to legal investigations. Insect evidence has been helpful in determining PMI or site of human death, to link a suspect to the scene of crime, to prove moving of the corpse to a different location and to determine drug levels in a deceased person [1]. Urban forensic entomology includes such things as litigations and civil law actions involving arthropods in dwelling or as house and garden pests. Law suits dealing with the misuse of pesticides are included in this subfield. Stored product forensic entomology generally deals with arthropod infestation or contamination with a wide range of commercial products (e.g. beetles or their parts in candy bars, flies in ketchups, or spiders in bathroom tissue). The third category, medicolegal forensic entomology is the focus of present study and most popularized aspect of the science. It deals with arthropod involvement in events surrounding felonies, usually violent crimes such as murder, suicide and rape but also includes other violations such as physical abuse and contraband trafficking [2,3]. A more accurate name for this category is medicocriminal forensic entomology [3,4]. Insects can be found associated with carcasses throughout both the early and late stages of decomposition [5].

In the first account that appeared in a 13th century Chinese Manual of Forensic Medicine, the victim died of wounds inflicted by a sickle. The inquest officer assembled the neighboring farmers before him with their sickles laid on the ground. In the hot weather, flies flew to and landed on one sickle whose owner then confessed his guilt [6].

Critical for the estimation of PMI is the correct identification of insects [7,8]. Incorrect identification may result in the application of inappropriate developmental data in estimation of insect age, a significant problem considering the great variability in growth rates between (and within) various taxa [9,10]. The relatively similar species C. vicina and C. vomitoria, of the same genus, may both be found on carrion, however, their developmental rates vary greatly. At 26.7˚C, C. vicina requires 508 hours to complete egg to adult emergence, while C. vomitoria requires 854 hours [11]. An error in identification may result in a difference of several days in the ultimate PMI estimate. There are many techniques currently being developed to differentiate between the various species of forensically important insects. Scanning electron microscopy (SEM) is one of those techniques which can be employed to identify key morphological features of eggs and maggots. Some of the morphological differences that can help identify the different species are the presence/absence of anastomosis, the presence/absence of holes, and the shape and length of the median area.

II. SEM IN FORENSIC ENTOMOLOGY

The SEM method provides an array of morphological features for use in identifying fly eggs; however, this method does have some disadvantages. The main disadvantage is that it requires expensive equipment and can take time to identify the species from which the egg originated, so it may not be useful in a field study or to quickly identify a particular egg. The SEM method is effective provided there is ample time and the proper equipment and the particular fly eggs are plentiful. The ability to use these morphological differences gives forensic entomologists a powerful tool that can help with estimating a PMI, along with other relevant information, such as whether the body has been disturbed post mortem.

Greenberg and Singh [12] used SEM to identify calliphorid individuals, but found several confounding factors like conspecific interpopulation variability, high similarity

between eggs of congeneric species and similarity between certain species of different genera.

Sukontason et al. [13] recovered the third instar of an unidentified sarcophagid fly from a mummified body of 32 years old Thai male and examined it using SEM. Although the morphological features of this larva are similar to the other sarcophagid larvae, some features including number and arrangement of papillae on the anterior spiracle, structure of spines, and size of circumspiracular tubercles at caudal segment and branching peculiarity of the posterior spiracular hairs could be helpful for species identification.

Sukontason et al. [14] presented Chrysomya nigripes Aubertin as a blow fly species of forensic importance in Thailand, and used SEM for the morphological observation of fly puparia. Morphologically, they focused on the characteristics of puparia used to accurately identify fly species. Numerous puparia of C. nigripes were found aggregated, adhering side by side, on the tibia of a skeletonized corpse, which was recovered from a forested area of Chiang Mai, northern Thailand. In the triangular shape of the anterior end of the puparia, three thoracic segments and broad hairy patches beginning dorsolaterally at the sixth segments were distinguishing characteristics. Their study showed pupariation of the flies along the bone of a corpse as well as morphological features and provided important guidance in identifying C. nigripes puparia. They have also given a key to differentiate puparia of C. nigripes from the other flies of forensic importance in Thailand.

Mendonca et al. [15] used SEM to identify eggs of species of forensic importance, such as Chrysomya megacephala, Chrysomya putoria, Lucilia cuprina, Lucilia eximia and Ophyra aenescens. C. megacephala had no anastomosis or holes at the top of the islands and C. putoria had few anastomoses and no holes, whereas L. eximia and O. aenescens were found to have anastomoses and holes and L. cuprina had only anastomoses. The median area was bifurcated anteriorly in C. megacephala, L. eximia and O. aenescens and rounded in C. putoria and L. cuprina. Also the sculptures observed in the chorionic cells, the length and the way that median area ends up posteriorly are characteristics of great diagnostic value to identify muscoids of forensic importance. Again Mendonca et al. [16] used SEM to identify eggs, larvae, and puparia of C. albiceps. They showed that eggs were elongated with the anterior region ending in a "Y" shape and the posterior end was tapered. The micropyle was a well-adorned orifice with some projections around it. The first instar larva was composed of 12 segments separated by spines. Only one spiracular opening could be seen at the posterior spiracle. Body tegument was smooth and tubercles were not seen. Antennae and maxillary palps were visible. Second and third larval instars were very similar to first instar, except for the presence of anterior spiracle. However, body tegument was composed of net-like patches and tubercles were visible. Tubercles present at the third instar larvae were robust and erect. Puparia showed a retracted cephalic region and curved tubercles.

Szpila and Villet [17] presented images of first instar of forensically important species Calliphora croceipalpis, Chrysomya chloropyga, Chrysomya marginalis and Chrysomya putoria using SEM and also provided a key based on these morphological characteristics.

In 2012, Mendonca et al. [18] identified larvae and puparia of C. putoria using SEM. The first instar larvae were composed of 12 smooth segments separated by spines. Antennae and maxillary palps were visible. Anterior spiracle was absent and only one spiracular opening could be seen at the posterior spiracle. Second and third larval instars were similar to first instar, except for the presence of anterior spiracle that is composed by 11-12 spiracular ramifications. At the anal segment, two spiracular openings were found in second instars and three openings in third instar larvae. Puparia showed a retracted cephalic region and none of the head structures were visible. Mendonca et al. [19] again identified larvae and puparia of C. megacephala using SEM. The larval instar body of C. megacephala is similar at all instars. The integument is smooth with small spines located at the limit of all segments. The cephalic region has a group of robust spines with one or two tips. The puparia are very similar to third instar larvae, except for the cephalic structures that are retracted. The integument shows the wrinkles from the third instar larvae and posterior spiracle disc with three spiracular openings localized on the top of an elevation. They concluded that SEM provided some characteristics to distinguish among Chrysomya species that could help entomologists to identify immature found on corpses.

Singh et al. [20] also studied morphological features of larvae of the species Parasarcophaga ruficornis on the basis of SEM. The principal diagnostic characters included are, the cephalopharyngeal apparatus, the cephalic segment, structure and orientation of spines, pupal respiratory horns, the structures of both anterior and posterior spiracles.

Grzywacz et al. [21] used SEM and provided an identification key for third instar larvae, which covers the full set of cadaver-colonising species of Muscidae from the western Palaearctic (Europe, North Africa, Middle East). The carrion-visiting Muscidae worldwide are catalogued, and those

species breeding in animal carrion and dead human bodies are briefly discussed with regard to their forensic importance.

Da-Silva-Xavier et al. [22] described and analyzed the morphological characteristics of the larvae stages L1, L2, L3 and the puparium of R. belforti by scanning electron microscopy (SEM). Ten specimens of each stage were analyzed. Larvae of R. belforti follow the typical muscoid vermiform pattern with 12 segments. The anterior region is pointed, while the posterior region is thicker. The spines of the cephalic collar are flattened and with double, triple or quadruple points, different from the spines along the body that only have a single point. In L2, the anterior spiracle is present with a varying number of papillae (16–22), differing from other species. The posterior spiracles are located within the peritreme. The spiracular cavity is internalized in the posterior region, following the pattern that differs Sarcophagidae from other families. L3 features more visible and developed spines around the cephalic collar, getting thicker and denser near to the first thoracic segment.

Szpila and Wallman [23] provided light microscopy photographs, line illustrations and scanning electron microscopy micrographs for first instar larvae of six Australian species of Chrysomya. All species have confirmed or potential in forensic investigations given their carrion-breeding habits. Morphology of the first instar larvae of Ch. nigripes, Ch. rufifacies, Ch. saffranea and Ch. varipes is revised, while larvae of Ch. incisularis and Ch. latifrons are described for the first time. The following morphological structures are documented: pseudocephalon, antennal complex, maxillarypalpus, facial mask,thoracic and abdominal spinulation, spiracularfield, posterior spiracles and cephaloskeleton. New diagnostic features of the cephaloskeleton and the spinulation of the abdominal segments are described.

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