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Focus stacking technique in identification of forensically important *Chrysomya* species (*Diptera*: *Calliphoridae*)

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Abstract Entomological evidence showed great role in death investigations during the last decades. This depends mainly on identification of insect samples by expert taxonomists who are not available among forensic investigator teams in Egypt which represents one of the obstacles that prevent the use of entomological evidence.

This study aimed to provide easily used key for identification of Egyptian *Chrysomya* species using focus stacking technique that can be applied in forensic investigation.

Materials and methods: Collection of flies was done using fly traps that were hung in urban districts of Cairo during June–September 2014. Continuous shooting photography of the collected flies was done using a Nikon Stereomicroscopy and camera of smart phone (Galaxy S5), stacking of partially focused images was done by Zerene Stacker software. *Chrysomya* species were identified and separated from other metallic calyptrate dipteral based on its characteristic morphological features.

Results: Seventy six *Chrysomya* specimens were collected; *Chrysomya albiceps* was the most abundant (39) with 16 male and 23 females, followed by *Chrysomya megacephala* (34) with 13 male and 21 females. Three *Chrysomya marginalis* were recovered; one male and two females.

Conclusion: This study presents simple method for photographing of insect samples that can be used by untrained personals for further identification by expert taxonomists.

Recommendations: Further studies on the blowfly species that occur in Egypt and documentation of their key for identification are recommended to facilitate the diverse applications of these important insects in forensic investigations.

1. Introduction

Forensic entomology refers to the application of the study of insects and their arthropod for forensic and legal purposes.[1] During the last decades, entomological evidence showed great

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role in death investigations since it helps in estimation of the minimum time since death and determination of the season of death, geographic location of death in cases of body transport, movement or storage of the remains following death, time of decapitation and/or dismemberment and submersion interval. In addition, it can help the pathologist to identify sites of trauma and postmortem artifacts on the body; and to associate the victim and suspect to each other and to the scene.[2]

Furthermore, it can be utilized as an alternate toxicology sample, and human DNA can be extracted from it to be used in forensic testing.[3] It is also possible to determine the period of neglect of living persons with infested wounds by examination of the recovered insects from these wounds.[4]

Blow flies (*Diptera*: *Calliphoridae*) are the first colonizing insects, attracted to the carrion by the odor of putrefactive gases that are produced during decomposition.[5] These flies are most active and abundant throughout the year in many geographic locations. Due to their early arrival and colonization in large numbers as well as voracious appetite, consumption of most of the soft tissues of the cadaver by their larvae usually occurs within a few days.[3]

Identification of insect samples is a crucial step in the interpretation of forensic evidence that should be conducted by an expert in insect taxonomy since it is a highly skilled procedure.[4] This represents one of the obstacles that prevent the use of entomological evidence in forensic investigations in Egypt because there are no expert taxonomists among forensic investigator teams who are responsible for examination of the death scene.

In addition, taxonomists are mainly depending on pictorial keys that describe insects' morphology or two dimension pictures that don't illustrate the same color as the original sample. Also, these two dimension photos don't show the fine structure clearly.[6] This is due to a low depth field, shallow focus and diffraction effect that cause reduction of optical resolution and rendering high quality photographing of the complete object in the same field impossible.[7]

Recently, with photography entering new era of computational technology, several software were developed aiming at overcoming these difficulties. Focus stacking software is one of photographing software that allows a combination of pictures with multiple depths of field to get the whole object in focus.[7]

This study aimed to provide an easily used key for identification of Egyptian *Chrysomya* species using focus stacking technique that can be applied in forensic investigation.

2. Materials and methods

2.1. Flies collection

Commercially available reusable transparent top traps plastic Fly traps were used for collection of flies. The 2 piece polypropylene molded traps were filled with 250 mL commercially available fresh liquid bait, poured into the yellow moat-base of the trap. When the moat became full, they were carefully emptied, cleaned and refilled with another bottle of the lure. Traps were hung in urban districts of Cairo during June–September 2014. Sites were chosen to be in close contact to people. The traps were suspended in trees about 2 meters from the ground.

Metallic flies were separated from other gray, collected, counted and preserved in boxes till identified. It is common to preserve collected flies using a pin but this was not the case here as the pin can interfere with the photography.[6] Cotton pad was used for the flies to rest on and tilting was done by the help of a paint brush until the desired pose was obtained, glue was not used for fixation in this study to allow the reuse of the samples in teaching purposes.

2.2. Image stacking

Continuous shooting photography was done using a Nikon Stereomicroscopy and camera of smart phone (Galaxy S5). PMax image stacking technique by Zerene Stacker software, a commercially available stacking, was used to stack large number of images. Stacking of partially focused images captured at different distances from the mobile camera was combined into single focused image to overcome the shallow depth of focus of this lens.

2.3. Morphological description

Chrysomya species were separated from other metallic calyptrate diptera by the presence of row of bristles on the meron which is a thoracic plate between the bases of the 2nd and

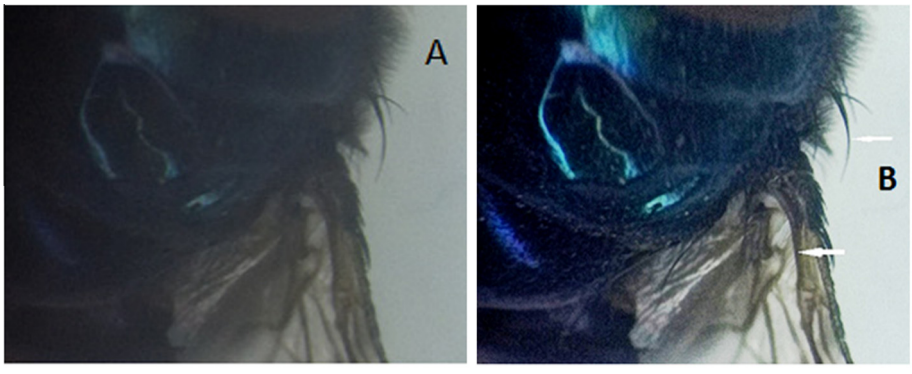

Figure 1 Row of hairs above the stem-vein at the base of the wings, greater ampulla with thick hairs (A) before stacking, (B) after stacking.

3rd legs,[8] the absence of a prominent subscutellum, having a plumose feather-like setae on the arista and sharply bent wing vein M.[9] Identification of the morphological features and description were based on the previous reports and keys provided by Whitworth (2006)[10]; Szpila et al. (2008)[11]; Marshall et al. (2011)[9], Ramaraj et al. (2014)[12]; Yang et al. (2014)[13] and Akbarzadeh et al. (2015).[14]

3. Results

Chrysomya species were identified by having row of hairs above the stem-vein at the base of the wings, greater ampulla with thick hairs (Fig. 1), as well as densely haired lower calypter and by having dark transverse bands on the dorsum of the III and IV abdominal segments. Head is holoptic in males while dichoptic in females. Then, *Chrysomya marginalis* was identified according to Akbarzadeh et al. (2015) based on the anterior wings margins darkness (Fig. 2).[14] By the color of the anterior respiratory spiracle, *Chrysomya megacephala* was differentiated from *Chrysomya albiceps* (dark brown in *C. megacephala* while (bright, creamy in *C. albiceps*). Also the color of genal dilation and postgenal helped in differentiation; it is bright yellow, with yellow hairs in *C. megacephala* (Fig. 3) while pale dusting whitish genae covered with dense white hairs characterizes *C. albiceps* (Fig. 4). In males, the parafrontal is nearly obliterated with narrow frons as the eyes are almost touching, while in females the head had separated eyes containing wider frons than those in males and uniformly small facets in all *Chrysomya* species.

Seventy-six *Chrysomya* specimens were collected; *C. albiceps* was the most abundant (39) with 16 male and 23 females, followed by *C. megacephala* (34) with 13 male and 21 females. Three *C. marginalis* were recovered; one male and two females. Body length of *C. albiceps* ranged from 0.7 to 0.8 cm in male and 0.6 to 0.8 cm in females. The characteristic sharply demarked large eye facets in the upper 2/3 were prominent in *C. megacephala* male, with a sharp change over to small ones in the lower 1/3 as shown in (Fig. 5), all females and other species male lack this character. The body length of *C. megacephala* male ranged from 0.8 to 0.9 cm whereas females ranged from 0.8 to 1 cm. Finally, the only recovered male of *C. marginalis* was one cm while the body length of both recovered females was 1.2 cm.

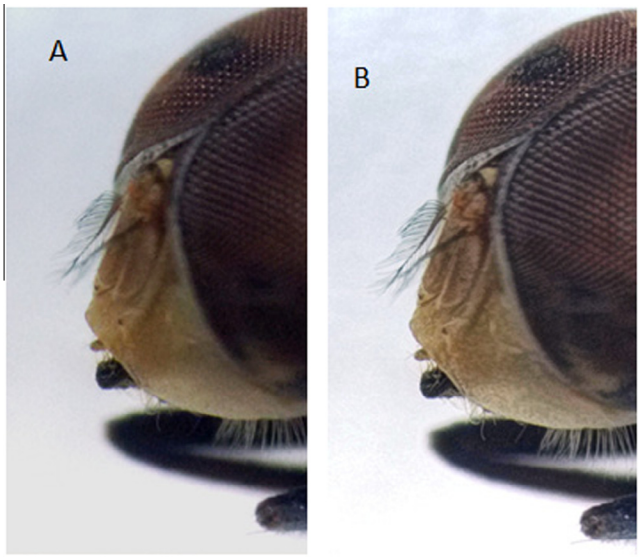

Figure 3 Yellow color of genal dilation and postgenal in *C. megacephala*. (A) before stacking, (B) after stacking.

4. Discussion

Studying taxonomic character of forensically important species as *Chrysomya,* will help in acceleration and amplification of the use of entomological evidence in death investigations. Moreover, availability of a picture gallery with good resolution of photos will indeed help in flies' identification and making Egyptian species photos available for researcher all over the world for exchange of information. Stacked

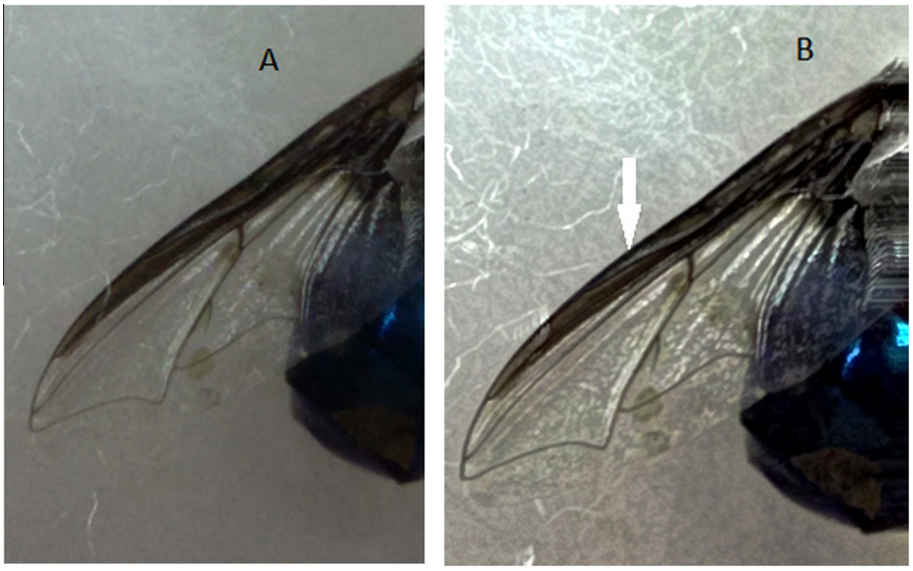

Figure 2 Anterior wings margins darkness *C. marginalis*, (A) before stacking, (B) after stacking.

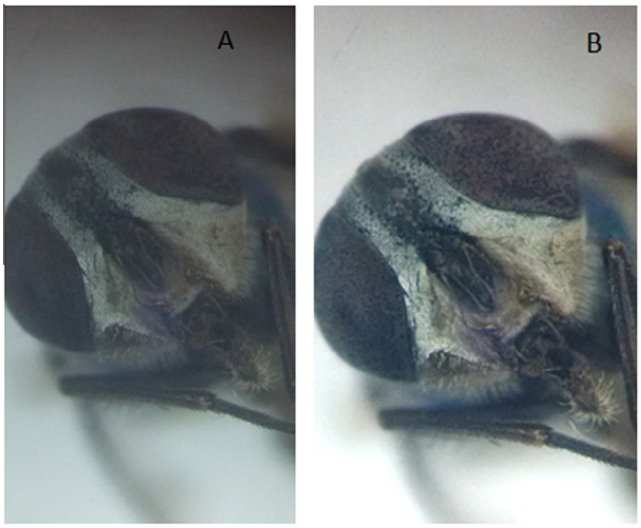

Figure 4 Pale dusting whitish genae covered with dense white hairs in *C. albiceps* (A) before stacking, (B) after stacking.

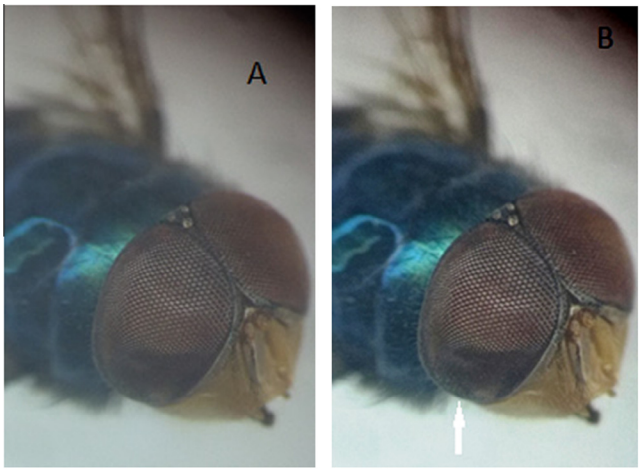

Figure 5 Characteristic sharply demarked large eye facets in *C. megacephala* male. (A) before stacking, (B) after stacking.

macrophotography or microphotography of insects helps to render high resolution, sharp full color images at a cheaper price and with sample preservation for reuse. Zerene system was recommended by Brecko et al. (2014)[7] and Friedrich et al. (2014)[15], particularly in case of dried specimens as color changes of the specimen may occur.[7]

Focus stacking technique allowed combining good focused shots of all parts of the fly to be clearly visualized in the final photo. Expensive microscopy used to auto-focus and photograph images is available.[15] However, handy items could also do well except for the need to standstill while continuous shooting with hand hold of the smart phone that needed a lot of patience.

5. Conclusions

Taking into consideration unavailability of taxonomic experts among forensic investigators in the legal system in Egypt, this study presents a simple method for photographing insect samples that can be used by amateur untrained personals. Using handy tools as mobile camera with a magnifying lens or simple stereomicroscopy allows pictures or even videos to be taken on remote crime scenes to be sent to professional laboratories to be identified using simple photo key on the spot. Thus, this may be a rapid linking tool between forensic investigators and expert taxonomies in universities to share in identification of these forensically important species.

Further studies on the blowfly species that occur in Egypt and documentation of their key for identification are recommended to facilitate the diverse applications of these important insects in forensic investigations.

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Conflicts of interest

None declared.

Ethical approval

Necessary ethical approval was obtained from the institute ethics committee.

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