Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2016; 4(1): 338-341

Received: 19-11-2015
Accepted: 21-12-2015

Jose A Nuñez
Departamento de Ciencias Morfológicas y Forenses. Escuela de Ciencias Biomédicas y Tecnológicas. Facultad de Ciencias de la Salud, Universidad de Carabobo. Valencia. Venezuela.

Jonathan Liria
Laboratorio Museo de Zoología, Facultad Experimental de Ciencias y Tecnología, Universidad de Carabobo. Valencia. Venezuela.
Actual address: Universidad Regional Amazónica IKIAM, km 7 vía Muyuna. Napo. Ecuador.

Correspondence
Jonathan Liria
Laboratorio Museo de Zoología, Facultad Experimental de Ciencias y Tecnología, Universidad de Carabobo. Valencia. Venezuela.

Cephalopharyngeal geometric morphometrics in three blowfly species (Diptera: Calliphoridae)

Jose A Nuñez, Jonathan Liria

Abstract
The blowflies (Diptera: Calliphoridae) comprise a group of medical and forensic importance because some species are responsible for myiasis and immature stages of several species feed on corpses and show preference for certain stages of decomposition. In both cases, the correct taxonomic determination is a crucial aspect for a medical or forensic investigation. The geometric morphometrics is a recent tool that describes the shape variation, although this technique has been used in adult blowfly, it has not been evaluated in immature. The goal of this study was to analyze the variations on cephalopharyngeal morphometrics, to support the identification of forensic immature flies. For this we photographed a total of 101 cephalopharyngeal skeletons from *Chrysomya albiceps* (n=34), *C. megacephala* (n=34) and *Lucilia cuprina* (n=33). Landmark coordinate (x, y) configurations were registered and aligned by Generalized Procrustes Analysis. Canonical Variates Analysis (CVA) were implemented with proportions of re-classified groups and MANOVA. Statistical analysis of variance found significant differences in centroid size (Kruskal-Wallis). The CVA showed significant separation, and *a posteriori* re-classification was 100% correctly assigned. The main differences between the three species were localized on: base of parastomal bar, apical tooth, clipeal arc, concavity of the pharyngeal sclerite, and union between hypostomal sclerite and the mouth hook. These differences could be useful as additional tools for larvae taxonomic identification; however, more studies are needed that include a more complete species representation in Calliphoridae.

Keywords: Mouth parts; *Lucinae*; Chrysomyinae; landmarks; forensic entomology.

1. Introduction

The blowflies (Diptera: Calliphoridae) comprise a group of public health importance, because some species are responsible for myiasis [1-3]. On another hand, are relevant from the forensic point of view, because the immature stages of several species feed on corpses and some show preference for certain stages of decomposition [4]; also the larvae development and species succession are used as tool in determining the postmortem interval [5-7]. In both cases, the correct taxonomic determination is a crucial aspect for medical or forensic investigation. Sukontason *et al.* [8] and others, stated that the immature identification can be accomplished with taxonomic keys that rely on a variety of distinct morphological (internal and external) features: overall body appearance, anterior and posterior spiracles, spine bands along the body, the mouth parts, among others. The mouth parts, called cepahalopharyngeal skeleton, are formed by several sclerites (labial, dental, hypostomal, pharyngeal, etc.); the shape of these structures differs between species and stages [9-12].

The Geometric Morphometrics (GM) is a recent tool that describes the shape variation, by separating it in shape configuration and size. This tool is powerful and popular because information regarding the spatial relationship among landmarks on the organism is contained within the data. This gives the ability to draw evocative diagrams of morphological transformations or differences, offering an immediate visualization of shape and the spatial localization of shape variation [13, 14]. Recently, the GM was used in adult blow flies, demonstrating the importance of wing shape, to support the identification of forensic flies [15, 16]. However, studies have not been performed using GM in the immature stages, so in this article we described the variation of cephalopharyngeal skeleton configuration and size in three blow flies species.

2. Material and Methods

2.1. Specimens and data acquisition: Between January to March 2012, we collected adults of three blow flies species in the surroundings of Hospital Adolfo Prince Lara (10° 28' 24.58" N, 68° 01' 48.82" W), Puerto Cabello Municipality, Venezuela: *Chrysomya albiceps* (Wiedemann

1819), *C. megacephala* (Fabricius 1794) and *Lucilia cuprina* (Wiedemann 1830). Each species (50 males and 50 females) was remained in the insectary until they reproduced, and females laying the eggs. Then, the larvae develops until the third-instar, and 101 specimens (34 = *C. albiceps*; 34 = *C. megacephala*; 33 = *L. cuprina*) were sacrificed to dissect the cephalopharyngeal skeleton; for clearing and mounting we follow Sukontason *et al.* [17] protocol. Were photographed, selected and digitized, eight anatomical landmarks (LM1-LM8), all according Bookstein [18] type I and II criteria: 1) Clipeal arc, 2) dorsal cornu, 3) concavity of pharyngeal sclerite (tentorial phragma), 4) ventral cornu, 5) union between hypostomal sclerite and the mouth hook, 6) apical hook, 7) dorsal apodeme of mouth hook, and 8) base of parastomal bar (Figure 1).

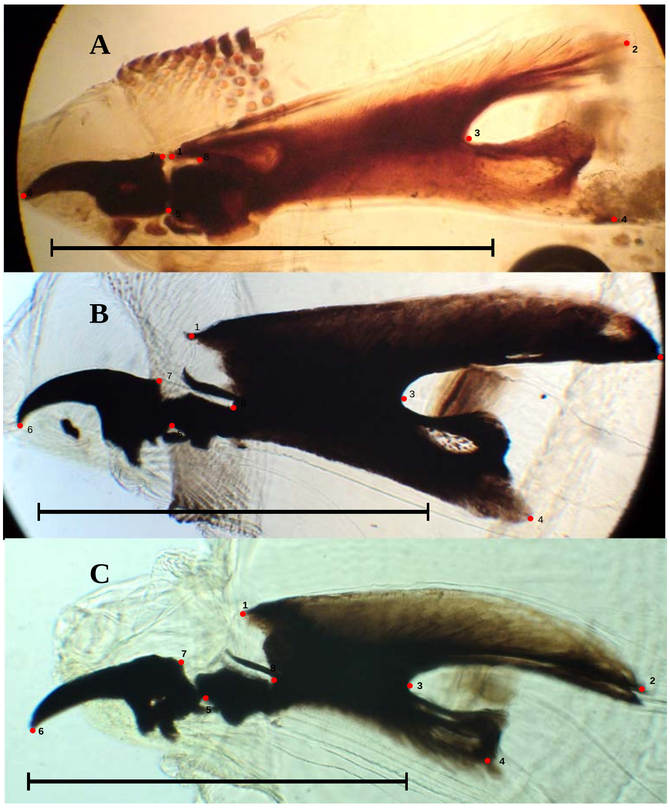

Fig 1: Cephalopharyngeal skeleton of A) *Chrysomya albiceps*, B) *C. megacephala* and C) *Lucilia cuprina*, showing the landmarks (1-8) disposition. The polygon enclosed by the points conform the configurations analyzed. Scale bar equivalent to 1 mm

2.2. Morphometric analysis: From 101 matrix configurations geometric coordinates of eight landmarks, we perform the Generalized Procrustes Analysis, with Coord Gen program [19] for Procrustes superimposition and then was extract a matrix variables conformation (Partial warps = Pw) and centroid size (CS). The Pw matrix was used for an Canonical Variates Analysis (CVA) with CVA Gen [20] to determine whether pre-defined groups (species) can be statistically distinguished based on multivariate data. The effectiveness of the CVA in assigning specimens to groups is typically determined using a cross validation procedure in which a small number of specimens are omitted from the initial calculation of the CV axes and used as a test set; the omitted specimens are then treated as unknowns and assigned using the CV axes [14, 21]. Finally, we analyzed the CS differences by means of a non-parametric ANOVA with Kruskall-Wallis test (*P* 0.05), using Bonferroni correction, with PAST statistical program [22].

3. Results and Discussion

3.1. Centroid size: We found significant differences (Kruskal-Wallis: $\chi2$ = 69.21, $p<0.001$) in the cephalopharyngeal skeleton isometric size, between the three species: *C. albiceps* (2.590 mm ± 0.015), *C. megacephala* (2.457 mm ± 0.037), and *L. cuprina* (2.091 mm ± 0.034).

3.2. Differences in cephalopharyngeal conformation: We present the assignation test results, based on *a priori* group definitions from morphological identification, and *a posteriori* assignment based on Mahalanobis distances between each specimen and the species mean. Axis 1 Λ = 0.0029, $\chi2$ = 540.467, df 24, $p<0.0001$ and Axis 2 Λ = 0.2280, $\chi2$ = 136.758, df 11, $p<0.0001$; the specimens were 100% correctly reclassified in each group. The Figure 2, show the two axes diagram form CVA, the three Calliphoridae showed high separation; *C. megacephala* and *L. cuprina* are more similar than *C. albiceps*. The thin-plate spline deformation grid show the differentiation between species (Figure 3): *C. albiceps* can be differentiate from *C. megacephala* in the displacement of the base of parastomal bar (LM8) and the displacement of the concavity of the pharyngeal sclerite (LM3); *C. albiceps* differ from *Lucilia cuprina* in the displacement of the base of parastomal bar, the displacement of apical tooth (LM6), displacement of clipeal arc (LM1), displacement of the concavity of the pharyngeal sclerite, and displacement of union between hypostomal sclerite and the mouth hook (LM5); *L. cuprina* and *C. megacephala* can be differentiate in the displacement of clipeal arc, displacement of base of parastomal bar, and the displacement of union between hypostomal sclerite and the mouth hook.

Sukontason *et al.* [20] stated that the immature stage of the fly is the stage most frequently collected from corpses and/or crime scenes, and the correct identification of the species is an initial and essential step for use as entomological evidence in a forensic investigation. There are various approaches to identify fly larvae, based on either morphology [9, 11, 24-25], immunology [26], or molecular [27-29]. Each approach has advantages and disadvantages, and the application should be based largely on the available equipment or researcher expertise.

Recently, the Geometric Morphometric has proven to be an important tool for description of the immature mouth parts in several insects groups. Pizzo *et al.* [30] studied the epipharynx shape in Scarabeidae sister species, and found that the epipharynx conformation to be a better tool than genitalia for discriminating close related species. Arambourou *et al.* [31], describing the shape in chironomid mentum larvae from exposed specimens to lead or Nonylphenol. Later, Laurito *et al.* [32] exploring the dorsomentum configuration in four *Culex* species, and concluding that the larval characters are high informative for discriminating *C. tatoi* Casal and García 1971 from *C. mollins* Dyar and Knab 1906. Bai *et al.* [33] described the mandible evolution and coprophagous habits in Scarabeinae, mapping the configurations over the preferred phylogenetic trees. However, in Calliphoridae immature the morphometrics studies has been focused only to linear measures (width or length) of the entire body for age estimation and subsequently in Postmortem Interval determination [34, 35]; in other studies the traditional morphometrics of posterior spiracle has been used for species differentiation [23, 36, 37]. In our study we found differences in the cepahalopharyngeal shape of three blowfly species that could be useful as additional tools for taxonomic identification; however, more studies are needed that include a more species representation in Calliphoridae.

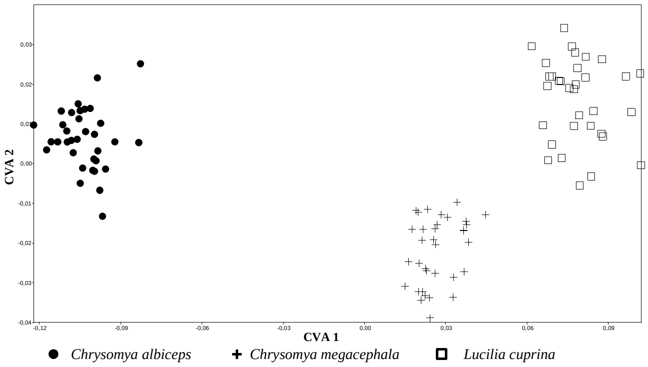

Fig 2: Canonical Variants Analysis diagram of two canonical axes from 101 Calliphoridae specimens

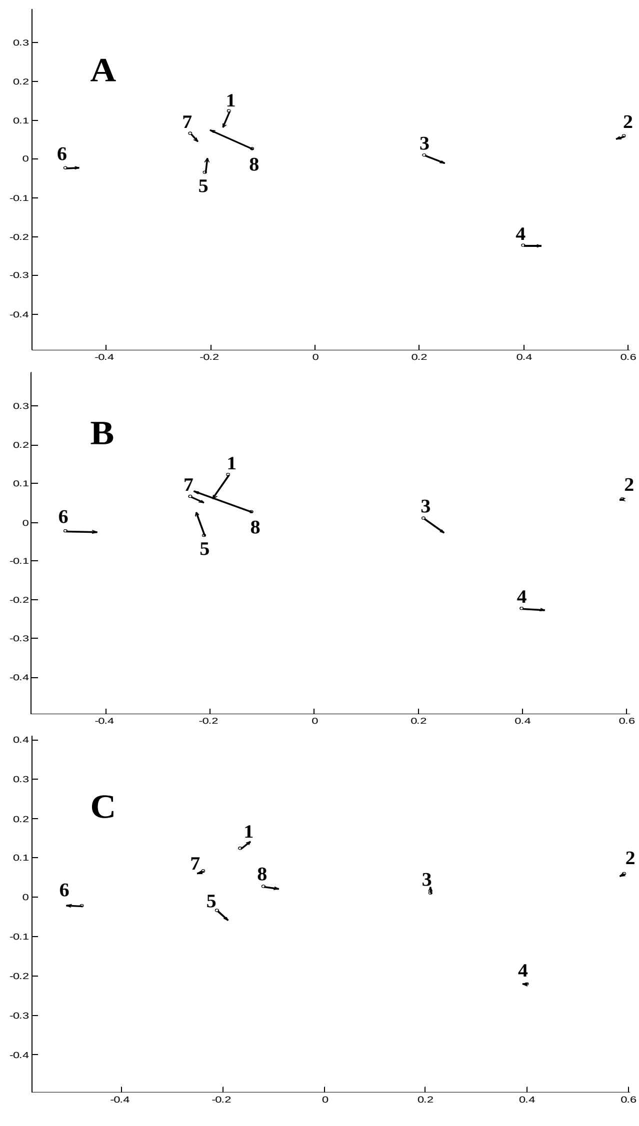

Fig 3: Grid deformation showing differences, as vector displacements, between the mean configuration of A) *Chrysomya albiceps* and *C. megacephala*, B) *C. albiceps* and *Lucilia cuprina*, and C) *L. cuprina* and *C. megacephala*

4. Acknowledgement
We appreciate the collaboration of the staff of Hospital Adolfo Prince Lara, in particular Dr. Napoleón Tocci D, for allowing the blowflies collections.

5. References

1. Stevens JR. The evolution of myiasis in blowflies (Calliphoridae). International Journal for Parasitology. 2003; 33(10):1105-1113.
2. Mariluis JC, Mulieri PR. Calliphoridae, califóridos, In Salomón OD (ed) Actualizaciones en artropología sanitaria argentina, Fundación Mundo Sano, 2005; 302:95-100.
3. Francesconi F, Lupi Myiasis O. Clinical Microbiology Reviews. 2012; 25:79-105.
4. Smith KG. A manual of forensic entomology, Trustees of the British Museum (Natural History) and Cornell University Press London, 1986, 105.
5. Catts EP, Goff ML. Forensic entomology in criminal investigations. Annual Review of Entomology. 1992; 37:253-272.
6. Liria J. Insectos de importancia forense en cadáveres de ratas, Carabobo–Venezuela. Revista Peruana de Medicina Experimental y Salud Pública. 2006; 23(1):33-38.
7. Nuñez-Rodriguez J, Liria J. Sucesión de la entomofauna cadavérica a partir de un biomodelo con vísceras de res. Salus. 2014; 18(2):35-39.
8. Sukontason K, Sukontason KL, Ngern-klun R, Sripakdee D, Piangjai S. Differentiation of the third instar of forensically important fly species in Thailand. Annals of the Entomological Society of America. 2004; 97(6):1069-1075.
9. Liu D, Greenberg B. Immature stages of some flies of forensic importance. Annals of the Entomological Society of America. 1989; 82(1):80-93.
10. Carvalho-Queiroz MM, Pinto de Mello R, Lima M. Morphological aspects of the larval instars of Chrysomya albiceps (Diptera, Calliphoridae) reared in the laboratory. Memórias do Instituto Oswaldo Cruz. 1997; 92(2):187-196.
11. Wells JD, Byrd H, Tantawi I. Key to third-instar Chrysomyinae (Diptera: Calliphoridae) from carrion in the continental United States. Journal of Medical Entomology. 1999; 36(5):635-611.
12. Florez E, Wolff M. Descripción y clave de los estadios inmaduros de las principales especies de Calliphoridae (Diptera) de importancia forense en Colombia. Neotropical Entomology. 2009; 38(3):418-429.
13. Adams DC, Rolhf FJ, Slice DE. Geometric morphometrics: Ten years of progress following the ‘revolution’. Italian Journal of Zoology. 2004; 71(1):5-16.
14. Webster M, Sheets HD. A practical introduction to landmark-based geometric morphometrics. Pp. 163-188 in J. Alroy and G. Hunt (eds.), Quantitative Methods in Paleobiology. Paleontological Society Papers, 2010, 16.
15. Lyra ML, Hatadani LM, De Azeredo-Espin AML, Klaczko LB. Wing morphometry as a tool for correct identification of primary and secondary New World screwworm fly. Bulletin of Entomological Research. 2010; 100(1):19-26.

16. Vásquez M, Liria J. Geometric wing morphometrics for Chrysomya albiceps and C. megacephala identification (Diptera: Calliphoridae) from Venezuela. Revista de Biología Tropical. 2012; 60(3):1249-1258.
17. Sukontason K, Methanitikorn R, Sukontason KL, Piangjai S, Olson JK. Clearing technique to examine the cephalopharyngeal skeletons of blow fly larvae. Journal of Vector Ecology. 2004; 29(1):192-195.
18. Bookstein FL. Morphometric tools for landmark data: Geometry and Biology. Cambridge, EEUU. 1991, 435.
19. Sheets HD. CoordGen7, Coordinate Generation program for calculating shape coordinates. 2011; Available at: http://www3.canisius.edu/~sheets/imp7.htm.
20. Sheets HD. CVAGen7, Canonical Variates Analysis program for the analysis of shape, based on partial warp scores. 2011; Available at: http://www3.canisius.edu/~sheets/imp7.htm.
21. Zelditch M, Swiderski DL, Sheets HD, Fink W. Geometric morphometrics for biologists: a primer. Boston: Elsevier Academic Press. 2004, 444.
22. Hammer Ø, Harper DAT. PAST: Palaeontological Statistics, version 2011; 2:10. Available at: http:// folk.uio.no/ohammer/past.
23. Sukontason K, Sribanditmongkol P, Ngoen-klan R, Klong-klaew T, Moophayak K, Sukontason KL. Differentiation between Lucilia cuprina and Hemipyrellia ligurriens (Diptera: Calliphoridae) larvae for use in forensic entomology applications. Parasitology Research. 2010; 106(3):641-646.
24. Erzinclioglu YZ. The larvae of some blowflies of medical and veterinary importance. Medical and Veterinary Entomology. 1987; 1(2):121-125.
25. Omar B. Key to third instar larvae of flies of forensic importance in Malaysia. In: Greenberg B, Kunich JC (eds) Entomology and the law: flies as forensic indicators. Cambridge University Press, Cambridge, 2002, 120-127.
26. McDonagh L, Thornton C, Wallman JF, Stevens JR. Development of an antigen-based rapid diagnostic test for the identification of blowfly (Calliphoridae) species of forensic significance. Forensic Science International: Genetics. 2009; 3(3):162-165.
27. Vincent S, Vian JM, Cariotti MP. Partial sequencing of the cytochrome oxidase b subunit gene I: a tool for the identification of European species of blow flies for postmortem interval estimation. Journal of Forensic Sciences. 2000; 45(4):820-823.
28. Wallman JF, Adams M. The forensic application of allozyme electrophoresis to the identification of blowfly larvae (Diptera: Calliphoridae) in southern Australia. Journal of Forensic Sciences. 2001; 46(3):681-684.
29. Rolo EA, Oliveira AR, Dourado CG, Farinha A, Rebelo MT, Dias D. Identification of sarcosaprophagous Diptera species through DNA barcoding in wildlife forensics. Forensic Science International. 2013; 228(1-3):160-164.
30. Pizzo A, Lorenza A, Macagno M, Roggero A, Rolando A, Palestrini C. Epipharynx shape as a tool to reveal differentiation patterns between insect sister species: insights from Onthophagus taurus and *O.* illyricus (Insecta: Coleoptera: Scarabaeidae). Organisms, Diversity & Evolution. 2009; 9(3):189-200.
31. Arambourou H, Beisel JN, Branchu P, Debat V. Patterns of Fluctuating Asymmetry and Shape Variation in Chironomus riparius (Diptera, Chironomidae) Exposed to Nonylphenol or Lead. PLoS ONE 2012; 7(11):e48844. doi:10.1371/journal.pone.0048844
32. Laurito M, Almirón WR, Ludueña-Almeida FF. Discrimination of four Culex (Culex) species from the Neotropics based on geometric morphometrics. Zoomorphology. 2015; 134(3):447-455.
33. Bai M, Li S, Lu Y, Yang H, Tong Y, Yang X. Mandible evolution in the Scarabaeinae (Coleoptera: Scarabaeidae) and adaptations to coprophagous habits. Frontiers in Zoology. 2015; 12(1):30-40.
34. Vélez MC, Wolff M. Rearing five species of Diptera (Calliphoridae) of forensic importance in Colombia in semicontrolled field conditions. Papéis Avulsos de Zoologia. 2008; 48(6):41-47.
35. Sharmaa R, Garga RK, Gaurb JR. Various methods for the estimation of the post mortem interval from Calliphoridae: A review. Egyptian Journal of Forensic Sciences. 2015; 5(1):1-12.
36. Amorim JA, Ribeiro OB. Distinction among the puparia of three blowfly species (Diptera: Calliphoridae) frequently found on unburied corpses. Memórias do Instituto Oswaldo Cruz. 2001; 96(6):781-784.
37. Sukontason KL, Ngern-Klun R, Sripakdee D, Sukontason K. Identifying fly puparia by clearing technique: application to forensic entomology. Parasitology Research. 2007; 101(5):1407-16.