Geometric Morphometrics Sexual Dimorphism in Three Forensically- Important Species of Blow Fly (Diptera: Calliphoridae)[1]

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Abstract: Forensic entomologists use adult and immature (larvae) insect specimens for estimating the minimum postmortem interval. Traditionally, this insect identification uses external morphology and/or molecular techniques. Additional tools like Geometric Morphometrics (GM) based on wing shape, could be used as a complement for traditional taxonomic species recognition. Recently, evolutionary studies have been focused on the phenotypic quantification for Sexual Shape Dimorphism (SShD). However, in forensically important species of blow flies, sexual variation studies are scarce. For this reason, GM was used to describe wing sexual dimorphism (size and shape) in three Calliphoridae species. Significant differences in wing size between females and males were found; the wing females were larger than those of males. The SShD variation occurs at the intersection between the radius R_1 and wing margin, the intersection between the radius R_{2+3} and wing margin, the intersection between anal vein and CuA_1, the intersection between media and radial-medial, and the intersection between the radius R_{4+5} and transversal radio-medial. Our study represents a contribution for SShD description in three blowfly species of forensic importance, and the morphometrics results corroborate the relevance for taxonomic purposes. We also suggest future investigations that correlated shape and size in sexual dimorphism with environmental factors such as substrate type, and laboratory/sylvatic populations, among others.

Key Words: Geometric morphometric sexual dimorphism, wing, shape, size, Diptera, Calliphoridae, Chrysomyinae, Lucilinae

Introduction

In determinig the minimum postmortem interval (PMI), forensic entomologists use blowflies (Diptera: Calliphoridae) and other insects associated with body corposes (Bonacci et al. 2009, González et al. 2011, Nuñez et al. 2016). Habitually, blowfly species identification comprises taxonomic dichotomous keys for adults (Carvalho and Mello-Patiu 2008, Amat et al. 2008, Solano and Ramírez 2015) or immature stages (Florez et al. 2009, Szpila et al. 2013), to provide reliable information to reconstruct the event involving the perpetrator in court

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trials (Ramos et al. 2012). The blowflies have been the target of forensic entomology studies for PMI estimation because they are among the first insect group that colonizes the body (Ahmed and Joseph 2016).

In some cases, however, the insects collected comprise shattered and fragmented specimens, thus complicating identification. Newer techniques, such as molecular identification, could be useful for solve these problems (Mazzanti et al. 2010; Boehme et al. 2010, 2012; Rolo et al. 2013). Molecular tools have been demonstrated to have high specificity and sensitivity, allowing taxonomic classification without waiting until adult emergence, or require specific knowledge of traditional taxonomy (GilArriortua et al. 2015). Nevertheless, DNA identifications have some disadvantages: high cost, low species database records representation, and the time necessary to obtain results, as many cases demand immediate information that can contribute to the justice system (Solano et al. 2013, Pérez and Carvajal 2013). New tools, like geometric morphometrics, play a key role because they easily and quickly establish species identification based on anatomical points, like fingerprints in biometric studies. The only requirement of geometric morphometrics is a morphometric database against which to compare the specimens. Geometric morphometrics is a technique that quantifies phenotypic differences among groups (Toro et al. 2010), and recently demonstrated its importance for blowfly adult and immature taxonomic identification (Vásquez and Liria 2012, Nuñez and Liria 2016).

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Benitez (2013) stated that sexual dimorphism is the most interesting source of phenotypic variation in an organism. Sexual differences in morphological characters are common phenomena in many taxa. Recently, some studies are focused in the Sexual Shape Dimorphism (SShD) investigations (Benítez et al.,

2013), that use geometric morphometrics techniques for describing sexual shape variation. In Calliphoridae, SShD investigations are scarce (Lyra et al., 2010), although they comprise an important research area in evolutionary biology (Gidaszewski et al. 2009) and medical entomology (Dujardin et al. 1999; Dujardin and Slice 2007). Due to the scarcity of SShD data, we used geometric morphometrics to describe the wing sexual dimorphism (size and shape) in three forensically important blowfly species.

Methods
Sampling and data acquisition

Between January and March 2012, we collected *Lucilia cuprina* (Wiedemann, 1830) and *Chrysomya megacephala* (Fabricius, 1794) in the surroundings of Hospital Adolfo Prince Lara (10°28'24.58" North Latitude and 68°01'48.82" West Longitude), Puerto Cabello Municipality, Venezuela. Later, in January 2016, *Chrysomya albiceps* (Wiedemann, 1819) was collected in Valencia City (10°13'78.00" North Latitude and 68º00'32.00" West Longitude), Valencia Municipality, Venezuela (Figure 1). For each species, we sorted the specimens into males and females: *L. cuprina* (21 females and 19 males), *C. megacephal*a (21 females and 19 males) and *C. albiceps* (35 females and 35 males). The right wing was dissected, slide mounted, photographed, including eight type I (e.g., tissue juxtaposition or intersection of wing veins) digitalized landmarks, Figure 2) following Booskstein (1991) definitions and McAlpine (1987) for morphological descriptions. The landmarks (LM) are, as follows: intersection between costa and subcosta veins (LM1), intersection between radius R_1 and wing margin (LM2), intersection between radius R_{2+3} and wing margin (LM3), media vein curvature (LM4), intersection between anterior cubital (CuA_1) and discal medial-cubital (LM5), intersection between anal vein and CuA_1 (LM6), intersection between media and radial-medial (LM7), intersection between radius R_{4+5} and transversal radio-medial (LM8).

Morphometric analysis

The 150 specimens x, y coordinate matrix configurations was analyzed in CoordGen (Sheets 2011a) for Procrustes superposition and extracting the conformation variables (Partial warps) and the Centroid Size (CS); the Partial warps matrix was used in a Canonical Variate Analysis (CVA) and Multivariate Analysis of Variance with CVAGen program (Sheets 2011b), for determining whether pre-defined groups (species - sex) can be statistically distinguished based on multivariate data (Webster and Sheets 2010). Finally, the CS was analyzed by means a Kruskal-Wallis test with Bonferroni correction in PAST program (Hammer and Harper 2011).
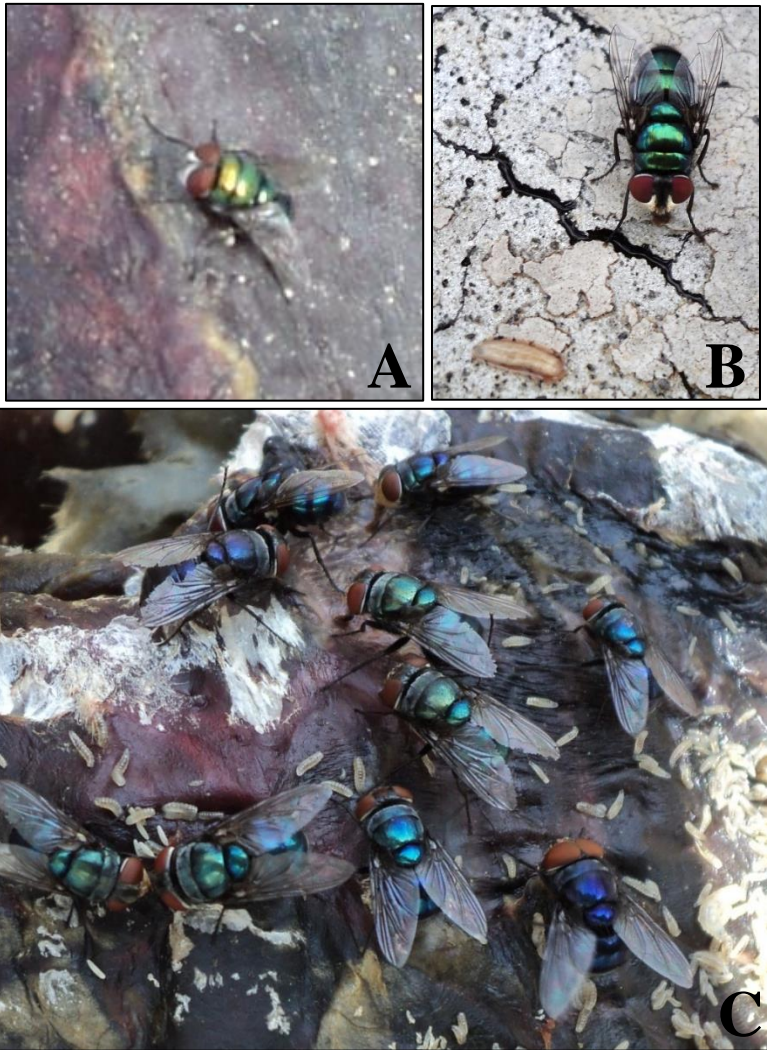
Figure 1. Representative flies of forensic entomology importance used in this study, all photographed in nature over carrion. A. *Lucilia cuprina* (Wiedemann, 1830). B. *Chrysomya albiceps* (Wiedemann, 1819). C. *Chrysomya megacephala* (Fabricius, 1794). All images taken by José Antonio Nuñez-Rodríguez.
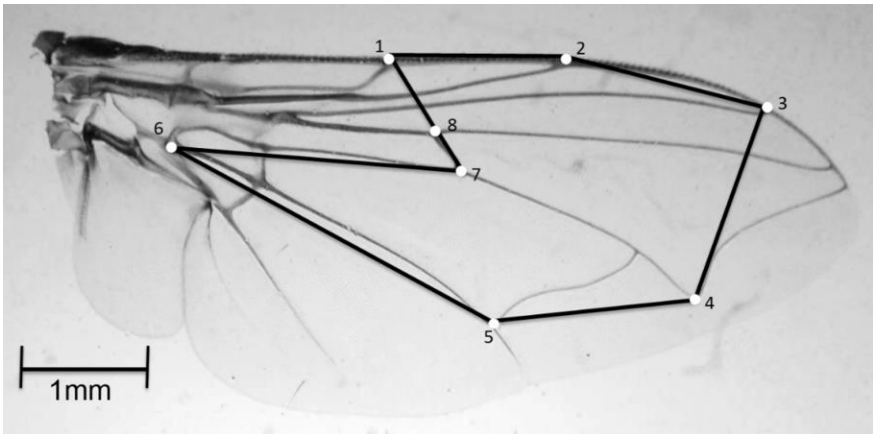
Figure 2. Wing of *Lucilia cuprina* showing the eight-landmark disposition. See text for definitions. The polygon enclosed by the landmarks correspond with the conformation studied.

Results

Sexual Size Dimorphism

Lucilia cuprina specimens were larger (3.62-4.76 mm), followed by *C. megacephala* (3.38-4.40 mm) and *C. albiceps* (2.43-4.22 mm); the female wings were significantly (Kruskal-Wallis $X^2 = 90.58$, df = 5, p < 0.001) larger than males: *Lucilia cuprina* females (mean $4.39 \pm$ SD 0.22) and males (4.06 ± 0.19), *C. megacephala* females (4.09 ± 0.13) and males (4.08 ± 0.26), and *C. albiceps* females (3.54 ± 0.44) and males (3.48 ± 0.30).

Sexual Shape Dimorphism

The first four CVA axes explained 47.59% of variance (CV1 = 43.80%, CV2 = 2.81%, CV3 = 0.65% and CV4 = 0.33%), all axes showed significant discrimination with lambda Wilks (Λ): Axis 1 $\Lambda_1 = 0.0024$, $X^2 = 844.00$ df = 60 p < 0.001, $\Lambda_2 = 0.1079$, $X^2 = 311.67$ df = 44, p < 0.001, $\Lambda_3 = 0.4121$, $X^2 = 124.12$, df = 30, p < 0.001, and $\Lambda_4 = 0.6782$, $X^2 = 54.35$, df = 18, p < 0.001. Table 1 shows the assignment *a posterior*i re-classification; the best assignment was in *C. megacepahala* females (100%), *C. megacephala* males (100%), *L. cuprina* females (95%), and *C. albiceps* males (94%), followed by and *C. albiceps* females (88%) and *L. cuprina* males (84%).

Table 1. Results of statistical testing of group assignments for total sample of 150 blowfly specimens, based on a posteriori assignments using the CV axes and Mahalanobis distances between each specimen and the group (species - sex) mean.

A priori (before morphometric analyses) assignments	A posteriori (after the morphometric analyses) assignments					
Lucilia cuprina females	20	1	0	0	0	0
L. cuprina males	3	16	0	0	0	0
Chrysomya megacephala females	0	0	21	0	0	0
C. megacephala males	0	0	0	19	0	0
C. albiceps females	0	0	2	0	31	2
C. albiceps males	0	0	0	0	2	33

The two axes diagram form CVA for three species of blow flies and sex showed (Figure 3) the separation into two mainly groups: one comprises *Lucilia cuprina* (male and females), and the another with the remaining species; finally, C*hrysomya megacephala* its separate from *C. albiceps*. The thin-plate spline deformation grid show differentiation between species (Figure 4): The main differences between *L. cuprina* and the *Chrysomya* species occur in the displacements of LM2, LM5, LM7 and LM8 (Figure 4A, 4B), and between *C. megacephala* and *C. albiceps* occurs short displacements in LM7 and LM8 (Figure 4C). Also, Figure 4 shows the thin-plate spline deformation grid SShD differences; *Lucilia cuprina* differences between males and females occurs in LM2, LM3 and LM7 (Figure 4D). The intersection between radius R_1 and wing margin (LM2) move to LM8, then the intersection between radius R_{2+3} and wing margin (LM3) displace forward, and the intersection between radius R_{4+5} and transversal radio-medial (LM7) displacement to LM8. In *C. megacephala* the changes between males and females occur in LM2, LM3, LM6, LM7 and LM8 (Figure 4E). The LM2 move to backward, LM3 displace to LM4, the intersection between anal vein and CuA_1 (LM6) move to backward, LM7 displace to LM3, and the intersection between radius R_{4+5} and transversal radio-medial (LM8) move to LM2. Finally, in *C. albiceps* the main differences between males and females occurs in LM2, LM3 and LM7 (Figure 4F). The LM2 displace backward, LM3 move forward, and LM7 displace to LM8.
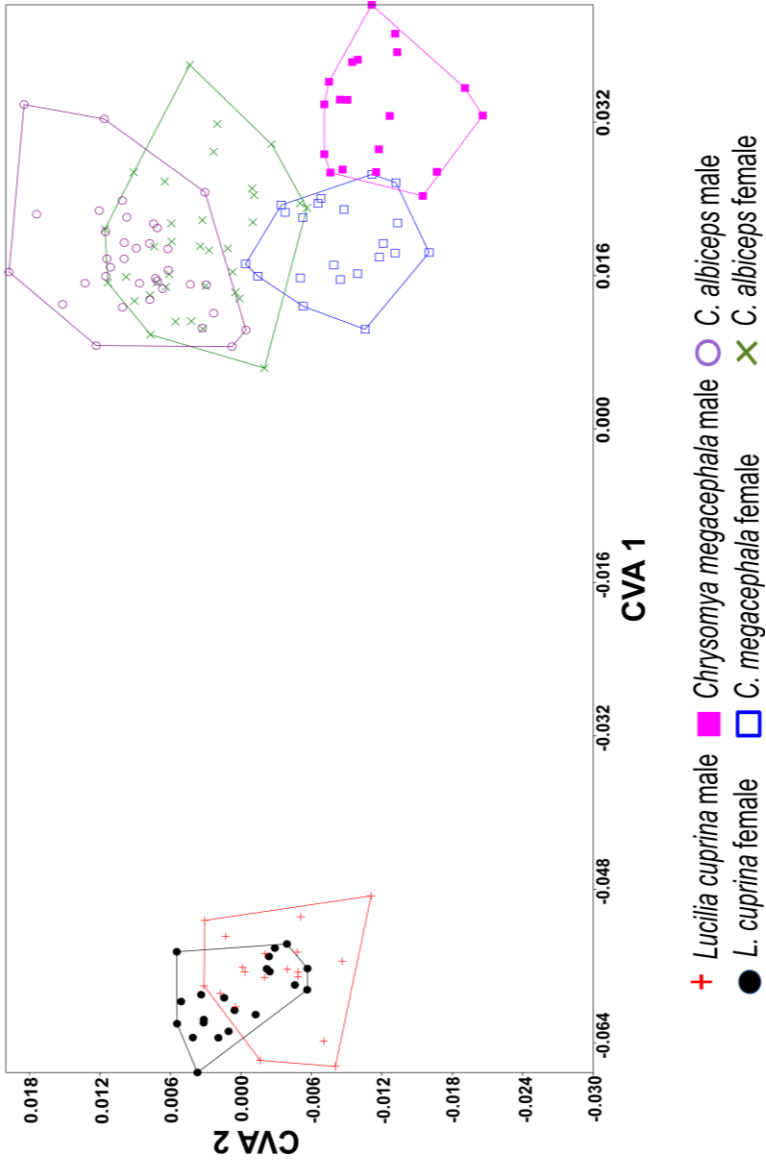
Figure 3. Canonical Variates Analysis diagram of two canonical axes from 150 blowflies (Calliphoridae) specimens by species and sex.
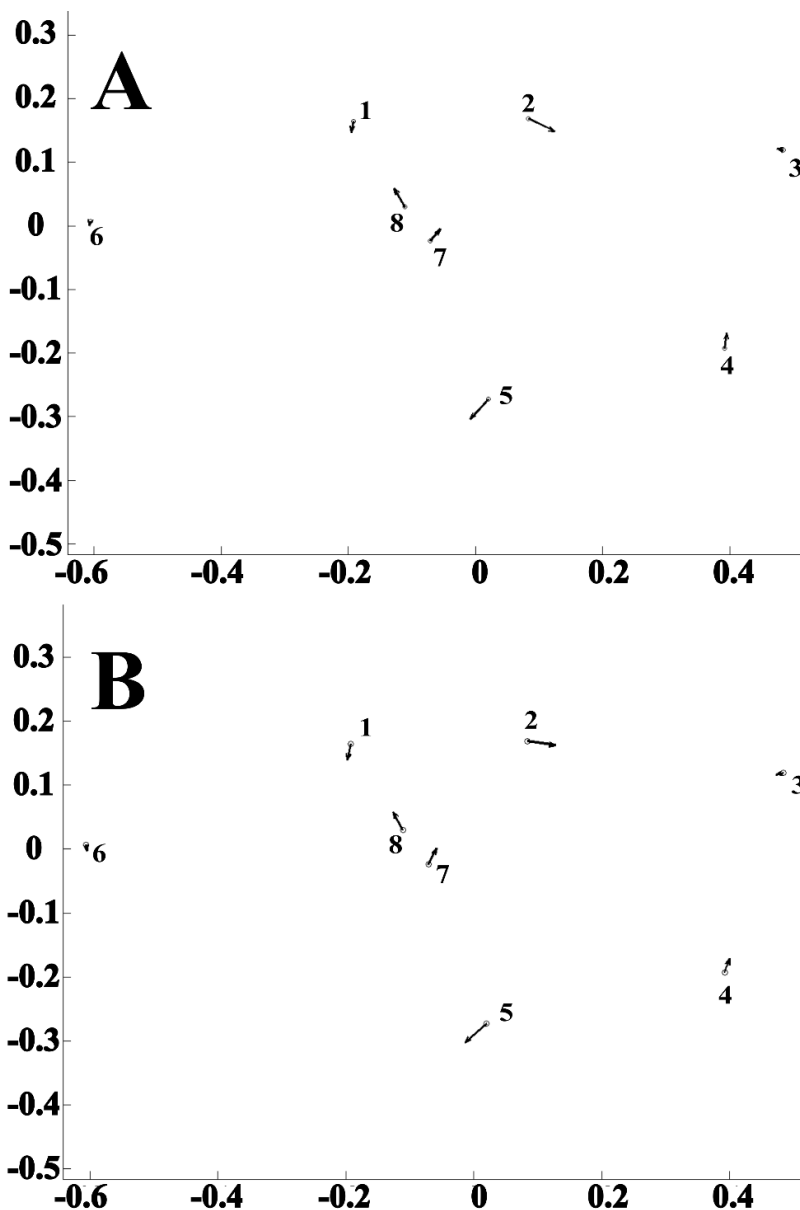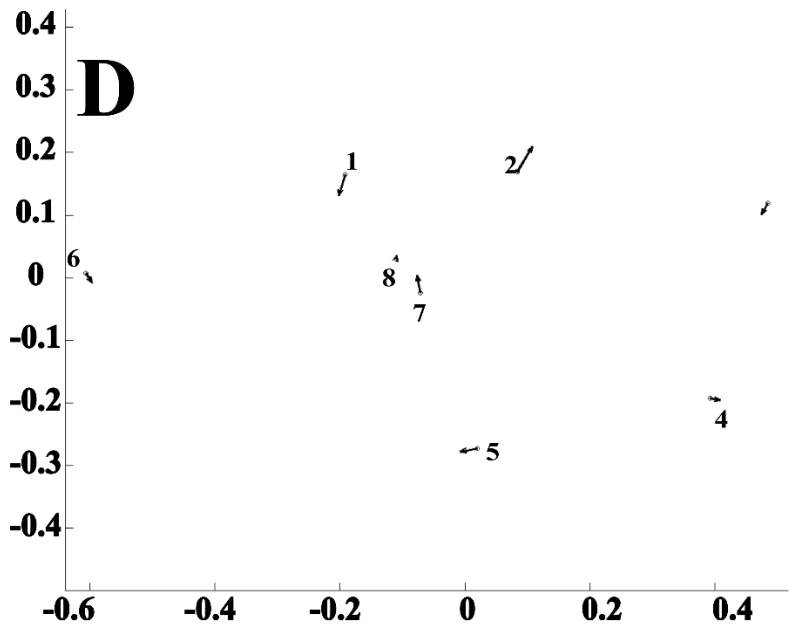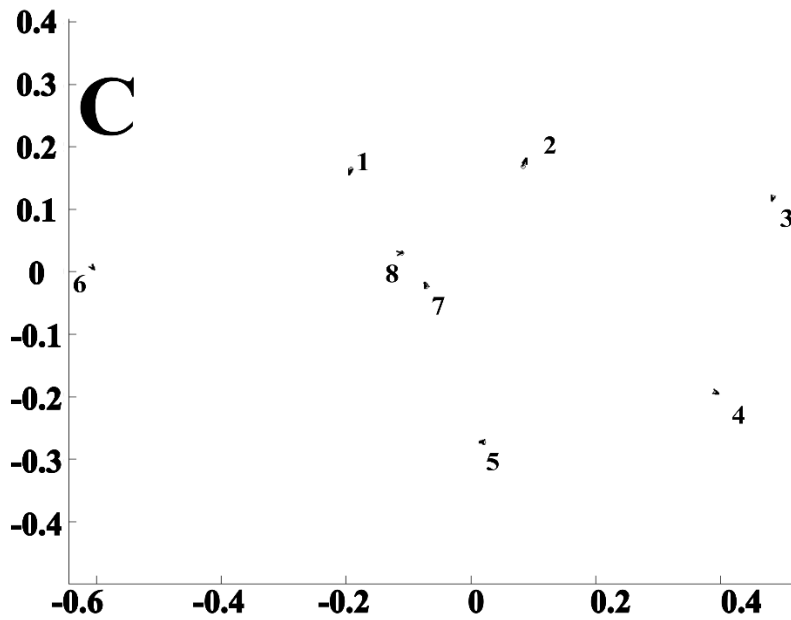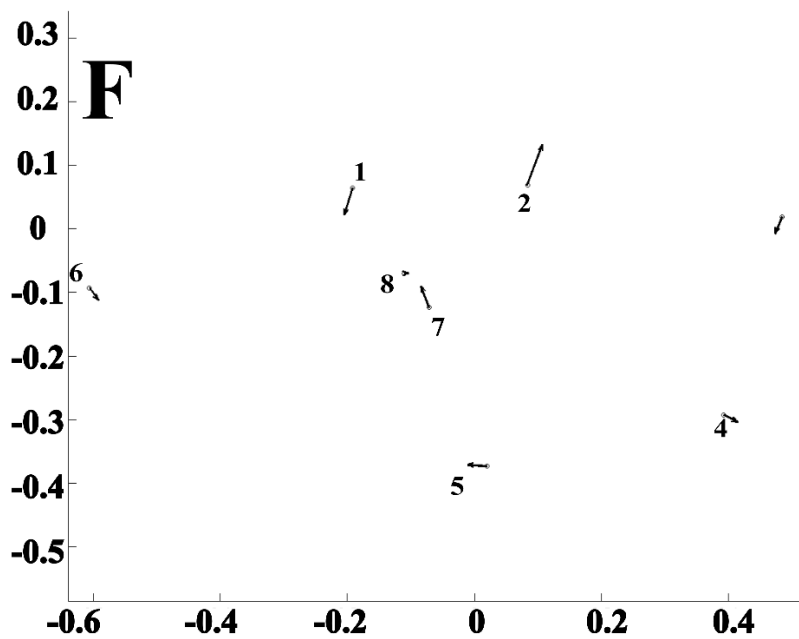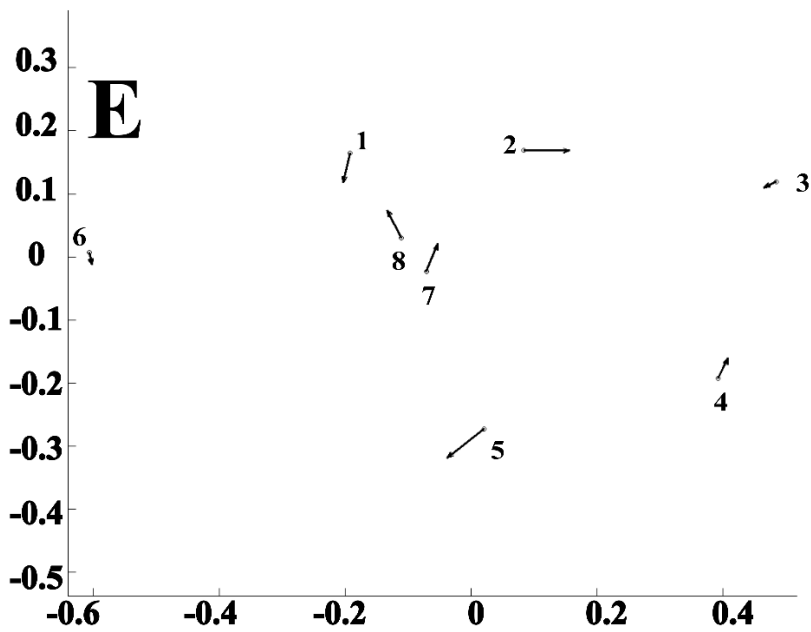
Figure 4.   Thin-plate spline grid deformations between: A) *Lucilia cuprina* and *Chrysomya megacephala*, B) *L. cuprina* and *C. albiceps*, C) *C. megacephala* and *C. albiceps*, D) *Lucilia cuprina* male and female (20x exaggeration), E) *Chrysomya megacephala* male and female (10x exaggeration), and F) *C. albiceps* male and female (20x exaggeration). Figure C-F in the following two pages.

Discussion

The geometric morphometrics is shown to have value for describing sex shape and size differences among several organisms. In forensic anthropology, it is a reliable tool for sex determination in complete bone or fragmented pieces. Recently, GM was used in skulls (Menéndez and Lotto, 2013), clavicle (Alcina et al. 2015), and pelvis (Huseynov et al. 2016). In entomology, Dujardin et al. (1999) demonstrated for Triatominae bugs, that wild female specimens are larger, in contrast to those reared in laboratory. Aldana et al. (2011) determining wing SShD in *Panstrongylus geniculatus* (Latreille, 1811) specimens collected in sylvatic ecotopes. In other insects, Vergara et al. (2014) found in *Chiasognathus grantii* (Stephens, 1831) sexual dimorphism variations in body shape and mandibles size. These authors consider that differences could be attributed to selective pressure due to intrasexual competition for food resources, or due to environmental variations during the ontogenetic development. In blowflies, we found only two SShD investigations: Lyra et al. (2010) using 16 landmarks in *Cochliomya macellaria* (Fabricius, 1775) and *C. hominivorax* (Coquerel, 1858) wings. *Cochliomya macellaria* females were larger than males, and this size variation could be interpreted as a fecundity advantage. However, in *C. hominivorax* males were significantly larger than females, suggesting the existence of selective pressures probably related to male behavior. Recently, Espra et al. (2015) using 21 landmarks for describing wing conformation; found significant shape differences among males and females of *Lucilia sericata* (Meigen, 1826). In forensic studies, Pinilla et al. (2010) consider that carcasses size and availability and environmental factors associated to the site of perimortem wounds, are a primary determinant promote sexual dimorphism differences. Our study represents a contribution to the SShD description in three Venezuelan blowfly species of forensic importance, and the results corroborate the value of geometric morphometrics for taxonomic purposes and suggests future investigations that correlated shape and size sexual dimorphism with environmental factors such as the substrate type, laboratory/sylvatic populations, among others.

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