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Wing geometric morphometrics as a tool for taxonomic identification of two fly species (Diptera: Muscidae) of forensic relevance

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Abstract

The taxonomic identification of fly species through wing geometry is a helpful tool for entomologists and officials involved in forensic research, who not necessarily require expertise on insect taxonomy. Members of the Muscidae family are relevant sources of evidence in forensic entomology; however, developing countries often lack experts in the taxonomical identification of essential species for the assessment of aspects such as the minimum postmortem interval (mPMI). Our study proposes a low-cost, fast, and technologically-accessible quantitative tool for the identification of *Atherigona orientalis* and *Ophyra aenescens*, associated with human corpses at advanced states of decomposition. We propose a tool that is based on the geometric variability observed in eight homologous landmarks on wing veins and the interpretation of morphometric estimates after a generalized Procrustes analysis. The use of a geometric approach for effective discrimination between *Atherigona orientalis* and *Ophyra aenescens* was supported by statistically significant differences in wing conformation and size. The evidence presented in this study shows that the analysis of geometric variability in the wing morphology of species of forensic relevance can contribute to simple and objective species identification. Geometric morphometrics is a simple and readily available tool for forensic science.

Keywords: *Muscomorpha, Calyptratae, forensic entomology, landmarks.*

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Introduction

Bacterial activity drives physico-chemical changes during the decomposition of cellular tissues in a lifeless human body, that attract scavenging species on specific brackets of time and at delimited anatomical parts of the host. The tempo and mode of corpse colonization by scavenging species has an inherent ecological complexity, which is associated to the death, decomposition, and putrefaction of the human body, and which is the subject of forensic entomology. Several factors further affect the decomposition process. For example, the larger the volume and mass of the decomposing body are, the greater the abundance and complexity the cadaveric entomofauna has (Matuszewski *et al*., 2016). Wells and LaMotte (2017) defined the term "forensic entomology" as all activities associated with the use of insects to estimate the moment of death. The former authors also defined two common sources of information for postmortem interval estimation. The first was based on the development of an individual insect, particularly blow flies (Calliphoridae). The second was related to changes in the composition of insect communities in a corpse (succession).

The minimum postmortem interval (mPMI) is frequently used in forensic entomology and allows the establishment of time intervals between corpse discovery and time of death; both are sources of evidence that can be used to verify the testimonies of witnesses and defendants. It is essential for forensic procedures to understand the ecological succession, life cycles and taxonomy of the species associated to corpses, mainly because the use of the entomofauna as evidence depends on various circumstances which are often specific to each investigative case (Keshavarzi *et al*., 2016). The presence of external and environmental factors, such as clothing and temperature, affects the mPMI due to the extended time that insects take to colonize the host (Matuszewski *et al*., 2016).

As active decomposers of corpses, members of the Muscidae family are essential to forensic science and are present in tropical and subtropical regions of the world (Grzywacs and Pape, 2014). The large number of flies and the diversity of Muscidae species that are found in corpses at tropical regions requires significant taxonomical effort and entomological expertise, which is not always available to forensic cases in developing countries. Latin America has traditionally lacked the interest to develop new knowledge and technologies in forensic science that could be adapted to local conditions and which could use the available entomofauna as an indicator of time of death (Ramos-Pastrana *et al*., 2012; García-Ruilova and Donoso, 2015; Rodríguez-Olivares *et al*., 2015). Based on empirical evidence, Ramírez (2012) has remarked on the scarcity of experts in the study of Muscidae as indicators of the mPMI, which has a negative influence on the development of forensics in the South American region.

As is the case of *Atherigona orientalis* Schiner, 1868 and *Ophyra aenescens* (Wiedemann, 1830), certain Muscidae species can represent considerable challenges for taxonomy, despite their usefulness for forensics, as both species can be used as source material for fast and low-cost alternatives for mPMI estimation, but due to their small size, these species are often discarded during forensic processes and analyses (Grzywacz *et al*., 2017a; Ren *et al*., 2018).

Other studies in forensic entomology have demonstrated the importance and benefit of using the morphology of scavenging species for taxonomical identification and the subsequent use of determined species as markers of the mPMI (Lyra *et al*., 2010; Vásquez and Liria, 2012; Nuñez and Liria, 2016a,b; Macedo, 2017). The wing structure in adults and the cephalopharyngeal skeleton in larvae have been shown to have useful information for taxonomical classification (Lyra *et al*., 2010; Vásquez and Liria, 2012; Nuñez and Liria, 2016b). By employing homologous features in biological organisms, geometric morphometrics serves to quantify phenotypic variation and explore changes in morphological shape (Bookstein, 1991). Our study presents quantitative tools in geometric morphometrics for the identification of *Atherigona orientalis* and *Ophyra aenescens*, both species were proposed as relevant to the determination of the mPMI in human corpses at advanced states of decomposition.

Materials and Methods

Specimens and data

Bovine meat was left to rot for five days. This advanced state of decomposition served as bait to collect a total of 64 specimens in *Atherigona orientalis* (n=32) and *Ophyra aenescens* (n=32). Individuals were classified into either of both species by taxonomic keys (Carvalho *et al*., 2002; Patitucci *et al*., 2013). We sampled for flies at an urban zone in the city of Valencia (Carabobo State, Venezuela, 10º13’78’’ N and 68º00’32’’ W). The right wings of each specimen were dissected and fixed on microscope slides with Faure’s aqueous fixing medium and prepared according to the protocol by Martín (1994), which includes distilled water (50 ml), Arabic gum (30 gr), glycerol (20 ml), and chloral hydrate (50 gr). We photographed the 64 wings with a digital camera (Sony Cyber Shot 16.2) mounted on a microscope (Nikon Eclipse E100) and assisted by a tripod. We digitized each wing image on x and y coordinates with the TPSDig digitizing program (Rohlf, 2008). We established a total of eight homologous landmarks on wing veins, which correspond to type I landmarks according to Bookstein (1991) and named after the anatomical definitions by McAlpine (1987). These homologous landmarks (LM) were: intersection of the subcoastal cell with the wing margin (LM1), intersection between the R1 vein with the wing margin (LM2), intersection between the R_{2+3} vein and the wing margin (LM3), intersection between vein R_{4+5} and the wing margin (LM4), intersection of the cubital-median transversal discal vein with the median vein (LM5), intersection of the median vein with the transversal radio-median vein (LM6), intersection of the radial vein (R_{4+5}) with the transversal radio-median vein (LM7) and intersection at the bifurcation of the radian vein at R_{2+3} and R_{4+5} (LM8).

Morphometric analysis

We used a generalized Procrustes analysis on MorphoJ (Klingenberg, 2011) to estimate shape variables and centroid size (CS) from the landmark coordinates obtained from the 64 specimens. We subsequently used the shape variables for a discriminant analysis (DA) that provided insights on the quantitative classification of specimens into either *Atherigona orientalis* or *Ophyra aenescens*. Differences between classified individuals on CS were analyzed by a Kruskal-Wallis test, with a Bonferroni correction on PAST (Hammer and Harper, 2011).

Results

There were significant differences (x^2=47.26, df=1, P < 0.001) on wing geometric size *Atherigona orientalis* ($\overline{x}$=1294.83 pixels (px), sd=101.97 px) and *Ophyra aenescens* ($\overline{x}$=1794.30 px, sd=98.33 px). As shown by the first function inferred from the DA, the shape of the wing, as quantified by the selected landmarks, is a significant discriminator of both *Ophyra aenescens* and *Atherigona orientalis* (Fig. 2). The totality of studied individuals is correctly classified into their corresponding species on the first discriminant function. The first discriminant function on wing shape is statistically significant as inferred by a Hoteling's T-square test (T^2=2553.16, *P*<0.0001). An interpolation of the average thin-plate splines estimated for both *O. aenescens* and *A. orientalis* shows the main features that differentiate these two species in terms of the general geometry of the wing at the established landmarks (LM1-LM8). The overall geometry for *O. aenescens* is much constricted or compressed than that for *A. orientalis*. In this same sense, the latter species has a broader and more squared wing than the former.

Discussion

Atherigona orientalis is a pantropical species, frequently found on forensic cases, and therefore the individuals of this species are relevant to criminalistic studies. Proper identification of this species is essential to the study of ecological succession on human corpses in urban areas of Venezuela and other countries such as Ecuador (Salazar and Donoso, 2015) and Colombia (Uribe *et al*., 2010). *Atherigona orientalis* shows a preference for diverse substrates such as feces, entrails, urban residues, corpses, fruits, and plant organic material (D'Almeida and Pinto, 1996; D'Almeida and Almeida, 1998; Salazar *et al*., 2012). The presence of this species on laboratory rat corpses (*Rattus norvegicus* Berkenhout, 1769), rabbits [*Oryctolagus cuniculus* (Linnaeus, 1758)] and monkeys (*Macaca fascicularis* Raffles, 1821) has been reported as early as the first day of death (Azwandi *et al*., 2013). This species has also been found in corpses at advanced states of decomposition, or that have been partially carbonized (Oliveira *et al*., 2014; Mashaly, 2016). Its presence has also been reported in Venezuela on pig corpses after the third day of death (Centeno, 2016). *A. orientalis* can be confused with *A. reversura*, the Bermudagrass stem pest, which was recorded for the first time in Argentina (Patitucci *et al*., 2016) and later in Brazil (Ribeiro *et al*., 2016). However, both species can be differentiated by the following characteristics: 1) In *A. orientalis* the wing r-m crossvein is beyond the middle dm cell, as well as beyond the intersection of the subcostal and costal veins. 2) In *A. reversura* the r-m crossvein is always present in the basal half of the dm cell and anterior to the intersection of the subcostal and costal veins (Ribeiro *et al*., 2016).

Ophyra aenescens is a widespread species, which is originally from the Neotropics. This species has been reported on human corpses during exhumation, therefore it is valuable for the study of taphonomic processes (Mariani *et al*., 2014) and mPMI estimation on corpses at advanced states of decomposition, regardless of season or time of year (Rocha *et al*., 2009; Battán *et al*., 2010). The presence of this species has been reported from Venezuela in morgues and urban zones of the Carabobo state (Nuñez *et al*., 2016). This species can be recognized from other members of the genus by the presence of yellow-orange palpi and a long and wide ocellar triangle, with a rounded apex that reaches the lunule (Carvalho *et al*., 2002; Patitucci *et al*., 2013).

As shown in the present study, the use of geometric landmarks on insect wings and the geometric assessment of biological shape allow for objective and accurate taxonomical identifications on quantitative grounds and

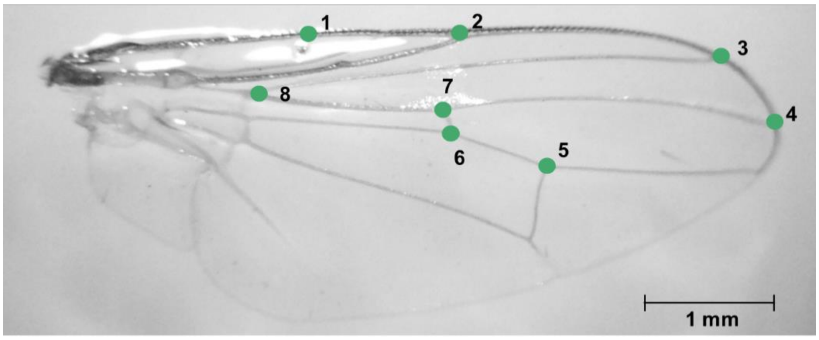

Figure 1. Wing of *Ophyra aenescens*, showing the arrangement of landmarks (LM1-LM8).

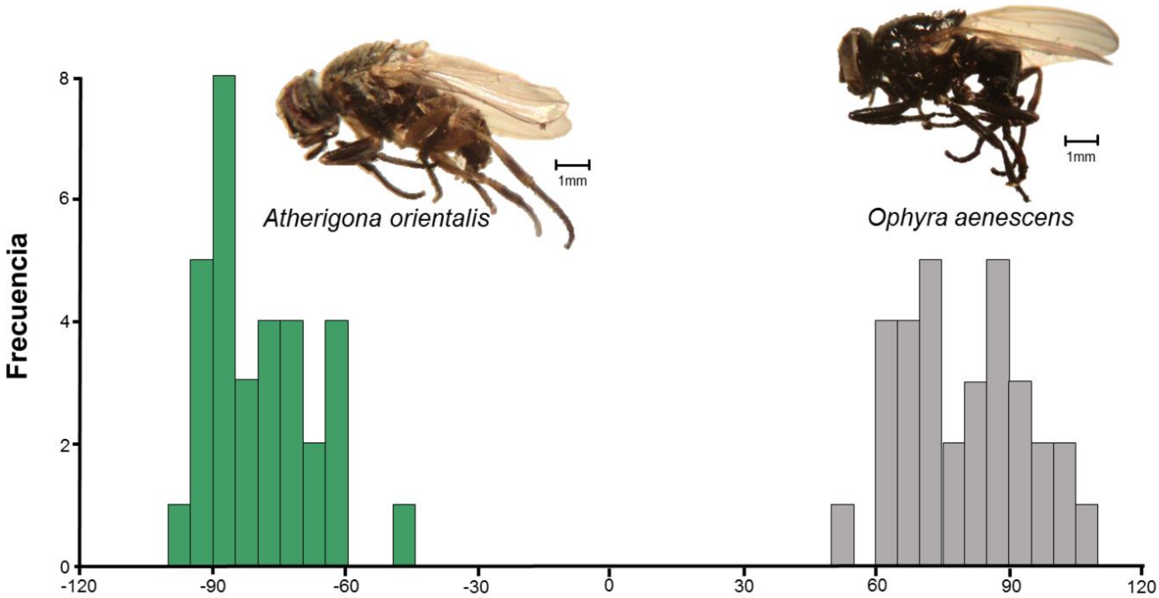

Figure 2. Histogram of the first canonical axis after a discriminant analysis on the wing morphology of *Atherigona orientalis* and *Ophyra aenescens*.

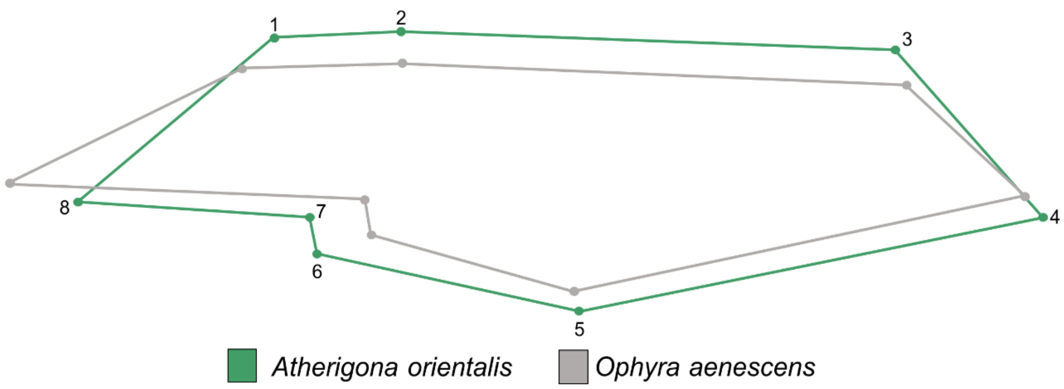

Figure 3. Thin-plate spline showing the average differences in wing shape between *Atherigona orientalis* and *Ophyra aenescens*.

with an estimate of statistical error. For 13 genera of forensic relevance in the Muscidae family, the use of geometric morphometrics on wing landmarks can have a 99.8% taxonomic accuracy (Grzywacs *et al*., 2017b). Our study differs from Grzywacs *et al*. (2017b) in that we used a set of eight landmarks, instead of fifteen, and we also included size as a relevant factor; also, Grzywacs *et al.* (2017b) did not provide details on those landmarks which were more critical to establish differences between genera or species. Grzywacs *et al*. (2017b) used a relatively low sample size to represent four genera (*Azelia*, *Graphomya*, *Mydaea,* and *Polietes*), and this may preclude a robust estimation of the necessary covariance matrix for CVA/MANOVA, especially considering that sample size must often be larger than the number of analyzed variables. The differences in methods between Grzywacs *et al*. (2017b) and our research make comparison of both studies difficult.

As a tool, the taxonomical identification via wing geometry is an advantage to both entomologists and forensic officials who are involved in forensic research, and who do not necessarily require proved expertise on insect taxonomy. However, the use of quantitative tools for the taxonomical identification of entomofauna of forensic relevance should always follow an initial qualitative approach on taxonomy, often supported by taxonomical keys. Quantitative assessments of morphology are particularly necessary for genera such as *Hydrotaea, Ophyra,* and *Muscina*.

Quantitative tools, such as the geometric analysis of insect wings, could serve to evaluate the presence and magnitude of sexual dimorphism. An assessment of wing geometry through quantitative methods can also be used for determining the relation of morphological variation and community structure to environments and substrates such as corpses. The application of geometric morphometrics has been applied with success in the Calliphoridae and Piophilidae families, both groups have forensic relevance (Nuñez and Liria, 2016b; 2017; Sontigun *et al*., 2017).

The evidence presented in this study showed that the analysis of the geometric variability of the wing structure of forensically relevant species is a simple and affordable tool. This proposed tool can also contribute to the development of techniques and procedures that will allow an objective and efficient estimate of the mPMI. Researchers in forensics should work together to construct a morphological database, which should include the largest possible collection of individuals and species of forensic relevance, including their morphological characteristics (e.g. wing photographs and landmarks), and associated geographic and ecological information (e.g. substrate availability and preferred temperatures). This morphological database will allow forensic science in Venezuela and the Latin American region to develop methods and capabilities adapted to local conditions for efficient forensic processes. It is in this context that forensic science in Latin America must be strengthened on three fundamental aspects for effective use of entomofauna and the estimation of the mPMI, which are: 1) traditional taxonomy, 2) molecular techniques and 3) geometric morphometrics.

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