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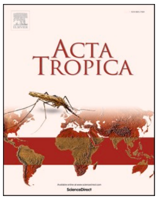

Size does matter: intraspecific geometric morphometric analysis of wings of the blowfly *Chrysomya albiceps* (Diptera: Calliphoridae)

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ABSTRACT

Blowflies have forensic, sanitary and veterinary importance, as well as being pollinators, parasitoids and ecological bioindicators. There is still little work with real data and from experiments assessing the relationship between blowflies' morphologic features and environmental and demographic factors. The present work tests whether the variation, in the shape and size, of *Chrysomya albiceps* (Wiedemann, 1819) wings is influenced by the following factors: 1) time; 2) temperature; 3) sex and; 4) different types of carcasses (pig, dog/cat and whale). Male and female wings from four different sites collected in six different years were used to obtain wing size and shape of *C. albiceps*. Analyses between wing shape and the variables tested had low explanatory power, even though they had statistical support. However, it was possible to identify differences in wing shape between males and females, with good returns in sex identification. The comparison between wing size and the variables tested showed that wing size has a negative relationship with temperature, significant differences between sexes, slight variation over time and no influence by carcass types. Furthermore, wing size influenced wing shape. Understanding population-specific characteristics of *C. albiceps* provide important insights about how the species reacts under specific conditions.

1. Introduction

Insect wings are membranous structures with functions ranging from flight to protection from external shocks (Parchem et al. 2007; Salcedo and Socha, 2020). Furthermore, they are important structures in the morphological distinction of groups (Gullan and Cranston, 2014). One of the ways to study morphology of these structures and that has grown in recent years is Geometric Morphometry - GM - (Cooke and Terhune 2015; Tatsuta et al. 2018). GM consists of a set of multivariate statistical techniques to visualize and analyze the morphology of insects through landmarks in an orthogonal space (Dujardin 2008). The landmarks generate 'x,y' coordinates, used to generate shape and size variables to be associated with covariates of interest (Bookstein 1991). Geometric Morphometry applied to wings is used to elucidate evolutionary effects, influence of environmental factors and ecological relationships (Chazot et al. 2016; Ivorra et al. 2019; Lemic et al. 2020), in addition to serving as a complement to molecular analyses (Sauer et al. 2020). Wing shape has been widely used to identify differences between species (Sontigun et al. 2019; Limsopatham et al. 2021) and between populations (Limsopatham et al. 2018). The GM of wing size is studied along with shape (Garzón and Schweigmann 2018), as it can influence wing shape, an association known as allometry (Benítez et al. 2013). Recently, wing size

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has been used to answer how competition can be a limiting factor between two blowflies under experimental conditions (Carmo et al. 2018; Ivorra et al. 2022).

Insect wing shape is less influenced than size by environmental factors (Gómez et al. 2014; Gómez and Correa, 2017). This demonstrates that shape is a more useful trait than size in species identification, so there are works that focus only on variations in shape (e.g., Grzywacz et al. 2017; Sontigun et al. 2019). Research that focus on wing size seek to provide answers about how the development of the studied model may respond to environmental factors such as temperature and diet (Klingenberg, 2010). Temperature, for instance, may favor the population rise of some insects, increasing the likelihood of developing more generations in their seasons of occurrence (Lehmann et al. 2020; Abarca and Spahn, 2021). However, with population increase, intraspecific competition for resources increases, which can generate a negative relationship between wing size and temperature, as seen in the blowfly *Chrysomya megacephala* (Fabricius. 1794) (Reigada and Godoy, 2005). Furthermore, seasonality is a factor that does little to explain the body size of califorids (Gião and Godoy, 2006).

Blowflies have forensic, sanitary and veterinary importance, as well as being pollinators, parasitoids and ecological bioindicators (Zumpt, 1965; Ramírez and Davenport, 2016; Wolff and Kosmann, 2016). Wing shape in blowflies has already been used to study adaptation of invasive populations (Laparie et al. 2016), develop tools for taxonomic identification (Sontigun et al. 2017; Macleod et al. 2018; Szpila et al, 2019), analyze population differences (Hall et al. 2014), evaluate responses to interspecific competition (Macedo et al. 2020) and characterize sexual dimorphism (Brown 1979; Lyra et al. 2009; Vásquez and Liria 2012; Hall et al. 2014; Laparie et al. 2016; Sontigun et al. 2017; Macedo et al. 2018; Szpila et al. 2019; Jiménez-Martín et al. 2020). In *Chrysomya albiceps* (Wiedemann, 1819), wing size does not differ between sexes (Jiménez-Martín et al. 2020). However, in similar species such as *Chrysomya rufifacies* (Macquart, 1842) and other species of the same genus, differences were detected (Sontigun et al. 2017).

C. albiceps is one of the best studied forensic indicators in the world (Grassberger et al. 2003, Corrêa et al. 2019; Williams and Villet 2019; Al-Qahtni et al. 2021). It is a species distributed in Africa, the Iberian Peninsula, the Mediterranean and the Middle East (Séguy 1930-1932; Holdaway 1933). In recent decades, *C. albiceps* is invasive in South America and responsible for the displacement of species such as *Cochliomyia macellaria* (Fabricius, 1775) (Faria et al. 1999), due to its predatory behavior in the larval stage (Grassberger et al. 2003; Rosa et al. 2006; Faria et al. 2007). Food resources of *C. albiceps* in nature are rich, but temporary and unevenly distributed, so it is expected to find variations in some morphological characters and body size of the species (Battán-Horenstein and Peretti, 2011). The species has already been collected using pig, dog/cat, rabbit and rat carcasses, (Early and Goff, 1986; Moura et al. 1997; Carvalho et al. 2000; Grassberger et al. 2003; Velásquez, 2008; Prado e Castro et al. 2011; Mashaly et al. 2020). However, it is still unknown how the difference in carcass types may affect wing size and wing shape in blowflies. In Portugal, the species generally occurs throughout the mainland and Madeira Island, appearing from late spring to autumn (Prado e Castro et al. 2009a; Farinha et al. 2014). Continuous monitoring of the occurrence of species such as *C. albiceps* is of fundamental importance to prevent health emergencies (Vanin et al. 2009). This includes work on how morphological traits may vary according to different conditions.

The present work tests whether there is variation in the shape and size of *C. albiceps* wings: 1) over time; 2) by temperature influence; 3) by sex and; 4) by types of carcasses (pig, dog/cat and whale), using Portuguese populations.

2. Methods

2.1. Specimen collection and identification of species

C. albiceps specimens were obtained from six different years and come from four sites in Portugal (Table 1). The specimens were kindly provided by the second (2004, 2006 and 2007), the fourth (2014) and the third (2017) authors. The 2021 specimens were collected by the first author. The collections in 2004, 2006, 2007, 2014 and 2021 were made through Forensic Entomology experiments. In the 2004, 2006 and 2007 trials, an adapted Schoenly trap (Prado e Castro et al. 2009b) was used to collect the flies. The trap surrounded the carcass, while the flies were captured in collecting cups attached to the trap. In 2014, flies were also collected in cups attached to a small cage constructed from metal and wood that enclosed the carcass. In 2021, flies were collected via deadly jar, using metal tweezers. The carcasses were surrounded by a cage built from wood and cloth that allowed the entrance of the collector, to facilitate the collection of the insects. In all these experiments, collection was done daily, at least during the period when *C. albiceps* was present in the carcass. The 2017 flies were collected manually, via metal forceps, and deposited directly into a flask with 70% alcohol. The specimens were given for this work already identified and had the identification confirmed by the first author using the works of Rognes (1990) and Grella et al (2015).

2.2. Slide preparation and capture of images

The right wing of each specimen was detached by using a combination of fine-tipped forceps. Each wing was positioned in ventral face on a slide and a drop of mounting medium was added before the apposition of a coverslip. The slides were left at room temperature for 7 to 10 days for drying and avoiding bubbles. Images of the slides were taken using a digital camera (Zeiss Stereo Lumar v.12) attached to a stereomicroscope with magnification at 14.8x and objective at 1.2x.

2.3. Wing morphometrics landmarks

The set of images was transformed into a TPS file using the tps Utility program software (tpsUtil32, v. 1.78, Rohlf 2019) to avoid biases in the landmarks digitization process. To obtain the wing coordinates, the software tpsDig2 v. 2.31 (Rohlf 2017) was used. In total, 16 landmarks were digitized on the wings of the specimens (Fig. 1), which were used to obtain the 'x,y' coordinates and subsequent size and shape metrics.

2.4. Variables used in statistical analysis

In the statistical analyses, two variables were mainly used: the

Table 1
Source data and sample size of the specimens of *Chrysomya albiceps* used in this study.

City	Location	Year	Method	n Male/ Female
Coimbra	40°12′42.64"N; 8°27′10.56"W	2004	Pig carcass	95/95
Lisbon	38°42′27.46"N; 9°10′56.30"W	2006	Pig carcass	97/97
Lisbon	38°42′27.46"N; 9°10′56.30"W	2007	Pig carcass	100/100
Lisbon	38°42′56.10"N; 9°11′37.23"W	2014	Dog/Cat carcass	26/73
Lisbon	38°43′7.31"N; 9° 9′1.36"W	2017	Whale carcass	37/7
Lisbon	38°42′56.10"N; 9°11′37.23"W	2021	Dog/Cat carcass	98/100

Note: Although not related to the objectives of the study, the presence of *C. albiceps* in whale carcasses, *Balaenoptera acutorostrata* (Lacépède, 1804), in Portugal has been recorded for the first time.

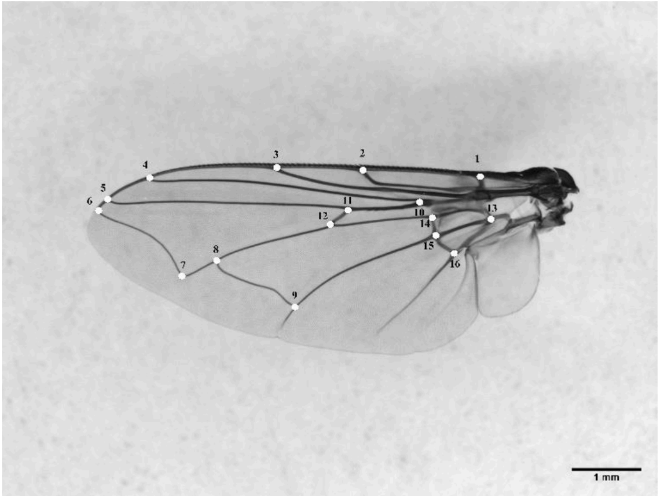

Fig. 1. Landmarks on the wing of *Chrysomya albiceps*.

Centroid Size (CS), used to perform the analyses with the wing size; and the Procrustes Coordinates (PC), used for the analyses of the wing shape. The procedure is done by importing the TPS file in MorphoJ (Klingenberg, 2011) and running in the software a Procrustes Fit following the path "Preliminaries → New Procrustes Fit →Align by Principal Axes →Perform Procrustes Fit". This step is necessary not only to generate the variables used in the analyses, but also to remove variations due to differences in scale, position, and orientation of the coordinates (Sontigun et al, 2017). The output of the procedure is the maintenance of the raw coordinates, a data matrix with the PC and CS. In shape analysis from MorphoJ, the variables "Sex" (Male or Female), "Year" (Year of Collection), Type of Carcass = "Pig" + "DogCat" + "Whale" were used as classifiers; and as covariates, the maximum (TMAX), minimum (TMIN) and mean temperature (TAVG) of the month in which the specimens were collected. All temporal variables were obtained at https://www.ipma.pt/pt/oclima/series.longas/ (IPMA, 2022). Moreover, "TMAX", "TMIN", "TVG", "Sex", "Year", "Pig", "DogCat" and "Whale" were also used as independent variables in the wing size analyses (more details in later sections).

2.5. Allometry

The effect of allometry, when wing size influences shape variation, was evaluated by simple linear regression in MorphoJ. For this, the CS was used as independent variable and the PC as dependent variable. Complementarily, it was tested if this relationship could be influenced by sexual dimorphism, by grouping the sex variable in a multiple regression with 10,000 rounds of randomization. The residuals of the PC *vs* CS regression were subjected to the Discriminant Function Analysis (DFA) to find out whether sexual dimorphism is altered when excluding the allometric effect. The DFA underwent cross-validation test with Mahalonobis Distance (MD) associated with permutation test with 10,000 rounds. Furthermore, it was also tested the relationship of the residuals with TMAX, TMIN and TAVG.

2.6. Wing shape analysis

The variation in wing shape was analyzed in the MorphoJ. To test the difference between wing shapes by sex, DFA was used with permutation test with 1,000 permutation runs and MD. The DFA further underwent a cross-validation test to assess whether the difference was adequately evaluated. To test whether there was a difference between wing shapes by sex and year, Canonical Variable Analysis (CVA) was used. To test for variation in wing shape by type of carcass (Pig, Dog/Cat and Whale), a CVA was performed too. In both CVA, the significance level of permutation tests with MD was obtained from 10,000 permutation rounds. To test whether the variation in *C. albiceps* wing shape can be explained by temperature variation, a simple linear regression with 10,000 permutation rounds was performed in MorphoJ: PC *vs* TMAX, PC *vs* TMIN and PC *vs* TAVG.

2.7. Wing size analysis

The difference between wing size was tested by Sex, Year of Collection and Type of Carcass (CS *vs* Sex, CS *vs* Year and CS *vs* Pig, Dog/Cat, Whale). In this step, the RStudio Program (RStudio Team 2021) was used. The data did not reach normality (Shapiro-Wilk Test) nor homogenized variance (Levene Test). Therefore, the Wilcoxon rank sum test with continuity correction was used to assess the size difference by Sex. The difference in wing size per Year was assessed using the Kruskal-Wallis rank sum test. As a post hoc test, a pairwise evaluation was performed using Wilcoxon rank sum test with continuity correction with Bonferroni correction. The wing size difference by Type of Carcass was tested in the same way (CS *vs* Pig, Dog/Cat and Whale). Kernel regressions were used to find out whether the wing size of *C. albiceps* can be explained by temperature variation (CS *vs* TMAX, CS *vs* TMIN and CS *vs* TAVG). This method is used to identify non-linear relationships between two random variables. The estimator used in Kernel Regression was Local-Constant, the type of Regression was Second-Order Gaussian and the Bandwidth Selection Method was Least Squares Cross-Validation.

2.8. Error measurement

A sub-sample of 30 wings digitized twice was separated to find out if there was measurement error of the landmarks. A Procrustes ANOVA was then performed combining the two coordinate datasets. This procedure is described by Benítez et al. (2020).

3. Results

3.1. Error measurement results

When the square mean value exceeds the error value, it means that there was no measurement error during landmarking. Consequently, it is assumed that the analyses were not biased by any kind of procedural error in the measurements. (Table 2).

3.2. Allometry results

Wing size explained the changes in wing shape, PC *vs* CS= 10,6591% (p<0.0001). Considering this influence, all further analyses were performed when removing the allometric effect. Even so, some results are shown to highlight the practical differences of removing the allometric effect (Table 3). It was evidenced that allometry influences sexual dimorphism in *C. albiceps* (predicted= 5, 6919%, p<0.0001). Furthermore, the explanatory predictions between PC and temperatures decreased (values not shown). Allometry also caused changes in sex

Table 2
The Procrustes ANOVA result comparing two subsamples of *Chrysomya albiceps* wing images to assess measurement error. If MS > Error I, it is assumed that the analyses were not biased by any kind of procedural error in the measurements.

Centroid size	Wings				
Effect	SS	MS	df	F	p
Individual	11,002012	0,379380	29	65811,8	<0.0001
Error 1	0,00017	0,000006	30		
Shape					
Effect	SS	MS	df	F	p
Individual	0,02034992	0,0000250615	812	274,3	<0.0001
Error 1	0,00007674	0,0000000914	840		

Table 3
Allocation of groups by Sex using Discrimination Function Analysis in MorphoJ (DFA, $p<0.0001$). The values in bold show how the correct identification of sexes is improved after removing the influence of wing size on wing shape.

DFA	Allocated to		DFA	Allocated to	
Group	Female	Male	**Group**	**Female**	**Male**
Female	467	5	**Female**	**470**	**2**
Male	2	451	**Male**	**1**	**452**
Cross-Validation	Allocated to		**DFA**	**Allocated to**	
Group	Female	Male	**Group**	**Female**	**Male**
Female	467	5	**Female**	**469**	**3**
Male	2	451	**Male**	**2**	**451**

Note: Bolded values are the DFA result after removing allometric effect.

discrimination (Table 3, MD= 5.4159, $p<0.0001$). Cross-validation confirmed the values found in the DFA - T-Square p <0.0001.

3.3. *Wing shape results*

DFA by Sex confirmed variation in shape - MD= 4.9262, $p<0.0001$ - (Fig. 2) and allocated the two groups (Table 3). Cross-validation confirmed the values found in the DFA -T-Square p <0.0001. The success in group identification reached 99.36% for females and 99.55% for males. Variation was also found between sexes in the same year as evidenced by CVA. Despite the high overlap between points on the graph, the first two canonical variables were responsible for explaining 80.70% (Fig. 3), which denotes that the analysis identified dimorphism not only by Sex among all specimens, but also dimorphism among specimens by Year, which is confirmed in Table 4. The MD distance between the groups analysed by CVA also showed that the wing shapes of flies collected at the same site are less distant than flies collected between different sites. There was also a lot of overlap of points in the CVA of the wing shape by type of carcass (Fig. 4). The MD distance was low between each of the methods (Pig *vs* DogCat= 1.4681, Pig *vs* Whale= 2.6016, DogCat *vs* Whale= 1.9885), but the result had statistical support at $p<0.0001$. Little variation in shape as a function of temperatures was found by regression analysis, even the results had statistical support (Table 5) ($p<0.0001$).

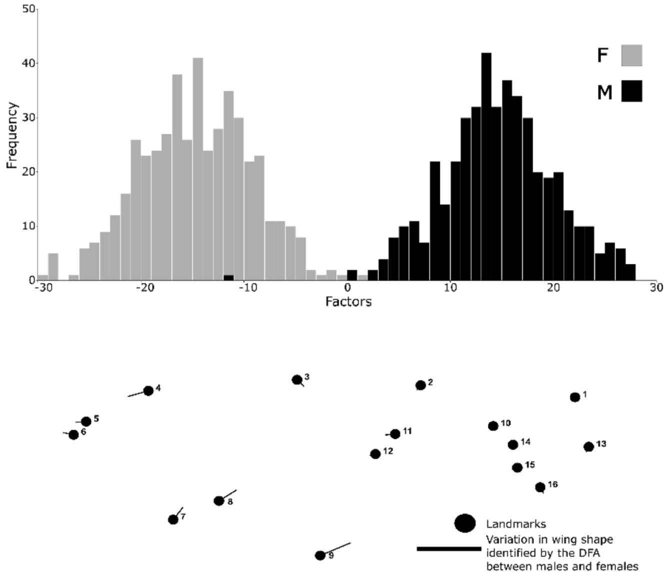

Fig. 2. Confirmed variation in wing shape between males and females of *Chrysomya albiceps* (analysis made in MorphoJ). At the top: histogram representing values of the discriminant scores for the original data variation. At the bottom: diagram indicating shape differences between the two group means (the most variable landmarks are at more apical points). F= Female; M= Male; DFA = Discriminant Function Analysis.

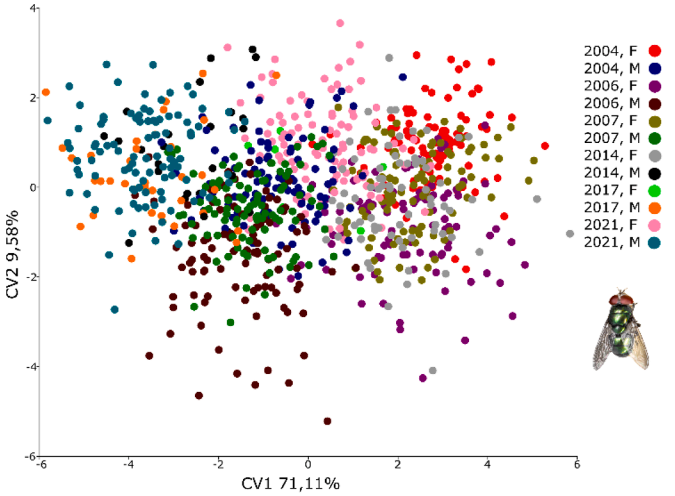

Fig. 3. Scatterplot representing Canonical Variate Analysis in MorphoJ discriminating the wing shape of *Chrysomya albiceps*, considering the variables Sex and Year together. Each color represents a different combination of the variables Year and Sex in which *C. albiceps* was collected. F= Female; M= Male, CV = Canonical Variate.

3.4. *Wing size results*

Variation in wing size by Sex was found by the difference in medians detected by the Wilcoxon rank sum test (Fig. 5). The W value is not an estimate of how different the medians are, but rather the number of times the median of one group is smaller than that of the other. Variations was also found in wing size by Year (Fig. 6) and between the types of carcasses (Fig. 7). When testing whether wing size can be influenced by temperature, a non-linear relationship by Kernel regression (Fig. 8) was obtained. All relationships had statistical support $p< 2.22e-16$ and similar values in R^2. However, in other outcomes of the analysis, the relationship between wing size and TMAX ($R^2= 0.2800388$, Residual Standard Error= 0.605526, h= 0.4862875), TMIN ($R^2= 0.3369224$, Residual Standard Error= 0.5811118, h= 0.03400968) and TAVG ($R^2= 0.3367556$, Residual Standard Error= 0.5811856, h= 0.4910393) showed different results. The h value is a smoothing parameter known as Bandwidths. Very high or small values indicate that the model did not work very well to describe the relationship between two variables. Apparently, this is what happened in CS *vs* TMIN. On the other hand, CS *vs* TAVG proved to be a good description of the relationship between wing size and temperature.

4. Discussion

4.1. *A brief discussion about sample size issues*

Some studies in the literature that evaluated sexual dimorphism in Calliphoridae did not detect intraspecific sexual dimorphism for some species, either in wing shape, wing size, or both (Sontigun et al. 2017; Szpila et al. 2019; Jiménez-Martín et al. 2020). In all these studies, a common point is the low sample size for some species, an experimental limitation that can generate ambiguous interpretations and hide existing relationships in nature (Bissonette, 1999). For instance, there is no way of knowing whether the failure to detect sexual dimorphism in wing size or shape in *C. albiceps* in previous works is the result of low sample size, regional differences between populations, or the method of obtaining the landmarks. However, the sample size in the present work was greater than in previous works. Therefore, sexual dimorphism may have been detected in the present work by this factor. Modern geometric morphometry is indeed a cheap and very useful tool for integrative taxonomy (Schlick-Steiner et al. 2010). A low sample size can return

Table 4
Mahalonobis distances found by Sex and Year analyzed together by Canonical Variate Analysis and permutation test with significance obtained at 10,000 rounds ($p < 0.05$). M= Male, F= Female.

Mahalonobis Distance Values Years & Sex	2004, F	2004, M	2006, F	2006, M	2007, F	2007, M	2014, F	2014, M	2017, F	2017, M	2021, F
2004, M	3,8391										
2006, F	2,277	3,4579									
2006, M	5,0628	2,2536	3,8399								
2007, F	2,2809	3,5137	2,0858	4,5293							
2007, M	4,6211	2,0814	3,9482	2,2698	3,9204						
2014, F	2,4595	3,4222	1,8028	4,0584	2,1249	4,1156					
2014, M	5,5501	2,8115	5,1181	3,062	5,2641	2,7816	4,9655				
2017, F	4,5794	3,7606	3,9488	4,2036	4,0093	3,7295	4,0813	4,5023			
2017, M	6,6413	3,5545	6,047	3,2792	6,152	3,2992	5,9257	2,6319	4,4375		
2021, F	2,8806	2,0949	2,7511	3,2719	2,8182	2,8781	2,6723	3,4747	3,0308	4,3114	
2021, M	6,5506	3,3241	6,0523	3,2686	6,2562	3,2498	5,8767	1,7265	4,9814	2,0113	4,2051

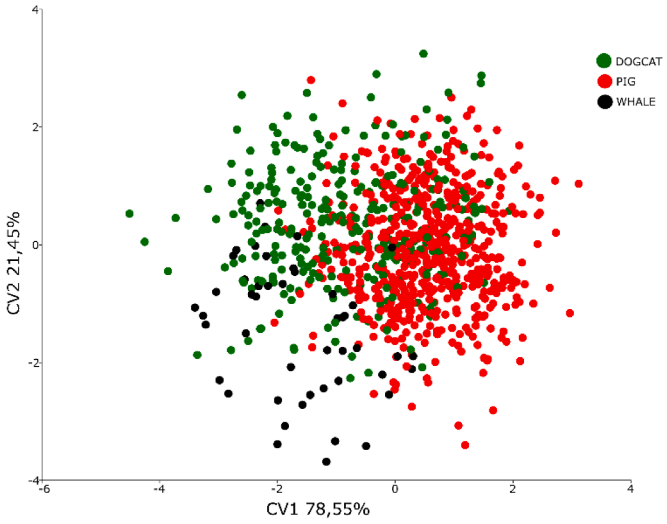

Fig. 4. Scatterplot representing Canonical Variate Analysis in MorphoJ discriminating the wing shape of *Chrysomya albiceps*, considering the type of carcass. Each animal and its respective color represents the type of carcass in which the *C. albiceps* individuals were collected. CV = Canonical Variate.

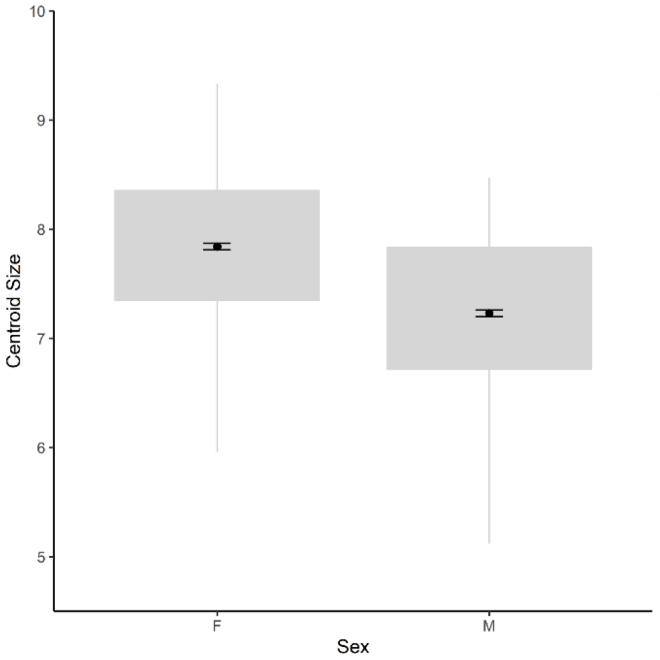

Fig. 5. Boxplot representing CS (Centroid Size) vs Sex: Difference in wing size between F= females and M= males of *Chrysomya albiceps*. W = 160563, $p < 2.2e\text{-}16$. CS female= 7.84; CS male= 7.22.

Table 5
Values from the regression analysis in MorphoJ between Procrust Coordinates (PC) and temperatures (TMAX, TMIN and TAVG).

Regression Test	Total SS	Predicted SS	Residual SS	% predicted
PC *vs* TMAX	0,41349661	0,00666912	0,40682749	**1,6129**
PC *vs* TMIN	0,41349661	0,00900937	0,40448724	**2,1788**
PC *vs* TAVG	0,41349661	0,00765520	0,40584142	**1,8513**

Note: bolded values are the percentage of explanatory prediction of one variable as a function of the other.

high assertiveness values in species identification (Sontigun et al. 2017; Szpila et al. 2019). Thus, specific investigations on how sample size may interfere with the detection of patterns in wing shape and wing size are needed.

The Figs. 4 and 7 show the differences between wing shape and wing size as to the Type of carcass in which the flies were collected. These results should be interpreted with caution because of the disparity in sample size between the groups tested. This was an effort to start a discussion on the subject, but the low sample size did not allow a robust analysis.

4.2. Allometry discussion

The present work found allometric effect on wing shape variation of *C. albiceps* with good statistical support (see Section 3.2). However, this effect did not influence so much the sex discrimination of the species (see Table 3), but it influenced the results of the temperature effect on the wing shape (results not shown). Possibly, this happened because the size of insect structures is directly influenced by temperature, which potentiates the effect of size on shape. In other Diptera species, allometry was also identified (Sontigun et al. 2019, Oliveira-Christe et al. 2020, Limsopatham et al. 2021). Sontigun et al. (2017) found a low allometric effect that did not impact the identification of blowflies. However, allometry can play an important role in detecting sexual dimorphism. For practical purposes, it is suggested that all work involving phenotypic variation should remove the allometric effect before any analysis, as recommended by Sontigun et al. (2017).

4.3. Wing shape discussion

Changes in the wing shapes of blowflies have already been identified due to seasonality and biological invasion (Laparie et al. 2016), competition (Macedo et al. 2020) and allometry (Szpila et al. 2019). In the present study, effects with good statistical support were identified, however, with low explanatory power. Furthermore, a strong effect of

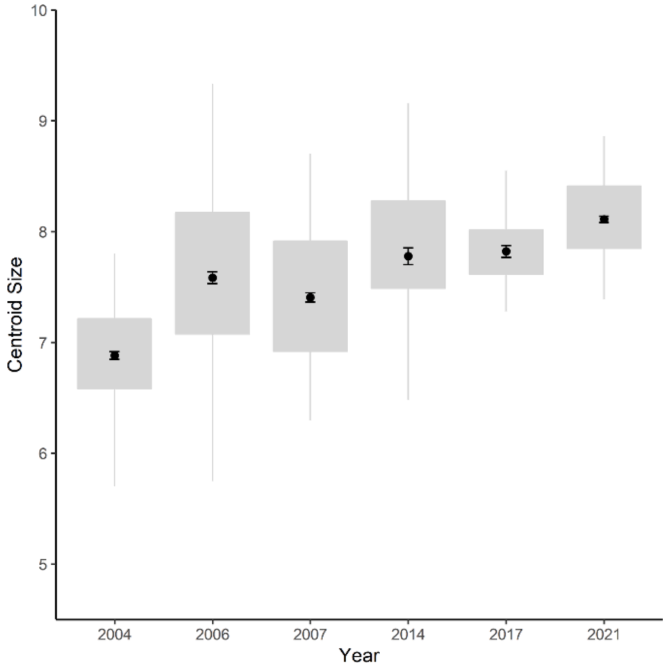

Fig. 6. Boxplot representing CS (Centroid Size) vs Year: Difference between wing size of *C. albiceps* over time in the years sampled for this study. $H = 331.76$, $df = 5$, $p < 2.2e-16$. The values of Pairwise comparisons using Wilcoxon rank sum test with continuity correction was: 2004 vs 2006= $p < 2.2e-16$; 2004 vs 2007= $p < 6.0e-15$; 2004 vs 2014= $p < 2.2e-16$; 2004 vs 2017= $p < 2.2e-16$; 2004 vs 2021= $p < 2.2e-16$; 2006 vs 2007= $p < 0.05739$; 2006 vs 2014= $p < 0.24193$; 2006 vs 2017= $p < 0.95188$; 2006 vs 2021= $p < 8.3e-13$; 2007 vs 2014= $p < 5.4e-06$; 2007 vs 2017= $p < 0.00034$; 2007 vs 2021= $p < 2.2e-16$; 2014 vs 2017= $p < 0.99999$; 2014 vs 2021= $p < 0.00558$; 2017 vs 2021= $p < 5.3e-05$.

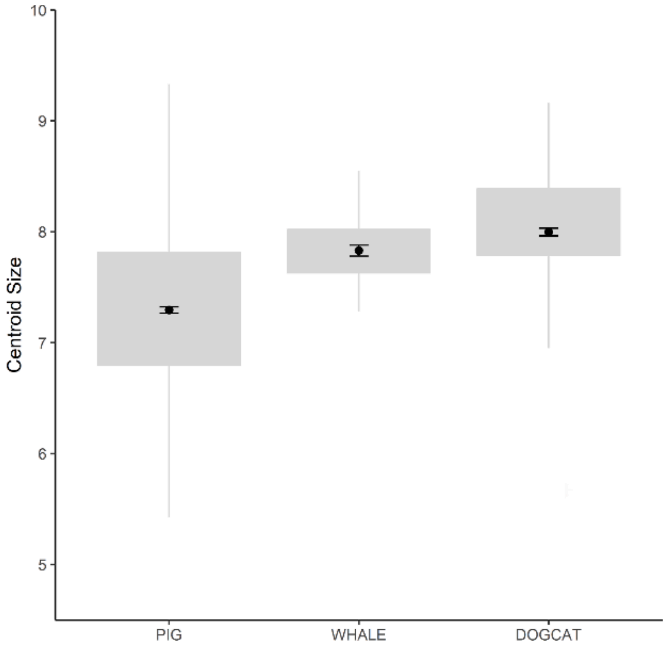

Fig. 7. . Boxplot representing CS (Centroid Size) vs Type of Carcass (Pig, Whale and Dog/Cat). Each animal represents the type of carcass in which the *Chrysomya albiceps* individuals were collected. $H= 215.89$, $df = 2$, $p < 2.2e-16$. The values of Pairwise comparisons using Wilcoxon rank sum test with continuity correction was: Pig vs Whale= $p < 2e-08$; Pig vs Dog/Cat= $p < 2e-16$; Whale vs Dog/Cat= 0.0012.

wing size on wing shape (allometry) was observed (see Section 3.2), a similar conclusion to that of Szpila et al. (2019). This is the reason for presenting the DFA results before and after removing the allometric effect (see Fig. 2).

Discrimination Function Analysis identified sexual dimorphism in *C. albiceps* with a high success rate (Fig. 2), which is encouraging for scholars less experienced with Calliphoridae taxonomy. Dimorphism in wing shape is identified for several species of blowflies (Szpila et al. 2019), including other species of the genus *Chrysomya* (Sontigun et al. 2017). However, some studies have not found sexual dimorphism in *C. albiceps* (Vásquez and Liria, 2012; Szpila et al. 2019; Jiménez-Martín et al. 2020). In the present work, this may have occurred because it reflects regional variation in flies (Hall et al. 2014), an allometric effect sufficient to cause differences in wing shape (Szpila et al. 2019), or divergent niche between sexes (Shine, 1989; Fryxell et al. 2019). Females have a competitive advantage for finding animal carrion (Campobasso et al. 2001). Examining Fig. 2, the wing shape between the two sexes is more stable at the base, while the most variable points are at more apical points, a detail also noted by Jiménez-Martín et al. (2020) in the species of blowflies from the Iberian Peninsula. The possibility of using the wing shape of *C. albiceps* as a tool for sex identification in the species was confirmed, provided that allometry is identified. This does not exclude the use of traditional taxonomic criteria, nor the importance of considering geographic variation to complement identification. These results are valuable for Forensic Entomology, for instance, when dealing with damaged specimens that are found at crime scenes.

The variations in wing shape by Year/Sex (see Fig. 3) and by Type of Carcass (see Fig. 4) are not clear. In Fig. 3, the sum of the two main covariates was 80.69%. Considering the 12 groups tested, this figure shows that wing shape does not vary by Year and Sex, when analyzed together. The non-detection of variation in wing shape over the six years sampled indicates that there is a strong genetic component driving these changes in *C. albiceps*, which makes wing shape a stable criterion for taxonomic identification. In Fig. 4, the two main covariates explained the variation in shape by 100%, which denotes that the analysis did not separate the three groups tested in the CVA. As seen in Section 4.1, this may have happened because of the sampling disparity between the groups tested, mainly because of the low sample size of specimens collected on the whale carcass.

4.4. *Wing size discussion*

In this work, there is a clear difference in wing size by sex in *C. albiceps* (see Fig. 5). The identification of sexual dimorphism by size is contrary to another work with the same species in the Iberian Peninsula (Jiménez-Martín et al. 2020). In that work, the differences the wing size is not as variable and do not have statistical support. This difference between the two results may have occurred because of the difference in sample size (see Section 4.1), or natural differences between local populations. For instance, the females of blowflies need protein nutritional sources for the development of their eggs (Shewell, 1987). It is expected that due to this factor, females need to move over a larger distances in their habitats, since guilds of carrion-feeding species are donor-controlled, i.e., oviposition of these species depends on the random supply of resources in nature (Polis and Strong 1996). Consequently, female flies accessing animal carcasses are expected to be larger and better competitors.

Size variations in insects are often associated and correlated to environmental factors (Battán-Horenstein and Peretti 2011; Gallesi et al. 2016). A negative relationship between wing size and temperature has been found elsewhere (Garzón and Schweigmann 2018), including *C. megacephala* (Reigada and Godoy, 2005). In the present work, the same conclusion was reached: the higher the temperature, the further the wing size decreases, in a non-linear relationship (Fig. 8). Although *C. albiceps* has a competitive advantage in carcasses environments, in temperate environments it is a seasonal species. The species range is

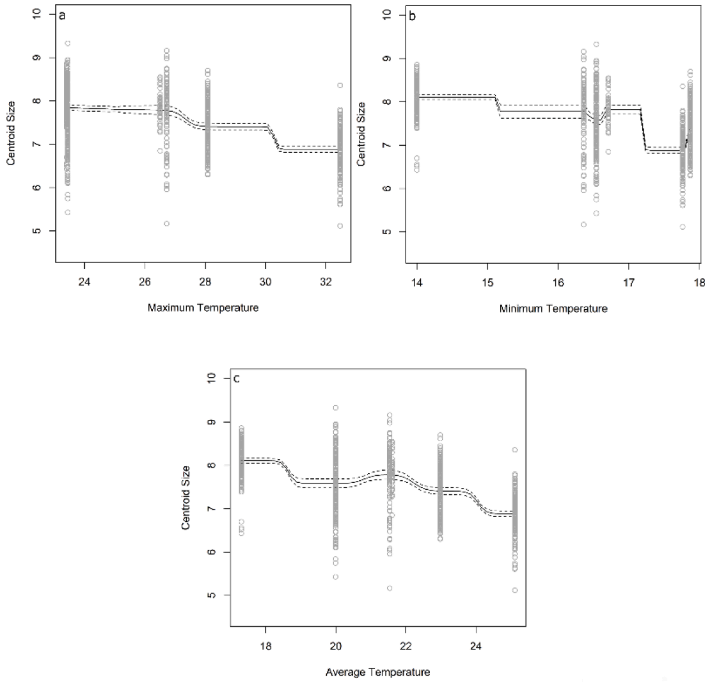

Fig. 8. Scatterplot representing CS vs TMAX (top, left), CS vs TMIN (top, right) and CS vs TAVG (bottom, mid): Relationship between wing size (Centroid Size = CS) of *Chrysomya albiceps* and maximum (TMAX), average (TAVG) and minimum temperature (TMIN) of the insect sampling periods, estimated by Kernel regressions.

restricted in Portugal to the warmer months of the year (Prado e Castro et al. 2009a, 2012, 2016), so population size increases with temperature. This generates greater competition for resources and consequent trade-offs in population parameters such as size.

C. albiceps is a species that competes voraciously for resources in the larval stage (Faria et al. 2007). It is recorded feeding on other species and on itself, especially in environments where larval density is high, such as in animal carcasses (Ullyett 1950). In Forensic Entomology experiments using animal carcasses, *C. albiceps* usually appears in the adult stage in two moments. The first, when eggs are laid, in the first days of colonization, and the second at the birth of new individuals from the initial egg laying (since this species does not migrate from cadavers to pupate). As adult size in these flies basically depends on the life history of their larval stage, it was expected that different Carcass Types could influence the wing size of *C. albiceps*. However, this is not what the present work found, as seen in Fig. 7. On the other hand, population parameters such as weight and body size are density dependent characteristics and influenced by factors as temperature (Reis et al. 1994; Tarone et al. 2011). Moreover, it is not excluded that, as with the wing shape, no difference was detected because of the lack of sample size.

Over the years, a slight difference in wing size was noted. However, it is notable that the greatest difference expressed is between the years 2004 and 2021 (Fig. 6). This indicates that other factor may have caused the increase in wing size in *C. albiceps*, as the relationship with temperature was negative. Riback and Godoy (2008) found no relationship between seasonality and wing size and tibia size of *C. albiceps* over two years, indicating no influence of temperature change on some morphological traits of the species. Under experimental conditions, the wing size of *C. albiceps* is larger in the presence of *Lucilia sericata* (Meigen, 1826) than when the species interacts with itself (Ivorra et al. 2022), which confirms the results obtained by Ullyett (1950). Therefore, it is plausible that the most important factor for the body size and structures of *C. albiceps* is its peculiar larval behavior, which not only feeds on animal carcasses, but also preys on other species.

5. Conclusion

Unlike what was previously known, *C. albiceps* has sexual dimorphism in both wing shape and wing size. Both were influenced by temperature, although this influence was low in the case of the wing shape. One of the reasons for this is the relationship between wing size and wing shape, a phenomenon already well known. Studies with information on species populations in different locations demonstrate how the same species can have different developments, so it is important to assess regional differences in species such as *C. albiceps*. The results of this work can be used as a standard for future studies and provide further information on the natural history of this species, as well as being useful for fields such as Forensic Entomology.

Authorship statement

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Furthermore, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication before its appearance in the *Acta Tropica*.

Ethics approval and consent to participate

Ethical approval for this research project was given by CEIE (Ethics Commission for Research and Teaching from Faculty of Veterinary Medicine, University of Lisbon). Written and oral consent for cat/dog/pig were obtained for every case from owners or responsible shelter centre personnel.

CRediT authorship contribution statement

Sérgio J.M. Rodrigues-Filho: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Project administration. **Catarina Prado e Castro:** Resources, Writing – review &

editing. **Luís Filipe Lopes:** Resources, Writing – review & editing. **Isabel Pereira da Fonseca:** Resources, Writing – review & editing. **Maria Teresa Rebelo:** Conceptualization, Methodology, Resources, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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