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Research Article

Geometric morphometrics analysis of forensically important *Sarcophaga* species

Carina I. C. Reis[1,*], Paulo Mascarenhas[2], Ana C. Farinha[3], Marco A. Menezes[4] and Maria Teresa Rebelo[1]

[1]Centro de Ecologia, Evolução e Alterações Ambientais (CE3C); Centro de Estudos do Ambiente e do Mar (CESAM), Faculdade de Ciências da Universidade de Lisboa (FCUL), Lisboa, Portugal
[2]Centro de Investigação Integrada Egas Moniz (CiiEM), Egas Moniz School of Health & Science, Almada, Portugal
[3]Centro de Estudos Florestais, Instituto Superior de Agronomia, Lisboa, Portugal
[4]Departamento de Entomologia, Museu Nacional/Universidade Federal do Rio de Janeiro - UFRJ, Rio de Janeiro, Brasil

*Corresponding author. E-mail: carinareis77@gmail.com

Abstract

Geometric morphometrics is a method that offers a mathematical description of biological forms based on geometric definitions of their size and shape, enabling the discrimination between species. In this study, we aimed to utilize geometric morphometrics as a valuable tool for identifying Sarcophagidae species, a family of flies that are challenging to distinguish morphologically. We collected a total of 80 wings (both left and right) from male individuals representing nine species (*Sarcophaga africa*, *Sarcophaga argyrostoma*, *Sarcophaga hirticrus*, *Sarcophaga jacobsoni*, *Sarcophaga lehmanni*, *Sarcophaga melanura*, *Sarcophaga pandellei*, *Sarcophaga portschinskyi*, and *Sarcophaga teretirostris*). These wings were removed, mounted on microscope slides, photographed, and digitized using 15 landmarks. The results demonstrated effective differentiation among seven species, representing significant progress in the expedited identification of *Sarcophaga* species. Due to its speed, affordability, and user-friendly nature, the wing landmark-based geometric morphometrics, when combined with taxonomy, can enhance the robustness of Sarcophagidae analyses and prove to be useful for discriminating flesh flies in forensic contexts.

Keywords: geometric morphometry; Sarcophagidae; forensic entomology; wings

Introduction

Certain species of Sarcophagidae, commonly referred to as flesh flies, are categorized within the group of sarcosaprophagous dipterans due to their larvae's necrophagous or necrophilous feeding habits. Consequently, they serve as crucial aids in criminal investigations [1–5].

The examination of arthropod succession on cadavers has demonstrated utility in forensic investigations by aiding in the estimation of the postmortem interval [6]. However, its application is constrained by limited ecological, biological, and taxonomic understanding [7–10]. Sarcophagidae play a crucial role within the sarcosaprophagous community [11], with their third-stage larvae regarded as secondary consumers [12].

With over 3 000 described species [13–15], the Sarcophagidae family is typically identified by the presence of three longitudinal black stripes on the thorax and a checkered or spotted abdomen [16, 17]. Predominantly, they are ovoviviparous, meaning the eggs hatch into larvae during or shortly after oviposition. This trait likely serves as an adaptation for exploiting ephemeral food sources or evasive hosts, particularly in the case of parasitoid species [18].

Rigorous species identification is essential for implementing effective control measures when dealing with public nuisances and accurately determining the postmortem interval in forensic investigations [6, 19, 20]. However, this task is frequently challenging, either due to the difficulty in observing diagnostic morphological characteristics, their fragility, or due to the limited presence of DNA [21]. Consequently, in recent years, there has been a growing reliance on morphometric techniques to discern species diversity and delineate relationships among various structures [22, 23]. Morphological characteristics of wings have emerged as a crucial tool for identifying various families of dipterans, which are pertinent to criminal and forensic investigations [24–26]. Geometric morphometrics entails utilizing a set of reference points or landmarks to facilitate the morphological comparison of any bodily structure. This process eliminates extraneous information, such as the position and orientation of specimens, and focuses solely on their shape [27, 28]. In the context of dipterans, geometric morphometric analysis typically employs intersections of wing veins as landmarks, aiding in specimen identification [28]. This approach is gaining traction and finding application in identifying significant forensic families like Sarcophagidae, as it offers a dependable, straightforward, rapid, and cost-effective methodology [29–33].

Geometric morphometry, when applied to wings, serves as a potent tool for elucidating evolutionary effects, the influence of environmental factors, and ecological relationships

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[26, 34]. Moreover, it complements molecular analyses [35]. Wing shape analysis has been widely utilized to discern disparities between species [25, 30] as well as among populations [36].

Hence, the objective of this study is to assess a landmark-based geometric morphometric analysis of wings for species identification of Mediterranean flesh flies that are medically and forensically significant.

Materials and methods

Specimen collection and identification of species

The specimens utilized in this study were gathered in Aroeira (a coastal region; 38.574841N, 9.195791W) and Sertã (a central mountainous region of Portugal; 39.772335N, 7.987741W) using baited traps and preserved in 70% ethanol, following the methodology outlined by Farinha et al. [37]. Species identification (*Sarcophaga africa* Wiedemann 1824, *Sarcophaga argyrostoma* (Robineau-Desvoidy, 1830), *Sarcophaga hirticrus* Pandellé, 1896, *Sarcophaga jacobsoni* (Rohdendorf, 1937), *Sarcophaga lehmanni* Müller, 1922, *Sarcophaga melanura* Meigen, 1826, *Sarcophaga pandellei* (Rohdendorf, 1937), *Sarcophaga portschinskyi* (Rohdendorf, 1937), and *Sarcophaga teretirostris* Pandellé, 1896) was conducted using morphological keys [38–41].

Slide preparation and capture of images

Forty male individuals from nine species were included in the analysis. Both left and right wings were photographed, utilizing entomological forceps, and mounted on semi-permanent slides with glycerin. Wing images were captured using a digital camera (LEICA DFC450; Wetzlar, Germany) attached to a stereomicroscope (LEICA M205C), with a magnification of 16.0× and an objective lens set at 0.8×.

The multifocus function of the digital camera was utilized for all wings. This function enables the capture of a series of images at different focal planes, which is especially beneficial for examining specimens with depth or intricate structures. By taking multiple photographs with slight adjustments in focus, the camera ensures that all parts of the specimen remain sharp and clear across the entire depth of field. This produces a composite image that offers a comprehensive view of the specimen, simplifying detailed analysis and documentation.

In this particular case, a sequence of 30 photographs was captured using the multifocus function, likely with evenly spaced intervals along the depth of the specimen. This meticulous approach ensures comprehensive coverage and facilitates a thorough examination and documentation of the wing morphology.

Wing morphometrics landmarks

Fifteen anatomical landmarks on the wings of the specimens were selected (Figure 1), distinguished by their connections and intersections that yield insights into wing configurations [42]. These landmarks were chosen based on prior research conducted with geometric morphometry of Diptera wings [43–46].

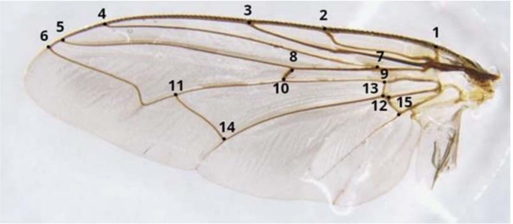

Figure 1 Wing of *Sarcophaga jacobsoni* male. The numbered points indicate the landmarks used for wing measurements. Landmarks: 1. intersection between the humeral transverse vein and costal vein, 2. intersection between the subcostal and costal veins, 3. intersection between the anterior branch of the anterior radius vein (AR) and the costal margin (C), 4. intersection between the first post-anterior branch of the radius vein (PA1R) and the C, 5. intersection between the second post-anterior branch of the radius vein (PA2R) and the C, 6. intersection between the medial vein (M) and the C or wing periphery, 7. second bifurcation of the AR resulting the creation of this vein's PA1R and PA2R branches, 8. anterior intersection between the radius-medial transverse vein (R-MT) and the PA2R, intersection between the cubital vein (Cu) and wing periphery, 9. anterior intersection between the distal medial–cubital transverse vein (M-CT1) and the medial vein (M), 10. posterior intersection between the R-MT vein and the M, 11. anterior intersection between the distal medial–cubital transverse vein (MCT2) and medial vein (M), 12. posterior intersection between the proximal M-CT1 and the Cu, 13. landmark 12 and the Cu, 14. posterior intersection between the proximal M-CT2 and Cu, 15. posterior inter-section between the anal-cubitus transverse vein (A-CT) and the first anal vein (A1).

The anatomical landmarks on each wing were acquired using tpsUTIL64 software [47], tpsRELW32 software [48], and tpsDIG32 software [49]. The sequence of landmark introduction remained consistent for each individual, as this is essential for establishing spatial homology of the structures *via* their coordinates. Each landmark corresponds to a coordinate on a Cartesian plane. A matrix represented the anatomical landmarks for the left and right parts of the wings, comprising 30 *XY* coordinates in the Cartesian plane for the nine selected Sarcophagidae species.

Statistical analysis

Statistical analyses were conducted using MorphoJ software (version 1.08.01 for Windows) [50]. After loading the morphometric matrix data into MorphoJ, outliers were inspected, and a Procrustes fit was performed. Procrustes is a statistical shape analysis technique employed to analyse the distribution of a set of shapes. MorphoJ performs a comprehensive Procrustes fit and projects the data to the tangent space *via* orthogonal projection [51]. This procedure places less weight on observations that are distant from the average shape, thereby enhancing the procedure's robustness against the influence of outliers. The Procrustes fit entailed several steps, including the alignment of the specimens relative to the coordinate system for graphical output and the pairing of landmarks. Subsequently, the Procrustes fit incorporated a data matrix comprising centroid coordinates and size into the wing shape dataset, enabling the computation of a covariance matrix and the implementation of allometric correction of the wing shape data against the centroid size. The allometric correction was implemented through a pooled within-group regression, which separated the component of variation predicted by the centroid size from the residual component of variation, which was uncorrelated with the centroid size. The allometric effect-corrected wing shape data (residual component) and the original uncorrected wing shape data were employed in the subsequent procedures. The Mahalanobis and Procrustes distances between the Sarcophagidae species were calculated using discriminant analysis (Tables 1 and 2).

Table 1. Mahalanobis distances among Sarcophagidae wings landmarks (without allometric correction).

	S. africa	*S. argyrostoma*	*S. hirticrus*	*S. jacobsoni*	*S. lehmanni*	*S. melanura*	*S. pandellei*	*S. portschinskyi*
S. argyrostoma	6.9							
S. hirticrus	21.2	22.9						
S. jacobsoni	15.6	16.1	17.0					
S. lehmanni	9.9	11.9	17.3	11.0				
S. melanura	14.9	15.2	11.7	13.9	14.7			
S. pandellei	20.5	21.1	27.0	24.2	23.2	24.1		
S. portschinskyi	10.4	12.2	17.9	12.7	12.5	12.6	18.3	
S. teretirostris	12.1	12.6	12.0	11.4	11.7	5.8	21.7	9.8

All distances were nonsignificant.

Table 2. Mahalanobis distances among Sarcophagidae wings landmarks (with allometric correction).

	S. africa	*S. argyrostoma*	*S. hirticrus*	*S. jacobsoni*	*S. lehmanni*	*S. melanura*	*S. pandellei*	*S. portschinskyi*
S. argyrostoma	7.8							
S. hirticrus	25.5	25.2						
S. jacobsoni	18.6	17.3	17.5					
S. lehmanni	10.7	11.9	20.0	12.6				
S. melanura	17.0	15.8	13.2	14.0	15.2			
S. pandellei	22.0	21.5	27.7	24.3	23.5	24.1		
S. portschinskyi	10.6	13.7	24.4	17.9	14.2	16.5	21.1	
S. teretirostris	13.7	12.9	14.3	11.9	11.9	6.0	21.8	13.3

All distances were nonsignificant.

Dendrograms were constructed by hierarchical clustering of the Procrustes distances matrix data, from which a cophenetic correlation was derived [52]. The cophenetic correlation coefficient assessed how accurately the displayed dendrogram preserved the pairwise distances between the original unmodeled data points. Canonical variate analysis was conducted to identify the wing shape features that best differentiated among the Sarcophagidae species (Figure 2). The specimens were then depicted in a scatter plot with the first two canonical variates as coordinates (Figure 3). Ellipses representing the 95% confidence interval were plotted for each species with three or more specimens. All statistical findings were deemed significant if the associated P-value < 0.05.

Results

Species discrimination

The first and second components of the canonical variate analysis (CVA) explained 53.8% and 20.1% of the total variance, respectively in allometry uncorrected data and 57.6% and 16.6% in allometry-corrected data (Figure 3). The wing landmarks that primarily elucidated the shape distinctions along the CVA first variate display were those nearest to the wing's attachment nodes. These landmarks include the right-wing points 3–6 (R1; R2 + 3; R4 + 5; M1), 11, and 14, which correspond to the maxima of curvature in the distal portion of the M. Moreover, the landmarks with significant

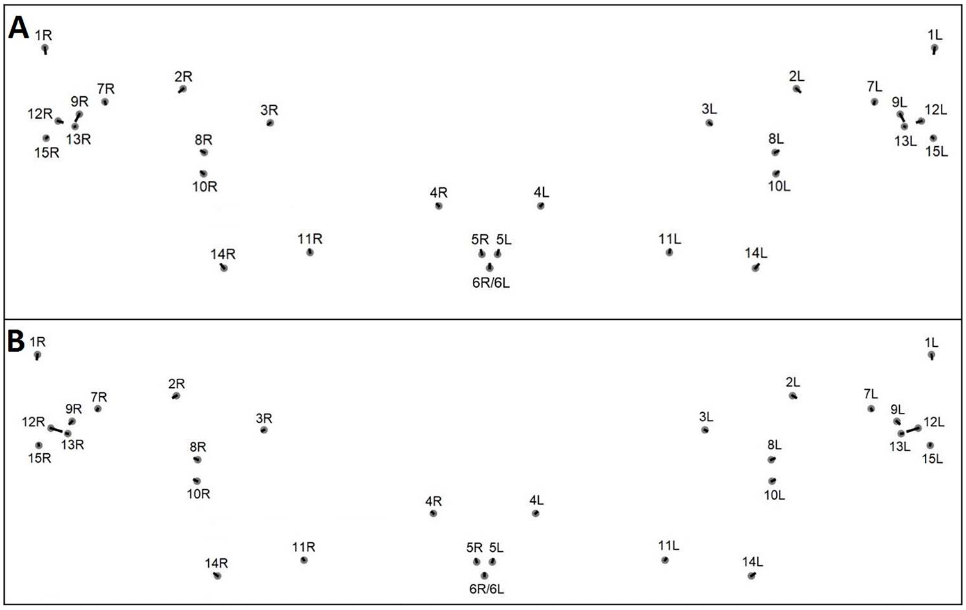

Figure 2 Canonical variate 1 displaying allometry uncorrected (A) and allometry-corrected (B) shape change from average conformation for wing landmarks. Left wing landmarks (1L–15L) and right wing landmarks (1R–15R).

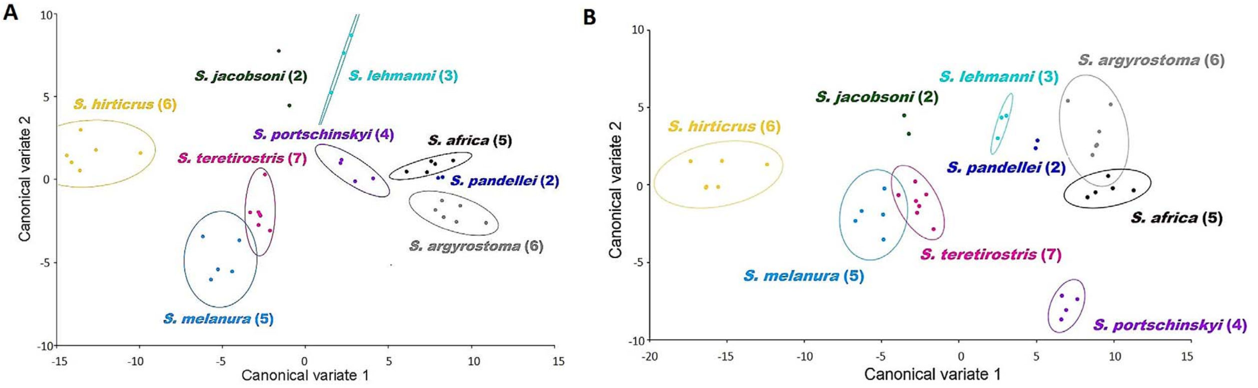

Figure 3 Canonical variate analysis (allometry uncorrected data). Ellipses represents 95% confidence intervals calculated for species represented with three or more points. (A) The variance explained for the first and second variates was 53.8% and 20.1%, respectively. (B) The variance explained for the first and second variates was 57.6% and 16.6%, respectively.

shape changes are at points 9, the anterior intersection of the distal M-CT1 and the medial vein (M) and 12, the posterior intersection of the proximal M-CT1 and the cubital vein (Cu), as illustrated in Figure 2, along with their corresponding counterparts on the left wing.

The scatter plot (Figure 3A) demonstrates a distinct separation between *S. hirticrus* and *S. argyrostoma*, as well as a clear differentiation between *S. portschinskyi* and *S. africa*. In general, seven species (*S. africa*, *S. argyrostoma*, *S. hirticrus*, *S. jacobsoni*, *S. lehmanni*, *S. pandellei*, and *S. portschinskyi*) exhibit noticeable differentiation, while two species (*S. melanura*, and *S. teretirostris*) partly overlap. Mahalanobis distances resulting from pairwise comparisons among the nine studied species with values ranging from 5.8 (between *S. teretirostris* and *S. melanura*) to 27.0 (between *S. pandellei* and *S. hirticrus*) (Table 1).

With the allometric correction, the scatter plot (Figure 3B) illustrates a distinct segregation between *S. portschinskyi*, *S. hirticrus*, and *S. lehmanni*. Four species (*S. melanura*, *S. teretirostris*, *S. argyrostoma*, and *S. africa*) are also distinguished despite partially overlapping with each other. Mahalanobis distances resulting from pairwise comparisons among the nine studied species ranging from 6.0 (between *S. teretirostris* and *S. melanura*) to 27.7 (between *S. pandellei* and *S. hirticrus*) (Table 2).

Wing morphometry

The dendrogram illustrating Procrustes distances depicted four primary branches among Sarcophagidae species (Figure 4A). The initial branch encompassed *S. pandellei*. The second branch featured *S. teretirostris*, while the third branch comprised *S. africa*, *S. argyrostoma*, and *S. portschinskyi*. The fourth branch encompassed *S. hirticrus*, *S. jacobsoni*, *S. lehmanni*, and *S. melanura*.

In Figure 4B, with allometry correction, we observe three primary branches within Sarcophagidae species. The first group comprises the species *S. pandellei*, the second group

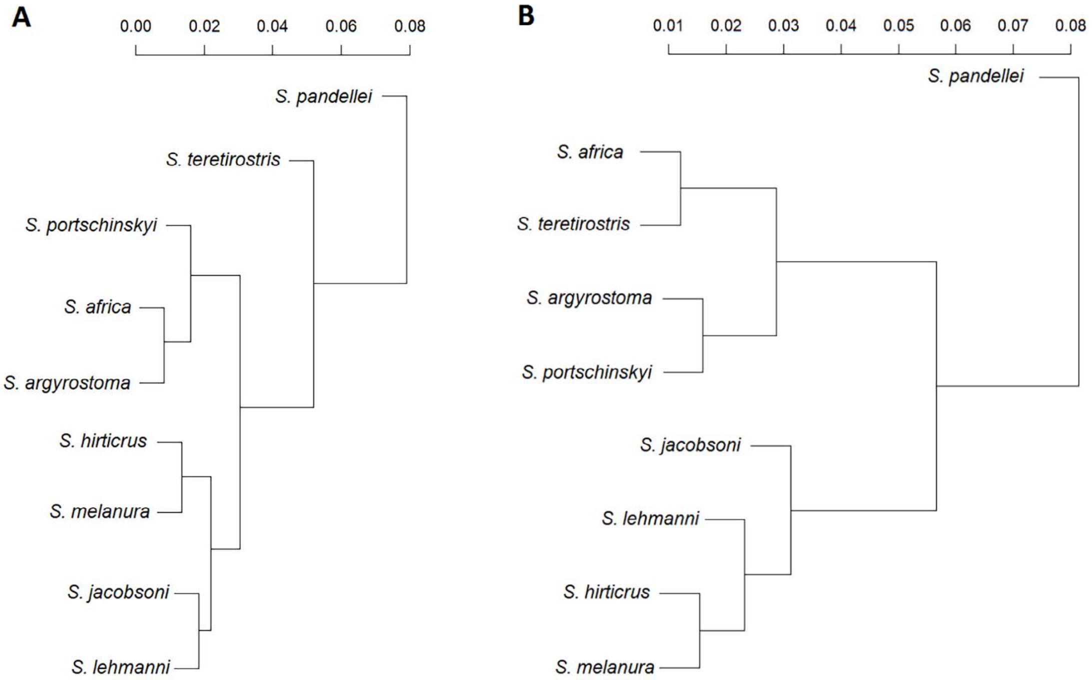

Figure 4 Procrustes distances radial dendrogram (allometry uncorrected data). (A) Cophenetic correlation coefficient = 0.98. (B) Cophenetic correlation coefficient = 0.93.

includes *S. africa, S. argyrostoma*, *S. portschinskyi*, and *S. teretirostris*, and finally, the third group consists of *S. hirticrus*, *S. jacobsoni*, *S. lehmanni*, and *S. melanura*.

Discussion

The CVA of wing shape enables the differentiation of Sarcophagidae species; however, distinguishing the *Sarcophaga* group remains challenging, as demonstrated by the recent study of Szpila et al. [39].

The definitive confirmation of species within the Sarcophagidae family typically relies on the analysis of male genitalia, as the distinct characteristics are often unique to each species, offering a more reliable basis for precise identification [53]. However, our study did not include molecular identification, a commonly used method to confirm species identity. In the absence of this molecular confirmation, only males were used for morphological identification as a precautionary measure to ensure accuracy. Despite the majority of specimens captured being females, only 40 males were utilized for morphological identification. This decision was based on the fact that male genital characteristics provide a more reliable and unequivocal basis for species identification. Although this approach may result in uneven sex sampling, it was chosen to ensure the accuracy of species identification in the study [54].

The effect of wing size on its shape change, known as allometric growth effect, is an important consideration in the morphometric analysis of insects, including the Sarcophagidae family. While there is a general understanding of this phenomenon in insects, it is crucial to specifically evaluate whether wing size influences its shape in flesh flies (Sarcophagidae) to determine its relevance for forensic studies. Several studies explore the allometric growth effect in insects, although not all focus specifically on the Sarcophagidae family. However, these studies provide a solid foundation for understanding the phenomenon in other insect groups, which can be applied to flesh flies. For instance, Rohlf and Marcus [55] discuss advancements in morphometrics and emphasize the importance of considering size effect on shape in morphometric studies. Mitteroecker and Bookstein [56] explore statistical methods for analysing the relationship between size and shape in morphometric data. Klingenberg [57] reviews the concepts of allometry in geometric morphometrics, addressing how size affects shape in different organism groups. While these studies do not specifically focus on Sarcophagidae, they provide a solid theoretical basis for investigating the effect of wing size on its shape in this family of flesh flies. It is recommended to conduct specific studies with Sarcophagidae to confirm and quantify the allometric growth effect in this group, which may have important implications for forensic insect analysis.

In the present study, the landmark-based geometric morphometrics approach (with allometric correction) proved effective in discriminating among seven out of the nine studied *Sarcophaga* species (three species *S. hirticrus*, *S. lehmanni*, and *S. portschinskyi* unequivocally, and other four, *S. africa*, *S. argyrostoma*, *S. melanura*, and *S. teretirostris* with less robustness). This discrimination was achieved primarily using the wing veins R1, R2 + 3, R4 + 5, and M1 (landmarks 3–6). Additionally, the curvature at the distal portion of the M (landmarks 11 and 14), as well as the anterior intersection of the distal M-CT1 with the M at landmark 9, and the posterior intersection of the proximal M-CT1 with the Cu at landmark 12, provided further insight into the observed differences.

Nevertheless, wing morphometry failed to differentiate between, *S. pandellei* and *S. jacobsoni*, which remained distinguishable solely through genitalia analysis. It should be noted that *S. pandellei* is the species that join at a high height on the dendrogram, which emphasizes its difference from other species.

It is evident that the methodology still requires improvement, particularly in terms of increasing the number of specimens and expanding the studied geographical area. These areas should be addressed in future studies to enhance the methodology's effectiveness. However, despite these limitations, the findings of this study provide valuable insights into the use of wing geometric morphometrics in Sarcophagidae species. By narrowing down the number of likely species for a particular wing, the study significantly reduces the time required for the identification process.

Conclusion

In this study, we employed a landmark-based approach to analyse the geometric morphometrics of wings from nine different species of Sarcophagidae, a family of flies commonly known as flesh flies. Geometric morphometrics involves the quantitative analysis of shape and size using landmarks, which are specific points identified on biological structures.

The results demonstrated that this landmark-based approach effectively distinguished between seven out of the nine *Sarcophaga* species examined. By analysing the positions of specific landmarks on the wings, we could identify unique patterns or shapes that differentiated one species from another.

This method proves particularly valuable in forensic scenarios where ambiguity or a loss of diagnostic characteristics in specimens may occur. For instance, if a forensic investigator discovers a fly specimen at a crime scene but it is damaged or lacks distinct features necessary for traditional morphological identification, geometric morphometrics of the wing can still provide valuable information for species identification.

Moreover, this approach accelerates the identification process and complements traditional morphological identifications. Instead of relying solely on time-consuming and sometimes subjective visual examinations of physical characteristics, quantitative measurements of wing shape and size can support or confirm species identification. This can be especially beneficial in cases where rapid identification is crucial, such as in forensic investigations.

Authors' contributions

Carina I. C. Reis and Maria Teresa Rebelo conceived and designed the research. Ana C. Farinha, Marco A. Menezes and Maria Teresa Rebelo conducted the fieldwork and identified the specimens. Carina I. C. Reis performed image acquisition, processed data, and designed the figures. Carina I. C. Reis and Paulo Mascarenhas conducted statistical and data analysis; Carina. I. C. Reis, Paulo Mascarenhas and Maria Teresa

Rebelo wrote the first draft of the manuscript, and all authors provided comments on previous versions. All authors have read and approved the final manuscript.

Compliance with ethical standards

Not applicable.

Disclosure statement

All authors declare no conflict of interest.

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