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Wing Morphometric Analysis of Some Species of The Genus *Sarcophaga* **(Diptera: Sarcophagidae) in Egypt**

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ARTICLE INFO ABSTRACT

 Species of the genus *Sarcophaga* have significant medical, veterinary, and forensic importance. They can act as mechanical carriers of enteropathogens in humans, cause myiasis in cattle, and aid in determining human remains and the Post-Mortem Interval (PMI) in forensic cases. Therefore, accurate identification of flesh fly species is crucial. Despite their importance, adult *Sarcophaga* species are difficult to identify correctly due to their similar appearances, with species identification typically relying on the morphology of male genitalia. In this study, we conducted wing morphometric analysis on 92 flesh fly specimens, comprising 12 species from Egypt. Each specimen's right wing was removed, photographed, mounted on a microscope slide, and digitized using 19 landmarks. The study analyzed the wing shape variation among different subgenera and species through canonical variate analysis, which separated the subgenera into distinct groups with a high percentage of correct classification. The analysis also showed some overlaps in wing shape among species within each subgenus, and most comparisons resulted in a high percentage of correct classification, except for *S*. *jacobsoni* and *S*. *mennae*. The phylogenetic tree based on the wing morphology of species largely placed each species into its respective subgenus. Our findings suggest that wing shape can serve as a reliable tool for distinguishing between different subgenera and species of flesh flies. This research provides valuable insights into improving the identification process of these important insects.

INTRODUCTION

 The genus *Sarcophaga*, comprising approximately 890 species organized into 169 subgenera globally, (Buenaventura *et al.,* 2017; Ramos *et al.,* 2022). In Egypt, this genus represented 28 species belong 9 subgenera (El-Ahmady *et al*., 2018). Of these, some species have substantial medical and forensic importance. Adult flies serve as mechanical carriers of enteropathogens, transmitting pathogens to humans (Graczyk *et al*., 2005 Getachew *et al*., 2007). Additionally, certain species can cause myiasis in humans and cattle, depending on their larval feeding habits (Ferraz *et al*., 2010; Chaiwong *et al*., 2014; Giangaspero *et al*., 2017). Moreover, *Sarcophaga* species are significant in forensic investigations as they are attracted to and feed on decaying human carcasses (Catts, 1992; Wells & La Motte, 2010).

Their presence can be helpful in determining human remains in forensic cases. The Post-Mortem Interval (PMI), such as *S. (B.) africa* (Wiedemann), *S. (Liop.) argyrostoma* Robineau-Desvoidy, *S. (Liop.) ruficornis* (Fabricius), *S. (Lios.) aegyptica* Salem, *S. (Lios.) dux* Thompson, *S. (Lios.) tibialis* Macquart, and *S. (Par.) hirtipes* Wiedemann (Sukontason *et al.,* 2007; Kavitha *et al.,* 2013).

 Identifying the correct species of flesh flies is crucial for forensic purposes and to determine the minimum Post-Mortem Interval (PMI). Traditionally, the morphology of the external male genitalia has been used to identify most fleshfly species (Kurahashi & Chaiwong, 2013; Meiklejohn *et al.,* 2013; Vairo *et al.,* 2014). Additionally, molecular characteristics have been employed for species identification (Giroux, 2007; Tan *et al.,* 2010; Guo *et al.,* 2012; Jordaens *et al.,* 2013). However, identifying male genitalia can be challenging for non-taxonomists due to its complex structure. Furthermore, female identification is also problematic, as there are few available identification keys (Richet *et al.,* 2011; Vairo *et al.,* 2015). While DNA identification is a reliable method, it is often expensive and requires advanced equipment (Sontigun *et al.,* 2019). Emerging techniques, such as geometric morphometric analysis based on landmarks on the wings, have become valuable tools for distinguishing between different species (Sontigun *et al.,* 2017) and studying geographic variation within species (Hall *et al.,* 2014; Carvajal *et al.,* 2016).

 Geometric morphometric analysis of wings has been widely used in the study of various dipteran taxa, including flesh flies (Sarcophagidae) (Sontigun *et al.,* 2019), fruit flies (Drosophilidae) (Bubliy *et al.,* 2008; Perre *et al.,* 2014), mosquitoes (Culicidae) (Jaramillo-O *et al.,* 2015; Wilke *et al.,* 2016), black flies (Simuliidae) (Pepinelli *et al.,* 2013), midges (Ceratopogonidae) (Muñoz-Muñoz *et al.,* 2014), blow flies (Calliphoridae) (Lyra *et al.,* 2010; Sontigun *et al.,* 2017), muscids (Muscidae) (Grzywacz *et al.,* 2017), and tabanids (Tabanidae) (Cárdenas *et al.,* 2013).

 The present study represents the first attempt at using wing morphometric analysis for species identification of medically and forensically important flesh flies in Egypt.

MATERIALS AND METHODS

Specimen Collection:

 A total of 92 flesh fly specimens were collected from different localities across Egypt, representing most of the ecological zones in the country, between January 2022 and June 2023. Decayed meat bait traps and aerial nets were used to collect the flesh flies. The collected samples were killed in the field using ethyl acetate and immediately pinned. The identification of samples was carried out using a stereo microscope (NOVEL), based on the taxonomic key of El-Ahmady *et al*. (2018) (Table 1).

Slide Preparation:

 The wings of the fly specimens were carefully removed using fine forceps, targeting the basicostae region. To prepare the wings for analysis, they were placed in xylene, which helped in mounting them and prevented the formation of bubbles. The wings were then carefully mounted on microscope slides using DPX Mountant, covered with coverslips, and allowed to dry at room temperature for a week.

 Photographic images were captured for each wing using a Canon EOS 6D camera. The TpsUtil V. 1.81 software (Rohlf, 2013) was used to construct TPS files from the images, which helped to minimize potential bias during the digitization of landmark locations (Fig. 1). The TpsDig2 V.2.31 software (Rohlf, 2015) was utilized to digitize 19 landmarks on each wing. To ensure accuracy, each wing was digitized twice to minimize measurement errors, as recommended by Sontigun *et al.* (2017).

Subgenus	Scientific name	Collection site	Specimens number	
Bercaea	S. africa (Wiedemann, 1824)	Abo Swuier, Aga, Awlad Saqr, Bilbeis, El- Qantara, El-Salam, Kafr Saqr, Nasr City, Sharm El-Sheikh, Wadi El-Natroun, Kom Oshem	24	
Liopygia	S. argyrostoma Robineau-Desvoidy, 1830	Al-Salam, Aga, El-Qantra, Kafr Saqr, Marsa Matruh, Nasr City, Sharm El-Sheikh.	12	
	S. ruficornis Fabricius, 1794	Abbasia	3	
	S. aegyptica Salem, 1935	Abo Suweir, Al-Salam, Kafr Saqr, Kom Oshem, Nasr City, Sharm El-Sheikh, Wadi El-Natroun	20	
	S. dux Thomson, 1869	Al-Salam, Bilbeis, Kafr Saqr, Marsa Matruh, Nasr City,	17	
	S. jacobsoni Rohdendorf, 1937	Kom Oshem, Wadi El-Natroun	6	
Liosarcophaga	S. mennae Al-Ahmady, 2018	Kom Oshem,	2	
	S. parkeriRohdendorf, 1937	Awlad Saqr, Al-Salam, Wadi El-Natroun	3	
	S. redux Walker, 1849	Sharm El-Sheikh	$\overline{2}$	
	S. rohdendorfi Salem, 1936	Geneifa	2	
	<i>S. tibialis</i> Macquart, 1851	Marsa Matruh	\overline{c}	
Parasarcophaga	S. hirtipes Wiedemann, 1830	Abo Suweir, Al-Salam, Bilbeis, Kafr Saqr, Marsa Matruh, Nasr City, Sharm El-Sheikh, Wadi El-Natroun	14	

Table 1: list of the *Sarcophaga* specimens studied.

Fig. 1: The right wing of *S. aegyptiaca.*

Geometric Morphometric Analysis:

 The raw landmark coordinates of all specimens were aligned and superimposed using the Procrustes Fit function to eliminate variation caused by differences in scale, position, and orientation (Bookstein, 1991). Further statistical analyses were conducted using the centroid size (the square root of the sum of the squared distances between the centre

of the configuration of landmarks and each landmark) and Procrustes coordinates derived from the landmark data.

 Following a generalized Procrustes analysis in MorphoJ, the Procrustes coordinates of each specimen were averaged to determine potential measurement error. Additionally, the centroid size was averaged for each specimen.

Allometry:

 To account for the potential influence of wing size on wing shape variation, we assessed the allometric effects by analyzing the regression of Procrustes coordinates (the dependent variable) against centroid size (the independent variable) across the different subgenera and species. This was done using a permutation test with 10,000 rounds, which was conducted using the MorphoJ software version 1.06 (Gidaszewski *et al.,* 2009; Sontigun *et al.,* 2019; Klingenberg, 2011).

Shape Variation:

 The wing shape variation between the subgenera and species was evaluated using canonical variate analysis (CVA). This analysis was based on Mahalanobis distances, and the statistical significance of the differences was assessed through a permutation test with 10,000 rounds, conducted using the MorphoJ software. Additionally, to determine the reliability of the classification based on Mahalanobis distances, a cross-validation test in discriminant function analysis (DFA) was performed. The significance of the classification results was also tested using a permutation test with 10,000 rounds.

Phylogenetic Relationships of Wing Shape Among Species:

 The relationships between the 12 flesh fly species were analyzed using their wing morphometric data. The UPGMA (unweighted pair-group method with arithmetic averages) was the method employed for this analysis, which was carried out using the PAST software version 4.03 (Hammer et al., 2001). The UPGMA dendrogram was constructed using the Mahalanobis distances calculated through pairwise comparisons of the species, as derived from the canonical variate analysis (CVA).

RESULTS

1-Allometry Analysis:

 Based on the statistical analysis, the Procrustes coordinates regressed on centroid size showed a significant difference among subgenera and species. Allometry accounted for 11.64% of total shape variation for subgenera and 6.72% for species (permutation test with 10,000 rounds in MorphoJ: P < 0.05), except for *S*. (*Lios.*) *dux*, *S*. (*Lios*.) *jacobsoni*, *S*. (*Lios*.) *mennae* and *S*. (*Lios*.) *redux* (Table 2).

Table 2: Percentage of prediction indicating the amount of size-related shape variation of wings in each flesh fly species of each species.

2-Shap Variation:

 The analysis of wing shape, after accounting for size effects, revealed significant differences among the examined subgenera and species. The canonical variate analysis (CVA) at the subgeneric level identified three main axes of shape variation, with the first two canonical variates (CV1 and CV2) explaining 87.4% of the total variation. The scatter plot visualisation (Fig. 2) clearly demonstrates that the specimens group into distinct clusters corresponding to the different subgenera, indicating substantial shape divergence between them.

 Further quantitative analysis using Mahalanobis distances confirmed that the shape differences between the subgenera were highly statistically significant ($P < 0.0001$). The greatest distance was observed between *Parasarcophaga* and *Bercaea* (13.0339), while the smallest distance was between *Liosarcophaga* and *Bercaea* (7.1729) (Table 3). Importantly, the cross-validation test showed very high correct classification rates, ranging from 96.4% to 100.0% (Table 4). This indicates that the wing shape features captured by the geometric morphometric approach are highly effective for distinguishing and identifying the different subgenera based on their unique morphologies.

Table 3: The Mahalanobis distances between subgenera obtained from CVA using a permutation test with 10,000 rounds in MorphoJ.

Groups	Bercaea	Liopygia	Liosarcophaga			
Liopygia	8.4384					
Liosarcophaga	7.1729	7.6179				
Parasarcophaga	13.0339	9.5776	8.9557			

Table 4: Percentage of correct classification obtained from pairwise comparison of analyzed subgenera with cross-validation test using a permutation test with 10,000 rounds in MorphoJ.

Fig. 2: A scatter plot that displays the variation in wing shape among six subgenera of *Sarcophaga* based on the first two canonical variates (CV1 and CV2).

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 The canonical variate analysis (CVA) conducted at the species level identified 11 primary axes of shape variation. The first two canonical variates (CV1 and CV2) accounted for 61.6% of the total shape variation, with CV1 explaining 42.8% and CV2 explaining 18.8%. The scatter plot visualization using CV1 and CV2 (Fig. 3) revealed some interesting patterns among the species. Most of the *Liosarcophaga* species were found to overlap in morphospace, except for *S. rohdendorfi*, which appeared distinct. Similarly, within the genus *Liopygia*, *S*. *argyrostoma* and *S*. *ruficornis* were observed to overlap. In contrast, *S*. *hirtipes* (*Parasarcophaga*) and *S*. *africa* (*Bercaea*) were clearly separated in the morphospace. The quantitative analysis of Mahalanobis distances between all pairwise species comparisons confirmed that the shape differences were highly statistically significant ($P < 0.0001$). The distances ranged from 5.0831 (between *S*. *jacobsoni* and *S*. *mennae*) to 21.6657 (between *S*. *parkeri* and *S*. *rohdendorfi*) (Table 5). Accordingly, the cross-validation testing showed that the majority of species could be correctly classified based on their wing shape features, with classification accuracies ranging from 41.7% to 100.0% (Table 6). This suggests the geometric morphometric approach provides a robust tool for discriminating and identifying these sarcophagid fly species based on their unique wing shape characteristics.

Fig. 3: A scatter plot showing that displays the variation in wing shape among 12 species of *Sarcophaga* based on the first two canonical variates (CV1 and CV2).

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		$\overline{2}$	3	4	5	6	7	8	9	10	11	12
S. aegyptiaca												
S. africa	7.84											
S. argyrostoma	8.46	9.07										
S. dux	6.29	11.03	10.74									
S. hirtipes	10.63	14.93	11.38	11.37								
6. S. jacobsoni	7.64	9.01	8.92	9.72	11.58							
S. mennae	8.52	9.24	9.97	10.44	12.85	5.08						
S. parkeri	8.18	11.37	12.59	9.39	13.22	10.68	10.95					
S. redux	9.58	11.65	11.73	12.16	15.08	13.31	12.86	11.24				
S. rohdendorfi	17.41	20.34	19.29	16.16	15.11	18.27	18.90	21.66	20.90			
S. ruficornis	13.06	11.58	10.09	14.39	15.36	11.15	11.61	17.29	16.74	21.35		
S. tibialis	10.87	14.88	13.77	9.39	12.23	13.60	13.47	9.84	13.68	17.87	18.77	

Table 5 :The Mahalanobis distances between species obtained from CVA using permutation test with 10,000 rounds in MorphoJ.

Table 6:Percentage of correct classification obtained from pairwise comparison of analyzed species with cross-validation test using a permutation test with 10,000 rounds in MorphoJ.

						Group 2							
		1	2	3	$\overline{4}$	5	6	7	8	9	10	11	12
Grou p1	1. S. aegyptiaca		100	100	100	100	91.7	100	83. 4	100	100	75	100
	2. S. africa	100		100	100	100	91.7	100	100	100	100	100	100
	3. S. argyrostoma	100	100		100	100	100	100	100	100	100	100	100
	S. dux 4.	100	100	100		100	91.7	100	100	100	100	100	100
	5. S. hirtipes	100	100	100	100		91.7	100	100	100	100	100	100
	6. S. jacobsoni	97.5	100	100	100	100		48.6	100	100	100	100	100
	S. 7. mennae	100	100	100	100	100	45		100	100	100	100	100
	S. 8. parkeri	100	100	100	100	100	91.7	100		75	100	100	100
	9. S. redux	100	100	100	100	100	91.7	100	100		100	100	100
	10. S. rohdendorfi	100	100	100	100	100	100	100	100	100		100	100
	S. 11. ruficornis	100	100	100	100	100	100	100	100	100	100		100
	12. S. tibialis	100	100	100	100	100	91.7	100	100	100	100	100	

3-Phenetic Relationships Of Wing Shape Among *Sarcophaga* **species:**

 The UPGMA dendrogram analysis divided the 12 flesh fly species into two distinct groups (Fig. 4). The first group contained the monotypic subgenus *Bercaea*, represented by the single species *S*. *africa*, as well as the subgenus *Liopygia*, represented by the two species S. argyrostoma and S. ruficornis. This group also included two species from the *Liosarcophaga*subgenus, namely *S*. *jacobsoni* and *S*. *mennae*. The second group consisted primarily of species from the Liosarcophaga subgenus, including *S*. *aegyptiaca*, *S*. *dux*, *S*. *parkeri*, *S*. *redux*, *S*. *ruficornis*, and *S*. *tibialis*. Additionally, this group contained a single representative of the *Parasarcophaga* subgenus, *S*. *hirtipes*. The phylogenetic tree analysis revealed that the *Liosarcophaga* subgenus is a polytypic and paraphyletic group, as its members are distributed across the two main clades. In addition, *S*. *jacobsoni* and *S*. *mennae* (*Liosarcophaga*) formed a monophyletic relationship, as *S*. *aegyptiaca* and *S*. *parkeri*, and *S*. *dux* and *S*. *redux*. The *Bercaea* subgenus, represented solely by *S*. *africa*, was found to be a monotypic group and a sister clade to the *Liosarcophaga* and *Liopygia* subgenera. Similarly, the *Parasarcophaga* subgenus, with only *S*. *hirtipes*, was also monotypic and formed a monophyletic sister group to *Liosarcophaga*. Finally, the *Liopygia* subgenus, containing the two species *S*. *argyrostoma* and *S*. *ruficornis*, was identified as a polytypic group supported by a sister species and monophyletic relationship.

Fig. 4: UPGMA dendrogram showing phenetic relationships of wing morphology among *Sarcophaga* species constructed based on the Mahalanobis distances between species.

DISCUSSION

 Geometric morphometric analysis of the wing has been successfully used for species identification across various dipteran taxa. This method has been applied to study flesh flies (Sarcophagidae) (Sontigun *et al.,* 2019), fruit flies (Drosophilidae) (Perre *et al.,* 2014), mosquitoes (Culicidae) (Wilke *et al.,* 2016), black flies (Simuliidae) (Pepinelli *et al.,* 2013), midges (Ceratopogonidae) (Muñoz-Muñoz *et al.,* 2014), blow flies (Calliphoridae) (Lyra *et al.,* 2010; Sontigun *et al.,* 2017), muscids (Muscidae) (Grzywacz *et al.,* 2017), and tabanids (Tabanidae) (Cárdenas *et al.,* 2013). These studies demonstrate the effectiveness of wing morphometrics as a reliable technique for species identification across a wide range of dipteran families.

 Estimation of allometric effects is crucial in morphological studies, as it can influence taxonomic analyses of morphometric variation (Gidaszewski *et al.,* 2009; Sontigun *et al.,* 2017, 2019). In the present study, allometric analysis indicated that variation in wing shape among species is partly explained by differences in wing size. To study shape variation without the size effect, size correction was performed using residuals from the regression of shape on size, which accounted for approximately 6.72% of total shape variation among species (Sidlauskas *et al.,* 2011; Lorenz *et al.,* 2012; Sontigun *et al.,* 2017, 2019).

The cross-validation analysis of wing morphometric variation at the subgenera level showed a high percentage of correct classification, ranging from 96.4% to 100%. This is evident in the CVA scattered plot (Fig. 2), which separated the subgenera into distinct groups, accounting for 87.4% of the total variation. These results are similar to those reported by Sontigun *et al.* (2019).

Although there were some overlaps in wing shape among species within each subgenus in the morphospace of canonical variables, the majority of comparisons resulted in a high percentage of correct classification (45–100%), except for the pair *S. jacobsoni* and *S.*

mennae; whereas the two species are similar in the external morphological characters and the genitalia characters; *S. mennae* is characterized by Vesica truncate, dentated apically; harpes with a long spine-like process, about half style length (El-Ahmady *et. al.,* 2018). The CVA scattered plot (Fig. 4) shows partial overlap between some species of the subgenus *Liosarcophaga*, with *S. rohdendorfi* being distinctly separated.

The phylogenetic tree based on the wing morphology of the 12 flesh fly species largely placed each species into its respective subgenus. The phylogenetic relationships between subgenera and species detected here are in accordance with their morphological phylogenetic tree (El-Ahmady *et al.,* 2024).

The subgenus *Liosarcophaga* is a polytypic group and has a sister relationship with all other groups. The subgenus *Parasarcophaga* is a monotypic group and has a sister relationship with the subgenus *Liosarcophaga*, where *S*. (*Par.*) *hirtipes* and *S*. (*Lios*.) *rohdendorfi* are very close in the majority of the analyzed phylogenetic trees. Additionally, the subgenera *Liopygia* (polytypic) and *Brecaea* (monotypic) have a sister relationship with the subgenus *Liosarcophaga*, where *S*. (*B*.) *africa*, *S*. (*Liop*.) *argyrostoma*, *S*. (*Liop*.) *ruficornis*, *S*. (*Lios*.) *jacobsoni*, and *S*. (*Lios*.) *mennae* are very close in all the analyzed phylogenetic trees.

Our results suggest that wing morphology may reveal phylogenetic signals among *Sarcophaga* genus species. Landmark-based geometric morphometric analysis of wings can be a valuable tool in taxonomy and systematics, as it is simple, reliable, cost-effective, and only requires undamaged wings for analysis (Sontigun *et al.,* 2019). Compared to molecular methods, landmark-based wing analysis is much easier, faster, and cheaper. Additionally, the complex structure of male genitalia makes it challenging for non-taxonomists to identify using traditional taxonomic keys (Vairo *et al.,* 2015; El-Ahmady *et al.,* 2018).

In conclusion, the present study demonstrates that analyzing wings using geometric morphometrics based on landmarks is a dependable method for classifying flesh flies at both subgenera and species levels, even for non-taxonomists.

Declarations:

Ethical Approval: Ethical Approval is not applicable.

Authors Contributions: Prof. Dr. Ahmed M. Galhom and Porf. Dr. Metwaly M. Montaser designed the experiments, reviewed drafts of the article, and approved the final draft. Dr.Ahmed Badry and Mr. Ahmed El-Ahmady conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored and reviewed drafts of the article, and approved the final draft.Dr. Medhat I Abu-Soud collected and identified the collecting specimens, reviewed drafts of the article, and approved the final draft.

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ARABIC SUMMARY

التحليل المورفومتري لألجنحة لبعض أنواع جنس ساركوفاجا) ثنائية االجنحة: ساركوفاجيدي (في مصر

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 تتمتع أنواع جنس الساركوفاجا بأهمية طبية وبيطرية وطب شرعي كبيرة. يمكن أن تعمل كحامالت ميكانيكية لمسببات الأمراض المعوية لدى البشر، وتسبب مرض النغف في الماشية، وتساعد في تحديد الرفات البشرية والفاصل الزمني بعد الوفاة (PMI) في حالات الطب الشرعي. لذلك، يعد التحديد الدقيق لأنواع ذباب اللحم أمرًا بالغ الأهمية. على الرغم من أهميتها، يصعب التعرف على أنواع التابوت البالغة بشكل صحيح بسبب مظهرها المتشابه، حيث يعتمد تحديد الأنواع عادةً على شكل الأعضاء التناسلية الذّكرية. لذا كان من الضروريّ استخدام طرق ووسائل أخرى للمسهمة في تسهيل عملية تصنيف االنواع لذباب اللحم.

في هذه الدراسة، أجرينا تحليلًا مورفومتريًا للجناح على 92 عينة من ذباب اللحم تضم 12 نوعا من مصر . تمت إزالة الجناح الأيمن لكل عينة، وتصويره، وتثبيته على شريحة مجهرية، ورقمنته باستخدام 19 معلمًا. قامت الدراسة بتحليل تباين شكل الجناح بين الأجيال الفرعية والأنواع المختلفة من خلال تحليل التباين القانوني، الذي فصل الأجيال الفر عية إلى مجمو عات متميزة مع نسبة عالية من التصنيف الصحيح. أظهر التحليل أيضًا بعض التداخل في شكل الجناح بين األنواع داخل كل جنس فرعي، وأسفرت معظم المقارنات عن نسبة عالية من التصنيف الصحيح، باستثناء *.S jacobsoni* و *mennae .S*. إن شجرة النشوء والتطور المستندة إلى مورفولوجيا جناح األنواع وضعت كل نوع إلى حد كبير في جنسه الفرعي. وتشير النتائج التي توصلنا إليها إلى أن شكل الجناح يمكن أن يكون بمثابة أداة موثوقة للتمييز بين الأجيال الفر عية المختلفة وأنواع ذباب اللحم. يوفر هذا البحث رؤى قيمة لتحسين عملية تحديد هذه الحشرات الهامة.

الكلمات الدالة:ساركوفاجا)ذباب اللحم(, الجناح , القياس المورفولوجي , علم التصنيف , مصر.