Assessment of variation between two blowfly species, *Lucilia cuprina* (Wiedemann) and *Lucilia sericata* (Meigen) (Diptera, Calliphoridae) using geometric morphometrics and cuticular hydrocarbon profiling

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ABSTRACT. *Lucilia cuprina* and *Lucilia sericata* are two closely related species due to their similarity in morphology, habitat, distribution, and economic importance. Even though other methods have segregated the species, the aspect of comparative studies on geometric morphometrics and cuticular hydrocarbon composition in species variability is yet to be explored in these species. This study was conducted to assess variability between the two species and between the sexes. Wing shapes of 187 specimens of both species were analysed by geometric morphometric techniques. Landmarks 11, 10, 6 and 9, which corresponds to the intersection between the medial and the radial medial veins, medial and branched cubitus veins, distal end of radius vein (R2 + 3 vein) and curve point of medial vein, respectively, contributed significantly to the variability within and between species. Cuticular hydrocarbon profiles of four randomly collected individuals each of male and female *L. cuprina* and *L. sericata*, were assessed using GC-MS. Octadecene, Celidoniol, Hexatriacontane, Tetracontane and Tetracontane were identified as common for both species. 9-Octadecenal(*z*) and Tetracosane-11-decyl being recorded as the most abundant hydrocarbons in male and female *L. cuprina*, and 13-methylheptacosane and Tetratetracontane in male and female *L. sericata*, respectively. Diagnostic characters indicating the variabilities can be used for the identification of the species.

Key words: Geometric morphometrics; cuticular hydrocarbons; variability

INTRODUCTION

Lucilia cuprina (Wiedemann, 1830) and *Lucilia sericata* (Meigen, 1826) are blowflies of forensic importance belonging to the genus *Lucilia* and the family Calliphoridae. The importance of these species is also

seen in agriculture and veterinary. Larvae of both species are known to cause sheep strike, and myiasis of sheep (Hepburn, 1943; Vogt & Woodburn, 1979; Heath & Bishop, 2006). *Lucilia sericata* is also reported to cause sheep strike in Northern Europe where *Lucilia cuprina* is absent (Rose & Wall, 2011). *L. sericata* is known to be a good pollinator of mango, and is as effective as the honey bee (Dag & Gazit, 2000). In health, larvae of *L. cuprina* and *L. sericata* are used in maggot debridement therapy (Williams et al., 2008; Du Plessis & Pretorious, 2011; Williams & Villet, 2014). In Ghana, the genus *Lucilia* has been reported as insects of forensic importance (Combey et al., 2017). These two species show similarities in morphology and ecology (Lutz et al., 2018) and are often misidentified for each other (Williams & Villet, 2014). Identification is usually difficult due to their extent of similarities. Accordingly, specific keys and characters have been identified to distinguish between them (Holloway, 1991; Lutz et al., 2018). Geometric morphometrics capture possible variations in species and populations and are able to characterise features of shape variation between species and groups. Previous studies have successfully utilized this technique to capture variability between blowflies (Sharanya & Zuha, 2019) regarding sexual dimorphisms (Nuñez-rodríguez & Liria, 2017), wing shape dimorphism (Espra et al., 2015) and other identification features (Jimenez-Martin et al., 2020). Cuticular hydrocarbons influences close-range orientation in species (Blomquist et al., 1993) as well as sexual isolation as well mate location, and courtship behaviours (Peterson et al., 2007). Environmental factors play a major role in determining the kind of cuticular hydrocarbon present in insects (Khidr et al., 2013).

Studies have been conducted to determine cuticular hydrocarbons of *L. sericata* at different stages (Moore et al., 2014, 2017) and also as chemotaxonomic tool for identification of *L. cuprina* (Barbosa et al., 2017). However, information on cuticular hydrocarbons of adults of *L. serciata* and *L. cuprina* in Ghana is yet to be conducted. As such, assessing the variability of *L. sericata* and *L. cuprina* using hydrocarbon profiles is paramount for identification. The present study is based on the hypothesis that geometric morphometrics and cuticular hydrocarbon profiling will indicate the variability between the two *Lucilia sp*. This study was conducted to assess variability between *L. cuprina* and *L. sericata*, as variability between sexes as they occur in Ghana. The findings of this study can provide reliable techniques and characters upon which identification of these two species can be made.

MATERIAL AND METHODS

Data Collection – Blowflies were collected from the science botanical garden of the University of Cape Coast (5.11626°N – 1.29492°W) in January 2020. Fresh beef was obtained and allowed to decay after freezing to kill potential microbes. Beef carrion was then placed in net cages used as traps and randomly placed at vantage points in the garden to attract blowflies. The traps were closed after about 10 minutes of exposure. This was repeated thrice a week for four weeks. Trapped blowflies were collected and killed in soapy water and stored in 70% alcohol. Blowflies collected were identified to the species level using an identification guide as described by Lutz et al. (2018).

Geometric assessment – A total of 200 randomly collected *L. cuprina* and *L. sericata* specimens were studied. The right forewings of *L. cuprina* and *L. sericata* were extracted and mounted on slides. Images of all mounted wings were captured with the help of an external microscope digital camera and OMAX Toup view application software version x64, 3.7.9229.20170607 (Fig. 1A). Captured images were converted into tps files using tpsUtil (v1.76 x64). 16 landmarks were digitized on each wing image using tpsDig2 software (v2.31) (Fig. 1B). Resulting data (i.e., the raw x and y coordinates of the landmarks) was imported into MorphoJ (v1.02j) for analysis. Variations in shape of wings of the two species were assessed using Principal Component Analysis, Canonical Variate Analysis and Discriminant Function Analysis.

Principal Component Analysis (PCA) was used to display the major features of shape variation between the two species. Discriminant Function Analysis (DFA) was used to determine the probability

of correct and incorrect classification of specimen for each species and sex in pairs. Canonical Variate Analysis (CVA) was used to find the shape features that best distinguish the sexes of *L. cuprina* and *L. sericata*. Please refer to Klingenberg (2011) for detailed description on PCA, DFA and CVA for variability assessments.

Hydrocarbon Profiling – A total of four specimens, a male and female each of two species were used for cuticular hydrocarbon assessment. Cuticular hydrocarbons were extracted using 100μl of analytical hexane. Hydrocarbon extract was analysed using GC–MS under strict temperature conditions, at 50ºC (for 1 minute), 220ºC and held at 310ºC, with an ion source temperature of 210ºC. Identifications of hydrocarbons were accomplished by comparing retention times and mass spectra of unknowns with three referenced libraries of mass spectra (NIST-14s, NIST-14 and WILEY-8).
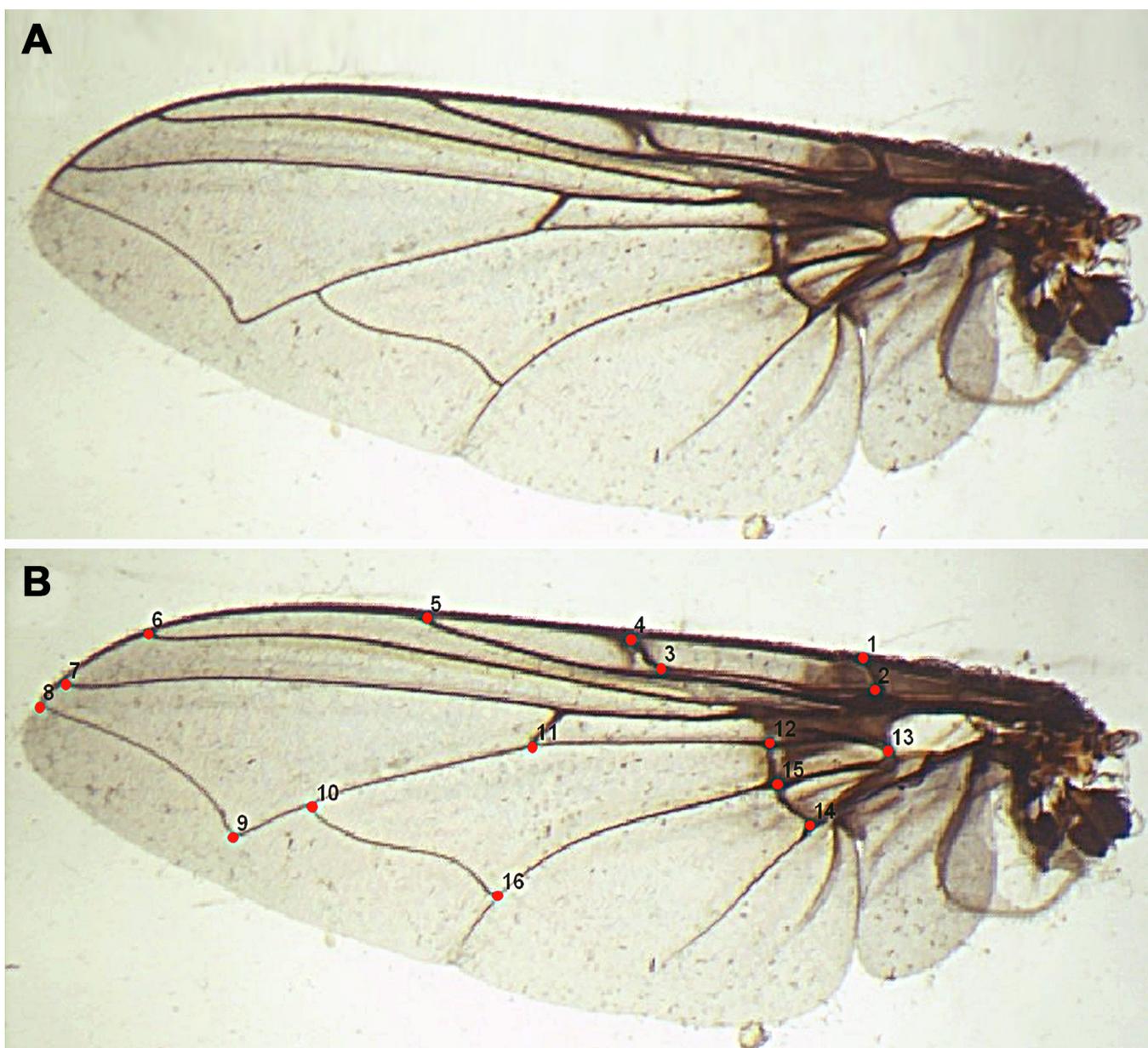
Figure 1. A. Image of wing of specimen captured under a digital microscope. **B.** Image of wing of specimen showing the 16 landmarks marked for each specimen.

RESULTS

Geometric Morphometric Variability – A total of 187 specimens were included in the analysis. 119 *L. sericata* females, 30 *L. sericata* males, 31 *L. cuprina* females and 7 *L. cuprina* males were identified. The average shape of the wing after rotation and translation to remove variation in size, orientation and position are shown in Table 1. Fig. 2 shows the deviations of the coordinates of each landmark for each specimen (shown as small blue dots) away from the mean cartesian coordinates (shown as deep blue circle at the center) of each landmark.

Principal Component Analysis (PCA) – Principal component analysis of *L. cuprina* and *L. sericata* specimens showed that landmark 11, which corresponds to the intersection between the medial vein and the radial medial vein, contributed most to the variability between the two species. Landmarks 10, 6 and 9 corresponding to the intersection between the medial vein and branched cubitus vein, distal end of radius vein (R2 + 3 vein) and curve point of media vein (Espra et al., 2015) respectively, contributed significantly to the variability between the two species as shown in Fig. 3A. A sum of the eigen values of the first three principal components (PC1 – 34.861, PC2 – 12.978, PC3 – 10.222) contributed to 58.061% of the total variability (Fig. 3B). Principal component analysis comparing the variability between males of both species and females of both species showed wide deviations from the centroid in landmarks 11, 10, 6 and 9 with landmark 11 contributing most to the variability (Fig. 3D). Unlike PCA for species, which had the first three components contributing the most, a sum of the eigen values of the first four principal components contributed to 57.739% of the total variability (Fig. 3E).

Principal component analysis of males and females of each of *L. cuprina* and *L. sericata* showed that landmark 11 contributed most to the variability (Fig. 4A) with landmarks 10, 6 and 9 showing significant deviations away from the centroid (Fig. 4A). The first four components contributed most to the variability of within species sexes (PC1 – 20.481, PC2 – 16.037, PC3 – 11.700, PC4 – 10.061) (Fig. 4B). For variability between males and females of each species, a scatter plot of the principal component analysis showed females of *L. cuprina* and *L. sericata* overlapped around the centroid while males of *L. cuprina* formed a subset of *L. sericata*. Males of *L. cuprina* and *L. sericata* pulled further away from the centroid. (Fig. 4C).
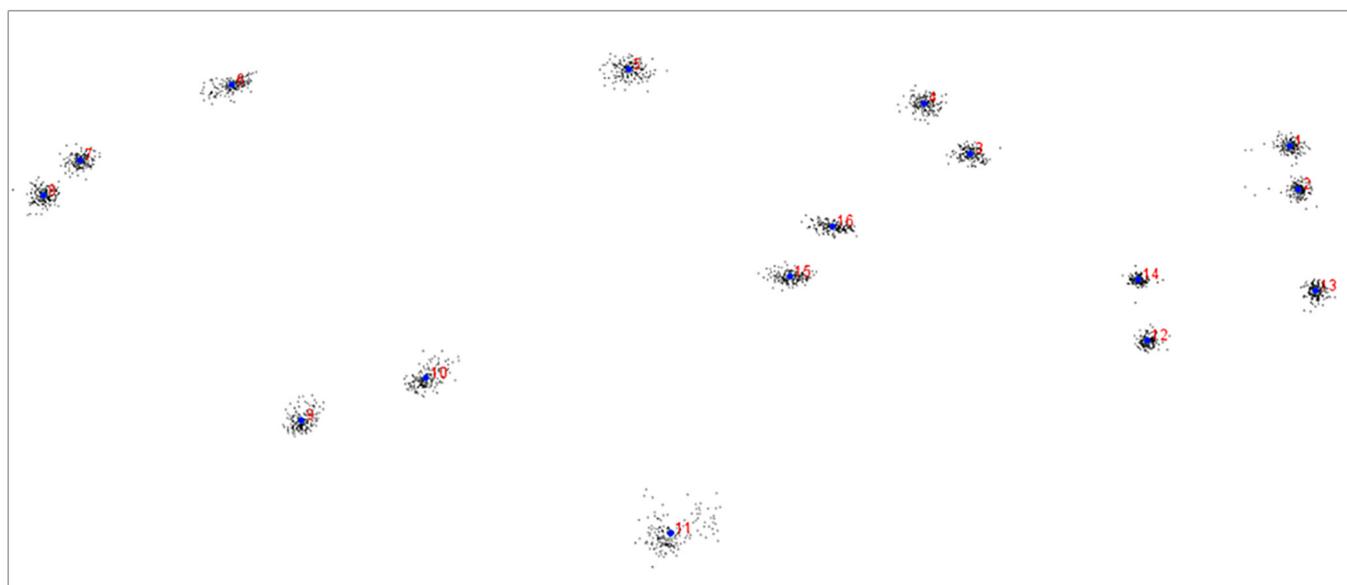

Figure 2. Procrustes fit of all 16 cartesian coordinates of landmarks on forewings of specimens showing the deviation of each specimen away from the mean coordinate.

Table 1. Table showing the average shape of the wing represented by cartesian coordinates of each landmark after Procrustes fit of all 187 specimens.

Landmark	Axis 1 (x)	Axis 2 (y)
1	0.30411014	0.05188027
2	0.30900255	0.02801148
3	0.12222234	0.04752995
4	0.09601838	0.07514295
5	- 0.07214887	0.09374541
6	- 0.29825039	0.08529739
7	- 0.38454265	0.04395278
8	- 0.40545400	0.02481924
9	- 0.25850266	- 0.09876061
10	- 0.18768903	- 0.07542318
11	- 0.04815861	- 0.16050840
12	0.22305178	- 0.05487750
13	0.31887833	- 0.02750544
14	0.21785181	- 0.02132049
15	0.01971232	- 0.01974152
16	0.04389855	0.00775768
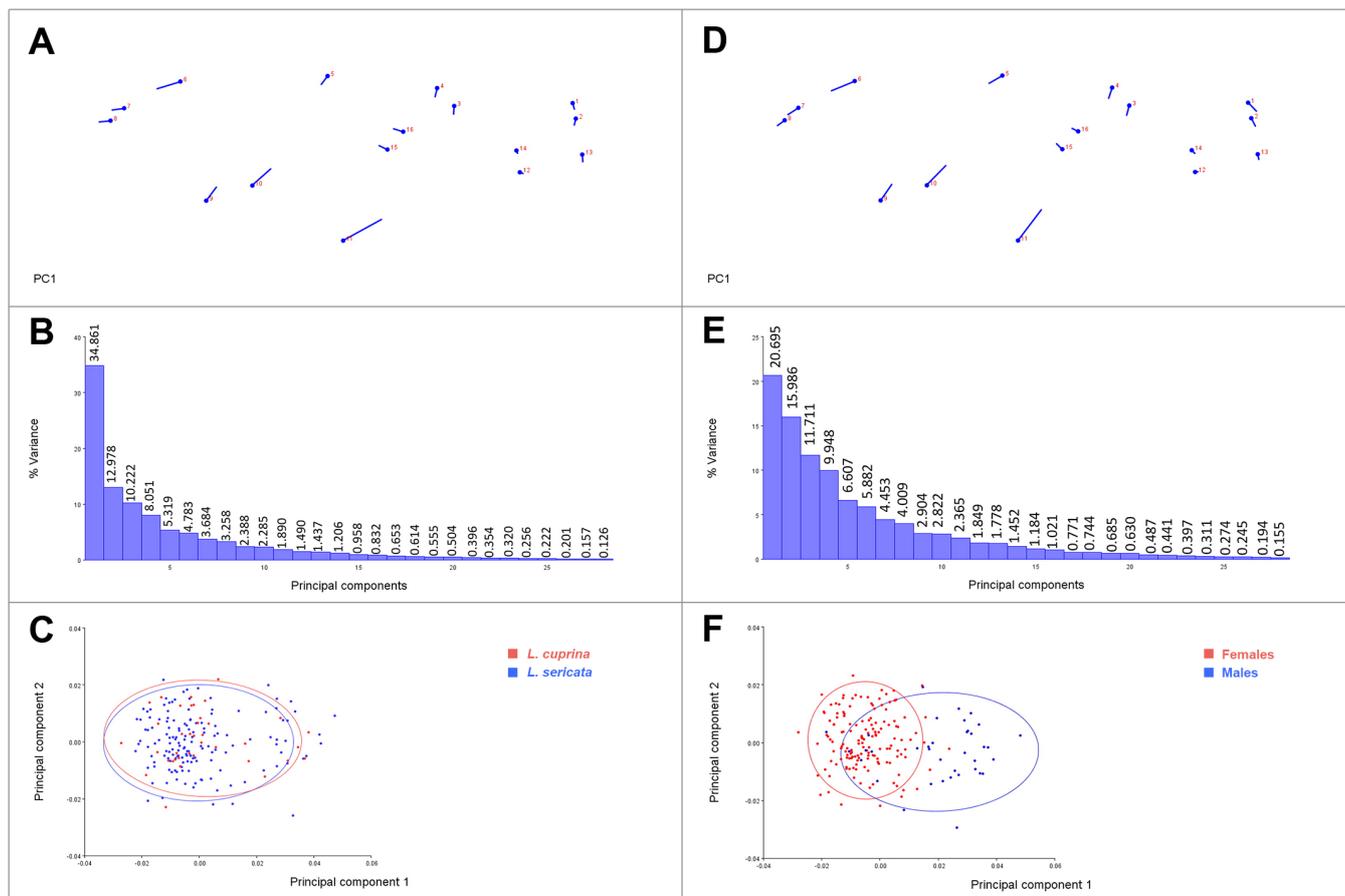
**Figure 3. A. & D.** Shape changes of principal components showing the amount of contribution of each landmark to the overall variability; **B. & E.** Eigen values of each principal component contributing to variability; **C. & F.** Scatter plot of principal components showing clustering; **A–C.** Between *L. cuprina* and *L. sericata*; **D–F.** Among all males and females of both *L. cuprina* and *L. sericata*.
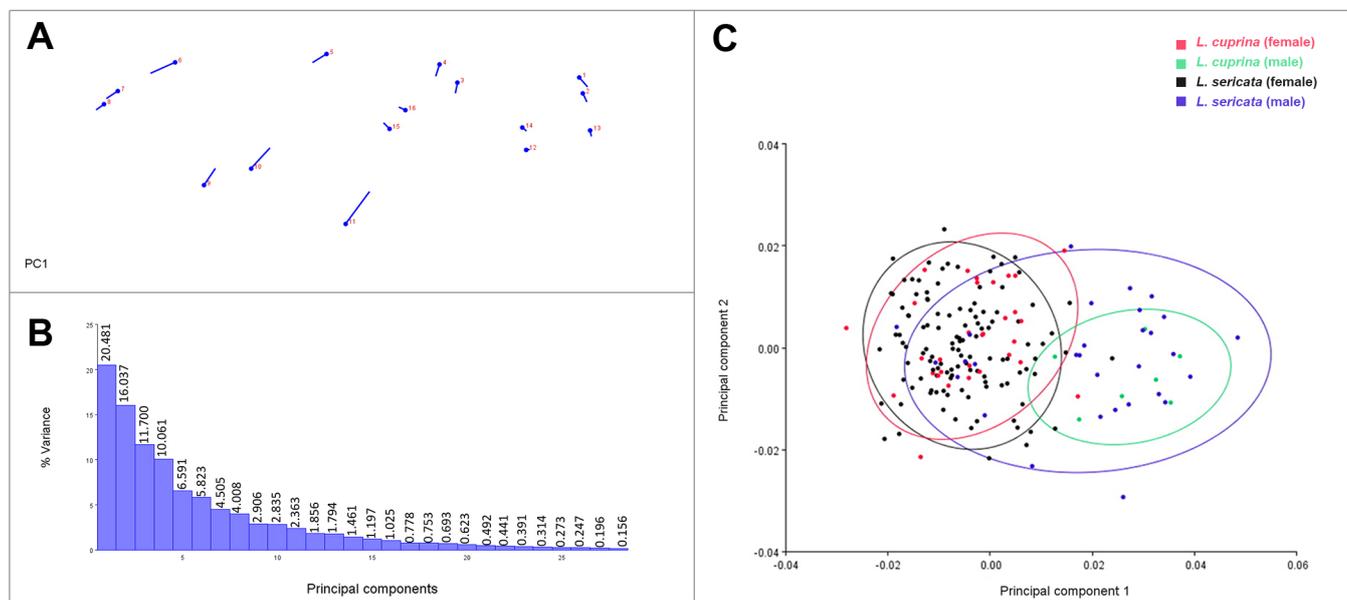
Figure 4. A. Shape changes of principal components showing the amount of contribution of each landmark to the variability between males and females of each of *L. cuprina* and *L. sericata*. **B.** Eigen values of each principal component contributing to variability among males and females each of *L. cuprina* and *L. sericata*. **C.** Scatter plot of principal components showing clustering of males and females each of *L. cuprina* and *L. sericata*.

Discriminant Function and Cross-Validation Analyses – Discriminant function analysis showed a non-significant comparative difference between *L. cuprina* and *L. sericata* (p = 0.7183). Discriminant function analysis scores distinguished *L. cuprina* from *L. sericata* by 71.1% (Fig. 5B) with 34.2 % accuracy in cross-validation (Fig. 5C). Discriminant function scores for *L. sericata* were however discriminated from *L. cuprina* by 69.8% (Fig. 5B) with 63.1 % accuracy in cross-validation (Fig. 5C). Shape changes after discriminant function analysis comparing the two species did not show any significant deviation of any of the landmarks from the centroid (Fig. 5A). Discriminant function scores distinguished males of both *L. cuprina* and *L. sericata* by 86.5% from females of both *L. cuprina* and *L. sericata* (Fig. 5E), with 81.1% accuracy in cross-validation (Fig. 5F). On the other hand, females of both *L. cuprina* and *L. sericata* were distinct from males of both *L. cuprina* and *L. sericata* by 97.3% (Fig. 5E) with 96% cross-validation (Fig. 5F). Deviations away from the centroid can be seen almost on all landmarks with more deviations seen on landmarks 11, 10, 6 and 9. These landmarks are features of interest based on which males and females were differentiated (Fig. 5D).

Shape changes after discriminant function analysis showed landmarks 10, 6, 9 and 10 were key in differentiating male and female *L. cuprina* (Fig. 6A) and male and female *L. sericata* (Fig. 6D). However, no observable change was seen in shape for discriminant function between female *L. cuprina* and female *L. sericata* (Fig. 7A) with slight change observed on landmark 10 and 11 for discriminant function between male *L. cuprina* and male *L. sericata*. (Fig. 7D). Comparing males and females of *L. cuprina* and *L. sericata*, Discriminant function scores showed a significant difference between males and females of both species (p<0.0001) however comparing males and females among species, significant differences were found between males and females of *L. cuprina* (LCF – LCM, p = 0.0097) and males and females of *L. sericata* (LSF – LSM, p<0.0001). Males and females of different species showed non-significant differences; *L. cuprina* female and *L. sericata* female (LCF – LSF, p = 0.7115), *L. cuprina* male and *L. sericata* male (LSM – LCM, p = 0.6263). Discriminant scores for males and females of the same species showed 100% distinction

of females of *L. cuprina* from males of *L. cuprina* (Fig. 6B) with 80.6% accuracy in cross-validation (Fig. 6C). On the other hand, males of *L. cuprina* showed 100% distinction from females of *L. cuprina* (Fig. 6B) with 57% cross-validation (Fig. 6C). It also showed 96.7% distinction of females of *L. sericata* from males of *L. sericata* (Fig. 6E) with 90.8% accuracy in cross-validation (Fig. 6F). On the other hand, males of *L. sericata* showed 83.3% distinction from females of *L. sericata* (Fig. 6E) with 70% cross-validation (Fig. 6F). Comparing males and females of different species, discriminant function scores showed 67.7% distinction of *L. cuprina* females from *L. sericata* females (Fig. 7B) with 38.7% accuracy in cross-validation (Fig. 7C). 71.4% distinction of *L. sericata* females from *L. cuprina* females (Fig. 7B) with 63% accuracy in cross-validation (Fig. 7C). 85.7% distinction of *L. cuprina* males from *L. sericata* males (Fig. 7E) with 28.5% accuracy in cross-validation (Fig. 7F) and 96.7% distinction of *L. sericata* males from *L. cuprina* males (Fig. 7E) with 63.3% accuracy in cross-validation (Fig. 7F).

Canonical Variate Analysis – Mahalanobis distances computed showed a significant difference between *L. cuprina* male and female (<0.0001), *L. sericata* male and female (<0.0001), *L. cuprina* male and *L. sericata* female (<0.0001), as well as *L. cuprina* female and *L. sericata* male (<0.0001). However, non-significant differences were seen in mahalanobis distances between *L. cuprina* female and *L. sericata* female (0.4654) as well as *L. cuprina* male and *L. sericata* male (0.8153) (Table 2). A similar trend was observed for calculated Procrustes distances among sexes of each species (Table 3). A Scatter plot of canonical variate analysis showed that females of each species were scattered around the centroid however, males have pulled away from the centroid. *L. cuprina* male formed a subset of *L. sericata* male (Fig. 8A). Shape changes showed that landmark 11 contributed most to the variability among groups followed by landmarks 10 and 6. (Fig. 8B).
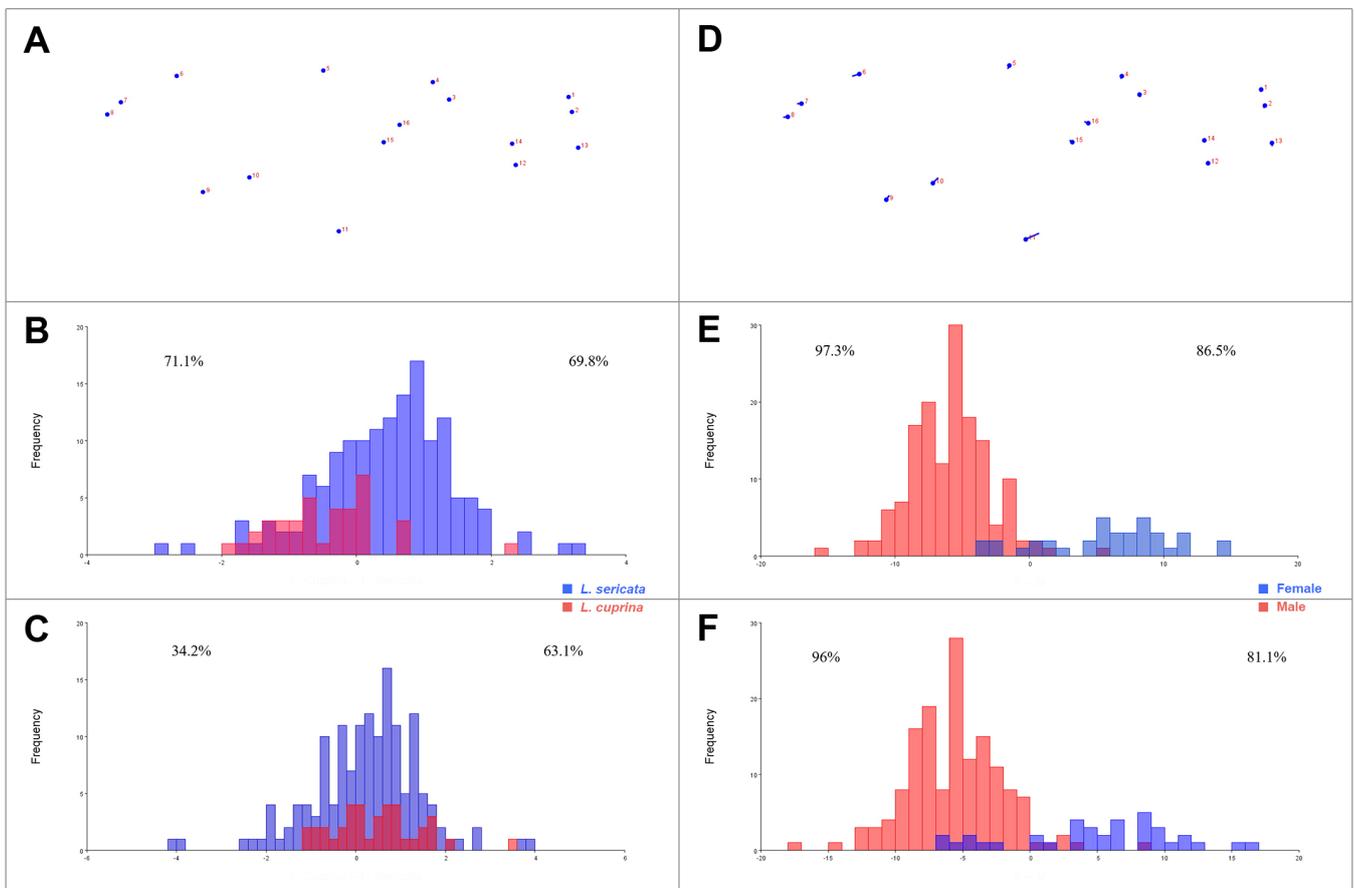
Figure 5. A. & **D.** Shape changes showing change in landmarks after discriminant function analysis. **B.** & **E.** Graph of Discriminant function scores. **C.** & **F.** Graph of Cross validation scores. **A–C.** Between *L. sericata* and *L. cuprina*; **D–F.** Among all males and females of both *L. cuprina* and *L. sericata*.

Table 2. Mahalanobis distances and corresponding *p*-values for pairwise comparison between male and females of each of *L. cuprina* and *L. sericata*.

	L. cuprina - female	*L. cuprina* - male	*L. sericata* - female
L. cuprina - **male**	4.3463 (<0.0001)		
L. sericata - **female**	1.0470 (0.4654)	4.2184 (<0.0001)	
L. sericata - **male**	3.5027 (<0.0001)	2.0583 (0.8153)	3.3287 (<0.0001)

Table 3. Procrustes distances and corresponding *p*-values for pairwise comparison between male and females each of *L. cuprina* and *L. sericata*.

|	*L. cuprina* - female	*L. cuprina* - male	*L. sericata* - female
L. cuprina – **male**	0.0358 (<0.0001)		
L. sericata - **female**	0.0050 (0.2612)	0.0359 (<0.0001)	
L. sericata - **male**	0.0279 (<0.0001)	0.0110 (0.4864)	0.0277 (<0.0001)
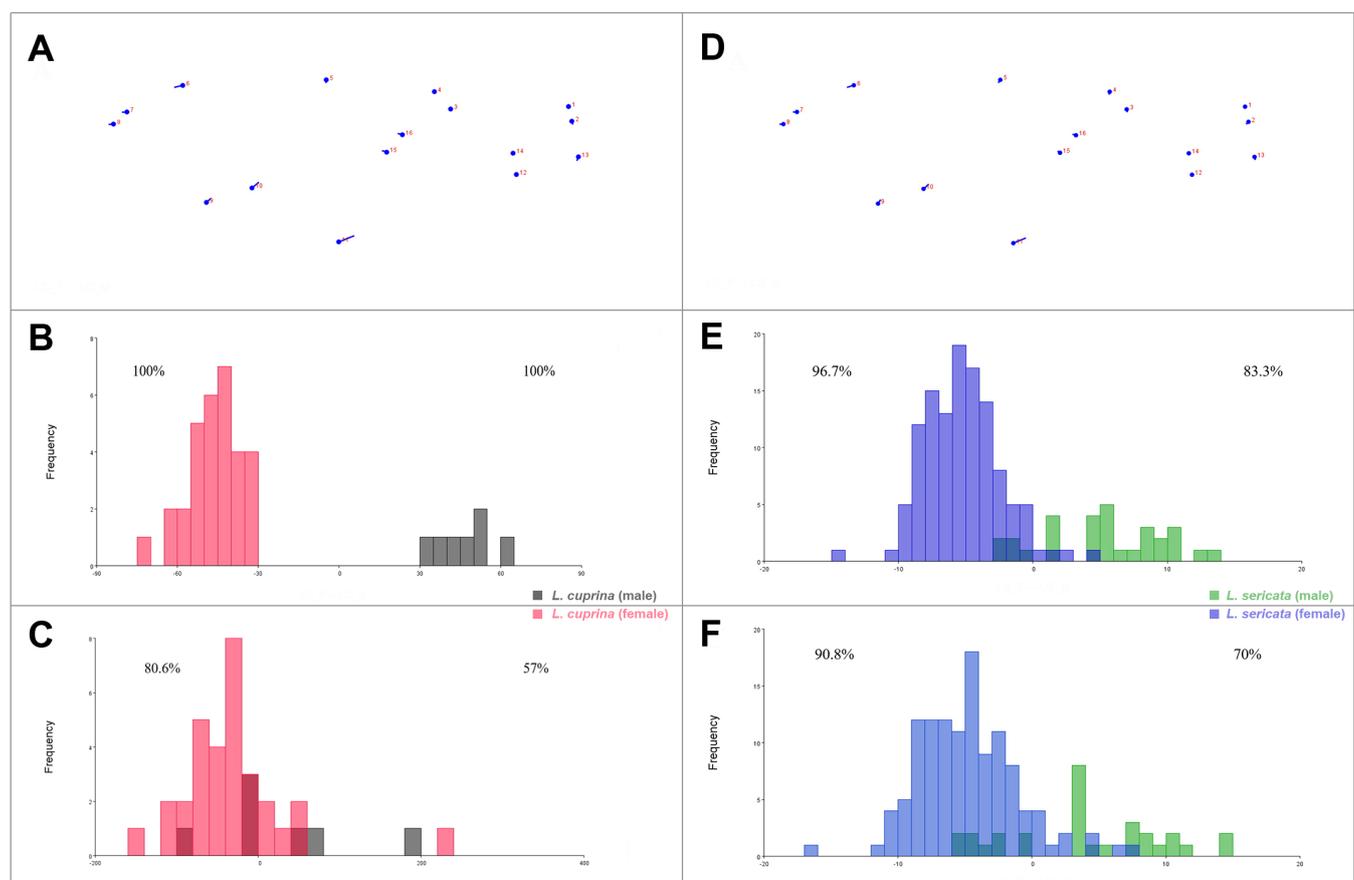
Figure 6. **A. & D.** Shape changes showing change in landmarks after discriminant function analysis. **B. & E.** Discriminant function scores. **C. & F.** Cross validation scores. **A–C.** between the male and female of *L. cuprina*; **D–F.** between the male and female of *L. sericata*.
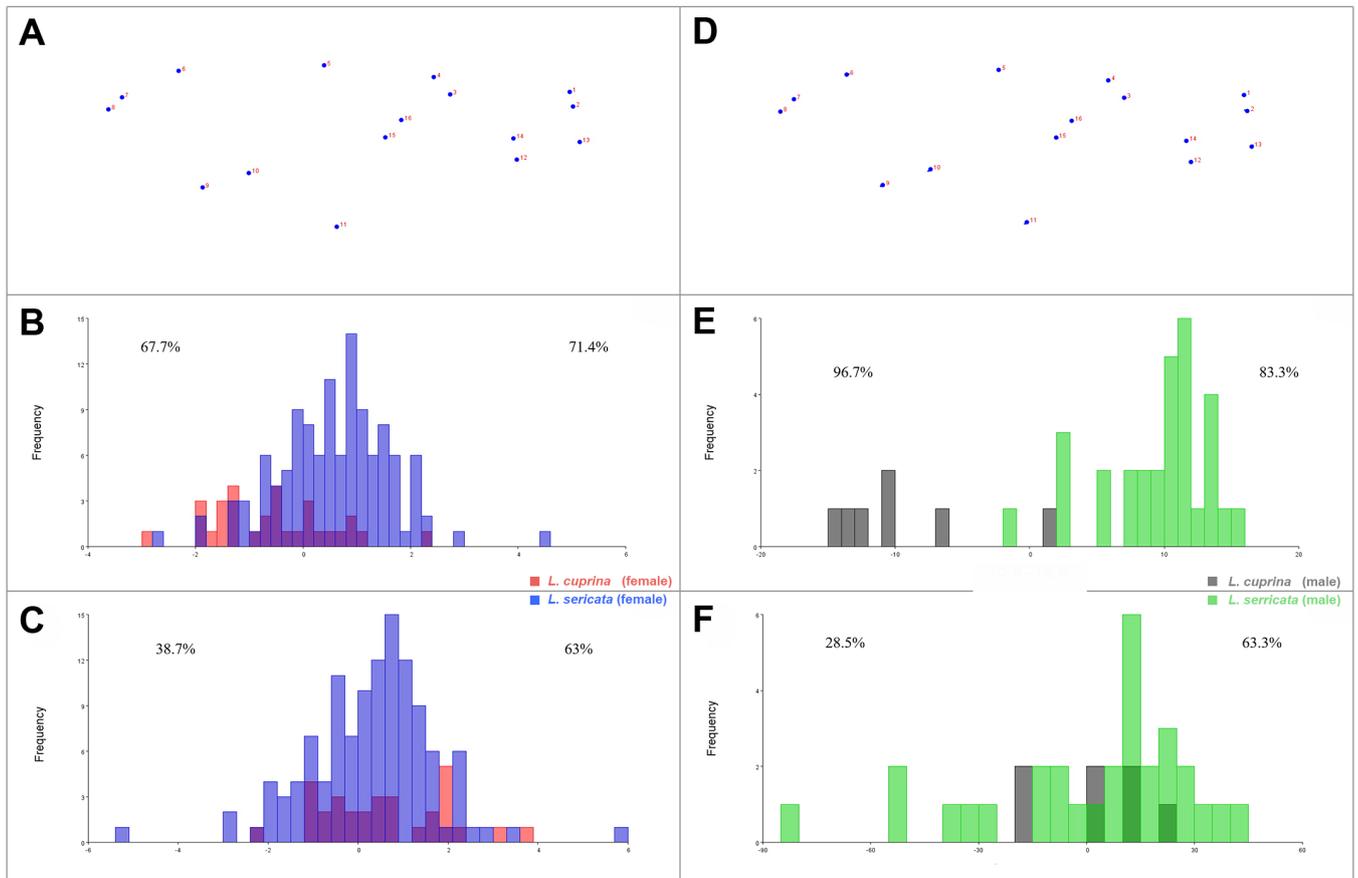
Figure 7. A. & **D.** Shape changes showing change in landmarks after discriminant function analysis. **B.** & **E.** Discriminant function scores. **C.** & **F.** Cross validation scores; **A–C.** between female *L. cuprina* and female of *L. sericata*; **D–F.** between the male *L. cuprina* and male *L. sericata*.
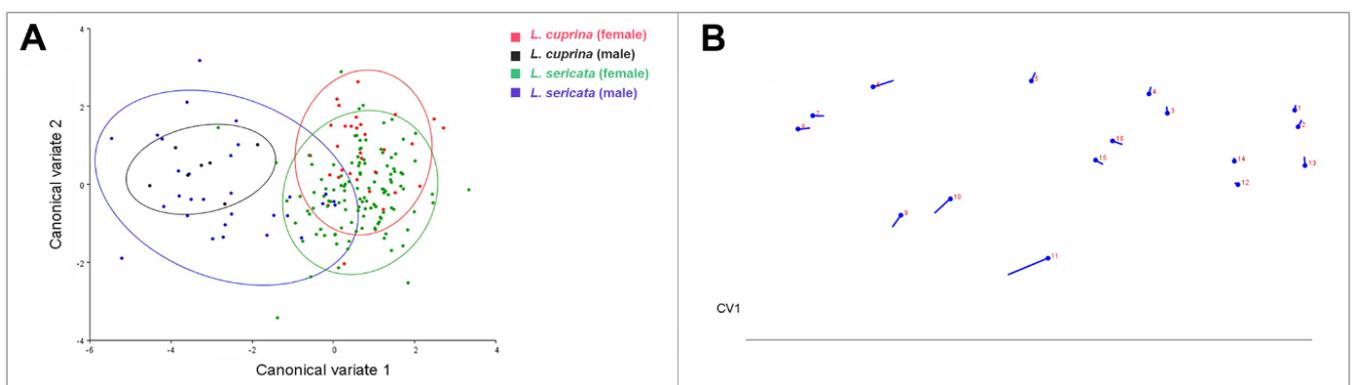
Figure 8. A. Scatter plot of canonical variate analysis between sexes of each specie. **B.** Shape changes canonical variate analysis of landmarks contributing to variability among sexes of each species.

Cuticular Hydrocarbon Profiling – A total of 38 cuticular hydrocarbons were identified by GC-Mass spectrometry from male and female *L. cuprina* and *L. sericata* specimens however, the number of hydrocarbons identified from each specimen varied. 20 cuticular hydrocarbons were identified from *L. cuprina* female, and 19 cuticular hydrocarbons were identified from both male and female *L. sericata* with *L. cuprina* male recording the least number of hydrocarbons (Table 4).

GC-Mass spectrometry revealed hydrocarbons in different concentrations for each specimen. For *L. cuprina*, 9-octadecenal was identified as the most abundant hydrocarbon in males while Tetracosane-11-decyl was identified as the most abundant hydrocarbon in females. Results also showed 13-methylheptacosane as the most abundant hydrocarbon in male *L. sericata* and Tetratetracontane as the most abundant hydrocarbon in female *L. sericata*. Major cuticular hydrocarbons in each specimen were identified as hydrocarbons having concentrations above 10% as shown in Table 5.

Table 4. Percentage concentration of cuticular hydrocarbons identified for male and female *L. cuprina* and *L. sericata*

Hydrocarbon	Empirical Formula	Concentrations (%)			
		L. cuprina (male)	*L. cuprina* (female)	*L. sericata* (male)	*L. sericata* (female)
11-Methylpentacosane	$C_{26}H_{64}$	2.52848	3.58193	1.82998	-
13-Methylheptacosane	$C_{28}H_{58}$	-	-	21.20329	1.10777
1-Decanol	$C_{10}H_{22}O$	-	-	0.07942	-
1-Dodecene	$C_{12}H_{24}$	-	0.16752	-	-
1-Heptacosanol	$C_{27}H_{56}O$	-		-	0.4135
1-Hexene, 5-Methyl-	C_7H_{14}	-	0.17984	-	-
1-Tridecene	$C_{13}H_{26}$	-	0.48706	-	-
2-Methylhexacosane	$C_{27}H_{56}$	-	1.94662	-	-
2-Methyltetracosane	$C_{25}H_{52}$	10.00045		-	-
2-Tetradecyl-1-Octadecene	$C_{32}H_{64}$	6.05168	16.3197	1.69784	9.67869
3-Methylpentacosane	$C_{26}H_{54}$	-	0.4635	-	-
4-Benzylbiphenyl	$C_{19}H_{16}$	1.21107	-	-	-
7-Oxabicyclo[4.1.0]Heptane, 1-Methyl-	$C_{10}H_{16}O$	-	1.86928	-	-
9-Octadecenal, (Z)-	$C_{18}H_{34}O$	18.37755	-	-	-
Celidoniol	$C_{29}H_{60}O$	11.01914	14.60326	1.77568	3.33289
Cyclohexane, 1,3-Dimethyl-, Trans-	C_8H_{16}	-	0.55781	9.58605	-
Docosane	$C_{22}H_{46}$	-	-	1.03294	1.10777
Docosane, 11-Butyl-	$C_{26}H_{54}$	-	3.58193	-	-
Dotriacontane	$C_{32}H_{66}$	-	11.19682	3.1874	1.87769
Eicosane	$C_{20}H_{42}$	-	1.39609	0.41594	1.02221
Heneicosane	$C_{21}H_{54}$	-	-	0.93036	0.79103
Hexacosane	$C_{26}H_{54}$	-	-	5.49696	0.79103
Hexane,3-Methyl-4-Methylene-	C_8H_{16}	-	0.34431	-	-
Hexatriacontane	$C_{36}H_{74}$	2.38029	2.11115	5.58359	14.56201
Nonacos-1-Ene	$C_{29}H_{58}$	-	-	-	0.4135
Nonacosane	$C_{29}H_{60}$	4.1017	-	11.23532	-
N-Tetracosanol-1	$C_{24}H_{50}O$	3.33095	-	-	-
Octacosane, 1-Iodo-	$C_{28}H_{57}I$	-	-	-	11.69565
Octadecane	$C_{18}H_{38}$	-	-	-	0.61428
Pentacosane	$C_{25}H_{52}$	11.01914	-	-	-
Pentadecane, 8-Hexyl-	$C_{21}H_{44}$	-	-	2.22732	1.91112
Pentatriacontane	$C_{35}H_{72}$	-	1.0385	-	-
Tetracontane	$C_{40}H_{82}$	4.23912	10.73622	7.97575	15.88354
Tetracosane	$C_{24}H_{50}$	-	-	3.6531	0.51439
Tetracosane, 11-Decyl-	$C_{34}H_{70}$	-	17.81333	-	-
Tetrapentacontane	$C_{54}H_{110}$	4.23912	9.56443	3.24423	12.66281
Tetratetracontane	$C_{44}H_{90}$	10.00045	-	1.5932	16.29806
Tetratriacontane	$C_{34}H_{70}$	-	1.22647	17.07179	1.91791

- : Cuticular hydrocarbon is absent in specimen.

Some cuticular hydrocarbons were present in all four specimens while some were unique for specific specie or sex in varying concentrations. In all four specimens, 2-Tetradecyl-1-octadecene, Celidoniol, Hexatriacontane, Tetracontane and Tetrapentacontane were common. 10 hydrocarbons were unique to the female of *L. cuprina* at very low concentrations except for Tetracosane-11-Decyl which recorded a relatively higher concentration. In females of *L. sericata*, 1-Heptacosanol, Nonacos-1-ene, Octacosane, 1-iodo and Octadecene were unique to the specimen. Male of *L. sericata* recorded 1- Decanol which was absent in all other specimens. Nonacosane was found to be present in males of both *L. cuprina* and *L. sericata*, but absent in females. 13-Methylheptacosane, Docosane, Heneicosane, Hexacosane, Pentadecane and Tetracosane were identified to be common to only male and female *L. sericata* however, hydrocarbons that were found to be common in male and female *L. cuprina* was either shared with both male and female *L. sericata* or either of the two.

DISCUSSION

The present study shows clear variations between the two species *L. cuprina* and *L. sericata*. This confirms that geometric morphometrics and hydrocarbon profiles are equally reliable in revealing these variations. Species usually vary from each other by their genetic make-up and overall morphological features however in this study, geometric morphometric measurements of the wings were able to show a distinct variation among *L. cuprina* and *L. sericata*. This was so in blowflies (Sharanya & Zuha, 2019) regarding sexual dimorphisms (Nuñez-rodríguez & Liria, 2017), wing shape dimorphism (Espra et al., 2015) and other identification features (Derstine et al., 2018). Eigen values of the first three principal components showed that there are distinct features of the wings that can be used to differentiate between *L. cuprina* and *L. sericata*. This is possibly due to a reflection of morphological and genetic variation in the wings of the two species. Samples of *L. cuprina* and *L. sericata* were collected from the same location indicating a similar environmental influence on both species therefore environmental factors could not have contributed to this variability. The variation can be solely said to have arisen from genetic influence on wing development.

Results from the study showed variations between the wing shape of *L. cuprina* and *L. sericata*. Discriminant function analysis distinguished individuals into two distinct groups with a few observable similarities. This may be due to the close relatedness between *L. cuprina* and *L. sericata* (Aubertin, 1933). Males and females of species generally vary in size, morphology, integuments and developmental time. Sexual dimorphism has been observed in size (Macedo et al., 2018) and wing shape (Espra et al., 2015) of *L. sericata* and also in the development of *L. cuprina* (Concha & Scott, 2009). The present study shows a clear sexual dimorphism of wing shapes of *L. cuprina* and *L. sericata*. A significant difference was observed between all males and females assessed in this study with high percentage differences in discriminant function and cross-validation analysis. Similar results was reported by Nuñez-rodríguez and Liria (2017). Interestingly, each species showed high percentage differences in discriminant scores and cross-validation scores for male and females' variability. This was confirmed by Canonical variate analysis, which showed significant differences between the Mahalanobis and Procrustes distances between males and females of the same species, as well as between male of *L. cuprina* and female of *L. sericata*. However, males of both species were similar likewise females of both species. Discriminant function analysis showed no significant difference between males of *L. cuprina* and *L. sericata* and between females of *L. cuprina* and *L. sericata*. Canonical variate anaylsis also showed no significant difference between the Mahalanobis and Procrustes distances. This was confirmed by scatter plots of canonical variate analysis which showed a clear distinction between the four groups with some overlaps. Males of both species are grouped whereas females of both species are also grouped. However, a distinction is seen between males and females of each species, confirming some form of relatedness or similarity between males and between females. Morphological characters for the identification of *L. cuprina* and *L. sericata* have shown that males of both species and females of both species have common structures between them for which can be assessed for variation.

Table 5. Major cuticular hydrocarbons (%conc > 10) identified for each of male and female *L. cuprina* and *L. sericata*.

Species		Hydrocarbon	Concentration (%)
L. cuprina	Male	9-octadecenal (Z)	18.38
		Pentacosane	11.02
		Celidoniol	11.02
		Tetratetracontane	10.00
	Female	Tetracosane-11-decyl	17.80
		2-Tetradecyl-1-octadecene	16.32
		Celidoniol	14.60
		Tetracontane	10.74
L. sericata	Male	13-methylheptacosane	21.20
		Tetratriacontane	17.07
		Nonacosane	11.24
	Female	Tetratetracontane	16.30
		Tetracontane	15.90
		Hexatriacontane	14.56
		Tetrapentacontane	12.66
		Octacosane-1-iodo	11.69

Such structures include; the extent of metallic sheen on parafrontal sclerites in females and the shape and vestiture of the surstyli and cerci in males (Holloway, 1991). Perhaps, specific characteristics of the wing shape are one of such structures that are more common between males and between females of both species than between males and females of each specie. Shape changes observed for discriminant analysis for the groups showed that landmarks 11, 10, 6 and 9 were key in discriminating between the two species and between the sexes of each species. No observable shape changes seen in shape changes of wings between males and between females of each species could mean that males share a similar wing shape likewise females.

Cuticular hydrocarbons function primarily to protect the insect against dessiccation (Blomquist, 2010), microorganism penetration, and parasitoid and predator attack (Koidsumi, 1957; David, 1967). The insect's genetic make-up and the environment determine the hydrocarbon composition of its cuticle. Results from this study showed common hydrocarbons for all specimens assessed. Of the 38 cuticular hydrocarbons identified, 5 hydrocarbons were present in all specimens in varying concentrations. 2-Tetradecyl-1- Octadecene, Celidoniol, Hexatriacontane, Tetracontane and Tetrapentacontane were identified in both male and female *L. cuprina* and *L. sericata*. Both species are closely related by virtue of being in the same genus hence these common hydrocarbons can be associated with the genus. The rest of the 32 hydrocarbons varied in concentration between the two species. Results for cuticular hydrocarbon profile assessments of *L. cuprina* and *L. sericata* showed more similarities between male and female

belonging to the same species as compared to similarities between same sexes. 19 hydrocarbons were recorded for each male and female *L. sericata* and out of these 19 hydrocarbons, 15 were shared between the two however, concentrations/abundance differed. Just like the variation in wing shape, variations in cuticular hydrocarbon compositions observed in this study can be attributed to differences in the genetic make-up of the specimens due to the similarity in the environment of the individual specimens. 13-methylheptacosane was identified as the most abundant hydrocarbon in male *L. sericata* but was relatively low in females (1.108). This compound has been identified as a male sex pheromone in males of *C. eyrytheme* (Sappington & Taylor, 1990) together with Nonacosane, which was identified in this study as unique hydrocarbons of males. Cuticular profile of female *L. sericata* revealed tetratetracontane as the most abundant hydrocarbon. Tetratetracontane was however low for male *L. sericata* (1.5932) and absent in female *L. cuprina* but it was found to be present in a relatively high concentration (10.00) in male *L. cuprina*. According to (Barretto & Vootla, 2018), tetratetracontane has antimicrobial properties as identified in the gut flora of Bombyx mori. Perhaps it plays a similar role in *L. sericata* and male *L. cuprina*. Male and female *L. sericata* showed variation in the concentrations of cuticular hydrocarbons identified. Major compounds identified in both species differed as shown in Table 5 even though they share a number of hydrocarbons. Similar observation was also made for male and female *L. cuprina*. No specie had one particular hydrocarbon dominating for both male and female. Nonacosane was identified as a male hydrocarbon however there was no unique hydrocarbon for females.

Male and female *L. cuprina* appear to be less similar to each other in terms of their cuticular hydrocarbon composition. 20 hydrocarbons were identified for female specimen while 13 hydrocarbons were identified for the male specimen. 6 hydrocarbons were however shared between the two. 9-octadecenal and Tetracosane-11-decyl were identified as the most abundant hydrocarbons for male and female *L. cuprina* respectively. Like *L. sericata*, *L. cuprina* also differed in the concentration of their hydrocarbons hence major compounds differed (Table 4). According to Blomquist (2010), hydrocarbons with fewer than 20 carbons may occur as pheromones and defensive compounds or as intermediates between pheromones and defensive compounds. 1-Decanol, 1-Hexene-5-methyl, 1-Tridecene, 7-oxabiclo [4.1.0] heptane-1-methyl, Hexane-3-methyl-4-methylene all are hydrocarbons with less than 20 carbons. Concentrations of these hydrocarbons were all <1%. Interestingly, all these hydrocarbons were identified in female *L. cuprina*.

Geometric morphometric techniques validate the size and shape differentiation of wing landmarks as strong taxonomic structures that can discriminate distinct due to genetic assimilation (Lutz et al. 2018). It appears that morphometric properties are originally produced in response to environmental condition or exposure to a teratologen and such exposure later becomes genetically encoded through natural selection. As organisms' genetics evolve to ensure that development proceeds in a certain way regardless of normal environmental variations. In the present study geometric morphometric analysis of the wings clearly showed distinct variation among *Lucilia cuprina* and *Lucilia sericata*. The first three eigen values of the principal components; PC1 - 20.095, PC2 - 15.986, and PC3 - 11.71 separated the two species. Moreover, landmark 11, 10, 6, and 9 which correspond to the intersection between the medial vein and the radial medial vein, medial vein and branched cubitus vein, the distal end of radius vein and curve point of media, respectively, contributed greatly into the variability between the two species. This was confirmed by the variation of their hydrocarbon's concentrations such as; 9-octadecenal, Tetracosane-11-decyl, 13-methylheptacosane, and Tetratetracontane. Environmental factors play a major role in determining the kind of cuticular hydrocarbon present in insects (Khidr et al., 2013). Studies have been conducted to determine cuticular hydrocarbons of *L. sericata* at different stages (Peterson et al. 2007; Khidr et al. 2013) and also as a chemotaxonomic tool for the identification of *L. cuprina* (Barbosa et al., 2017).

Results from this study show that *L. cuprina* and *L. sericata* show variability in wing shape and cuticular hydrocarbon profiles. Major wing shape characteristics that showed variability between the two species were intersection between the medial vein and the radial medial vein, intersection between the medial vein and branched cubitus vein, distal end of radius vein and curve point of the medial vein.

Hydrocarbons identified in both species were 2-Tetradecyl-1-Octadecene, Celidoniol, Hexatriacontane, Tetracontane and Tetrapentacontane. 9-Octadecenal (z) and Tetracosane-11-decyl were identified as the most abundant hydrocarbons in male and female *L. cuprina* while 13-methylheptacosane and Tetratetracontane were identified as abundant in male and female *L. sericata* respectively. These characters are important in distinguishing between the two species as they occur in Cape Coast, Ghana.

AUTHOR'S CONTRIBUTION

The authors confirm their contribution in the paper as follows: I.K. Badu: Data curation; I.K. Badu & R. Combey: Formal analysis; I.K. Badu & R. Combey: Conceptualization and Methodology; I.K. Badu & R. Combey: Writing and original drafting; R. Combey & P. Quandahor: Writing, review & editing. All authors read and approved the final version of the manuscript.

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AVAILABILITY OF DATA AND MATERIAL

The data presented in this study are available from the corresponding author on a reasonable request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this paper.

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ارزیابی تنوع بین دوگونه مگس لاشه، (Wiedemann) *Lucilia cuprina* و (Meigen) *Lucilia sericata* (Diptera, Calliphoridae) بر اساس ریخت‌سنجی هندسی و رخنمای هیدروکربن‌های جلدی

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چکیـده: مگس‌های *Lucilia cuprina* و *Lucilia sericata* دو گونه بسیار نزدیک از لحاظ ظاهری، زیستگاهی، انتشار و اهمیت اقتصادی هستند. اگرچه امکان تمایز این دو گونه به کمک سایر روش‌ها میسر شده، اما مطالعات مقایسه‌ای افتراقی بر اساس ریخت‌سنجی هندسی و ترکیب هیدروکربن‌های جلدی نیازمند بررسی بیشتر است. روش‌هایی برای تمایز این دو گونه وجود دارند، با این وجود شناسایی سایر روش‌های ارزیابی تنوع بالقوه بین آنها در کشور غنا که مطالعات کمتری در آن صورت گرفته، حایز اهمیت است. این تحقیق به منظور بررسی تنوع بین دو گونه مگس و بین حشرات نر و ماده آنها انجام شد. شکل بال در ۱۸۷ نمونه از هر دو گونه با روش ریخت‌سنجی هندسی تحلیل شد. لندمارک‌های شماره ۱۱، ۱۰، ۶ و ۹ که به ترتیب نشان‌دهندهٔ نقاط تقاطع رگبال‌های میانی با شعاعی میانی، میانی با انشعابات رگ بازویی، رگ پیرامونی با شعاعی (رگ‌های R2+3) و محل انحنای رگ میانی هستند، بیشترین تفاوت بین و داخل گونه‌ای را آشکار می‌کنند. رخنمای هیدروکربن‌های جلدی در چهار نمونه انتخاب شده به صورت تصادفی از نر و ماده دو گونه *L. cuprina* و *L. sericata* با استفاده از روش کروماتوگرافی گازی – طیف سنجی جرمی ارزیابی شدند. ترکیبات Octadecene، Celidoniol، Hexatriacontane، Tetracontane و Tetracontane در هر دو گونه وجود داشتند. دو ترکیب (z)9-Octadecenal و Tetracosane-11-decyl فراوان‌ترین مواد در حشرات نر و ماده گونه *L. cuprina* و ترکیبات 13-methylheptacosane و Tetratetracontane فراوان‌ترین مواد در حشرات نر و ماده گونه *L. sericata* بودند. خصوصیات افتراقی نشان‌دهنده تفاوت‌هایی است که می‌توان از آنها برای شناسایی دو گونه استفاده کرد.

واژگـان کلیدی: ریخت‌سنجی هندسی، هیدروکربن‌های جلدی، تنوع