Geometric Morphometric Study of Wing Shape in *Leva indica* (Acrididae: Gomphocerinae) from Different Environmental Sites of Coimbatore, India

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Abstract
A landmark based geometric morphometric study was carried out to describe the variation in wing of male and female Leva indica from different environmental sites of Coimbatore, Tamil Nadu, India. A total of 60 male and 60 female forewings were used in this study. To demonstrate the variation in wing shape, landmark data of each individual was examined through the morphometric softwares namely tps-Util, tps-Dig, tps-Relw, tps-Small and integrated software PAST. In total, 19 landmarks were identified in both male and female wings of L. indica. Based on the landmark coordinates, the first two relative warp analysis showed significant variation within and between the male and female wing shape from different sites.

Procrustes vs. tangent space distance showed that the distances were similar within males and females but significant differences appeared between the male and female wings of L. indica. Principal Component Analysis (PCA) between the male and female wings scatter plots also showed clear distinction from the result. The obtained results indicate that each sex of L. indica from different regions displays morphological differences in wing shape. These morphological changes could have occurred as a result of phenotypic plasticity.

Keywords: Geometric morphometric, Environmental sites, Relative warp, Principal component analysis.

Introduction

Many organisms can exhibit phenotypic variation as a result of environmental heterogeneity and ecological gradients. The understanding of how environmental heterogeneity influences organism phenotypic patterns, is a major focus of evolutionary ecology. Phenotype changes can increase organism fitness in certain environments[4]. Sexual dimorphism is one of the most remarkable sources of morphological variation which refers to the direction and degrees of sexual differences in body shape and size of the organism.

It has been recognized as a primary factor in determining phenotypic differences between species belonging to the same taxa and has become an increasingly important area of study in evolutionary biology[5]. The information concerning sexual dimorphism is crucial for better understanding of the ecology, behavior and life history of a species[2] and advances the process of identifying the male and female species that significantly differs in appearances (color, body shape and size)[11].

Geometric morphometrics is a powerful tool for taxonomic identification and functional analyses [14,15]. It also allows researchers to explore the geometric shape of a feature by characterizing it as a set of anatomical points known as 'landmarks'[8]. This technique allows the detection of differences in the shape, size of body structures and revealing intraspecific and interspecific variations that may be associated with evolutionary and ecological factors[9].

Therefore, this technique has become useful for the identification of variation among the populations of a given species[25]. The use of the landmark-based method was an efficient mechanism to illustrate the landmarks relative in the shape of organisms[19].

Wing shape morphology has been extensively studied in the field of Entomology to clarify the relationship between closely related taxa and helps in identifying populations within and between species of insects using geometric morphometrics[2,3,36]. Wings are a suitable structure for studying morphological variation in insects because their 2D flattened shape bears some useful landmarks[39]. Therefore, several studies use geometric morphometric as an effective mechanism to differentiate morphological variations and specifically wing morphology[5,20].

Morphometric features of grasshoppers have been widely used to study the evolution of body size, life history and color patterns[1]. *Leva indica* is a short-horned grasshopper belonging to the subfamily Gomphocerinae and tribe Dociostaurini. Gomphocerinae grasshoppers are the most diverse and species-rich subfamily of Acrididae, occurring in all continents except Australia and Madagascar[37]. Gomphocerinae grasshoppers may be identified by characters of their external morphology[26]. However, in this subfamily as well as in other grasshopper groups, individuals of the same species occupying different geographical locations may exhibit morphological variation in several structures of their external morphology[29].

This study aims to identify and compare the wing variation between the sexes of *L. indica* at different environmental sites using landmark-based geometric morphometrics.

Table 1
Information about the three different environmental regions of Coimbatore

Species	Sites	Locality	Latitude	Longitude	Number (n)	
					Males	Females
L. indica	I	Thenkarai	10°56′11.8″N	76°50′35.9″E	20	20
	II	Thudiyalur	11°04′48.7″N	76°56′29.4″E	20	20
	III	Karamadai	11°14′34.1″N	76°57′31.3″E	20	20

Material and Methods

Insects: Adults of both sexes of *L. indica* were collected from different environmental region (Table 1) of Coimbatore, Tamil Nadu, India during January to April, 2021. Geographically, Coimbatore lies at 11°01′00.5″N 76°57′20.9″E in South India at 411 m above sea level on the banks of the Noyyal River, in North Western part of Tamil Nadu. The mean maximum and minimum temperature in summer and winter vary between 35°C to 18°C. The average annual rainfall is around 700 mm (27.6 in) with the Northeast and the Southwest monsoons contributing to 47% and 28% respectively to the total rainfall.

Collection and identification of grasshoppers: The specimens were caught using the insect sweep net and the handpicking methods. Collected grasshoppers were identified based on the morphological characteristics under stereoscopic dissecting binocular microscope using the description available on the ‘‘Website (http:// orthoptera.speciesfile.org) Orthoptera Species File Online’’.

Wing preparation: In this study, only right forewings of male and female *L. indica* species were used to compare wing shape and co-variation between the populations. The male and female right forewings were carefully removed from the body of adult grasshoppers, *L. indica* using dissecting needles, scalpel and forceps. Thus, the wings removed were placed on a glass slide and examined under a microscope to describe the venation pattern of male and female *L. indica*.

Image processing: In order to capture the wing shape of *L. indica*, each wing image was photographed with the same scale using a digital camera attached to phase-contrast stereomicroscope (consistent magnification 10X/22) connected with the scope image version 9.0 image processing software with consistent resolution (1024×768 10 f/s). The digital images of forewings are taken to characterize their shape variation by geometric morphometrics.

Wing veins nomenclature: Wing veins nomenclature was identified using the published taxonomic keys[4,6,7,17,27,33]. The landmark locations on each wing were selected based on the wing veins intersection, wing margin, cross veins, veins branch point, major veins and termination of the wing veins.

Data acquisition: The wing photograph files were first converted in the tps-Util program version 1.76 to minimize bias in digitizing landmark locations of the specimen in tps file. The X, Y coordinates of landmark points on each species wing were subsequently digitized and measured by tps-Dig program version 1.40 to characterize the shape variation for this trait. The digitization and linear measurement (Pixel) in all wings were evaluated twice in order to reduce the quantity error. The scale bar was used to standardize landmark distances to the same absolute scale across all images. Based on the landmark points, morphometric variation in forewing shape was assessed separately for each sex by relative warp ordinations plots using the program of tps-Relw version 1.69 to explore the covariance between a set of variables and variance between two shapes.

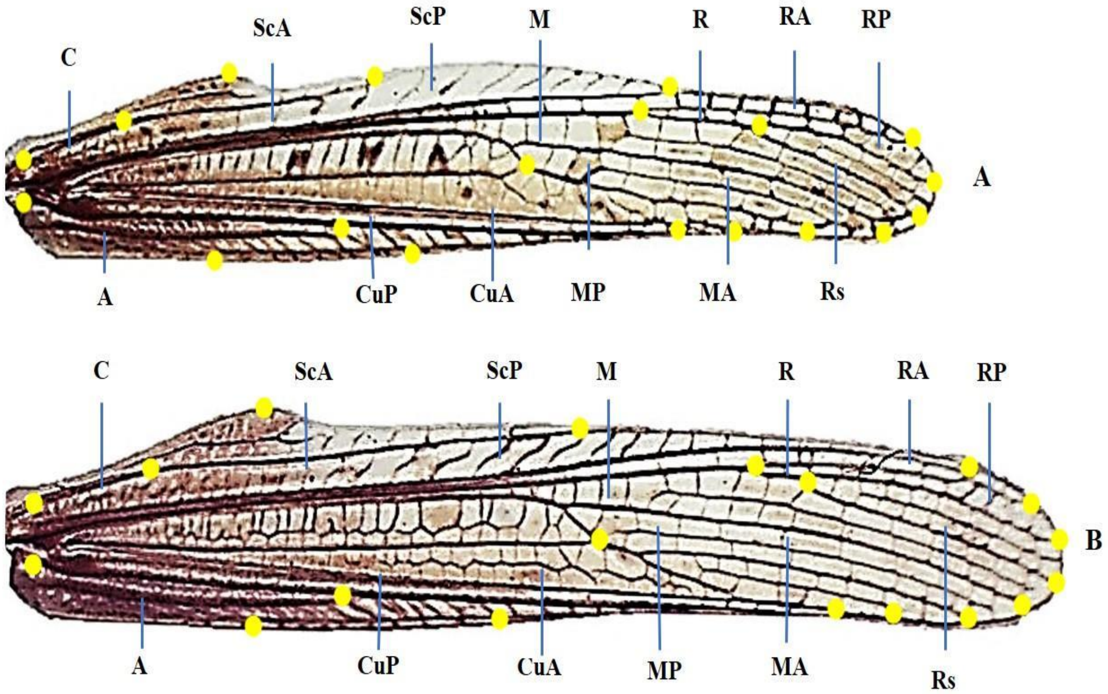
The relative warps were computed with the default weighting factor α = 0, in order to weigh all landmarks equally. Additionally, tps-small program version 1.34 was performed to estimate the correlation between procrustes and tangent space distance of the male and female wings, to ensure that the amount of shape variation in the data sets adequately represented after projection in the tangent space. Then, Principal Component Analysis (PCA) was performed on the landmark coordinates data in order to compare the correlation matrix and percentage of each principal component of the total variation in the wing of males and females using PAST (Paleontological Statistical Software) version 2.02.

Results and Discussion

Wing structure and wing vein nomenclature: We separately calculated the males and females from three locations in order to discriminate taxa at the interspecific level and to avoid the effect of sexual dimorphism. In this study, 19 homologous landmarks were identified in both male and female wings of *L. indica* to characterize the shape variation (Figure 1). The position of each landmark description was depicted in table 2.

The most informative variable areas between males and females were subcostal region (extended in females compared to males), radius extension on front edge of wing (it is relatively short in male compared to female), insertion point of radial sector vein (females have a smaller distance between the radial sector vein when compared with males) and edge of anal area which could be used to differentiate between the mentioned groups (Figure 1). The differentiation observed between male and female is based on the relative position of the landmarks. The males and females have the same approximate wing structure and wing veins nomenclature based on our data but subtle differences exist in the landmarks of the wing vein's location. The landmark location that appeared as a variability was used as morphological characteristics to separate females from males *L. indica*, they were visibly viewed in figures 2 and 3. The differentiation could be obtained based on the distance between the landmarks in each wing structure.

According to Laurito et al[21], detecting shape differences at a general structural level or between two or more landmarks can be translated to traditional morphometric characters for use in taxonomic keys. Therefore, geometric morphometrics would be used to discover areas of variability. Size and shape analysis of structure of the wing adults may allow discovery of new useful features to distinguish morphologically similar species and resolve taxonomic problems[13,16].



C: Costa, ScA: Sub costa Anterior, ScP: Sub costa Posterior, M: Median, MA: Median Anterior, MP: Median Posterior, R: Radial, RA: Radial Anterior, RP: Radial Posterior, Rs: Radial sector, CuA: Cubitus Anterior, CuP: Cubitus Posterior, A: Anal vein

Figure 1: Wing structure and position of the 19 landmarks in right forewing of *L. indica*.
A. Male B. Female

Table 2
Description of 19 landmarks location in wing structure of *L. indica* species

Landmarks	Description
1	Origin of subcostal vein, interaction between the radial and subcostal
2	Base of the precostal area
3	The tip of precostal vertex
4	The point of subcostal anterior margin with leading edge
5	The radius extension on front edge of wing
6-10	Distal edge of wing and interaction of radius branches
11	The point of median anterior with posterior border
12	Cross point of median vein with distal edge
13	The edge of anal area and cubitus anterior with posterior
14	Point of hind edge and vertex vertical line (Width of wing)
15	Insertion points of median with cubitus vein
16	Cross point of anal vein and the vertex vertical line
17	Interaction of cubitus anterior with posterior
18,19	Insertion points of radial sector vein

https://doi.org/10.25303/281rjce1330141

The link of each landmark (1-19) in male and female wings was illustrated in figure 2. The wing structure showed that the female wings bear a larger landmark distance than the male. In both male and female, maximum distance was observed in landmarks 19-1 (Subcostal vein to radial sector vein) and minimum distance was observed in landmarks 7-8 (distal edge of wing). The wing landmarks location had greater variation between the male and female species is 5, 18, 19 while only slight morphological variation was recorded at the landmark 4 which were sufficient to detect shape differences between male and female (Figure 2).

Landmarks distribution in the fore wing of *L. indica* sexes: Plots (red) and vectors (green) were represented with numbers. The figures 3 and 4 indicate the mean and distribution of the 19 landmarks of each individual wing of *L. indica* sexes from different sites. These landmarks distance of each population wing varied within and between the ecosystems. Suganya and Manimegalai [34] reported that the different morphometric parameters of *A. luteipes* population showed variation in one individual to another individual as well as one region to another. According to Bai et al[4], the body size and the wings of *Trilophidia annulata* demonstrated significant differences among the populations collected from different kinds of environments in China. At lower latitudes with higher temperatures, populations have smaller bodies whereas at higher latitudes with lower temperatures, they have larger bodies and wings.

Additionally, Cisneiros et al[10] reported intraspecific variations in the morphological characters such as head, pronotum, femur, body and wings on the populations of *Chromacris speciosa* collected from two locations in Pernambuco, Brazil. Variations in the wing morphology of *T. annulata* species may be linked to a variety of environmental factors[35].

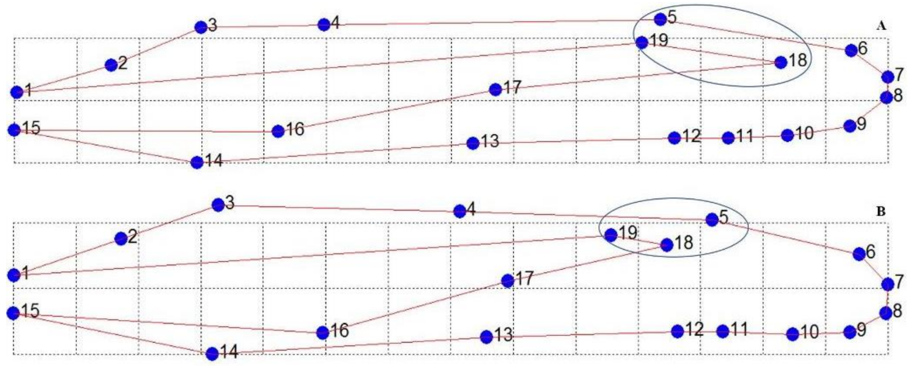


Figure 2: The link showing the distance of each landmark (1-19) and variation between male and female. A. Male B. Female

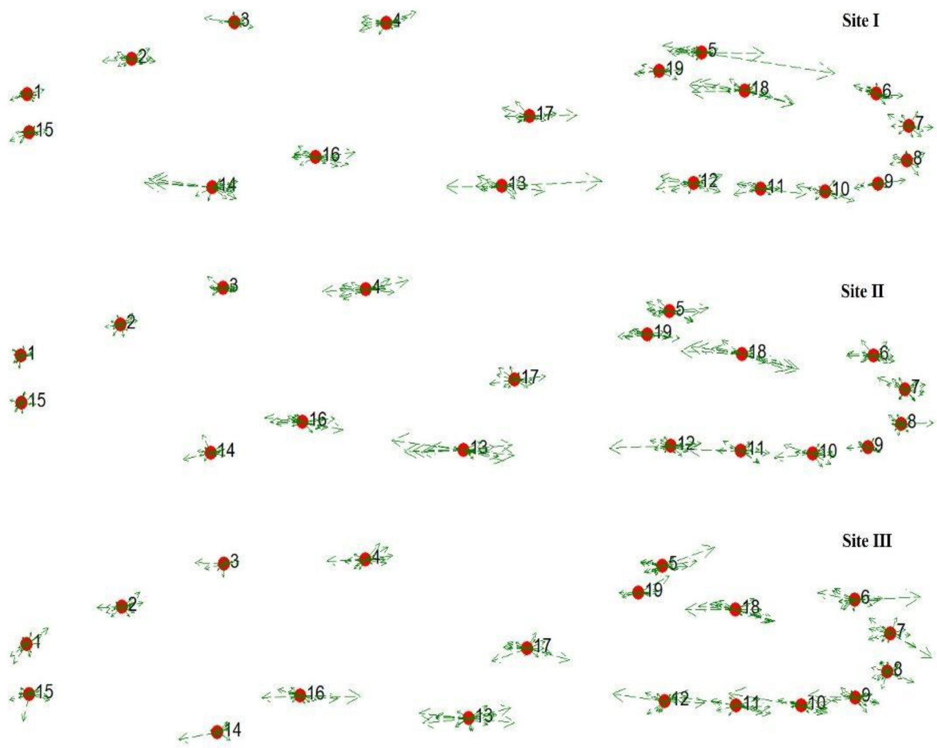


Figure 3: Alignment of 19 landmarks in the male wings of *L. indica*

In male population, landmarks 5, 13, 14 and 18 in site I, landmarks 4, 12, 13, 16 and 18 in site II and 5, 6, 7, 12, 13, 16 and 18 landmarks in site III showed more variation to the average wing shape of *L.* indica whereas, in female population, landmarks 4, 5, 13 and 18 showed more variation among all the three environmental sites. Variability was observed higher in female population compared to male population. The base and apex of the wings showed less variation in both male and female of *L. indica*. The forewing shape varies along with an environmental condition; demonstrating the importance of climatic variables in influencing morphological variations among populations.

Relative warp analysis: In tps-Relw program, the relative warp ordination plot was performed by using the unit, centroid size scaling method and orthogonal alignment projection method. Singular values; warp % and cumulative % were explained by 19 relative warps in both the males and the females as shown in table 3. The first two relative warps accounted for a high proportion of variance in both sexes. In the male population, first two warp values in site I, II and III were 50.94% (32.21% + 18.73%), 52.17% (28.51% + 23.66%) and 56.23% (38.83% + 17.40%) respectively. In the female population, first two warp values in site I, II and III were 53.86% (34.86% + 19%), 59.13% (41.10% + 18.03%) and 52.85% (30.61% + 22.24%) respectively. Site III warp value in male population wings and site II warp value in female population wings accounted for the highest variance across all the three ecosystems.

Relative warp ordination plots showed that both male and female wings of *L. indica* are distinct. We also identified non-affine shapes in the wings of *L. indica* populations among three sites. Manimegalai et al[23] reported that the method of relative warps has been used to identify the co-variation very efficiently. Relative warps are defined as linear combinations of affine and non-affine shape components that describe some portion of the variation observed in the specimens [32]. According to Bai et al[4], the forewing shape of *T. annulata* significantly changed among the 39 populations and wing shape deformation occurred mainly at the end of the forewing. In our study, wing shape deformation was noted mainly at the middle of the wing.

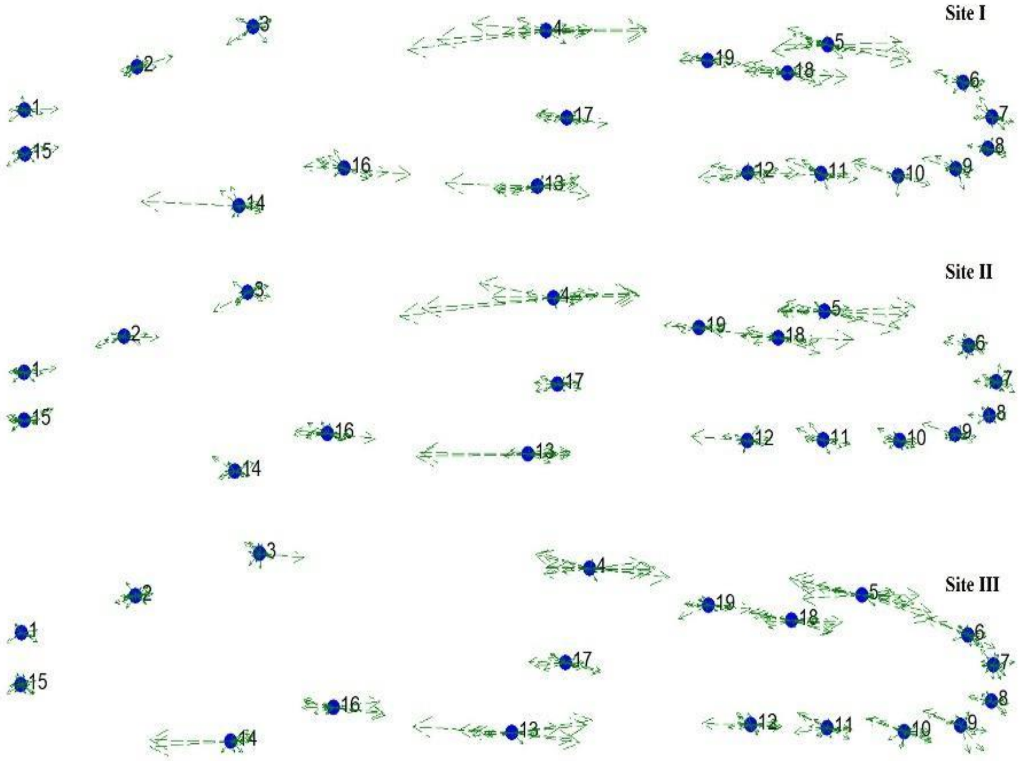


Figure 4: Alignment of 19 landmarks in the female wings of *L. indica*

Table 3
Relative warp explained the variation in male and female wing shape of *L. indica*

Sexes	Relative warp	Site I			Site II			Site III		
		Singular values	Warp %	Cum%	Singular values	Warp %	Cum%	Singular values	Warp %	Cum%
Males	1	0.16405	32.21%	32.21%	0.14264	28.51%	28.51%	0.17330	38.83%	38.83%
	2	0.12511	18.73%	50.94%	0.12994	23.66%	52.16%	0.11602	17.40%	56.23%
Females	1	0.20013	34.86%	34.86%	0.21459	41.10%	41.10%	0.18487	30.61%	30.61%
	2	0.14776	19.00%	53.87%	0.14215	18.03%	59.13%	0.15757	22.24%	52.84%

Table 4
Comparison of procrustes and tangent space distance in male and female wing of *L. indica*

Sexes		Site I		Site II		Site III	
		Procrustes distance	Tangent space distance	Procrustes distance	Tangent space distance	Procrustes distance	Tangent space distance
Males	Min	0.033656	0.033652	0.033259	0.033247	0.041273	0.041270
	Max	0.170813	0.170529	0.151901	0.151751	0.187135	0.186738
	Mean	0.090097	0.090040	0.084010	0.083969	0.086183	0.086125
	Slope	0.999244		0.999445		0.999097	
	Correlation	1.000000		1.000000		1.000000	
Females	Min	0.033439	0.033437	0.021056	0.021054	0.022526	0.022524
	Max	0.196893	0.196563	0.190720	0.190406	0.174873	0.174649
	Mean	0.105624	0.105534	0.103244	0.103156	0.105113	0.105042
	Slope	0.999041		0.999028		0.999280	
	Correlation	1.000000		1.000000		1.000000	

Procrustes and tangent space distance: In tps-small program, project the aligned coordinates orthogonally into tangent space, providing distances smaller than procrustes distances. In both male and female population, the mean values for procrustes distance and tangent space distance were 0.090097 and 0.090040, 0.105624 and 0.105534 respectively; it was observed higher in site I compared to other sites (Table 4). The distance between Procrustes and tangent space within males and females is relatively same in all the three sites, but a significant variation was observed between the male and female wings. The correlation between the two distances was found to be very strong.

According to Lesliee et al[22], correlations between these distances were always greater than 0.99. The ability to measure the degree of differences between shapes using Procrustes distance allows shape space to be defined and characterized[31].

Principal Component Analysis (PCA): The correlation distance between the wing shapes of each specimen variability clearly showed that the differences were highly significant based on the PCA wing variance results. In males, the accumulative contribution ratio of the first two PCs accounted together for 36.99 % (PC1 = 20.85% + PC2 = 16.14%) of the total shape variances and Eigen values were PC1 =70.44, PC2 =54.55 (Figure 5).

In females, the contribution ratio of the first two PCs summarized 42.22% (25.06+17.16) of the total variance and Eigen values were PC1=133.01, PC2= 91.12. In males, the correlation distance was the highest between the landmarks 12-13,13-14 and 19-1 whereas L4-5, 3-4 and 13-14 landmarks distance was the highest in females (Figure 6).

PC1 explained 65.16% of the variation in the parameters with an Eigen value of 21760.6. PC2 explained 11.37% of the variation in the parameters with an Eigen value of 3796.83. L3-4, 4-5 and 19-1 showed more variance between male and female. Two polygons distinctly illustrated the clear difference between the wing shapes of males and females. The scatter plots of PCA showed the females cluster on the right of the plots whereas the males cluster together on the left with overlap (Figure 7).

The non-intersection between the polygons in the wing shape analysis showed that wing vein characters are more informative to discover the variability area. The distribution of individuals along with the first two components represented the main shape variation of the wing of *L. indica*. PCA was helpful in finding the natural groupings of samples and such samples within a group are more similar to each other than the samples in different groups.

In addition, our findings indicated the importance of using different statistical methods, exhibiting sexual dimorphism of wing shape in *L. indica* populations and multiple sources of phenotypic information from the wing of two sexes to capture subtle patterns of differentiation characterization.

The observed variation within and between the males and females *L. indica* population collected from three geographical regions might be attributed to differences in environmental factors in the area such as location, altitude, longitude, climatic variables, habitat destruction, foraging habitat and vegetation.

Whitman[38] demonstrated that Orthoptera body size varies both within and between species, mainly as a result of environmental factors. Riget et al[30] observed differences on the wing morphology which might be influenced by random mating process within the individuals, greater population density, food preference, heat pressure, influence of parasites, diseases, sexual selection and some genetic components.

Also, the study showed that the wing variations might be attributed to the flight system and flapping kinematics[12]. Hence, the differences in the wing morphology could be associated with natural selection[18]. Microclimate and habitat characteristics are considered to be the main factors influencing grasshopper wing shape[24,28].

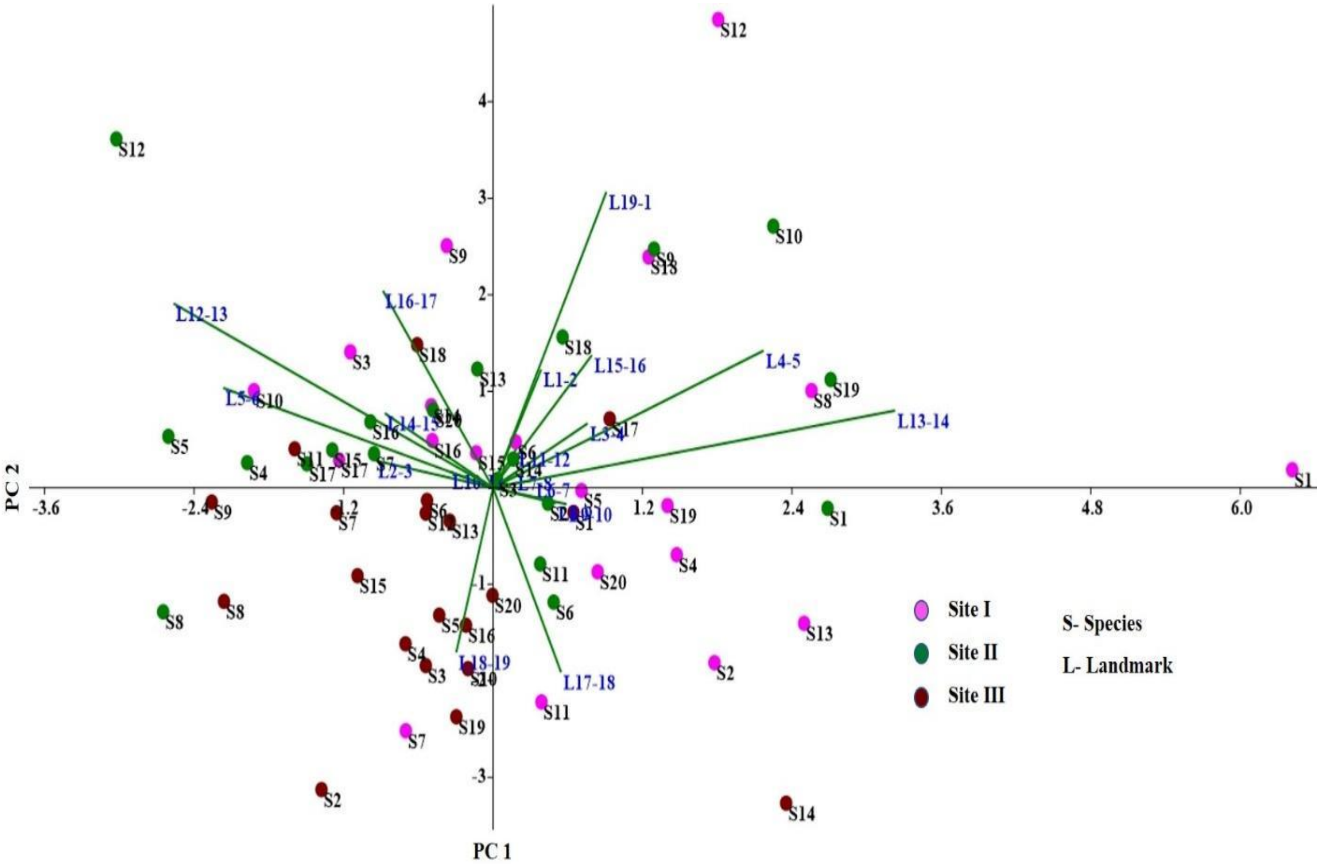


Figure 5: PCA scatter diagram of male wing of *L. indica* from different sites

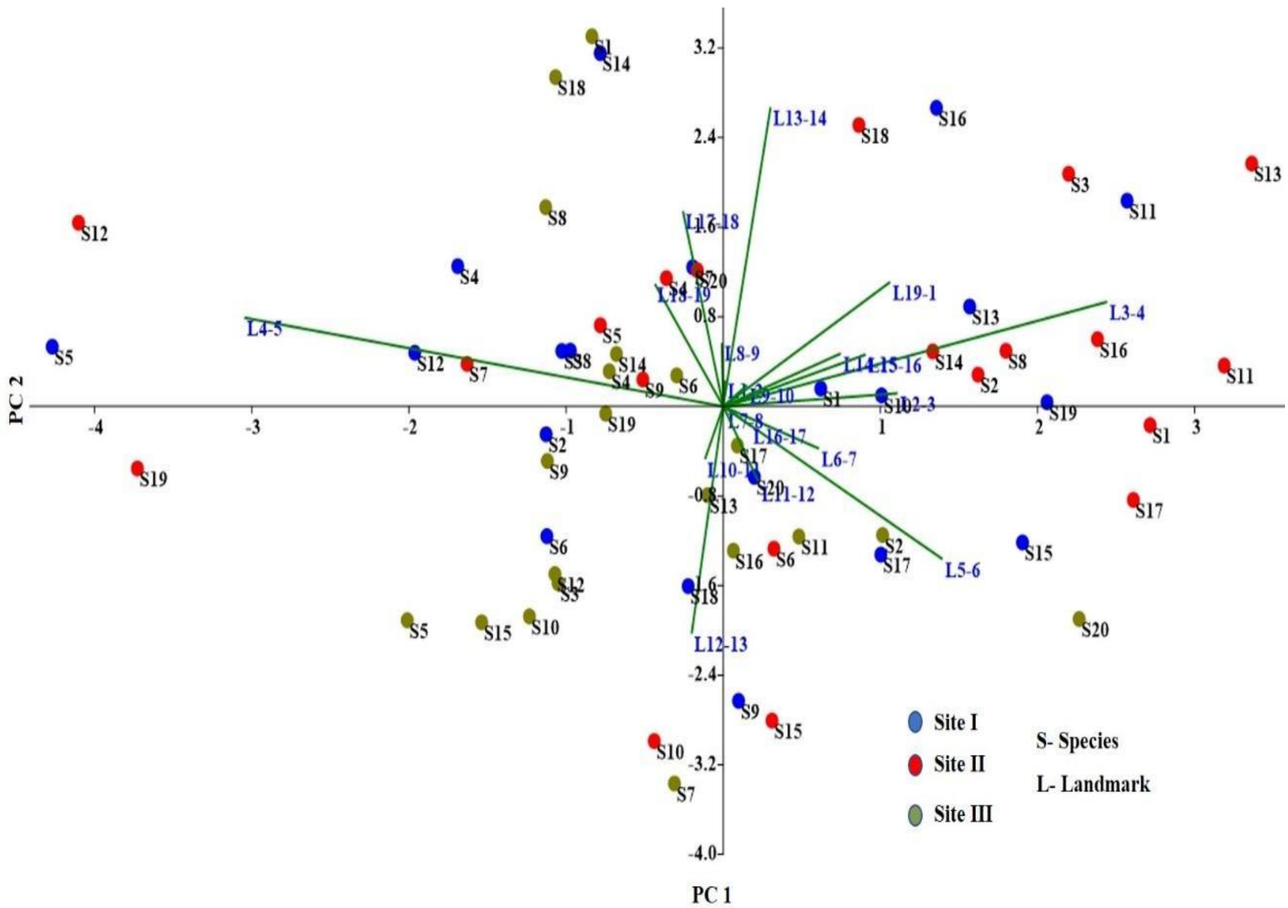


Figure 6: PCA scatter diagram of female wing of *L. indica* from different sites

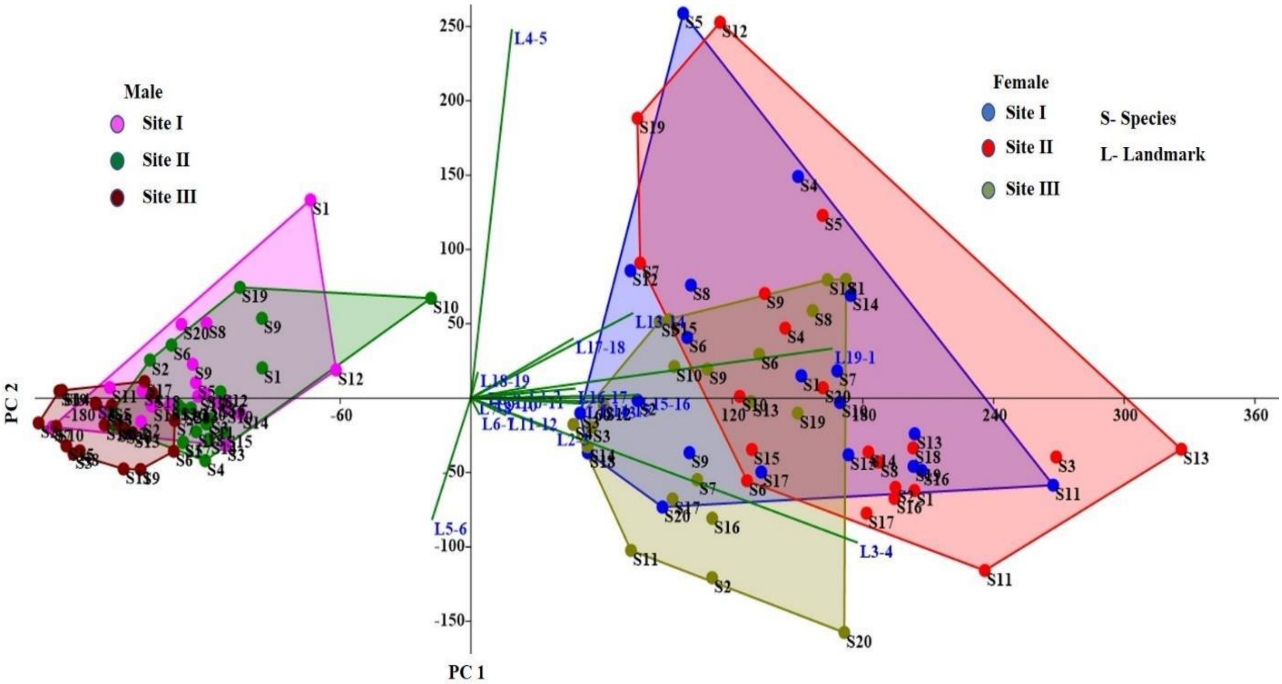


Figure 7: Wing deformation between male and female wing of *L. indica* using PCA

Conclusion

It is concluded that the differences in wing shape of male and female of *L. indica* vary among different environmental sites assessed via landmark based geometric morphometrics indicating the possible influence of environmental conditions on the variations in the morphology of the species. The geometric morphometric method contributed to a more quantitative way in describing the differences between male and female.

The present study will be helpful to identify variability areas between male and female populations of *L. indica*. Hopefully, this study will provide information about the taxonomy of *L. indica* based on their wing venation pattern.

Acknowledgement

We would like to thank the Department of Zoology, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, Tamil Nadu, India for providing the laboratory facilities and support for this study.

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(Received 20th November 2022, accepted 18th January 2023)