International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 18-07-2021, Accepted: 03-08-2021, Published: 19-08-2021
Volume 6, Issue 4, 2021, Page No. 180-185

Intraspecific morphometric variation among males of *Aulacobothrus luteipes* (Acrididae: Gomphocerinae) from two different geographical regions

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Abstract
The objective of the present study was to examine the variation in length of body, antenna, head, pronotum, forewing and hind femur in the males of *Aulacobothrus luteipes* from two different region of Coimbatore. Specimens were collected using sweep net method. Morphometric parameters were calculated using Excel and PAST program. The minimum and maximum length of body parts of male population from two different locations were statistically different from each other. The mean ± standard deviation revealed the length of measured parts (body, antenna, forewing, hind femur) varied with the two geographical sites, while head length and pronotum length showed a similarity with size. Regression analyses of different morphometric structures of male population showed strong positive correlation coefficient within both sites. The principal component analysis (PCA) and cluster analysis also revealed similarity and dissimilarity of male population from two regions. The statistical results of the study confirmed that the existence of some morphological differences of male population of *A. luteipes* from different locations.

Keywords: *Aulacobothrus luteipes*, morphometric parameters, correlation coefficient, principal component analysis

Introduction
Assessing the morphometric characteristics of insects has been very useful in population studies, in terms of communities and is used for the identification of nymph stages, features of sexual dimorphism [8, 12, 14, 3] as well as ecological studies of populations and communities [13, 28]. Insects show morphometric differences interrelated with the environment that may be the result of phenotypic and genotypic variation [1, 8]. Morphological variation provides a major source of characters and character states in traditional taxonomy [22, 23]. However, it is often difficult to determine whether variation is intraspecific or interspecific in taxonomic practice [6, 29]. Grasshoppers are characterized by diverse forms and colours and a range of ecological and economic importance [33] particularly due to their potential for causing damage to agriculture, as certain grasshopper species are phytophagous with defoliating behaviours [7]. Grasshoppers are widely spread and environmental sensitive species whose body sizes and shapes change dramatically over a large geographical range [26, 39]. The morphometric characteristics of grasshopper have also been very useful in the study of the evolution of body size, colour patterns and life history [2, 17]. *Aulacobothrus luteipes* is short-horned grasshopper, belongs to the family Acrididae and subfamily Gomphocerinae of order Orthoptera. Gomphocerine grasshoppers comprise perhaps the most diverse and species-rich subfamily of Acrididae, occurring on all continents except for Australia and Madagascar [38]. Gomphocerine grasshoppers may be identified by characters of their external morphology [25]. Previous studies conducted by Chitra *et al.*,[9], Mayya *et al.*, [20], Das *et al.*, [11], Sharma [32], Srinivasan and Prabakar [34], Arya *et al.*, [3], Kumar and Usmani [18, 19], Gupta [15], Usmani *et al.*, [37], Suganya *et al.*, [35] have added information on *A. luteipes* from different regions of India. However, there are currently no published reports of morphological variations of *A. luteipes* from India. In the present paper we study the intraspecific morphometric variation in males of *A. luteipes* belonging to populations collected from two different geographical region of Coimbatore, Tamil Nadu, India.

Materials and Methods
Study area
The population of *A. luteipes* used for this experiment was collected from two sampling points located within the Coimbatore, Tamil Nadu, India. Site I: Thenkarai (10°56'51"N 76°50'33"E) is located in the foot hills of western ghats about 17 km from the city of Coimbatore. Site II: Thudiyalur (11°04'32.3"N 76°56'00.8"E) is within the Coimbatore, about 10 km north of the city centre. The distance between the two sites chosen for collections is approximately 26 km.

Sampling
In order to perform the morphometric analyses, 10 adult male individuals from site I and 10 male individuals from site II were selected at random from the populations of *A. luteipes*. The grasshopper population sampling was done from two sites using sweep net and handpicking method.

Identification
The collected *A. luteipes* population were identified under stereoscopic dissecting binocular microscope using the keys of kirby (1914) and also referring to the Website (http://www.orthoptera.org) Orthoptera Species File Online''.

Morphometric measurements

For the study of intraspecific variations of *A. luteipes*, six morphometric characters were used in this study (Fig. 1). The parameters studied were body length (BL), antenna length (AL), head length (HL), pronotum length (PL), fore wing length (FWL) and hind femur length (HFL). The measurements of various parts in sampled specimen were obtained in millimetres (mm) using vernier calliper. The body parts length measurement (mm) in all population were evaluated twice in order to reduce the quantity error.

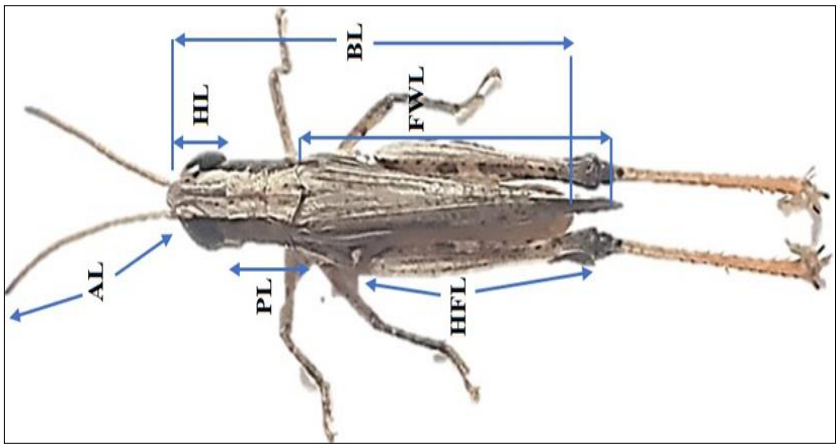

Fig 1: Structure and morphometric sites in *A. luteipes*. (BL-body length, AL- antenna length, HL- head length, PL- pronotum length, FWL- forewing length and HFL- hind femur length)

Data Analysis

The statistics parameters, mean ± standard deviation was employed to determine the morphometric variation between measured parts from two different geographical region using MS Excel software 2010. Pearson's correlations coefficient was performed using the same software in order to evaluate the relationships within and among population. Next, principal component analysis (PCA) was performed in order to compare the percentages of each principal component of the total variation in the morphometric parameter measurements using PAST software v. 2.16 [16]. Additionally, cluster analysis was performed using the same program to study the similarity of population from two region

Results

A total of 20 individuals of *A. luteipes* were selected among two sites for Morphometric analysis. The minimum and maximum body length, antenna length, head length, pronotum length, forewing length and hind femur length of *A. luteipes* in site I varied from 12.39 to 17.11, 5.5 to 8.11, 1.78 to 2.67, 1.96 to 2.83, 9.67 to 12.53 and 6.11 to 8.21 respectively (Fig. 2) and for site II ranged from 12.61 to 18.34, 5.79 to 9.18, 1.87 to 3.01 1.98 to 3.11, 9.73 to13.17 and 6.24 to 9.02 respectively (Fig. 2). The analysed parameters length was higher in site I compared than site II.

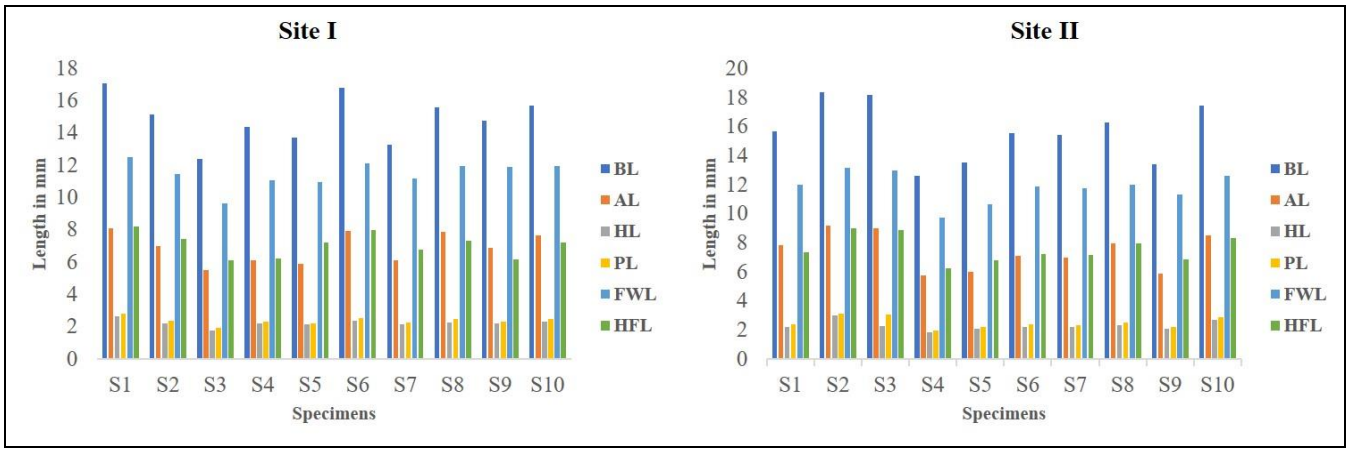

Fig 2: Morphometric measurement (mm) of six different parameters of each specimen of *A. luteipes* from two sites

The value of mean ± SD in length of body, antenna, head, pronotum, forewing and hind femur of *A. luteipes* from two sites were presented in Table 1. The minimum mean ± SD values were observed in site I and maximum mean ± SD values were observed in site II. Among the six parameters, maximum variance was noted in mean body length and minimum variance was noted in mean head length of *A. luteipes* from both sites. The mean length of head and pronotum showed similarity between site I and site II. Other morphometric parameters of *A. luteipes* showed slight variation between two sites (Table 1).

Table 1: Different morphometric parameters mean ± SD of *A. luteipes*

Parameters	Mean ± SD		Variance	
	Site I	Site II	Site I	Site II
BL	14.88± 1.49	15.65±1.99	2.24	3.99
AL	15.65±1.99	7.43±1.27	0.90	1.61
HL	2.24±0.22	2.311±0.32	0.04	0.10
PL	2.38±0.23	2.51±0.39	0.05	0.16
FWL	11.48±0.80	11.81±1.04	0.65	1.09
HFL	7.07±0.73	7.58±0.92	0.54	0.85

Based on the results of the regression, the value of R^2 in both site I and site II indicated that there is strong positive correlation within measured body parts (body, antenna, head, pronotum, forewing and hind femur) of *A. luteipes* (Fig. 3). Forewing length showed highest correlation coefficient within site I (R2 = 0.7959) and site II (0.7744) compared to other five parameters. The R^2 value of hind femur length showed highest similarity among two sites (site I = 0.7601 and site II = 0.761). Regression analysis showed *A. luteipes* species were more closely related within site I compared to site II.

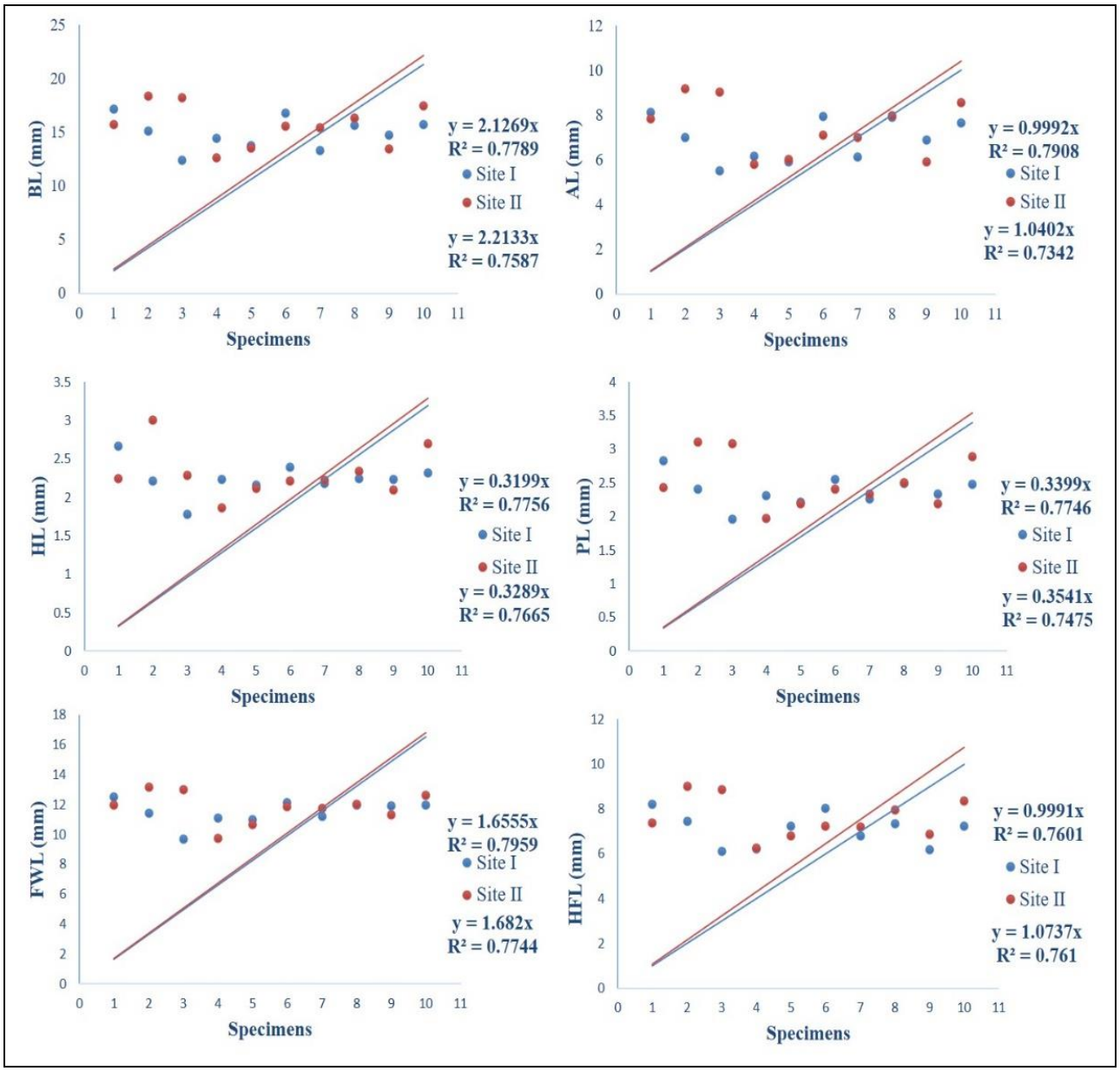

Fig 3: Relationship of morphometric parameters of *A. luteipes* population within and between geographical sites

Based on the PCA results, percentage of variance and eigen values were explained by six PCs. First two PCA (97.62%) showed highest variance and eigen value; other PCs had progressively less variances and eigen value. PC1 explained 95.54% of the variation in the parameters with eigenvalue of 5.86616. PC2 explained 2.08% of the variation in the parameters with eigen value of 0.127738 (Fig. 4.)

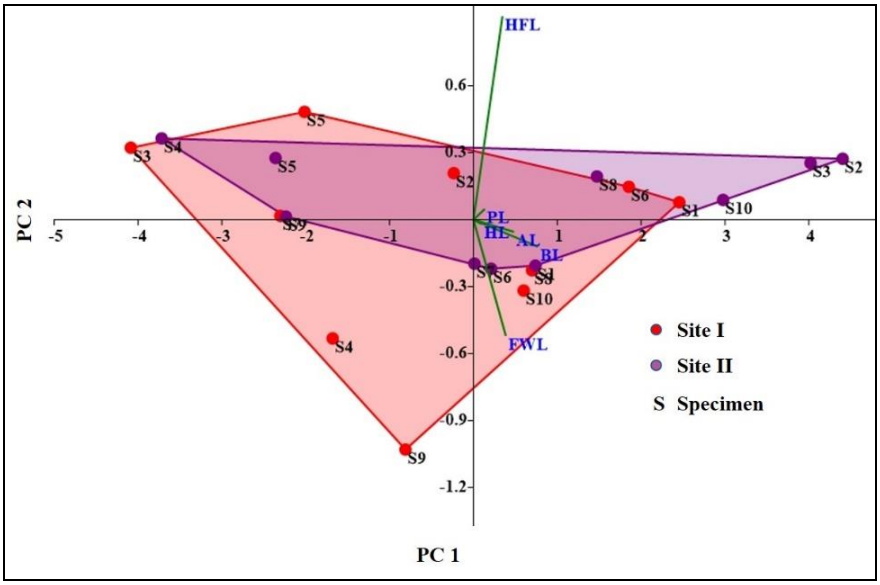

Fig 4: Scatter bi-plot from PCA showed morphometric variation of *A. luteipes* from two sites

Moreover, to study the similarity of morphometrics of *A. luteipes* population among two sites were performed on the basis of cluster analysis as presented in Fig. 5. Clusters were numbered in ascending order based on measurements. The similarity matrix from the quantitative data showed that most of the male population closely related to each other. The highest level of population similarity was grouped within site I compared to site II (Fig. 5).

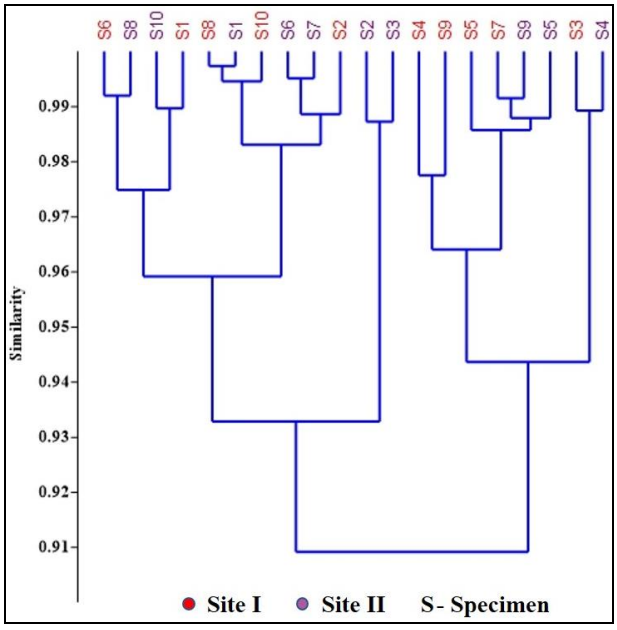

Fig 5: Cluster analysis showed similarity morphometric features among population of *A. luteipes* from different geographical sites

Discussion

In this study indicate that the different morphometric parameters of *A. luteipes* population showed variation in one individual to another individual as well as one region to other region. 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 higher latitudes with lower temperatures, they have larger bodies and wings. Additionally, Cisneiros *et al.*, [10] reported that 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. Whitman [40] demonstrated that body size in Orthoptera varies both between and within species, mainly as a result of environmental factors.

Adults of *A. luteipes* mean body length showed variation between site I (14.88 mm) and site II (15.65mm) (Table 1). The variation in the body size of *A. luteipes* is reflected in changes in length of antenna, head, pronotum, forewing and hind femur. Arya *et al.*, [3] reported that the body length of *A. luteipes* was 1.4 cm. Kumar and Usmani [19] examined body length of A. *luteipes luteipes* was 16.34. Body size is an important feature of organisms because it strongly correlates with numerous ecological, physiological, life history traits and influence fecundity, fitness and speciation [27].

The regression value was observed more than 0.7 (fig 3) in all the six parameters in both sites. In the present study we identified strong positive correlation for *A. luteipes* measure body parts within and among sites. Pearson correlation coeffcient, r, was used to determine the strength and direction of a linear relationship, between two variables. An r-value between 1.0 to 0.9 was considered very strong, 0.89 to 0.7 strong, 0.69 to 0.4 moderate and 0.39 to 0.1 weak linear correlation [30.36].

The PCA conducted in this study indicated that it is possible to separate the variation in populations of *A. luteipes* from two different region by the length of their various body parts. Based on the PCA bi-plot results, the distribution of individuals along the first two PCs showed highest variances and other PCs showed less variances. Cluster analysis is helpful in finding the natural groupings of samples, such that samples within a group are more similar to each other than the samples in different groups [5]. In our study, cluster analysis clearly revealed the similarity within and among population from two different geographical study area. Morphological variation is prevalent in wide-ranging species [20] and is related to phenotypic plasticity, physiological response to environmental factors and adaptation to local environments and divergent selection,

potentially leading to speciation through the evolution of reproductive barriers [22,24]. The present study demonstrated the intraspecific morphological variation of *A. luteipes* male populations in India.

Conclusion

The statistical results reported in the present study regarding the analyses of various morphometric measurements existence of differentiation in populations of *A. luteipes* from two locations. It is concluded that the different morphometric body parts of *A. luteipes* revealing intraspecific variation among two geographical sites, indicating the possible influence of different environmental conditions on the variations in the morphology of this species. In our knowledge, there is no report on morphology of *A. luteipes* from different parts in our country. Morphometric analysis of these species was done for the first time from this region. Hopefully this study will provide information about taxonomy of grasshopper in all over India.

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