Article

Morphometric variations of the head of rice ear bugs *Leptocorisa acuta* (Thunberg) populations

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Abstract
Leptocorisa acuta, sometimes known as the rice bug, is an agronomic pest that is well-known for causing damage to rice fields. The techniques of landmark-based geometric morphometrics are utilized in this work to assess the variance in head shape that exists across three groups consisting of both males and girls. A total of 35 landmarks were subjected to analysis using techniques such as analysis of variance and relative warp analysis. The findings demonstrated that there is a substantial amount of diversity not only between the three populations of male (p=0.02323) and female (p=3.705E-09) *L. acuta*, but also between male (p=1.36E-06) and female (p=0.06616) forms of the species. A significant proportion of the male *L. acuta* (RW=30.50%) and the female *L. acuta* (RW=41.6%) are located at the tips of the labrum and vertex, respectively. A number of causes, including genetics, ecological niche and dynamics, and evolutionary adaptation, might have been responsible for the appearance of variation. The knowledge of head shape diversity may be significant for the production of efficient pest management control in the future.

Keywords Aranea; attitudes; perspectives; conservation.

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1 Introduction

To a certain extent, the phenotype of an insect is determined not only by its genotype but also by the interplay between its genes and its environment (Whitman and Agrawal, 2009). According to West-Eberhard (1989) and Gadagkarand Chandrashekara (2005), this demonstrates that the final phenotype may be influenced by a variety of possible variables that affect gene expression. These factors include observable changes in development or life history, food choices, behavior, and other factors. The distinct feeding behaviors of each species are reflected in the various morphologies of insect heads, which include predaceous, sucking, rasping-lapping, and other similar behaviors (Albutra et al., 2012; Alegre et al., 2011; Cruz et al., 2011; Demayo et al., 2007; Demayo et al., 2011; Friedrich et al., 2013; Krenn, 2010; Labandeira, 2017; Manting et al., 2015; Ramirez and Gutierrez-Fonseca, 2014; Sepe et al., 2014; Tabugo et al., 2012; Torres et al., 2010). On the other hand, it has been demonstrated that populations within a species can exhibit morphological differences amongst themselves depending on their location and the host they employ (Albutra et al., 2012; Alegre et al., 2011; Ascaño et al., 2010; Chapman, 1998; Cruz et al., 2011; Demayo et al., 2011; Ferrari et al., 2007; Madjos and Demayo, 2017; Mahinay et al., 2014; Mahinay and Demayo, 2017; Sepe et al., 2019; Torres et al., 2011; Torres et al., 2013). It was believed that insects such as rice bugs belonging to the genus *Leptocorisa*, which are prevalent in many subtropical and tropical rice regions, exhibited changes in the morphometrical forms of their heads. These variations may have been connected to the type of host that the insect was feeding away from (Bernays, 1986). The agroecosystems of the Philippines are intricate because of the many diverse techniques that farmers take to manage their farms and the many different varieties of rice that are cultivated in various locations of the country. Since the head is used for herbivory and feeding, it is hypothesized that the rice host may have a significant influence on the likelihood of head shape variations among populations (Albutra et al., 2012; Alegre et al., 2011; Bernays, 1986; Cruz et al., 2011; Demayo et al., 2007; Demayo et al., 2011; Friedrich et al., 2013; Krenn, 2010; Labandeira, 2017; Manting et al., 2015; Ramirez and Gutierrez-Fonseca, 2014; Sepe et al., 2014; Tabugo et al., 2012; Torres et al., 2010). This is due to the fact that the head is utilized for both of these purposes. In terms of the consistency of the possible differences, it is presumed that the variability may be worsened due to the fact that rice varieties grown in different geographical locations also vary (Bernays, 1986; Clissold, 2008).

In this particular study, the head of the rice bug, also known as *Leptocoriza acuta*, was examined. The head was obtained from a number of different geographical places worldwide. Rice fields have allegedly suffered damage as a consequence of *L. acuta* draining the sap from developing grains (Grist and Lever, 1969; Srivastava and Saxena, 1967; Kobayashi and Nugaliyadde, 1988). These findings were published inthese three different studies. Consequently, the output is not up to par, and the grains are only half-filled with the product. Numerous rice varieties have been produced to be resistant to this insect despite the fact that studies indicate that its attack has caused serious damage to certain individuals. It is hypothesized that the morphological features of insect populations that may make use of the resistance factors in the rice variety would have been mirrored in the rice variety.

The application of geometric morphometric techniques is a well-established scientific method that has been utilized for the purpose of identifying and measuring possible differences that exist across populations of organisms (Rohlf, 2008). It is feasible that the investigation of head shape variation in *L. acuta* will be able to provide light on a possible relationship between head shape variation and the preferred feeding techniques of the organisms that were researched. The biological form of an organism makes a connection between the genotype and the environment, and it is one of the most significant aspects of the phenotype of an organism (Bernays, 1986; Clissold 2008; Thompson, 1992). In order to better understand how populations started to respond to the type of rice, it may be helpful to consider the relationship between the variety of head shapes and eating behavior. Changes in insect genotypes between geographical locations that are able to utilize the varied varieties of rice that are farmed by other factors might impact the shifts in insect populations (Clissold 2008; Nugaliyadde et al., 2000). The study of head shape variety may be of assistance in the process of developing potential pest management techniques that are helpful for the decrease, if not total eradication, of the agronomic pests that have been identified. It is also possible that it will shed light on the relationship between morphology and ecology.

2 Methodology

2.1 Collection and processing of samples

Samples for testing were collected from three neighboring barangays in Tambulig, Zamboanga del Sur, Western Mindanao, Philippines: Tungawan (T), Buos,Kapalaran (B), and New Village (NV). The collection was organized and carried out on September 7 and 8, 2015. As part of the process of collecting samples from paddy rice fields, sweep nets were utilized in the early morning and late afternoon. Samples were kept in bottles containing 70% ethanol and labeled with relevant information.

In the laboratory, samples were sorted according to gender using a Leica ES2 microscope, and they were identified down to the species level. For the head capsule dissection, only adult specimens were considered. To capture these images, a Canon camera with 16.2 megapixels was utilized.

Fig. 1 Map of the Philippines showing the municipality of Tambulig, Zamboanga del Sur, Western Mindanao.

2.2 Landmark assignment

Digital photography was performed on head capsules that had been dissected using the tpsDig ver. 2.10 program (Rohlf, 2006). When everything is said and done, there are thirty-five (35) landmarks, which results in seventy (70) coordinates in Cartesian space (x, y) (Fig. 2). It was decided to produce three duplicates in order to reduce the amount of measurement error (Dvorak et al., 2005). For convenience, the list of significant landmarks is shown below in Table 1.

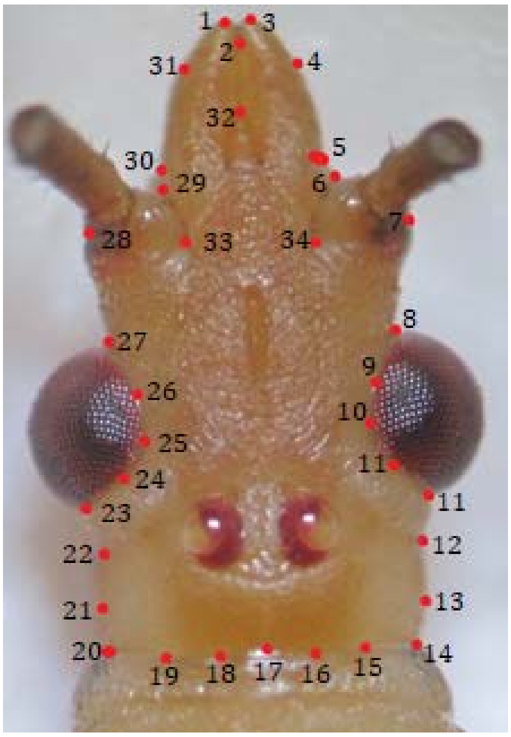


Fig. 2 Anatomical landmark (LM) points on the head of the rice bug, *Leptocorisa acuta.*

Table 1 Descriptions of anatomical landmark (LM) points on the head of the rice bug, *Leptocorisa acuta.*

Landmark	Descriptions
1	apex of the left labrum
2	the intersection between the left and the right labrum
3	apex of the right labrum
4,5	anterior lateral midline of the right labrum
6,7	orifice of the insertion of the right antennal joint
8,11	ends of the curvature of the right eye insertion
9,10,32	curvature of the right eye insertion
12	the junction between the frons and the vertex (right side)
13,14,15,16,17	contour of the vertex (uppermost sclerite)
27,28,29,30	contour of the vertex (dorsal surface)
18	the junction between the frons and the vertex (left side)
19,22	ends of the curvature of the left eye insertion
20,21,31	curvature of the left eye insertion
23,24	orifice of the insertion of the left antennal joint
25,26	anterior lateral midline of the left labrum
33,34,35	clypeus

2.3 Image analysis

Through the utilization of tpsRelW (Rohlf, 2008), a relative warps analysis was carried out in order to determine and compare the variations of the head capsule that were present throughout the populations in comparison to a consensus or mean shape. In the following step, thin-plate splines were utilized in order to show the transformation of every specimen. Box plots were developed in order to illustrate the population dispersion that was located at a distance from the mean form.

2.4 Statistical analysis

The mean differences in head centroid size among populations were estimated with the use of PaST software version 1.91 (Hammer et al., 2001), and then a One-Way Analysis of Variance (ANOVA) (Zhang and Qi, 2024) was performed on the data. It was determined that Tukey's test was necessary in order to establish substantial population-level interactions further. A cluster analysis using Ward's method (bootstrap = 1000) was carried out in order to study the degree of similarity across populations.

3 Results and Discussion

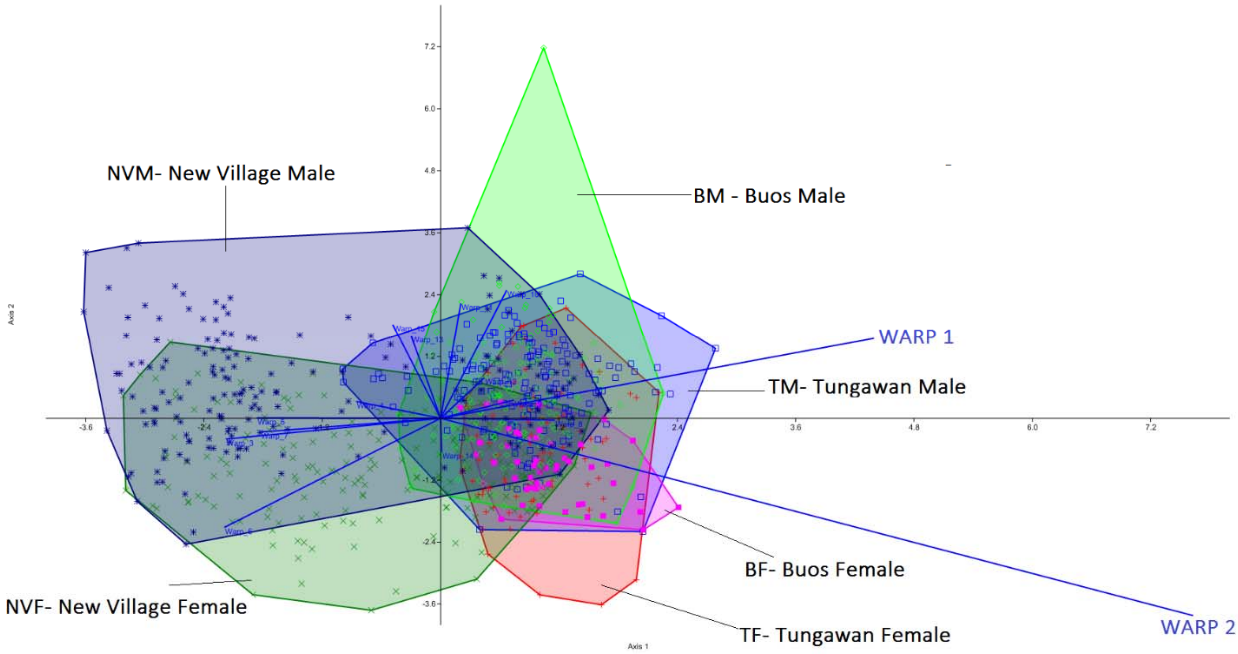
Within the *L. acuta* population, there were identified differences in the head morphologies of different populations (Table 2). On the basis of relative warps 1 and 2, populations from New Village were distinguished from those of other villages; however, these populations were not distinguished from one another individually. The demographic structures of different populations provide the basis for the disparities that exist across populations (Table 3). Additionally, it is possible to demonstrate, based on the findings, that there are significant sex disparities between the populations. On the basis of Warp 1, males were discriminated against, and on the basis of Warp 2, females were differentiated (Fig. 3, 4). According to the results of the discriminant analysis, more than eighty percent of the people are sexually distinct (Table 4, 5).

Table 2 *L. acuta* Head shape variation (based on 15 RW)

Wilk's Lambda	**0.2773**	**Pillai trace**	**1.021**
df1	75	df1	75
Df2	3903	Df2	4090
F	15.98	F	14
P(same)	**9.337e-173**	**P(same)**	**3.651E-150**

Table 3 CVA of variations between sexes and populations of *L. acuta.*

	S1TMA1	**S1BFA1**	**S1BMA1**	**S1NVFA1**	**S1NVMA1**
S1TFA1	5.06E-13	3.92E-06	2.69E-08	3.80E-24	2.21E-43
S1TMA1	-	2.64E-20	0.000166	3.27E-49	3.45E-55
S1BFA1		-	7.37E-15	8.15E-25	3.17E-38
S1BMA1			-	1.87E-41	9.01E-48
S1NVFA1				-	7.73E-38



B – Buos (Kapalaran); NV – New Village; T – Tungawan

Fig. 3 CVA analysis between populations of *L. acuta.*

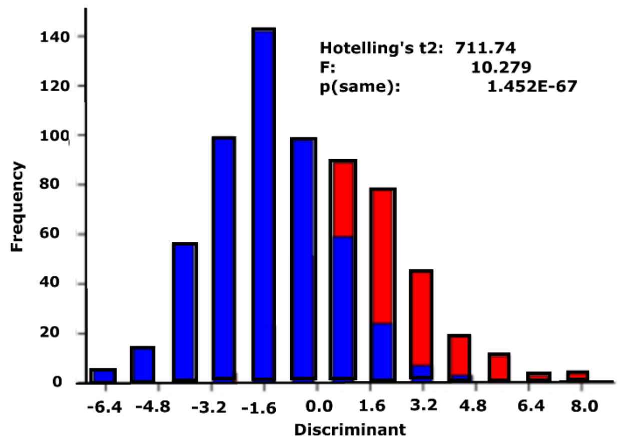


Fig. 4 Discriminant function analysis between populations of *L.acuta*.

Table 4 Sex differentiation in populations of *L. acuta*

	Female	Male		N
Female	264 (80.73%)	63 (19.27%)		**327**
Male	83 (14.97%)	507 (85.93%)		**590**
	%correctly classified	82.49%		

Table 5 Discriminant analysis between populations of *L. acuta.*

	TF	TM	BF	BM	NVF	NVM	Total
TF	47(55.95%)	11 (13.10%)	13 (15.48%)	10 (11.90%)	3 (3.57%)	0	84
TM	26 (15.76%)	92 (55.76)	5 (3.03%)	32 (19.39%)	4 (2.42%)	6 (3.64%)	165
BF	8 (15.69)	1 (2%)	40 (78.43%)	0	2(3.92%)	0	51
BM	28 (17.28%)	45 (27.78%)	16 (9.98%)	64 (39.51%)	8 (4.94%)	1 (0.62%)	162
NVF	29 (15.10%)	6 (3.12%)	21 (10.94%)	9 (4.69%)	97 (50.52%)	30 15.63%)	192
NVM	7 (3.89%)	17 (9.44%)	7 (3.89%)	19 (10.56%)	14 (7.78%)	116 (64.44%)	180
Total	145	172	102	134	128	153	834

Legend: B – Buos (Kapalaran), NV – New Village, T – Tungawan.

Transformation grids are utilized in order to provide descriptions of differences in head shapes that exist amongst male populations. These descriptions are based on the five significant relative warp analyses, which can be found in Table 6 and Figure 5. The first relative warp, which is responsible for 30.50 percent of the variance, is characterized by an unimodal distribution and a tilt toward the left of the mean. With regard to the labrum area, the Tungawan (T) population has expansion points, whereas the New Village (NV) and Buos (B) populations possess compression points.

Table 6 The significant relative warps and corresponding percentage variance explain the variation in the head between three populations of male *Leptocorisa acuta*.

RW	% Variance	Description
1	40.50	Variation in anterior margins
2	13.21	Variation in anterior margins
3	10.2	Variation in posterior margins
4	6.84	Variation in posterior margins
5	5.91	Variation in anterior and posterior margins

Both the second relative warp (RW2=13.21%) and the third relative warp (RW3=10.21%) have a distribution that may be described as bimodal. T is the one that is closest to the mean in the second relative warp, whereas NV has a substantial compression of the apex of both the left and right labrum. It can be observed that B is the one that is closest to the mean in the third relative warp. While the others are more inclined to the left of the mean, NV exhibits a conspicuous compression at the left side of the vertex's contour and an obviously lifted-up orifice of the left antennal joint's insertion. In comparison, the other curves are more inclined to the right of the mean position.

The fourth and fifth relative warps, which have RW4 values of 6.84% and RW5 values of 5.91%, have an unimodal distribution, much like the two important warps that came before them. In the fourth relative warp, NV is very near to the mean, whereas B showed compression of labral points and a slightly higher orifice where the right antennal joint enters. NV is pretty close to the mean. T displays expansion spots in the labrum and lateral surface, whereas B is very near to the mean in the fifth significant warp. There is a compression of the vertex in NV.

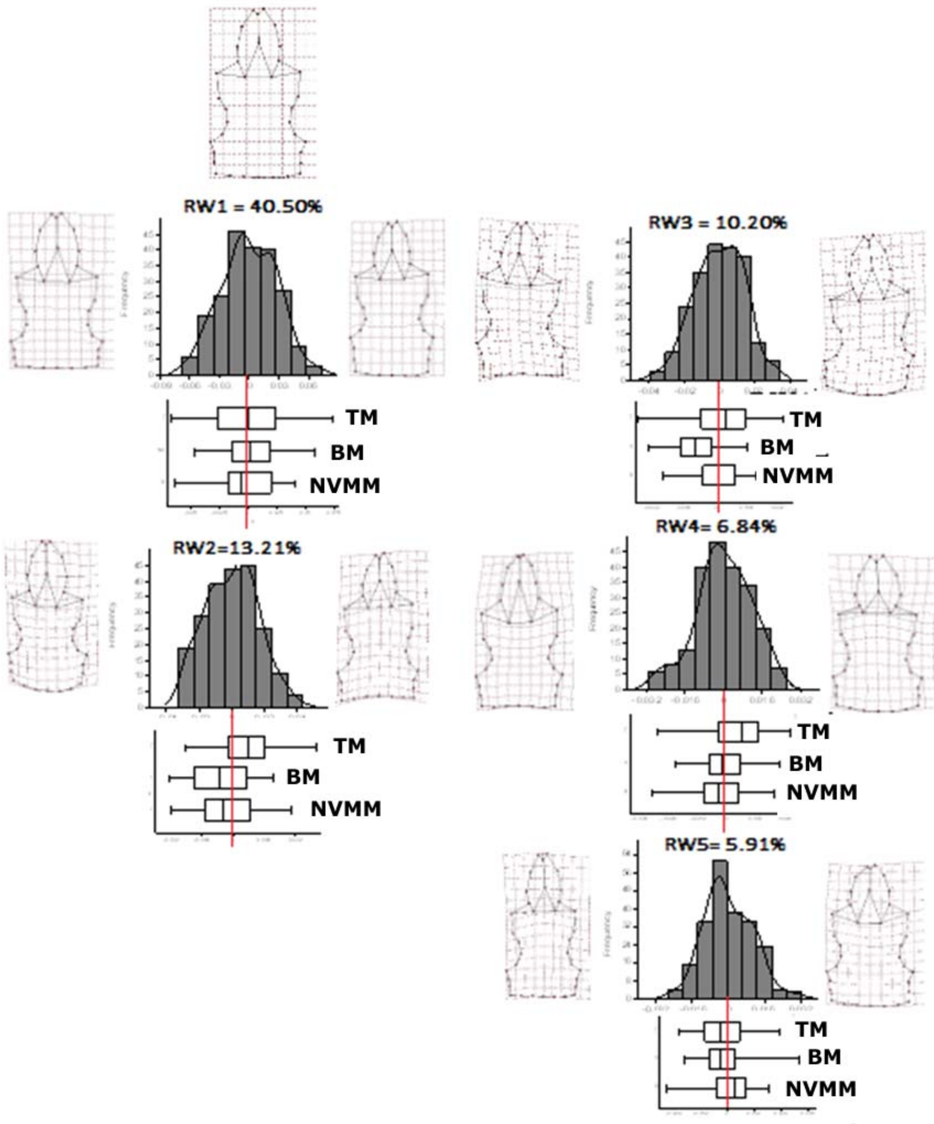


Fig. 5 Summary of the geometric morphometric relative warp analysis showing the consensus morphology (uppermost panel) and the variation in the shapes of the head among three populations of male *Leptocorisa acuta*.

Within the female *L. acuta* populations, the diversity in head morphology was analyzed using relative warp analysis, which resulted in the identification of five major relative warps. Each of these warps had an unimodal distribution, as shown in Table 7 and Figure 6. It was discovered through the first relative warp (RW1=41.62%) that population B is the one that is closest to the mean. While the other two populations are more inclined to the left, the T indicates compression of the vertex and on the orifice of the insertion of the left and right antennal joints. In contrast, the other two populations are more inclined to the right. With N populations having minor compressions in the posterior borders as well as compressed labrum and orifice of the left and right antennal joint, the second relative warps described a 14.71% deviation that is more likely to be to the right of the mean. This is because the second relative warps were able to explain the variance fully. When compared to B, which is the most closely aligned with the mean, T is tilted to the left and exhibits compression of the labrum and posterior margins.

A rightward slope is also present in the third relative warp, which has a value of 10.14 percent. On the other hand, this is most obvious in T populations that have a compressed lateral anterior midline of the right labrum, an upshifted orifice of the insertion of the left antennal joint, and shorter posterior margins. Both NV and B are comparable to the standard form.

The fourth and fifth significant relative warps, respectively, were inclined to the left of the mean (RW=7.01% and RW5=5.37%). This was the case for both of these instances. For example, populations of NV samples in the fourth relative warp show significant expansion in the vertex, labrum, and orifice of the insertion of the left antennal joint. This is in contrast to the behavior of T, which displays the reverse of the mean, and B, which is closest to the mean. Furthermore, B in the fifth relative warp has a compressed curvature of the left and right eye insertions, and it is more inclined to the left of the mean than the other two.

Table 7 Variation in the head between three populations of female *L. acuta*explained by the significant relative warps and its corresponding percentage variance.

Relative Warp	% Variance	Description
1	41.62%	Variation in anterior and posterior margins
2	14.71%	Variation in anterior and posterior margins
3	10.14%	Variation in anterior and posterior margins
4	7.01%	Variation in anterior and posterior margins
5	5.37%	Variation in anterior margins

Morphometrics is the quantitative study of biological shape, shape variation, and shape covariation with other biotic or abiotic variables or factors. Geometric morphometrics allows for the depiction of shape and the spatial localization of shape variation by enabling the creation of expressive diagrams representing morphological transformations or variations. It provides a wide range of tools for both statistically quantifying and visualizing form differences (Webster and Sheets, 2010). Statistical tools are being used to make it operate accurately and scientifically.

The statistical method known as analysis of variance (ANOVA) compares the means of several samples (Ostertagova and Ostertag, 2013; Zhang and Qi, 2024). The null hypothesis conveys the idea that all the data were sampled from populations with the same mean.

The ANOVA findings from all of the examined populations of both male and female *L. acuta* revealed substantial variations in head shape, as was shown in the preceding pages. Such variation is the outcome of a number of biotic and abiotic variables that collectively affected and affected to have created a morphological alteration.

Natural functions are reflected in the innate morphological structures of organisms, which most likely include shapes and sizes (Mayr, 1970). With such a conclusion, one may theoretically state that the outplay of ecological performance is one of the indisputable explanations for the presence of morphological variation between populations of organisms (Abrams, 1996; Chapman, 1998; Jablonka and Lamb, 2005).

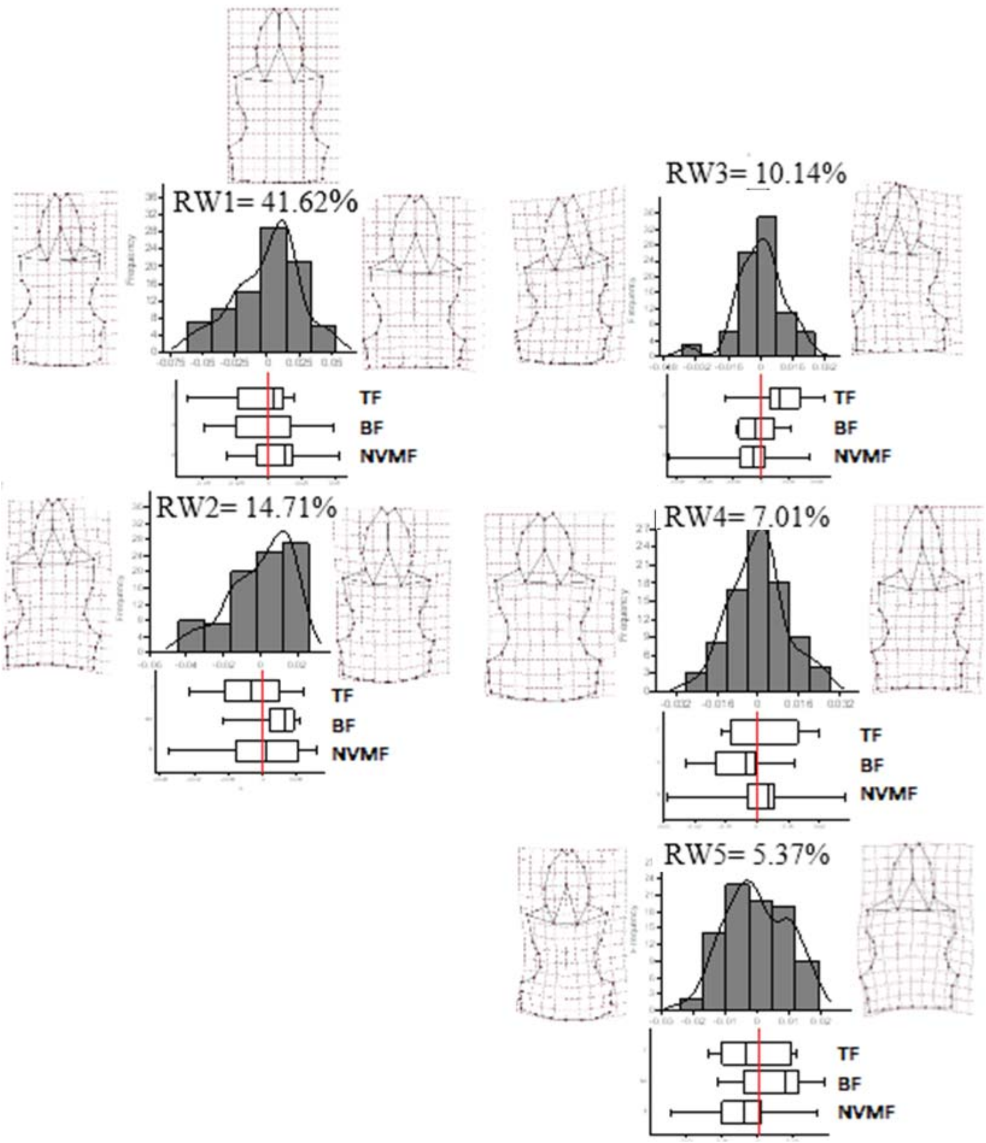


Fig. 6 Summary of the geometric morphometric relative warp analysis showing the consensus morphology (uppermost panel) and the variation in the shapes of the head among three populations of female *L. acuta*.

Additionally, different creatures have different ways of adapting to their surroundings (Mayr, 1970). The differences between the three populations of male and female samples of *L. acuta*may have resulted from an adapted trait that was passed down through previous generations as a result of natural selection. Natural selection could have affected current genotypic expressions, which could have resulted in the observed phenotypic variations (Jablonka and Lamb, 2005; Mayr, 1970). Such adaptation, however, is closely tied to the ecological niche of the organism, which may include food availability based on host selection, habitat selection, environmental diversity, and interactions between the organism and other species in its environment (Mayr, 1970). These deduced scientific hypotheses are confirmed by Mayr (1970) assertions that natural selection favors variability because of heterozygotes' propensity for superiority and the environment's diversity. However, this study does not address the length of time required for the genetic material to be impacted.

The mouthpart, or more specifically, the feeding mechanism, is located in the head of *L. acuta*. The relative warp analysis shows significant changes in the head morphology that may have been caused by herbivory and insect feeding. *L. acuta*is stated to breed on a range of grasses in addition to rice in research by Singh and Singh (2004). According to Nugaliyadde et al. (2000), *L. acuta*has been seen to consume the blooms of a variety of graminaceous weeds that grow in paddy environments. According to studies, rice is the only host plant that can support the entire community structure of paddy bugs, despite the fact that these insects tend to migrate to nearby host plants (Clissold, 2008; Price and Hunter, 2005). Although variations in the temporal and spatial quality and quantity of host plants have been used to explain patterns of herbivory and effects on insect populations (Clissold, 2008; Price and Hunter, 2005), the presence of nearby alternative hosts for the paddy bugs, such as the presence of graminaceous weeds, may also affect insect populations (Clissold, 2008; Price and Hunter, 2005). The relative warp analysis revealed variations in the head shapes of both male and female *L. acuta*in the three tested populations, which may be related to the insects' herbivory. According to a study by Bernays (1986), variations in head shape are a result of functional adjustments made to cope with strict diets. Thompson (1992) went on to say that the increase in head shape allowed caterpillars and acridids raised on harsh diets to enhance their rate of consumption. Therefore, we might entertain the notion that a change in head shape may have likely led to an increase in food intake and, consequently, a greater risk to farmers who may have anticipated developing significantly better pest management strategies (Bernays, 1986; Thompson, 1992). In addition to the genetic, ecological, and evolutionary adaptations that have been suggested as potential scientific causes of head shape variation, it is also important to consider ecological stressors like pest management practices (which may result in insecticide resistance), habitat destruction, and/or eradication as potential causes of variation over time (Pathak and Khan, 1994; Srivastava and Saxena, 1967). This is plausible given that developmental plasticity can lead to relative changes in head shape size (Clissold, 2008; West-Eberhard, 2003). Developmental plasticity is thought to have a significant role in adapting to changing settings (Clissold, 2008; West-Eberhard, 2003; Jablonka and Lamb, 2005; Thompson, 1992).

4 Conclusions

Using landmark-based geometric morphometric analysis, it was discovered that there were significant variations between the three populations that were sampled in terms of the head morphology of the male and female *L. acuta*. A variation of this kind may be seen in virtually every component of the skull, notably in the important markers of the anterior and posterior margins (the labrum and the vertex, respectively), as determined by relative warp evaluations.

It's possible that the interaction of several different factors, such as genetics, ecological niche and dynamics, and evolutionary adaptation, caused the changes observed.

A study on the variation in head morphology in *L. acuta* is believed to be necessary for future reference and might perhaps assist in the development of effective strategies for pest management.

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