

**Shapes of mandibles of white stemborer *Scirpophaga innotata* (Walker, 1863)
larvae associated with different rice varieties**

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ABSTRACT

Rice stem borers are considered the most serious insect pest of rice in Asia. The white stem borer *Scirpophaga innotata* (Walker, 1863) is the most prevalent among the different species that exist in the Philippines. This pest uses its strong mandibles to penetrate and feed on its plant host. Rice varieties of various level of resistance were deployed in different geographical locations in the Philippines but infestations were still observed and have contributed to economic losses to the farmers. Although differences in the resistance level among rice varieties makes an important factor in the control of the pest, no rice variety has been reported to be completely resistant against rice stem borer attacks. Several reports have indicated however that the level of damage caused by rice stem borer attacks differs. Thus it was the major objective of this study to determine if populations of white stem borer showed intraspecific variation between different geographical locations and between rice varieties. Since the mandible of the larvae is used in penetrating the plant, this morphological part of the insect larvae was investigated. Outline-based geometric morphometric analysis was used to test the hypothesis that the mandible shape of white stem borer differ with respect to different rice varieties and geographical location. Results of the principal component analysis showed variation in the shape of the mandible among populations of white stem borer taken from different rice varieties but not in the same variety collected from different geographical locations. Results of this study further confirm the general argument that host plant resistance play an important role in the evolution of pests.

Keywords: *Scirpophaga innotata*, Outline based geometric morphometric analysis, Principal component analysis, Kruskal-Wallis test.

INTRODUCTION

Rice is considered to be the most important staple food for millions of people in the Philippines (Rogel, 2004). The Philippines was considered as one of the largest rice producer in the world, accounting for 2.8% of global rice production. However, the country was also considered as the world's largest rice importer in 2010. The International Rice Research Institute (IRRI) in the Philippines has concentrated much of its research efforts toward developing management methods conducive to high yields of rice in the tropics. One of the major problems affecting rice yields and is receiving attention has been insect control. Rice stem borers are one of the insect pests considered to be the most serious insect pests of rice in the Asia (Chandler, 1967).

The white stem borer *Scirpophaga innotata* (Walker, 1863) is one of the Philippine stem borer species that feed exclusively on rice. For the past several years,

different rice varieties which have different set of genes coding for various plant characteristics responsible for host plant resistance were developed against rice stem borer's attacks. This strategy however was not very successful since infestations of the newly deployed variety still succumbed to the pest in various geographical locations. It is argued that if host plant species produce different selective regimes to herbivorous insects, genetic variations and host plant-associated local adaptation may also occur (Ruiz-Montoya *et al.*, 2003). Although no rice variety is completely resistant against rice stem borer's attack, differences in the level of resistance were observed and these sufficient differences among varieties still make varietal resistance an important factor in insect control (Chandler, 1967).

Since the most damaging of the stem borer developmental stage is the larva which bore inside the plant using its hard mandible, it is hypothesized that larvae surviving in rice varieties with different level of resistance may possess variations in the shape of the mandible. According to Caetano *et al.* (2008), the morphology of mouth parts, especially the mandibles, is directly related to the insect's diet. In the case of the rice stem borers, the strong mandible of the larva is associated not just on its diet but also in its penetration to the host plant. Its mandibles allow it to bore and to feed regularly on the plants causing the death of the affected plants. This study was therefore conducted to determine mandible shape variation among populations of white stem borers infesting rice varieties with different levels of resistance collected in different geographical locations using outline-based (elliptic Fourier) geometric morphometric (GM) analysis. Elliptic Fourier analysis (EFA) is the common tool used in quantifying outline based analysis. According to Tatsuta *et al.* (2004), elliptic Fourier analysis could be best performed in the analysis of the object's shape since it permit detailed examination of fine-scale morphological variation especially when the number of landmarks available can sometimes be insufficient to capture the shape of an object.

MATERIAL AND METHODS

Collecting of Samples: The white stem borer larvae were collected from different sampling sites in three provinces of Mindanao. They were Lanao del Norte, Misamis Occidental and Zamboanga del Sur respectively (Fig. 1). Aside from different sampling areas, samples were also obtained from different varieties of rice plant as shown in Table (1). A mature stage of rice plant was chosen in each of the sampling sites. Rice stalks which appeared white in colour and bearing no rice grains compared to the others were uprooted. With the aid of a knife, each stalk was longitudinally divided. Thus, revealing a colony of stem borer's larvae inside. The samples were then preserved in 95% ethyl alcohol.

Table 1: List of PSB/BPI/NSIC/IR rice variety and their corresponding characteristics.

Rice Variety	Ave. Yield (t/ha)	Growth Duration	Height (cm)	Susceptibility
IR 72	5	112	88	MS
PSB Rc 18	5.1	123	102	I
PSB Rc 26H	5.6	110	88	I
BPI Ri 10	4.7	108	84	MR
NSIC Rc 158	7	113	NA	MR

Legend: MR- Moderately Resistant; I – Intermediate; MS – Moderately Susceptible

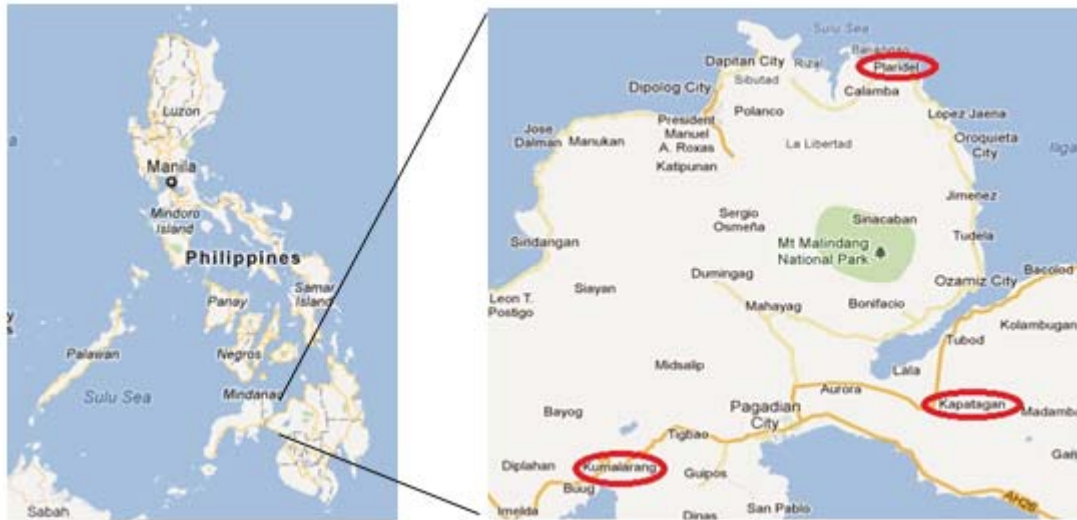


Fig. 1: Topographic view showing the sampling sites in Lanao del Norte (Kapatagan), Misamis Occidental (Plaridel) and Zamboanga del Sur (Kumalarang).

For simplicity, different populations were labeled as Kap158, Kap18, Kap10, Kum 26, Pla158, Pla26 and Pla72. Kap, Kum and Pla stands for Kapatagan, Kumalarang and Plaridel respectively where the samples were taken; 158, 18, 10, 26 and 72 stands for the NSIC Rc 158, PSB Rc 18, BPI Ri 10, PSB Rc 26H and IR 72 rice varieties respectively which serve as plant host of the white stem borer larvae.

Processing of Samples: Each larva was placed in a test tube that contains 5% sodium hydroxide. With the use of an electric stove, the larvae were indirectly boiled using a medium heat. After 2-4 hours, the body of the larvae appeared transparent and the mandible can then be distinguished due to its black appearance. Under the stereomicroscope, the mandibles were separated from the bodies using a dissecting needle and were then mounted on clear glass slides (Fig. 2). Glycerol was used to avoid accumulation of bubbles in the slides and finally sealed by transparent nail polish.

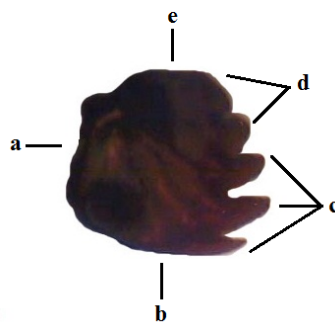


Fig. 2: Ventral view of the mandible of white stem borer *Scirpophaga innotata* (Walker, 1863) showing its different parts. Legend: a = mandible attachment site; b = external margin; c = incisor teeth; d = basal angle; e = basal margin; c – e = parts of internal margin.

Geometric Morphometric Analysis: The outline of the mandibles of white stem borers were analyzed in chain coding technique using the software package SHAPE v.1.3 (Iwata and Ukai, 2002) to examine the variation in shapes. Chain code is a coding system for describing geometrical information about contours in numbers from 0 to 7. The images produced by the MicronCAM attached to the stereomicroscope were converted into 24-bit Bitmap type. Chaincoder converts the full colour image

into a binary (black and white colour) image, reduces noise, traces the contours of objects and describes the contour information as chaincode.

The codes were then analyzed using Elliptic Fourier analysis, utilizing only the first 20 harmonics. It allows detailed analysis of fine-scale morphological variation in the outline of the mandibles of rice stem borer's larvae. This outline method automatically extracts and characterizes ordered series of harmonics, each harmonic being described by four new parameters called elliptical descriptors. Normalized elliptic Fourier descriptors (EFD) were used in accordance with the procedure suggested by Kuhl and Giardina (1982) to generate the shape descriptors via discrete Fourier transformation of the chain codes. Orientation and size resulted in each outline were represented by the 80 elliptic Fourier coefficients for starting normalization. Principal component analysis was used to summarize shape variation in the left and right mandibles based on the EFD coefficients. Principal component scores were further subjected to Kruskal-Wallis test, a non-parametric version of one way ANOVA, to determine if the populations differ significantly from one another based on the shape of its mandible. Box and whiskers plot was then used to visualize the distribution of different white stem borer populations. Multivariate and statistical analysis were done using the software PAST version 1.91 as platform (Hammer *et al.*, 2001).

RESULTS AND DISCUSSION

Geometric morphometric analysis in the left and right mandible of *S. innotata* showed morphological variation between seven different populations. Multivariate analysis of variance (MANOVA) showed significant shape variation in the left and right mandible between populations that host different variety of rice while non-significant variations were obtained between geographically separated populations. The results of MANOVA test were shown in Table (2). The canonical variates analysis (CVA) scatter plots in Figs. (3 and 4) show the distribution of different white stem borer population based on the shape of its left and right mandibles.

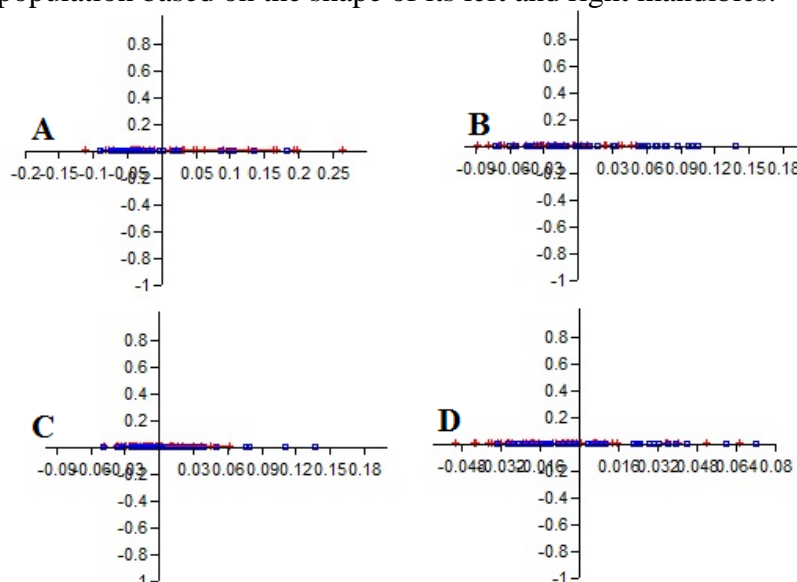


Fig. 3: CVA scatter plot showing the distribution of different populations obtained from different localities based on the mandible shape. Legend: A and B = variation in the left (A) and right (B) mandibles between Kap158 and Pla158 populations; C and D = variation in the left (C) and right (D) mandibles between Kum26 and Pla26 populations.

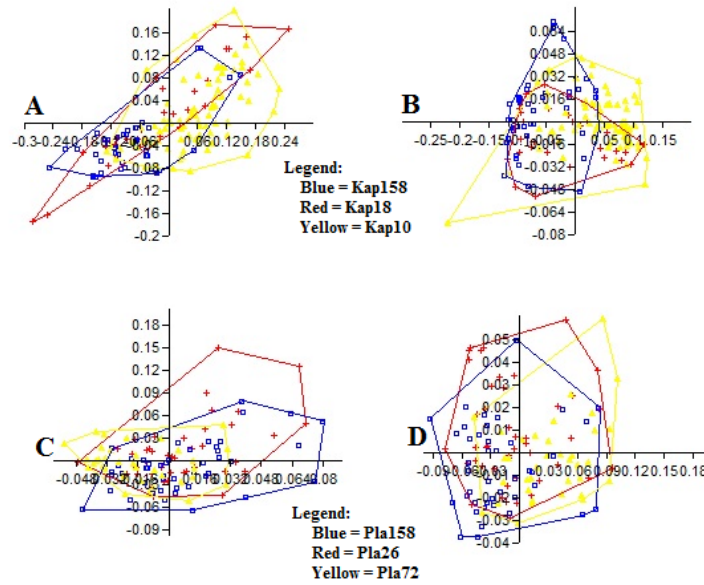


Fig. 4: CVA scatter plot showing the distribution of different populations that most different varieties of rice based on the mandible shape. Legend: A and B = variation in the left (A) and right (B) mandibles among Kap158, Kap18 and Kap10 populations; C and D = variation in the left (C) and right (D) mandibles among Pla158, Pla26 and Pla72 populations.

Table 2: Results of MANOVA test for significant variation in the shape of the left and right mandibles among populations of white stem borer.

		Wilk's Lambda	df1	df2	F	p(same)
Between Kap158 and Pla158 populations	Left	0.8291	9	63	1.443	0.1894
	Right	0.8628	10	65	1.034	0.4257
Between Kum26 and Pla26 populations	Left	0.8248	9	81	1.912	0.06171
	Right	0.8590	10	78	1.28	0.2564
Between Kap158, Kap18 and Kap10 populations	Left	0.5929	18	288	4.778	2.536E-09
	Right	0.5264	20	298	5.636	2.439E-12
Between Pla158, Pla26 and Pla72 populations	Left	0.7520	18	230	1.957	0.01298
	Right	0.6131	20	222	3.076	2.493E-05

Principal component analysis in the normalized elliptic Fourier descriptors of the left and right mandible showed 9 and 10 significant principal components respectively. The first principal component which account for the highest variability in the shape of the mandible contributes 62.68% of the overall shape variation in the left mandible and 59.66% of the variation in the right mandible. Tables (3 and 4) show the % variance contributed by each principal component in the left and right mandibles respectively. Specific shape variables in the left and right mandibles as explained by each of the significant principal component were shown in Table (5) and Fig. (5).

Boxplot was also used in order to visualize the reconstructed mandible shape of each of the seven populations of white stem borer. Analysis on the population distribution was done based on the overall variation in the shape of mandible. The principal component scores were further analyzed using the nonparametric version of one way ANOVA called Kruskal-Wallis test. The results of Kruskal-Wallis test done in each of the significant principal components were shown in Table (6) and Figs.(6&7).

Table 3: The Eigenvalues and percentage variance explain by each significant principal component for the overall, symmetrical and asymmetrical shape variation in the left mandible.

Principal Component	Eigenvalue	Proportion (%)	Cumulative (%)	Total Variance
Overall				2.883E-02 (100%)
1	1.81E-02	62.6789	62.6789	
2	2.26E-03	7.8269	70.5058	
3	1.63E-03	5.6531	76.1589	
4	9.91E-04	3.4368	79.5957	
5	7.91E-04	2.7445	82.3402	
6	6.21E-04	2.1522	84.4924	
7	4.60E-04	1.5968	86.0891	
8	4.50E-04	1.5596	87.6488	
9	3.86E-04	1.34	88.9888	
Symmetrical				1.951E-02 (67.66%)
1	1.50E-02	76.642	76.642	
2	1.03E-03	5.2961	81.9381	
3	8.92E-04	4.5746	86.5127	
Asymmetrical				9.323E-03 (32.34%)
1	4.67E-03	50.1324	50.1324	
2	9.55E-04	10.2412	60.3736	
3	7.75E-04	8.3088	68.6824	
4	6.47E-04	6.9406	75.623	
5	3.47E-04	3.722	79.3449	
6	2.95E-04	3.1629	82.5079	
7	2.74E-04	2.9346	85.4424	

Table 4 : The Eigenvalues and percentage variance explain by each significant principal component for the all, symmetrical and asymmetrical shape variation in the right mandible.

Principal Component	Eigenvalue	Proportion (%)	Cumulative (%)	Total Variance
Overall				1.626E-02 (100%)
1	9.70E-03	59.6557	59.6557	
2	1.35E-03	8.3046	67.9603	
3	9.21E-04	5.6615	73.6218	
4	5.51E-04	3.3898	77.0115	
5	4.84E-04	2.9764	79.988	
6	4.21E-04	2.5886	82.5765	
7	3.10E-04	1.9083	84.4849	
8	2.79E-04	1.7147	86.1995	
9	2.50E-04	1.5351	87.7347	
10	2.20E-04	1.3501	89.0848	
Symmetrical				1.207E-02 (74.19%)
1	8.92E-03	73.9595	73.9595	
2	9.20E-04	7.6229	81.5824	
3	4.83E-04	4.0008	85.5833	
Asymmetrical				4.198E-03 (25.81%)
1	1.31E-03	31.1621	31.1621	
2	6.06E-04	14.4246	45.5867	
3	4.47E-04	10.6552	56.2419	
4	3.79E-04	9.0358	65.2777	
5	3.36E-04	8.0126	73.2904	
6	1.81E-04	4.3025	77.5928	
7	1.73E-04	4.1175	81.7104	
8	1.37E-04	3.2747	84.985	

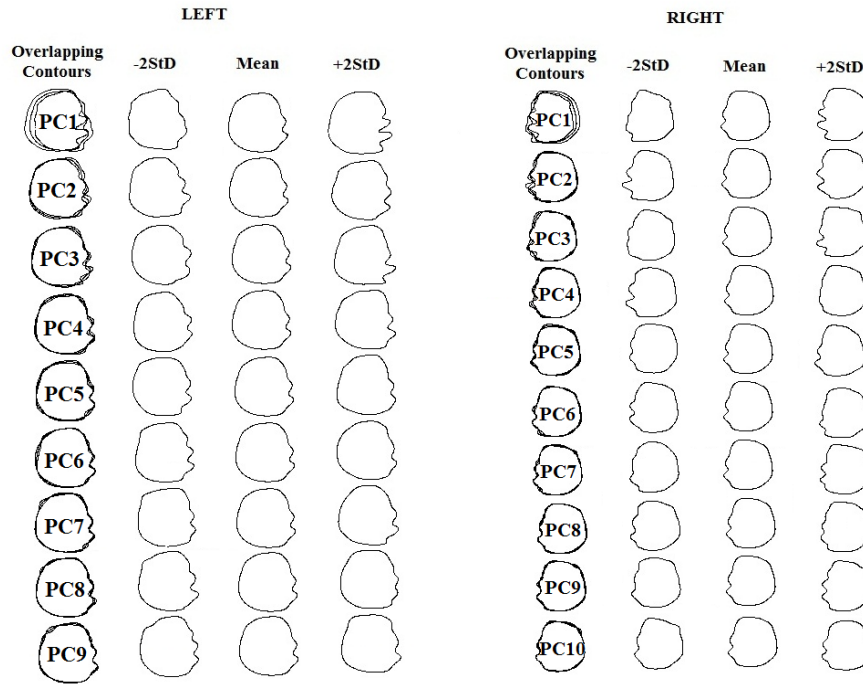


Fig. 5: Overall shape variation in the left and right mandibles of white stem borer *Scirpophaga innotata* (Walker, 1863) explained by each significant principal component.

Table 5: Percentage of variance and overall shape variation in the left and right mandibles as explained by each of the significant principal component.

PC	%	LEFT	%	RIGHT
	Variance		Variance	
1	62.68%	Variation in the site of attachment of the mandible to the head, distinctiveness of teeth, number of teeth and protrusion of teeth.	59.66%	Variation in the site of attachment of mandible to the head, distinctiveness of the teeth, number of teeth and length of teeth.
2	7.83%	Variation in the basal angle, position of the teeth and length of teeth.	8.30%	Variation in the site of attachment of mandible to the head, length of the teeth and basal angle.
3	5.65%	Variation in the position of the teeth and the length of teeth.	5.66%	Variation in the site of attachment of mandible to the head, position of the teeth and length of teeth.
4	3.44%	Variation in the basal margin and the site of attachment of mandible to the head.	3.39%	Variation in the distinctiveness of the teeth and length of the teeth.
5	2.74%	Variation in the site of attachment of mandible to the head and the protrusion of the teeth.	2.98%	Variation in the site of attachment of mandible to the head and length of the teeth.
6	2.15%	Variation in the site of attachment of mandible to the head and the distinctiveness of the teeth.	2.59%	Variation in the site of attachment of mandible to the head and the position of the teeth.
7	1.60%	Variation in the site of attachment of mandible to the head and the exterior angle.	1.91%	Variation in the basal margin and the distinctiveness of the teeth.
8	1.56%	Variation in the length of the teeth and the exterior angle.	1.71%	Variation in the site of attachment of mandible to the head, length of the teeth and exterior angle of the mandible.
9	1.34%	Variation in the basal margin and the basal angle.	1.54%	Variation in the distinctiveness of the teeth and the exterior angle of the mandible.
10			1.35%	Variation in the site of attachment of mandible to the head and the basal angle.

Table 6: Results of Kruskal-Wallis test for the significant differences in the mandible shape among different populations of white stem borer based on each significant principal component. Lower matrix – Left mandible, Upper matrix – Right mandible.

		Kap158	Kap18	Kap10	Kum26	Pla158	Pla26	Pla72
PC1	Kap158		0.04662	0.000673	0.000284	0.4353	0.000143	0.005784
	Kap18	0.0268		1.93E-12	0.02205	0.2324	0.002752	0.1929
	Kap10	0.0103	9.94E-11		1.63E-15	1.62E-12	6.56E-13	9.21E-07
	Kum26	0.03754	0.7505	7.75E-12		0.1824	0.2959	0.001644
	Pla158	0.08525	0.6103	1.82E-09	0.7869		0.01821	0.03527
	Pla26	0.01939	0.5683	3.42E-11	0.4937	0.2555		0.000254
	Pla72	0.01575	0.6124	3.03E-11	0.4354	0.1519	0.8798	
PC2	Kap158		0.8972	0.4756	0.4119	0.1091	0.07355	0.341
	Kap18	0.7792		0.2415	0.2769	0.0424	0.01972	0.2079
	Kap10	0.00206	0.00106		0.8272	0.3516	0.2783	0.9475
	Kum26	0.02385	0.02969	0.09946		0.2406	0.24	0.8618
	Pla158	0.494	0.6816	0.002665	0.06119		0.9188	0.3666
	Pla26	0.1876	0.3514	0.005019	0.155	0.4801		0.2532
	Pla72	0.2006	0.5055	0.006675	0.1561	0.4692	0.9452	
PC3	Kap158		0.005175	0.2937	0.001878	0.6162	0.6945	0.001624
	Kap18	0.9018		1.39E-05	0.3108	0.002166	0.001141	0.4029
	Kap10	0.765	0.7063		2.03E-06	0.9272	0.9032	1.36E-06
	Kum26	0.01867	0.00236	0.001325		0.000163	0.000168	0.6253
	Pla158	0.1896	0.07105	0.06773	0.2135		0.8351	0.000346
	Pla26	0.07083	0.02554	0.01934	0.399	0.6724		0.000145
	Pla72	0.08754	0.02549	0.02957	0.4173	0.7796	0.8907	
PC4	Kap158		0.06429	0.2609	0.009799	0.1893	0.9913	0.1437
	Kap18	0.7792		0.002167	0.608	0.4481	0.06468	0.6245
	Kap10	0.3786	0.06429		7.87E-05	0.008079	0.2783	0.006193
	Kum26	0.8816	0.551	0.245		0.1269	0.01109	0.2286
	Pla158	0.8182	0.407	0.2486	0.9556		0.1768	0.8418
	Pla26	0.2577	0.04515	0.4429	0.1228	0.1473		0.1264
	Pla72	0.4161	0.3644	0.001755	0.0913	0.04126	0.0025	
PC5	Kap158		0.7214	0.2596	0.00157	0.706	0.01662	0.5031
	Kap18	0.03858		0.5665	0.000723	0.9646	0.05598	0.827
	Kap10	0.1627	0.1938		2.19E-07	0.585	0.03174	0.6305
	Kum26	0.4659	0.000649	0.001938		0.000331	1.98E-07	0.000298
	Pla158	0.724	0.005866	0.02161	0.6764		0.05653	0.9054
	Pla26	0.5661	0.08468	0.4154	0.08668	0.2339		0.04039
	Pla72	0.2645	0.3087	0.9954	0.01611	0.07268	0.5036	
PC6	Kap158		0.4984	0.3245	0.1313	0.1313	0.006701	0.5031
	Kap18	0.3579		0.8705	0.3513	0.3513	0.0166	0.8455
	Kap10	0.07286	0.2444		0.4022	0.4022	0.00837	0.9931
	Kum26	0.006394	0.04644	0.3773		0.9968	0.1047	0.3323
	Pla158	0.09046	0.2727	0.942	0.3733		0.1047	0.3323
	Pla26	0.07953	0.4853	0.7886	0.1663	0.8922		0.02816
	Pla72	0.592	0.8019	0.1876	0.01711	0.2259	0.2145	
PC7	Kap158		0.4984	0.3245	0.1313	0.1313	0.006701	0.5031
	Kap18	0.9374		0.8705	0.3513	0.3513	0.0166	0.8455
	Kap10	0.9528	0.9917		0.4022	0.4022	0.00837	0.9931
	Kum26	0.9264	0.8425	0.8945		0.9968	0.1047	0.3323
	Pla158	0.8974	0.8833	0.9959	0.7505		0.1047	0.3323
	Pla26	0.2808	0.2659	0.1274	0.1561	0.1691		0.02816
	Pla72	0.2727	0.1862	0.1311	0.164	0.1214	0.851	
PC8	Kap158		0.7369	0.07885	0.4908	0.1035	0.8528	0.9071
	Kap18	0.8974		0.08504	0.7929	0.05953	0.8385	0.8904
	Kap10	0.5048	0.5889		0.2017	0.8666	0.04465	0.09005
	Kum26	0.6004	0.467	0.1768		0.1161	0.7297	0.809
	Pla158	0.8008	0.5116	0.2507	0.8023		0.01297	0.03527
	Pla26	0.03225	0.003247	0.000384	0.03024	0.01316		0.996
	Pla72	0.4125	0.1124	0.06206	0.5496	0.3644	0.1283	
PC9	Kap158		0.8241	0.5429	0.06638	0.8727	0.1467	0.8973
	Kap18	0.7156		0.5	0.02966	0.753	0.1093	0.7755
	Kap10	0.4678	0.05818		0.001371	0.133	0.00664	0.2894
	Kum26	0.02907	0.000462	0.03872		0.03566	0.4471	0.04478
	Pla158	0.851	0.4923	0.1758	0.003185		0.1851	0.8381
	Pla26	0.811	0.7118	0.08003	0.000555	0.7582		0.1289
	Pla72	0.2982	0.4633	0.01565	4.39E-05	0.1691	0.2817	

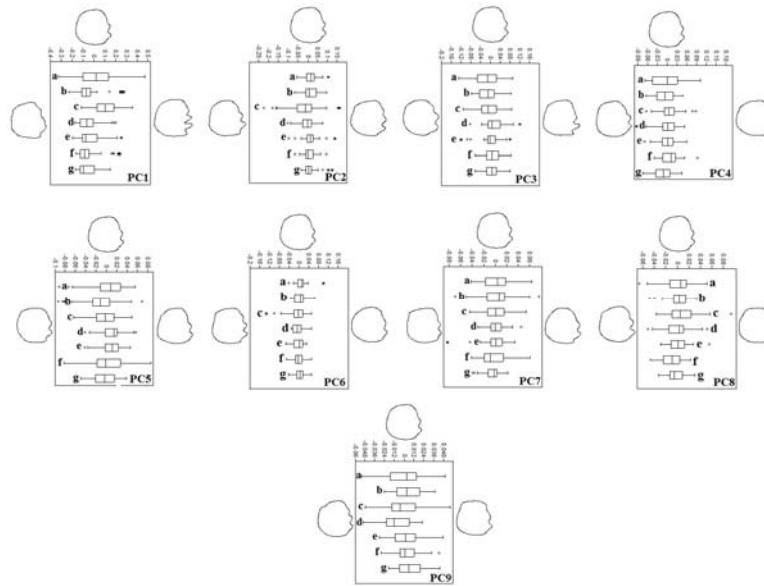


Fig. 6: Box and whiskers plot of the significant principal component in the left mandible of white stem borer *Scirpophaga innotata* (Walker, 1863). Legend: a = Kap158, b = Kap18, c = Kap10, d = Kum26, e = Pla158, f = Pla26, g = Pla72.

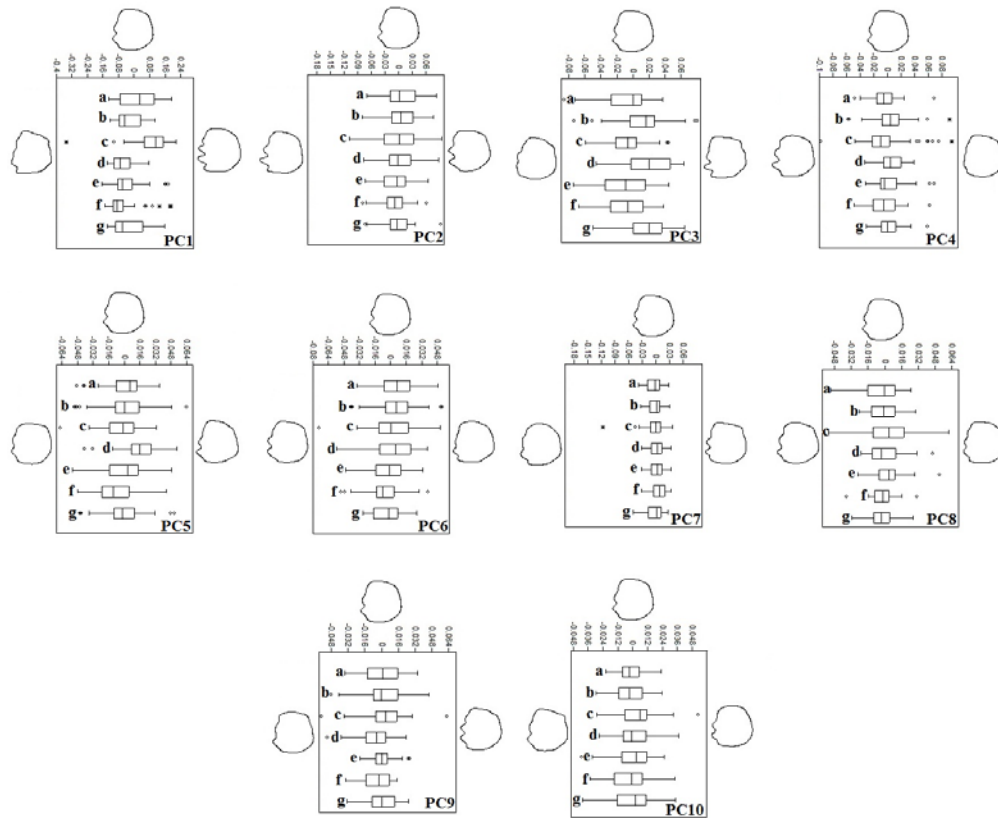


Fig. 7: Box and whiskers plot of the significant principal component in the right mandible of white stem borer *Scirpophaga innotata* (Walker, 1863). Legend: a = Kap158, b = Kap18, c = Kap10, d = Kum26, e = Pla158, f = Pla26, g = Pla72.

The overall sources of mandible shape variation in the white stem borers were separated into symmetrical and asymmetrical group. A total of 67.66% of the shape variation observed in the left mandible was contributed by the symmetrical variation while 32.34% was due to asymmetrical variation in the shape of the mandible. In the right mandible, 74.19% and 25.81% of the observed shape variation was explained by symmetrical and asymmetrical variation in the mandible respectively. Tables (7 & 8) and Fig. (8). show the percentage of variance contributed by each principal component

as well as the specific shape variables in the mandible explained by each of the significant principal component.

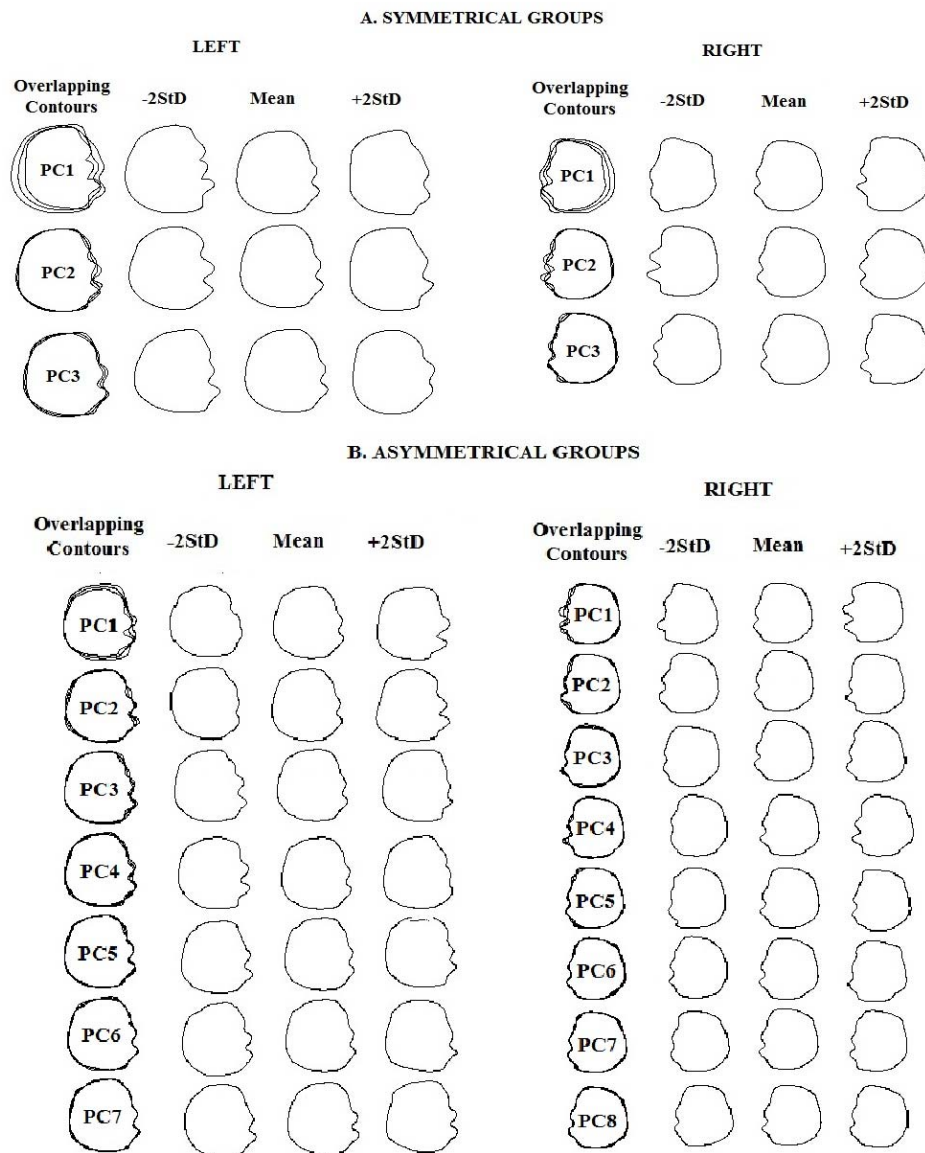


Fig. 8: Reconstructed mandible shape variation in white stem borer *Scirpophaga innotata* (Walker, 1863) explained by each significant principal component. (A) Symmetrical variations in the left and right mandibles and (B) asymmetrical variations in the left and right mandibles.

Table 7: Percentage of variance and symmetrical shape variation in the left and right mandibles as explained by each of the significant principal component.

PC	% Variance	LEFT	% Variance	RIGHT
1	76.64%	Variation in the site attachment of mandible to the head, number of teeth, basal margin and protrusion of teeth.	73.96%	Variation in the site attachment of mandible to the head, basal margin, distinction of teeth and length of teeth.
2	5.30%	Variation in the basal angle and position of teeth.	7.62%	Variation in the site attachment of mandible to the head, number of teeth, length of teeth and basal angle.
3	4.57%	Variation in the basal margin and length of teeth.	4.00%	Variation in the site attachment of mandible to the head, basal angle and number of teeth.

Table 8: Percentage of variance and asymmetrical shape variation in the left and right mandibles as explained by each of the significant principal component.

PC	% Variance	LEFT	% Variance	RIGHT
PC1	50.13%	Variation in the basal margin, external margin, distinction of teeth and protrusion of teeth.	31.16%	Variation in the site of attachment of the mandible to the head, number of teeth, distinction of teeth and length of teeth.
PC2	10.24%	Variation in the attachment site of the mandible to the body and length of teeth.	14.42%	Variation in the site of attachment of the mandible to the head and basal angle.
PC3	8.31%	Variation in the length of the teeth and basal angle.	10.66%	Variation in the site of attachment of the mandible to the head and basal margin.
PC4	6.94%	Variation in the length of the teeth, site of attachment of the mandible to the head and basal angle.	9.04%	Variation in the site of attachment of the mandible to the head, distinction of teeth and length of teeth.
PC5	3.72%	Variation in the basal angle and protrusion of the teeth.	8.01%	Variation in the site of attachment of the mandible to the head and basal angle.
PC6	3.16%	Variation in the basal margin.	4.30%	Variation in the site of attachment of the mandible to the head and basal angle.
PC7	2.93%	Variation in the site of attachment of the mandible to the head.	4.12%	Variation in the basal angle.
PC8			3.27%	Variation in the attachment of the mandible to the head.

The reconstructed shape of the mandible generated from each of the principal component showed that the variation in the mandible is attributed by the variation in the teeth and the attachment site of the mandible. The mandible attachment site ranges from having a rounded to pointed shape. Different aspect in the teeth of the mandible also showed great impact in the variation of the mandible shape. The mandibles have teeth that are either indistinct or apparent, have two or more in number, and diminutive or extensive. Looking at the box and whiskers plot, the distribution of different populations based on the first principal component separated the Kap10 population from the rest of the populations. The shape of the mandible characterized by the Kap10 population have pointed mandible attachment site and with the highest number of teeth that are more prominent and extensive compared to the other populations. Kruskal-Wallis test verified the observed variation in the shape of the mandible as described by the reconstructed mandible contour. Shape variation in the mandible of Kap10 population was highly significant among all the populations of white stem borer. Furthermore, the right mandible showed more significantly varied shape between populations compared to the left mandible.

Differences in the geographical location and variety of rice plant host were the two factors being considered which caused variation in the shape of mandible among populations of white stem borer. However, the result of this study showed non-significant shape variation in the mandible among populations taken from different localities. Kruskal-Wallis test showed non-significant p value ($p > 0.05$) between Kap158 and Pla158 populations and between Kum26 and Pla26 populations. Analysis was done between populations of white stem borers taken from different localities but host the same variety of rice plant to determine the influence brought by geographical

variation in the observed mandible shape variation. Conversely, the result of Kruskal-Wallis test conducted between populations that host different variety of rice showed significant p value ($p < 0.05$) which indicates significant shape variation in the mandible among these populations.

The adult rice stem borers are highly mobile in nature and could disperse from one location to another. The environment inhabited by the host plants can change dramatically over short geographic distances. Variation in environmental conditions can result in some populations being more suitable for insect growth, survival and reproduction (Kittelson, 2004). These may explain observations on the pest behaviour that showed intraspecific variation in the damage to same rice variety in different geographic locations (Zahiri *et al.*, 2006). The spatial structure of populations and its ecological and evolutionary consequences are central issues in theoretical ecology and evolution, and there has been a great interest in the spatial patterns of insect–host plant interactions (Gotthard *et al.*, 2004). According to Khiaban *et al.* (2010), the recognition of the intraspecific variation in damage caused by rice stem borers, its nature and scope, may aid in our understanding about the pest and would allow us to predict the spatial and temporal occurrence of its problems.

Mandible shape variation influenced by disparity of rice varieties is in turn related to the varying resistance level of each rice variety against the stem borer attacks. Thousands of different rice varieties from the world collection at the International Rice Research Institute have been screened for stem borer resistance and these studies revealed that the ability of the plant to resist rice stem borer attacks was derive from several morphological characteristics of rice plant (Chandler, 1967). In the study conducted by Patanakamjorn (1965), the results showed that the plant resistance against striped stem borer *Chilo suppressalis* Walker varied widely in thirty three varieties of rice and was consistent in most of the varieties in all the three field experiments conducted in three different planting seasons. Various morphological and anatomical plant characters were correlated with stem borer susceptibility. Tall plants with wide leaves and thick stems were more susceptible to stem borer damage. Whereas varieties with vascular bundles arranged closer than the width of the larval head offered resistance to larval boring (Chandler, 1967).

Hosseini *et al.* (2011) made emphasis that rice genotypes with lower plant height, higher tiller and thinner stem diameter is more tolerant against striped stem borer. Moreover, varieties with thick layers of sclerenchyma tissue were usually less heavily infested than those with thin layers (Chandler, 1967). Thickening of cell wall results from deposition of cellulose and lignin making the vascular bundle tightly enclosed by epidermis and cell wall becomes thicker. Thus, resistance is directly correlated with the solidness of the stem. As a consequence the tissue becomes tougher and more resistant to the tearing action of mandibles. The stem borer must possess stronger mandibles with larger and more define teeth to successfully penetrate its plant host that have thick layers of sclerenchyma tissue. This phenomenon might be responsible for the evolution of the mandible shape in response to the different rice varieties it hosts. Aside from the morphological characteristics of the rice plant, various internal factors which interfere in the development and survival of the larvae within the host could also contribute to plants resistance against rice stem borers. Factor such as silica content and other chemical may be involved in the ability of the rice plant to resist stem borer's attacks. Being the main feeding organ of rice stem borer, the mandibles are always exposed to different biochemical effects present in the plant resulting in its continuous wearing and tearing. The silica particles in the plant interfere with larval feeding, often causing excessive mandible wear which resulted in

functionless mandibles so that insects like stem borer would die without feeding. As cited by Alegre *et al.* (2011), amorphous silicon present in most species of Poaceae, plant family of *Oryza*, can serve as harsh abrasive that may cause a tearing or even to the extent of loss of mandibular teeth during feeding process (Schoonhoven *et al.*, 2006) and only those species with larger mandibles can overcome such defence (Klapper and Denno, 2001). A similar effect of silica on stem borer larvae was recorded when larvae were reared on varieties containing different percentages of silica (Khan *et al.*, 1991).

CONCLUSION

Outline based geometric morphometric analysis in the shape of the mandible in white stem borer larvae showed variation among different populations. Principal component analysis showed that the variation was mainly due to the difference in the attachment site of mandible to the head and the different aspect of its teeth. Geographical variation and differences in the variety of rice plant host were argued to cause mandible shape variation among populations. However, this study showed that the variation in the mandible shape among populations were only attributed by the differences in the rice variety that host white stem borer's larvae as shown by the significant ($p < 0.05$) results obtained from statistical analysis. Non-significant variations were obtained from geographically separated populations that host the same variety of rice. Varying resistance level of different rice variety against the stem borer attacks was associated to the shape variations in mandible. Morphological and biochemical characteristics of different rice varieties were responsible for the plant host resistance. More resistant variety have mandible with more define and extended teeth compared to the less resistant varieties.

ACKNOWLEDGEMENT

The authors would like to thank Prof. Sharon Rose M. Tabugo and Mr. Michael Muhmin E. Manting for the assistance during the analysis of data and especially to the DOST SEI (ASTHRDP) for the scholarship grant.

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