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MORPHOMETRICS OF MALE GENITALIA IN RICE YELLOW STEM BORER *SCIRPOPHAGA INCERTULAS* POPULATIONS FROM TELANGANA

Athulya R[1,2], A P Padmakumari[3*], T Uma Maheswari[1] and A Kalaisekar[4]

[1]College of Agriculture, PJTSAU, [3]ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad 500030, Telangana, India
[2]Institute of Wood Science and Technology, Bengaluru 560003, Karnataka, India
[4]ICAR-Indian Institute of Millets Research, Rajendranagar, Hyderabad 500030, Telangana, India
*Email: padmakumariento@gmail.com (corresponding author): ORCID ID 0000-0003-2318-2540

ABSTRACT

Morphometric characterization of male genitalia is an important aspect for studying intraspecific variation in Lepidoptera. In this study, size variation in the male genitalia of rice yellow stem borer (YSB) *Scirpophaga incertulas* (Walker) collected from nine locations through sex pheromone traps was examined. Measurements of male genitalial parts like uncus length (UL), uncus width (UW), valva length (VL), valva width (VW), saccus length (SL), saccus width (SW), aedeagus length (AD) were considered. Significant size variation was observed among the populations, and the measurements SL, SW, VL, VW, UL, UW and AD contributed to about 16% to location variability. The results showed that all characters except valva width was significantly different (p≤0.05) among the nine populations.

Key words: Rice *Scirpophaga incertulas*, male genitalia, intraspecific variations, uncus, valva, saccus, aedeagus, morphometrics, principal, components, cluster analysis

Rice (*Oryza sativa* L.) is an ancient and the most genetically diversified cereal crop. Rice harbours more than 100 species of insect pest. Among various stem borers reported from rice, yellow stem borer (YSB), *Scirpophaga incertulas* (Walker) shares >70% of stem borer pests (Gururaj Katti et al., 2011). YSB is majorly distributed in Southeast Asia, China, Afghanistan and India. Grain yield losses of 3 to 95% due to YSB damage had been reported in India (Muralidharan and Pasalu, 2006). It is the most destructive and widely occurring insect pest of rice that attacks all stages of the crop from the seedling stage to maturity (Bandong and Litsinger, 2005). The adult moths are always observed resting on the rice leaf blade in a field in the early hours of the day. It was also observed that there is a variation in the size of the adult moths. Hence, a study on the morphometrics in understanding the intraspecific variations is necessary. Morphometrics has become a widely used technique in taxonomy, with a special emphasis to geometric morphometrics (Rohlf and Marcus, 1993; Adams et al., 2004; Zelditch et al., 2004; Mutanen et al., 2007; TÓth et al., 2014). Geometric morphometrics proved to be suitable to uncover and quantify small intra- or interspecific differences (Garnier et al., 2005: TÓth and Varga, 2011). Insect genitalia are important for species identification and are key traits for the evolution of reproductive isolation. Among the many parameters, morphometric characterization of male genitalia is an important aspect for studying intraspecific variation in Lepidoptera. Male genitalia are widely recognized as the most stable and informative of all morphological structures. Male genitalia are considered one of the most important diagnostic traits in insect systematics (Tuxen, 1970). Carlos Cordero and Joaquín Baixeras (2015) opined that in Lepidoptera, male genitalia have been generally considered more informative in taxonomical terms than female genitalia. This study on the morphometrics of the male genitalia of adult moths of *S. incertulas* includes six populations collected from Telangana state, and one each from Palakkad, Kerala; Raichur, Karnataka; and Ludhiana, Punjab.

MATERIALS AND METHODS

Adult male moths of *S incertulas* were collected from nine locations viz., Medak (MDK), Warangal (WGL), Nizamabad (NZD), Jagtial (JGL), Rangareddy (RGD), Mehabubnagar (MBR), Palakkad (PKD), Raichur (RCR) and Punjab (PJB) using sex pheromone traps (YSB Detector^R Pheromone chemicals, Hyderabad) installed in the rice fields. Abdomen from adult male moths was detached by pressing it slightly with the help of forceps. Then, it was placed in 10% potassium hydroxide (KOH) for digestion overnight at room temperature. After digestion, the abdomen was transferred to a cavity block filled with distilled water and softly scrubbed hairs, scales, and the debris from the abdomen using camel hair brush.Then, the abdomen was placed into a watch glass with 20% ethanol and cleaned again. The abdomen was then placed into a cavity block containing 70% ethanol for final cleaning and genitalia were separated from the abdomen. For separation of male genitalia from the abdomen, inter segmental membrane of last abdominal segment was cut and the aedeagus was removed from the genitalia by holding the base of the valva with a pair of forceps and detached the aedeagus with another pair. The detached genitaliawas shifted to a cavity block containing absolute ethanol. Genitalia were kept in proper position and valva were stretched and spread apart by using fine needle and forceps. Valva were held in a stretched position for 1-2 min and then, glass chips were placed over it for 2 hr. The stretched genitalia were mounted in Canada balsam using clean microscope slide and covered with cover slide. The slides of male genitalia (N=10 from each location) were prepared and measurements of genitalial characters like uncus length (UL), uncus width (UW), valva length (VL), valva width (VW), saccus length (SL), saccus width (SW) (Fig. 1a) and aedeagus length (AD) (Fig. 1b) were taken with LYNX ISH500 5.0 MP camera mounted on Lawrence and Mayo Stereozoom microscope. Descriptive statistics was calculated using MS-EXCEL for all chosen traits. The analyses viz., univariate and multivariate ANOVA and principal component analysis (PCA) were done to determine intraspecific variation. All these tests were carried out using R package 'ggpubr'.

RESULTS AND DISCUSSION

The morphometrics was analysed from the nine field populations. The measurements of seven parameters pertaining to male genitalia were observed and given in Table 1 and Fig. 1. Descriptive and univariate analysis were conducted for each character. Tukey's Honest Significant Difference (HSD) Test was carried out to test differences among the means of locations. The results showed that all characters except valva width was significantly different ($p \leq 0.05$) among the nine populations. The length and width of uncus, length of valva, length and width of saccus and aedeagus length differed significantly among nine locations. The UL ranged from 604.80 (MBR) to 699.40µm (RCR). RCR population showed highest mean uncus length (699.40 µm), uncus width (378.20 µm) and saccus length (294.80 µm). The mean UW of MDK, WGL, JGL, RGD, MBR and PJB populations were not significantly different from each other, but different from RCR and JGL populations. The highest and lowest mean UW were reported from RCR (378.20 µm) and MDK (283.60µm) populations, respectively. The mean VL of populations ranged from 810.40 (JGL) to 949.40 µm (NZD). There were no significant differences among the populations in case of VW. The mean value of VW ranged from 443.40 to 497.20 µm. Saccus length varied from 224 µm (JGL) to 294.80 µm (RCR). Aedeagus length varied from 1171.60 µm (JGL) to 1590.20 µm (NZD). The study on *Drosophila mojavensis* supports a role for aedeagus size in the male-female mating interaction, with a secondary role for aedeagus shape.

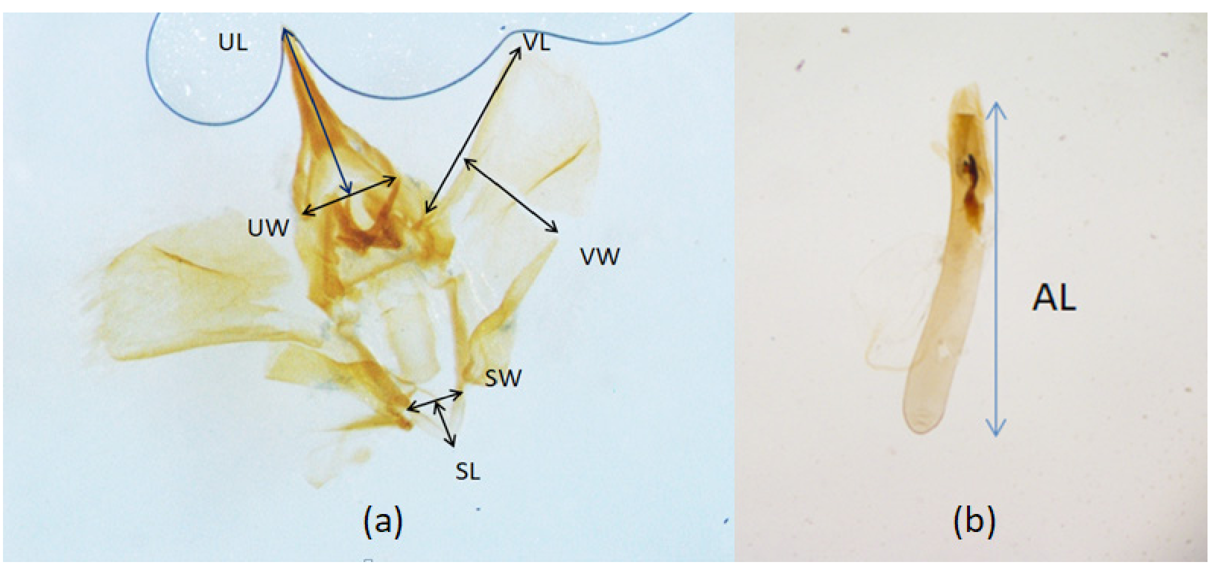

Fig. 1. *S. incertulas*. a. Male genitalia of. B. Aedeagus
(UL-uncus length, UW-uncus width, V-valva length, VW-valva width, SL-saccus length, SW-saccus width, AD-aedeagus length)

Table 1. Morphometrics of the male genitalia from populations of *S. incertulas*

Location with its Geographical Coordinates	Measurement (µM)						
	UL	UW	VL	VW	SL	SW	AD
MDK 17.5192° N, 78.2784° E	624.80^{bc}	283.60^{b}	890.20^{abc}	443.40	231.80^{bc}	150.80^{ab}	1242.40^{de}
WGL 18.0122° N, 79.5990° E	687.20^{ab}	311.00^{b}	917.80^{ab}	469.80	242.00^{bc}	185.20^{ab}	1344.00^{c}
NZD 18.67°N 77.9°E	650.20^{abc}	322.80^{ab}	949.40^{a}	478.80	237.80^{bc}	154.60^{ab}	1590.20^{a}
JGL 8.7895^{0} N 78.9120^{0}E	640.40^{abc}	300.80^{b}	810.40^{c}	448.60	224.00^{c}	151.20^{ab}	1171.60^{e}
RGD 17.3201° N 78.3939° E	641.20^{abc}	319.60^{b}	946.20^{a}	497.20	243.40^{bc}	161.60^{ab}	1455.60^{b}
MBR 17.2731° N 81.844° E	604.80^{c}	316.80^{b}	868.80^{abc}	480.60	249.00^{abc}	161.60^{ab}	1254.20^{d}
PKD 10.8088° N 76.1894° E	699.20^{a}	331.80^{ab}	835.80^{bc}	479.80	277.80^{ab}	168.60^{ab}	1222.60^{de}
RCR 16.2044° N 77.3341° E	699.40^{a}	378.20^{a}	938.80^{a}	489.20	294.80^{a}	184.60^{a}	1378.00^{bc}
PJB 30.9041°N, 75.8066° E	678.00^{ab}	317.80^{b}	943.60^{a}	483.40	236.40^{bc}	147.20^{b}	1382.60^{bc}
Min	604.80	283.60	810.40	443.40	224.00	147.20	1171.60
Max	699.40	378.20	949.40	497.20	294.80	185.20	1590.20
Mean	658.36	320.27	900.11	474.53	248.56	162.82	1337.91
SD	32.06	24.32	48.90	16.85	21.63	13.34	123.93
CV	0.05	0.08	0.05	0.04	0.09	0.08	0.09
F value	4.87*	4.36*	7.96*	0.72 NS	4.35*	3.64*	58.70*

Means with same alphabets within a column not significantly different; *Significant at ($p \leq 0.05$); (UL-uncus length, UW-uncus width, V-valva length, VW-valva width, SL-saccus length, SW-saccus width, AD-aedeagus length)

In natural populations, mating failure based on aedeagus size could serve as an important reproductive isolating mechanism resulting in failed insemination attempts after both the male and female show a willingness to mate (Richmond, 2014). The effect of location on the male genitalia morphometric characters was tested with MANOVA. The results registered that the effect of location was highly significant (Pillai's Trace 2.759, p=5.237e-09; Hotelling's -Lawley Trace 23.653, p= 2.2e-16; Roy's Largest Root 18.39, p= 2.2e-16; Wilks' Lambda 0.0032425, p= 2.2e-16) on the seven measured parameters of male genitalia suggesting significant differences between nine locations (Table 2).

PCA plots of male genitalia morphometric variables of first two dimensions with linear projections were made to see the major contributing characters for variability among male moth populations occurring in nine different locations. SL, SW, VL, VW, UL, UW and AD contributed about 16 percent to location variability in male moths (Fig. 2). Valval length, valval width and aedeagus length were highly correlated to each other. On the other hand, saccal length and saccal width were also correlated with uncus length and uncus width but correlation was weak as compared to valval and aedeagus correlation. VL, VW and AD were not correlated with SL, SW, UL and UW. Male

Table 2. MANOVA table for location effects on male genitalia morphometrics

Effect		Value	F Value	Num DF	Den DF	Pr (>F)
Locations (8)	Pillai's Trace	2.759	2.927	56	252	5.237e-09***
	Hotelling's Trace	23.653	11.947	56	198	< 2.2e-16***
	Roy's Largest Root	18.390	82.754	8	36	< 2.2e-16***
	Wilk's Lambda	0.003	5.657	56	166.87	< 2.2e-16***

Signif. codes 0 '****'; 0.001 '***'; 0.01 '*'

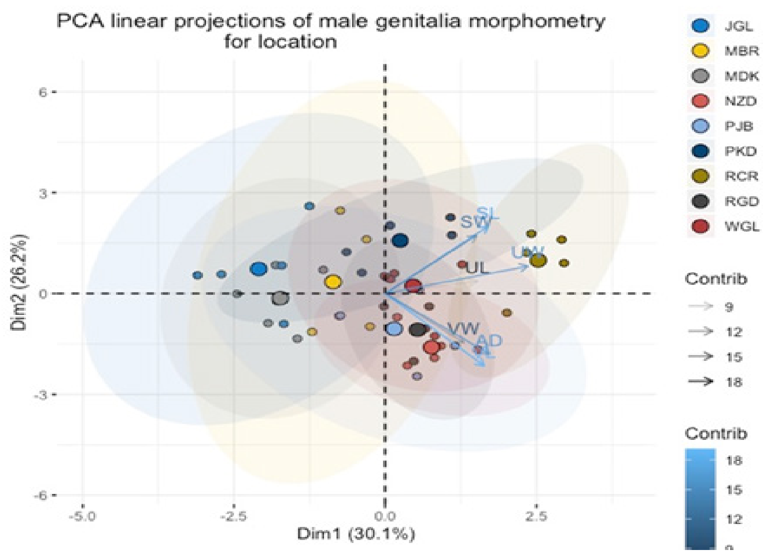

Fig. 2. PCA linear projections of male genitalia morphometry for locations

genitalia morphometry had very strong influence on the variability of male moth populations of WGL, NZD, RGD and PKD locations while the male populations of MBR, MDK and JGL were less influenced by the male genitalia morphometry. The saccal morphometry had high influence in variability of populations of PKD whereas uncus morphometry had high influence on populations of WGL. Contrarily, valval and aedeagus morphometry had high influence in variability of populations of WGL, NZD and PJB. Populations of JGL, MBR and MDK were completely devoid of any influence by male genitalia morphometry to their location.

The significant differences observed in the size of the male genitalia parameters were taken into consideration. Parts of male genital exhibited geographic variability, but it is not related to the geographic distance. It is also noted that there were no structural differences in male genitalia of *S. incertulas* populations from nine locations under study. Generally, the genital characters are known to be more stable and informative in taxonomic aspect than the wing patterns (Shapiro and Porter 1989; Mutanen, 2005). During the past half of the century, morphometrics has become a widely used method in taxonomy, with special respect to geometric morphometrics (Mutanen et al., 2007). Geometric morphometrics proved to be suitable to uncover and quantify small intra- or interspecific differences. Until now, no study has reported quantitative comparison genital traits of rice yellow stem borer. Some authors have concluded that variability may occur in the genital structures, mainly of the males, due to their complexity, which attains great importance for the taxonomy of a species (Singh-Pruthi,1926; Lent and Jurberg, 1966,). Due to possible correlation between size and shape, species with remarkable variation in overall size may consequently exhibit larger genital variability. Mutanen and Kaitala (2006) opined that genital shape rather than size that tends to evolve rapidly. So size explorations should not be ignored in genital variation studies. The results suggest that genital morphology is not constant and should therefore be used with caution in lepidopteran taxonomy, agreeing with the observations of Mutanen et al. (2007) on the male genital variation in a moth *Pammene luedersiana* (Lepidoptera: Tortricidae). Several studies show that genitalia vary a lot within species (Goulson, 1993; Eberhard et al., 1998; House and Simmons, 2003). Intraspecific variation in genital morphology reflects the outcome of the interaction between genes and environment (Willmore et al., 2007).

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AUTHOR CONTRIBUTION STATEMENT

APK and TU conceived and designed research. AR conducted experiments. APK, TU facilitated collection of populations, contributed new reagents and analytical tools. AR, APK and AK analyzed the data. AR and APK wrote the manuscript. All authors read and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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