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Forewing venation pattern and genital plate structure in a non-outbreak population of the Rice Black Bug (*Scotinophara coarctata* Stål) from Lala, Lanao del Norte, Philippines

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Abstract. Rice Black Bug (RBB) is small and cryptic pest species that are believed to have caused significant yield loss of rice production in the Philippines. Various studies pointed to the variability in the ecology and morphology of populations of RBB. The present study reports on the variability in the wing venation pattern and shapes of the male and female genital plates structure in a non-outbreak population of RBB from Lala, Lanao del Norte. Results showed immense variability in wing venation patterns particularly in the type and number of marginal cells and the number and pattern of longitudinal veins. The marginal cells were either closed or open. On the other hand, the longitudinal veins were sometimes crossed, looped forming a closed circular pattern or at times show a complex criss-crossed pattern. Landmark-based geometric morphometric analyses of the shapes of the male and female genitalia showed variations in the length-width aspect ratio, concavity of the anterior margins and shapes of the posterior and anterior protrusions. The results may imply a high genetic diversity in this population of RBB despite present in a non-outbreak level. This diversity may have implications to the control and management of this rice insect pest.
Keywords: Genitalia, wings, phenotypic plasticity.

Introduction. Rice remains to be a primary staple food for Filipinos and this explains the fact that more than 2.3 million hectares of arable lands in the Philippines has been devoted to its cultivation (Barrion-Dupo et al 2007). However, rice production in the Philippines has its own share of problems as a significant amount of yield is lost to insect pests such as the rice black bug (RBB) (*Scotinophara* spp.) Barrion et al (2007) described species of RBB that are known to attack rice plants in all of its growth stages. Increase in RBB densities in farm lands in the country have been reported by Redondo et al (2007). However, an important observation is that RBB is irregularly distributed in the Philippine archipelago and show abundance in only some areas (Demayo et al 2007).

The state of RBB taxonomy in the Philippines has been particularly confusing due to the fact that populations of this insect pest show immense variability in both reproductive and non-reproductive traits (Barrion et al 2007). There are various reasons for the occurrence of infra-specific variability in populations of pests. These include phenotypic plasticity, which is the ability of an organism with a given genotype to change its phenotype in response to changes in its habitat, or to its movement to a different habitat. Phenotypic plasticity is an emerging theme in studies that are aimed to understanding the true nature of pests and in determining relevant population control measures (Whitman & Agrawal 2009).

Virtually any morphological trait can show intra and inter-populational variation and certain degree of phenotypic plasticity (Whitman & Agrawal 2009; Schlichting & Pigliucci 1998). RBB have reportedly been shown to show certain degree of infra-specific variation in wing morphology among eighteen populations from the Philippines (Demayo et al 2007). Disparities in the shapes of the head and pronotum among these populations were also established (Torres et al 2010). The study also showed that geographically distant populations sometimes show higher morphological similarities than geographically close populations. Variation in supposedly stable characters such as the male and female genitalia was also observed (Demayo et al 2007). The studies established the degree of variability among reproductive and non-reproductive traits, important in the formulation of a proper pest management program.

One concept in phenotypic plasticity related to the study of population density is termed as density dependent phase polyphenism. Jannot et al (2009) stated that density-dependent phase polyphenism occurs when changes in densities during the early stages of the insect's development results in remodeling of their phenotypes. Morphological differences between outbreak and non-outbreak populations can be brought about by density-dependent phase polyphenism, which has been found to occur in populations of the migratory locust (*Locusta migratoria*) (Chapuis et al 2008). This is important since this study focuses two morphological structures in a non-outbreak population of RBB. Such studies have been particularly important in understanding variation in crop performance in relation to attacks by insect herbivores (Karban et al 2004).

The studies on RBB have so far been focused on variations among populations of the insect pests. However, studies should also be conducted to determine patterns of morphologic variation having a low density population of RBB since density dependent phase polyphenism hypothesizes that low density populations contain low levels of phenotypic diversity. Thus, this study was conducted to determine variability in wing (non-reproductive trait) and genital morphology (reproductive trait) from a non-outbreak population from Lala, Lanao del Norte, Philippines.

Materian and Methods

Collection of sample. Seventy-nine samples of RBB were collected from a rice field in Lala, Maranding, Lanao del Norte (Figure 1). Rice black bugs were hand-picked as they were generally located at the base of the plant. Thirty-five male and forty-four females were collected from the site. The RBB's were placed in a container half filled with 32% ethanol and then properly sealed.

Figure 1. Geographical presentation of the sampling site in Lala, Lanao del Norte Philippines.

Segregation of sexes. Each sample captured was segregated by their sex by observation under a stereo microscope. RBB's sex is distinguishable by means of their genital plates found at the tip of the anterior of the body. Male RBB's are distinguishable by means of a saddle shaped abdominal tip of the anterior of the body (Figure 2). Female RBB's are distinguishable by means of two triangular protruding warts located in the inferior tip of the abdomen (Figure 2). Each RBB specimen was placed in separate eppendorf tubes and half filled with 32.5% ethanol and then properly labeled by sex and specimen number. Each of the male and female genital plates was photograph using a digital camera.

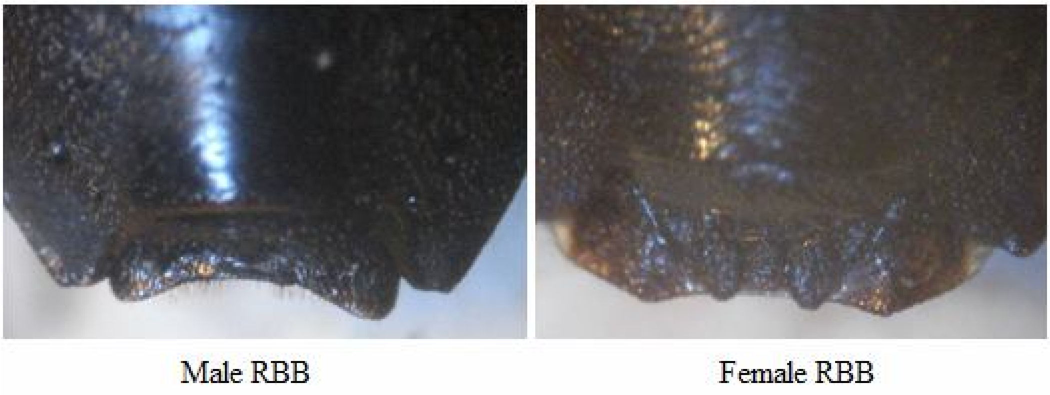

Figure 2. Male and female genital plates showing a saddle shaped abdominal tip for male and triangular warts for female.

Forewing separation and slide preparation. Forewings of the RBB were properly separated from the main body. A needle was inserted at the corium to expose the forewing and the right and left forewing was carefully separated from the body properly using tweezers. Right and left forewing of the RBB was mounted in one glass slide by pairs of six. One drop of glycerol solution was placed on the forewing to properly spread the hyaline portion of the wings. Another glass slide was placed on top of the wings and carefully pressed. The sides of the two compressed glass slides were sealed with a tape. Male and female wings were grouped, labeled and separated. The slide will be observed under a microscope and damaged wings were discarded from the samples.

Photo capture of forewings. Each glass slide containing the forewings was placed under a stereomicroscope. Images of each of the right and left forewing in both males and females was taken and labeled using a ten mega pixel camera (Figure 3). Images were transferred to the computer and labeled.

Figure 3. Sample of right and left forewing of RBB.

Outlining and comparison of wings. Photographed wings were edited using the Adobe Photoshop program. The pentool option was used to trace the outline of the corium and the hyaline portion of the wing. The longitudinal veins, closed and open marginal cells were also traced. Proper tracing of the angles and curves of the outlines were made. The brush choice in the stroke path option was used to enhance the outline. Each group was saved and properly labeled (Figure 4).

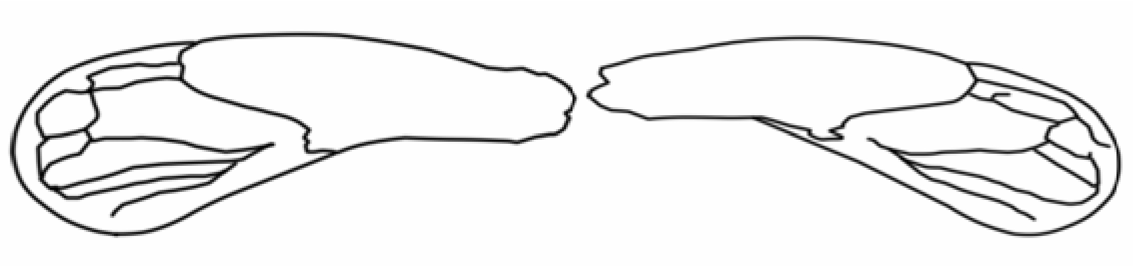

Figure 4. Sample outline of right and left RBB wing using Adobe Photoshop.

Wings were then observed by using the Microsoft Office Picture Manager program. Both female and male wing venation of the hyaline portion of the wing were observed and compared with the other wing venation of the other organism regardless whether it is the left or right wing. Wings will be grouped based on their wing venation pattern. Wings are grouped based on absolute differences and similarities. Exact images of the wing venation patterns were grouped together. Minor variation of the longitudinal and marginal cells was considered as a separate group.

Land-marking of genital plates. Two-dimensional Cartesian coordinates of 16 landmarks for male genital plates and 17 landmarks for female genital plates were digitized by tpsDig ver.2 software (Rohlf 2004) (Figure 5). Three replicates were obtained to reduce the measurement error of both male and female sample specimen (Dvorak et al 2005). Male and female samples were both run separately. X and Y coordinates of the landmark points were obtained by the software to be used for further analysis.

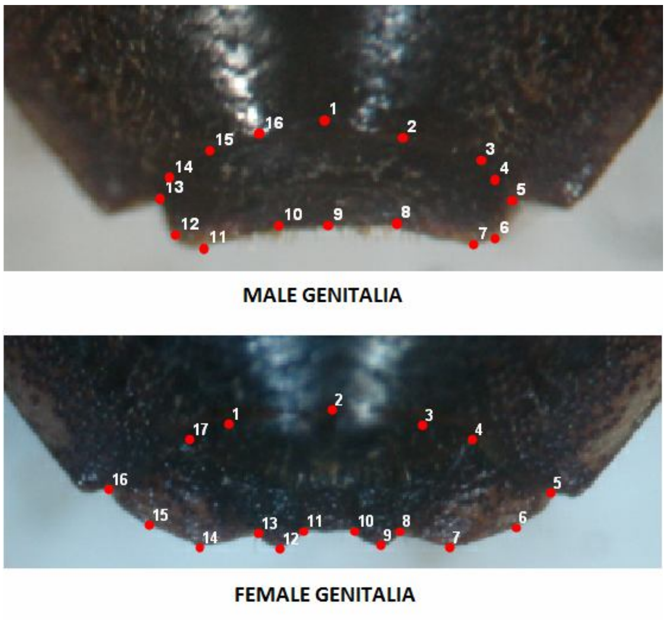

Figure 5. Designated landmarks and pseudolandmarks of the male and female genitalia.

Geometric morphometric analysis. Thin-plate splines were used in order to graphically illustrate patterns of shape variations based on the landmarks which represent the transformation of the reference to each specimen (Bookstein 1991). Male and female genital plates were run separately. Files were copied three times. The TpsDig2 program was used to landmark the variation in the shape of the genital plates (Rohlf 2004). The TpsUtil program was used to build tps file and make links files. Multivariate analysis was done using the Palaeontological Statistics (PAST) software (Hammer et al 2001). The relative warp analysis was the performed using the tpsRelw program version 1.46 (Rohlf 2004). Usually the most informative are the first and second scores of the relative warps (Hammer et al 2001).

Results and Discussion

Forewing. Based on the RBB's R+L wing structure, the R+L wing venation pattern outlines were evaluated for the similarities and differences. The venation patterns were based on the number of longitudinal veins including also its branches, the closed and open veins. Angles and shapes of the wing venation pattern were also included as basis for grouping. Forty-four female samples obtained eighty-eight wing types while thirty-five male samples obtained seventy groups giving an overall total of one hundred fifty eight wing venation patterns.

Assessment of all the venation patterns of both male and female RBB showed no similarities. Longitudinal veins showed variation in accordance to their number, angle and shape pattern. Forked longitudinal wings were also noted in their variation. The number of longitudinal veins ranged from 3–6. The observed branching ranged from 1-3 forked veins per wing. Marginal cells were either opened or closed. The number of closed marginal cells ranged from 1 vein to 6 veins per wing. Open marginal cells ranged from 1 vein to 3 veins per wing. Wings also differed in the number of layers for marginal cells. Likewise, the wing venation patterns were observed to vary in shape (Figure 6).

Marcus (2001) made an account of crossvein-less wings in *Drosophila melanogaster* and established the crucial role of the heat shock factor (Hsp90) in crossvein formation. This gene is sensitive to environmental perturbations and causes variations in crossvein pattern. Marcus (2001) posited that the lack of a stable wing pattern may indicate the inability of a species to regulate different factors in the development of the crossvein. Milkman (1960) stated the development of the posterior crossveins in *Drosophila melanogaster* is brought about by a polygenic system. Further studies are needed to determine the genetic basis of the development of the RBB wing.

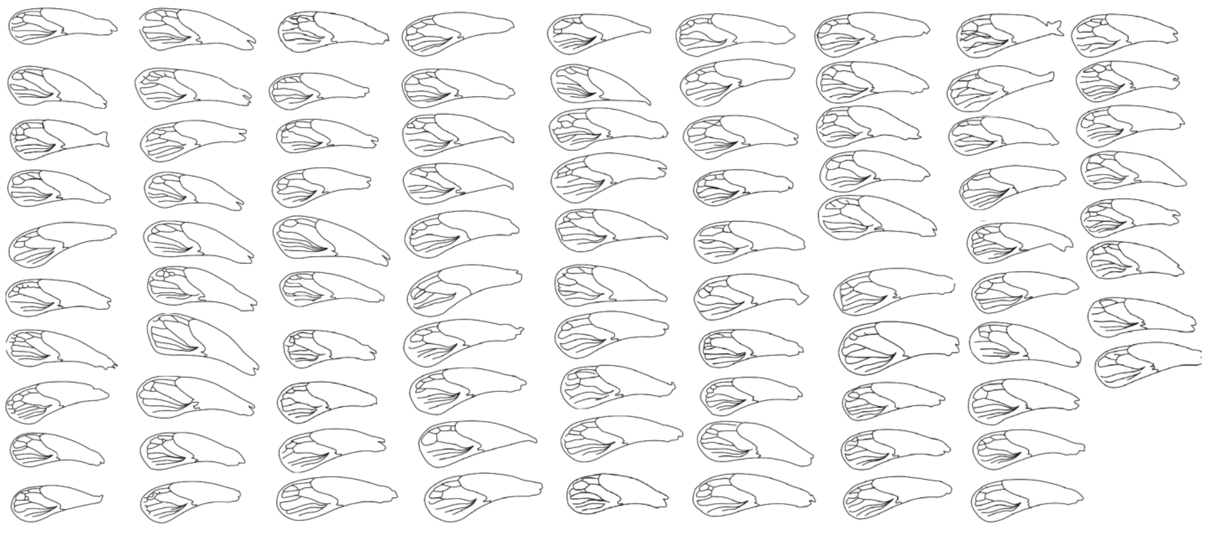

Figure 6. Variation in forewing venation patterns among the RBB, *Scotinophara* sp. from Lala, Lanao del Norte, Philippines.

Genital plates. Seventeen landmarks on the female genital plate of forty-four RBB samples indicated five significant relative warps (Table 1). Histogram indicated the samples came from one population (Figure 7). Relative warp one (RW1) indicated variation on the length width ratio (RW1>30%). RW1, RW2, RW3 and RW4 in the female RBB indicated the significant difference in the size and shape of the genital warts of the plate. RW2, RW4 and RW5 indicated the variation is attributed to the concavity of the anterior portion of the genital plate. Convex to those positively correlated (+) and concave to those negatively correlated (-). RW2 indicated the differences of RBB plates with symmetrical shaped genital plates with those that are asymmetrical, with the positively correlated indicating directional asymmetry in the female RBB. RW3 indicated the variation is attributed to the bilateral asymmetry of RBB genital plates.

Sixteen landmarks on the male genital plate of thirty-five RBB samples indicated four significant relative warps (Table 1). Histogram also indicated the samples came from one population (Figure 7). RW1 indicated variation of the genital plate is attributed to difference in their length-width ratio. RW2 and RW4 indicated the variation is attributed to the concavity of the anterior portion of the genital plate. Convex to those positively correlated (+) and concave to those negatively correlated (-). Variation is attributed to the bilateral asymmetry of the genital plates in RW3.

Table 1

Descriptions of the male and female genital plates as shown by their relative warps

RW	Female	Male
1	Variation is attributed to the difference in the length-width aspect ratio including shape and size of the triangular warts at the posterior margin. Variation in the lateral and postero-lateral margin of the margin of the genital plate. Illustrates asymmetry in the genital plate structures.	Variation of the genital plate is attributed to the difference in their length-width ratio and the antero-lateral margin.
2	Variation is attributed to the concavity of the anterior margin of the genital plate. Convex (+) and concave (-). Differences included the symmetrically shaped (-) genital plates with those that are asymmetrically shaped (+) which also are indicative of directional asymmetry. Differences including shape and size of the triangular warts at the posterior margin.	Variation is attributed to the concavity of its anterior margin. Convex to those positively correlated (+) and concave to those negatively correlated (-). Variations include difference in the antero-lateral margin of the genital plate.
3	Variation is attributed to the bilateral asymmetry of RBB genital plates with differences in the size and size of the triangular warts at the posterior margin.	Variation is attributed to the bilateral asymmetry of RBB genital plates.
4	Variation is attributed to the differences of RBB plates with symmetrical shaped (-) genital plates with those that are asymmetrically shaped including shape and size of the triangular warts at the posterior margin.	Variation is attributed to the concavity of its anterior portion. Convex to those positively correlated (+) and concave to those negatively correlated (-). Describes the asymmetry of the shape of the genital plate.
5	Variation is attributed to the concavity of its anterior portion. Convex to those positively correlated (+) and concave to those negatively correlated (-).	

The results suggest the presence of morphological diversification on a sample of RBB collected which may indicate possible genetic differentiation (Torres et al 2010). The relative warps analysis indicated possible polygenic control on the genital plate. Sasabe et al (2007) studied the biometric analyses on experimental F_1 and backcross population of carabid beetles of the *Carabus* sp. and revealed that inheritance of genital morphology is polygenic. A separate study by Monti (2008) using elliptic fourier analysis also showed the morphological differences among the genitalia of two closely related Noctuid moths *S. latifascia* and *S. descoinsi* which showed the trait is controlled by polygenic system. Further studies are still needed if the methods in these studies can be related to the RBB species. Further investigation is still needed if the variability indicates speciation or is influenced by either infrapopulation or geographic clines.

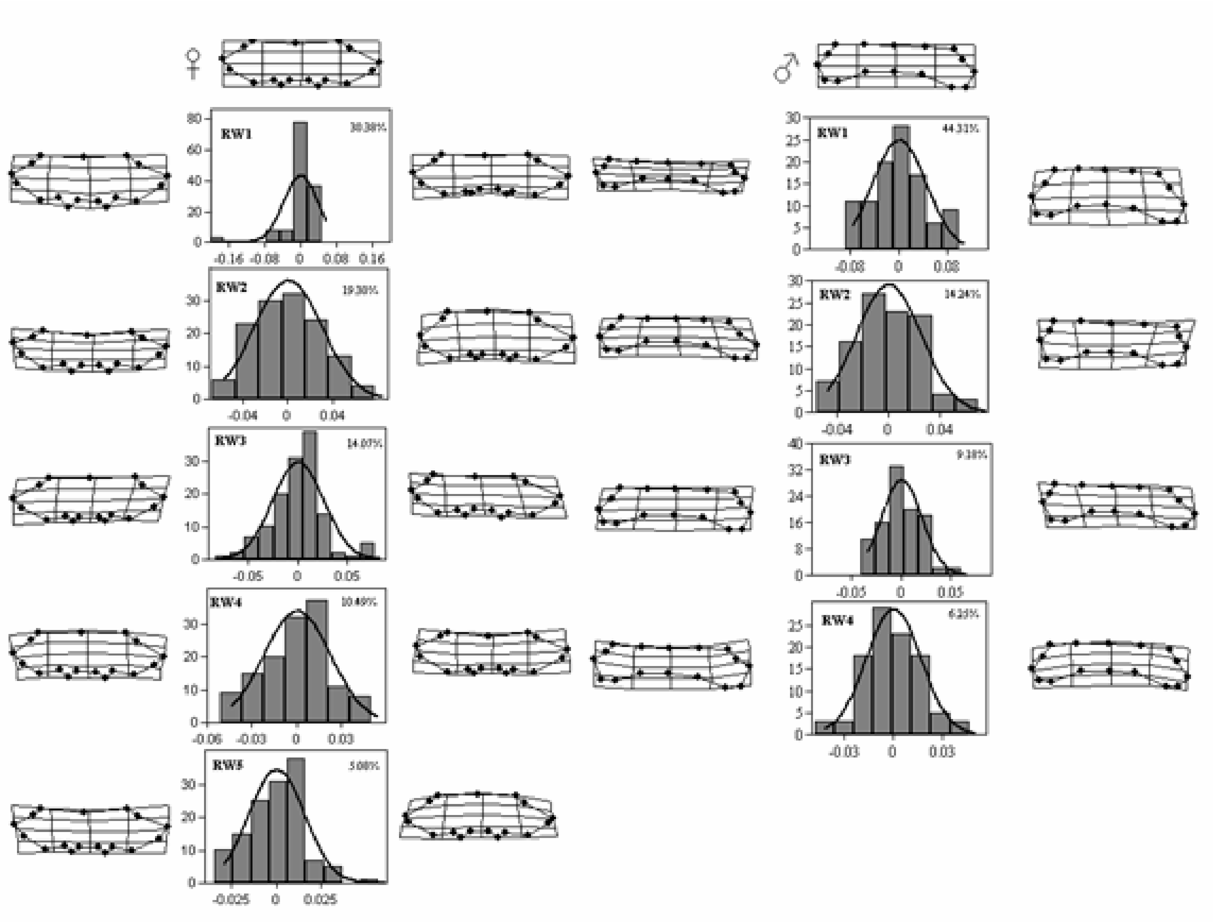

Figure 7. Summary of the geometric morphometric analysis showing the consensus morphology (uppermost panel) and the variation of genital plates among female and male populations of RBB found in Lala, Lanao del Norte.

In a non-outbreak population of the RBB, the samples showed immense significance in the inter-population variability on the wing venation pattern and the genital plate structure. The result supports the study that the RBB has a large degree of variability among its wing morphology but does not support the idea on the robustness of both male and female genital plates as evidenced by significantly high percentages of RW1 and RW2.

In the Philippines, populations of RBB can be found at varying densities (Litsinger 2007). Some populations can reach outbreak levels even at times when other populations are at low densities. Demayo et al (2007) found variations in morphological characters among outbreak and non-outbreak populations of RBB. This is advantageous to the concept of density dependent phase polyphenism as the results showed high variability in a low density population.

Knowledge on insect pest population variability and development of several resistant pests would indicate the success or failure of future pest management strategies (Torres et al 2010). Variability of the morphological trait would serve as a basis for further phylogenic studies thereby providing insights in the influence of phenotypic plasticity and the development of new pest management approaches. Thus, the overall variability on wing venation pattern and genital plate structure variation in this study is an informative contribution on the management of RBB as pests.

Conclusion. A total of 79 RBB samples showed great inter-individual variability among its wing venation pattern and genital plate structures. Male (35) and female (44) RBB samples differed in the type and number of marginal cells, number of longitudinal veins and the number of forks of the longitudinal veins. Some marginal cells differed in their shapes and are either closed or open. Longitudinal veins sometimes cross, run loops or show complex venation systems. Geometric analysis was used to determine the variability on the shape of the male and female genital plates. Relative warp analysis of male (RW1&RW2:>49%) and female (RW1&RW2:>58%) genital plates showed variations related to their length-width ratio and concavity of their anterior margins.

Even in low population densities, the RBB has shown great variability in both of its important morphological structures. The genitalia may have been controlled by a polygenic system while the wings may have been a result of an inability of the RBB to regulate its morphological structure against perturbation. This study implies that the RBB is among the insect species that is hard to implement a proper pest management plan due to the high variability of its important morphological characters.

It is recommended that further studies be conducted for the probability that genetic structuring among RBB has influenced the tendency of differing population densities and not strictly the influence of environmental factors.

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