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Variation in hindwing size and shape of *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae)

A. Moreno & A. Viloria

Abstract

Plutella xylostella (Linnaeus, 1758) has been claimed to be the most widely distributed Lepidoptera species. However, it is a highly polymorphic species, and there is evidence that what is known as *P. xylostella* is a species group, so it is possible that all records do not correspond to this species. Aim of the work was to evaluate the variability of hindwing size and shape of Venezuelan *P. xylostella* specimens and explore possible differentiation between groups of individuals. For this purpose, 126 specimens belonging to the entomological collections of the Museo del Instituto de Zoología Agrícola Francisco Fernández Yépez (MIZA, Central University of Venezuela) and the Instituto Nacional de Investigaciones Agrícolas (INIA), were sampled.

Variation patterns of wing size and shape were studied by means of landmark-based geometric morphometrics. The analysis revealed significant differences in wing shape between three groups separated by differences observed in the bifurcation of the M vein. These differences may be due to intra- or interspecific variation; there is not enough evidence to discern. In Venezuela, there are three morphotypes of *P. xylostella*, separated based on differences in shape and size of their hindwings. It is important to clarify the identity of these morphotypes by combining these results with further information such as molecular data and immature morphological taxonomy.
KEY WORDS: Lepidoptera, Plutellidae, geometric morphometrics, insect pest, taxonomy, Venezuela.

Variación en el tamaño y la forma del ala posterior de *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae)

Resumen

Plutella xylostella (Linnaeus, 1758) ha sido señalada como la especie de Lepidoptera con la más amplia distribución. Sin embargo, es una especie altamente polimórfica, existiendo evidencia de que lo que se conoce como *P. xylostella* es en realidad un complejo de especies, en este sentido, es posible que no todos los registros correspondan a esta especie. El objetivo de este trabajo fue evaluar la variabilidad en el tamaño y la forma del ala posterior de especímenes de *P. xlostella* colectados en Venezuela, y explorar una posible diferenciación entre grupos de individuos. Para este propósito, se utilizaron 126 especímenes pertenecientes a las colecciones entomológicas del Museo del Instituto de Zoología Agrícola Francisco Fernández Yépez (MIZA, Universidad Central de Venezuela) y el Instituto Nacional de Investigaciones Agrícolas (INIA).

Los patrones de variación en el tamaño y la forma del ala fueron estudiados mediante morfometría geométrica de hitos discretos. El análisis reveló diferencias significativas en la forma del ala entre tres grupos separados por diferencias observadas en la bifurcación de la vena M. Estas diferencias pueden ser atribuidas a variación intra o interespecífica, no se cuenta con la suficiente evidencia para discernir. En Venezuela, existen tres morfotipos de *P. xylostella* separados con base en diferencias en el tamaño y la forma de las alas posteriores. Es importante esclarecer la identidad de estos morfotipos, combinando estos resultados con información adicional como datos moleculares y taxonomía de inmaduros.
PALABRAS CLAVE: Lepidoptera, Plutellidae, morfometría geométrica, insectos plaga, taxonomía, Venezuela.

Introduction

Plutella xylostella (Linnaeus, 1758), is considered the most universally distributed of all Lepidoptera and the main insect pest of brassicaceous crops worldwide. It requires US$ 1.0 billion globally in estimated management costs in addition to the crop's losses (TALEKAR & SHELTON, 1993). The high incidence of DBM has been explained by the absence of effective natural enemies and its capability to develop resistance to insecticides, as well as, to adapt to very different climatic conditions (SALINAS, 1986; TALEKAR & SHELTON, 1993), which makes it important to study from economic and biological perspectives.

Plutella xylostella is highly variable from morphological, biological and genetic standpoints (JUSTUS & MITCHELL, 1999; CHACKO & NAYARANASAMI, 2002; PICHÓN *et al*., 2006; LANDRY & HEBERT, 2013), which can lead to confusion and misidentifications. In Australia, it was thought that the genus *Plutella* was represented by a single introduced species, *P. xylostella.* However, LANDRY & HEBERT (2013) found two genetically different lineages of this taxon, indistinguishable from each other in external appearance. One corresponds to *P. xylostella,* and the second lineage was described as the new species *Plutella australiana* Landry & Hebert, 2013. Considering this information and the fact that South America has the highest taxonomic diversity of the genus, it is worthwhile looking into Venezuelan specimens, where only *P. xylostella* has been recorded.

Traditionally, wing venation has been used in taxonomic studies of Lepidoptera. Currently, geometric morphometric analysis of insect wings has shown to be a valuable tool for species discrimination (PERRARD *et al.,* 2014), being useful across different taxa and taxonomic levels (VILLEMANT *et al*., 2007; FRANCOY *et al.,* 2008, 2009, 2011; MARSTELLER *et al.,* 2009; MÁRQUEZ *et al.,* 2011; FERREIRA, 2014; JERATTHITIKUL *et al.,* 2014; PERRARD *et al.,* 2014). Geometric morphometric provides powerful tools to quantify phenotypic variation by employing homologous features in biological forms (BOOKSTEIN, 1991). These tools are able to detect subtle differences that may not be conspicuous in comparative morphology studies and even in a classical morphometric analysis (FERREIRA, 2014).

The aim of this study was to investigate the variations among hindwings of Venezuelan specimens identified as *P. xylostella,* through landmark-based geometric morphometrics analysis, to explore differences between groups of individuals.

Methods

Specimens and data collection

Specimens used in this study come from the entomological collections of the Museo del Instituto de Zoología Agrícola Francisco Fernández Yépez (MIZA, Universidad Central de Venezuela) and the Instituto Nacional de Investigaciones Agrícolas (INIA). A total of 126 individuals were classified either of three groups based on differences that were observed in M vein of hindwing (G1, G2 and G3, see Fig. 1). The overview of the specimens included in the analysis is given in Table I. The right hindwings of each specimen were removed with fine forceps and cleared to visualize the venation with 5% KOH watery solution for ten minutes, then washed with distilled water and transferred to 70% ethanol (ROGGERO & PASSERIN, 2005) with a drop of red China ink. Scales were removed with a bent pin on a vise before mounting the wings on microscope slides with 80% glycerin and secured with a coverslip. Photographs of each wing were taken with a Nikon D5200® controlled by Zerene Stacker AutoMontage® software. A set of ten landmarks covering the wing surface were selected and digitized on x and y coordinates with the TPSDig 2.16® software (ROLHLF, 2010). All the landmarks are the intersections of the wing veins, or at the wing edge (Fig. 2) and correspond to type I landmarks according to BOOKSTEIN (1991).

Table I.– Numbers of specimens by group and sex used in the analysis.

Group	Sex	Number of specimens
1	♂	28
	♀	25
2	♂	14
	♀	15
3	♂	24
	♀	20
	Total	**126**

GEOMETRIC MORPHOMETRICS ANALYSIS

The tps file with raw coordinates of landmarks were loaded into R software (R CORE TEAM, 2018) and then superimposed with gpagen function from geomorph v 3.0.6® package (ADAMS *et al.,* 2018), which performs a generalized Procrustes analysis to estimate shape variables and centroid size (CS). The shape variables were subsequently used for a np-MANOVA using 10K permutations (ANDERSON, 2001; COLLYER *et al.,* 2015; ADAMS *et al.,* 2018) with the advanced.procD.lm function from the abovementioned package (ADAMS *et al.,* 2018). Because wing size and sexual dimorphism may affect wing shape variation, log (CS) and sex were included in the construction of linear models. The *post hoc* pairwise comparisons of group means were also performed using the same number of random permutations. To visualize shape variation patterns, a Principal Components Analysis (PCA) was used and thin-plate spline (TPS) results were added to the axes of the scatter plot. Finally, differences in size were assessed by using a two-way ANOVA on CS in the software GraphPad prism 6® (SWIFT, 1997), considering the group and sex as factors and using Tukey test for *post hoc* pairwise comparisons.

Results

Centroid sizes among the three groups were significantly different (F_{sex}= 13.56, p= 0.0003; F_{group}= 4.990, p = 0.0083), but only four of the pairwise comparisons were significant (Fig. 3). Females belonging to G3 accounted for the highest CS value (2.843 ± 0.199 mm), followed by G2 females (2.721 ± 0.288 mm), while the lowest being accounted for G2 males (2.531 ± 0.225 mm, Fig. 3).

The first two principal components (PC) accumulated 68.2% of the shape variation. The scatter plot of these two PC (Fig. 4) showed that G1 and G3 were clearly separated into distinct groups, while G2 showed overlap with the other two groups. These inter-group shape changes are visualized along first PC axis and are found with the bifurcation of M vein (landmark 10), and the locations where R2 and M1 meet the edge (landmarks 1 and 2, respectively). Results of np-MANOVA (Table II) showed significant differences in shape between these three groups, although allometric and sexual dimorphism effects were significant, their contribution to the overall variation was low (Table II, R^2). Post hoc test showed significant differences between all possible pairwise comparisons (p < 0.01 in all cases), except for males and females belonging to the same group (p = 0.5 in all cases).

Table II.– Results of np-MANOVA performed on shape variables.

	DF	SS	MS	R^2	F	Z	p
log(CS)	1	0,05091	0,05091	0,08331	18,5319	4,4633	0,0001*
Sex	**1**	**0,00751**	**0,00751**	**0,01229**	**1,9487**	**1,9487**	**0,0294***
Group	2	0,22116	0,110578	0,3619	40,2506	7,6854	0,0001*
Sex*Group	**2**	**0,00459**	**0,002297**	**0,00752**	**0,836**	**1,3263**	**0,086**
Residual	119	0,32692	0,002747	0,5168			
Total	**125**	**0,61119**					

Discussion and conclusions

The geometric morphometrics analysis showed variation in wing size and venation shape between three groups of Venezuelan *Plutella xylostella* specimens. Wing size and shape differences found in other lepidopteran species have been explained by interspecific variation due to hostplant (KHIABAN *et al.,* 2010; CAÑA-HOYOS *et al.,* 2014), environmental heterogeneity (BAI *et al.,* 2016) and geographical variation (MOZAFFARIAN *et al.,* 2007; KHAGHANINIA *et al.,* 2011). However, all the specimens used in this study had the same hostplant (all specimens recorded in *Brassica oleracea* L.) and ordination patterns among localities were not observed.

It has been claimed that pest species show higher variability than those that are not harmful; they are characterized by high morphological and ecological variation, ability to invade unbalanced landscapes such as crops, insensitivity to chemical control, genetic instability and high adaptability (BECHYNÉ & BECHYNÉ, 1970). According to the available information regarding different aspects of *P. xylostella* biology, ecology and taxonomy (ROBINSON & SATTLER, 2001; PICHON *et al.,* 2006; SUBRAMANIAN & LÖEHR, 2006; ROUX *et al.,* 2007; JANSSEN *et al.,* 2008; LANDRY & HEBERT, 2013; JURIC *et al.,* 2017; KARIYAWASAN, 2018), this species meets the abovementioned features, therefore, it is possible that the observed variability in figure 4 represents a continuum of intraspecific variation.

Alternatively, the geometric assessment of insect wings had allowed for accurate taxonomical identification of moth and butterflies species (ROGGERO & PASSERIN, 2005; FERREIRA, 2014; JERATTHITIKUL *et al.,* 2014; PRZYBYTOWICZ *et al.,* 2015). Wing venation has been long used for insect identification and may reflect the evolutionary history with a potential effect of other factors such as body shape, climate, and mimicry selective pressures (PERRARD *et al.,* 2004). According to PERRARD *et al.* (2014), it is a taxonomically relevant marker combining the accuracy of quantitative characters with the specificity required for identification criteria. Hence, the possibility that similar species coexist in Venezuela with *P. xylostella* should not be discarded. The case of *Plutella australiana* (LANDRY & HEBERT, 2013; KARIYAWASAN, 2018) represents evidence that *P. xylostella* is indeed a species-group.

Species identification allows attributing information to recognizable entities thus it is a first and crucial step in biological studies (PERRARD *et al.,* 2014). Misidentifications reduce the utility of applied investigations (such as pest control), because of different results regarding the same taxonomical entity (different species identified as the same) or similar results regarding different entities (one species identified as two or more). Venezuelan specimens identified as *P. xylostella* showed variability that may be taxonomically important, therefore it is important to clarify its source. It could be useful to link these results with additional information such as molecular data and immature morphological taxonomy.

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*A. M.
Museo del Instituto de Zoología Agrícola Francisco Fernández Yépez
Facultad de Agronomía
Universidad Central de Venezuela
Maracay, Aragua State
VENEZUELA / *VENEZUELA*
E-mail: abimoreno16@gmail.com
https://orcid.org/0000-0002-7516-6115

A. V.
Centro de Ecología
Instituto Venezolano de Investigaciones Científicas
Carretera Panamericana km 11
Apartado 20632
Altos de Pipe, Miranda state
VENEZUELA / *VENEZUELA*
E-mail: sebastianviloriacarrizo@gmail.com
https://orcid.org/0000-0002-5747-4747

*Autor para la correspondencia / *Corresponding author*

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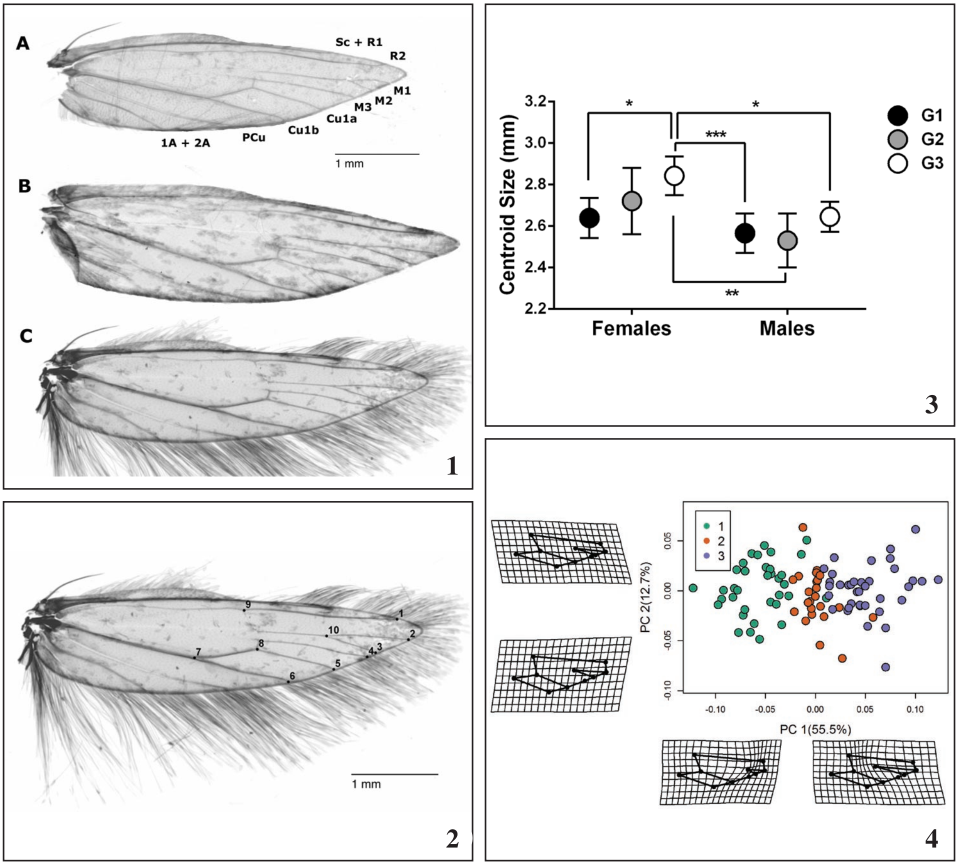

Figs 1-4.– 1. Groups of *P. xylostella* used in size and shape comparisons. Classification was done by differences observed in M vein. A: Group 1, M1 and M2 stalked over a section longer than the separate section of both veins. B: Group 2, M1 and M2 stalked over a section of the same length as the separate section of M1 and longer than the separate section of M2. C: Group 3, M1 and M2 stalked over a section shorter than the separate section of M1 and the same length as the separate section of M2. Condition in Group 3 matches the description of the species according to Baraniak (2007). **2.** Right hind wing of male *P. xylostella* showing the 10 chosen landmarks. **3.** Mean centroid size with IC 95% of hind wing for each group of *P. xylostella*. Asterisks indicate statistically significant differences between two groups. **4.** Scatter plot showing the variation in hind wing shape of three groups of *P. xylostella* along the first two principal components. Transformation grids illustrate changes from consensus shape along axes.