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Identity and genetic structure of eggplant fruit and shoot borer, *Leucinodes orbonalis* Guenée (Lepidoptera:Crambidae) populations in the Philippines inferred from morphological traits and *COI* sequence data

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ABSTRACT

Morphological and molecular analysis of 15 Philippine populations of eggplant fruit and shoot borer (EFSB), *Leucinodes orbonalis* Guenée were conducted to determine if these populations are constituted singly by *L. orbonalis* or by different species and to assess the level of variability among them. Morphometric analysis of five genital traits of 850 male adult EFSB from field populations and analysis of the *COI* gene sequence of 879 F_1 EFSB larvae from 15 main eggplant-producing provinces identified all individuals as belonging to *L. orbonalis*. Principal Component Analysis of five morphometric genital characters revealed high similarity among the EFSB populations regardless of geographic location. Thirteen (13) sequence variants (haplotypes) were identified, with one haplotype predominant and widespread throughout the country. The remaining haplotypes occurred rarely and differed from the widespread haplotype by one mutation. Overall, the EFSB populations from Philippines exhibited low nucleotide and haplotype diversity, indicating low genetic diversity. Topologies from a maximum likelihood tree indicate all thirteen haplotypes cluster in a single clade with EFSB populations from India and other South-East Asian countries. Further analysis with the Generalized Mixed Yule Coalescent (GMYC) method classified the different haplotypes into a single GMYC entity. Combined with morphometric analysis, differences between haplotypes are not suggestive of any subspecies. Negative values of Tajima's D and Fu's F_s tests combined with the phylogenetic analysis and overall low genetic diversity of Philippine populations support the hypothesis that EFSB is not endemic but introduced to the Philippines.

Introduction

The eggplant fruit and shoot borer (EFSB), *Leucinodes orbonalis* Guenée, is an insect widely distributed in the tropics and subtropics of Africa and Asia (Srinivasan, 2009). It is a serious pest of eggplant (*Solanum melongena* L.), where the larvae bore and feed inside the fruits and tender shoots. Damaged fruit produces holes leading to feeding tunnels, often filled with larval excrement (Alam et al., 2003; Srinivasan, 2009), causing even slightly damaged fruit to be unfit for consumption and marketing. In the Philippines, eggplant is an important commodity which accounts for 14% of the total volume of vegetables in 2016 (Philippine Statistics Authority, 2017). Damage from this pest presents the biggest threat to Philippine eggplant production, reducing yields by 51% to 73% (Francisco, 2009). Due to this, farmers have resorted to frequent application and the overuse of insecticides to kill larvae before they enter the fruit. Surveys of farmers have reported that almost all rely on frequent to heavy spraying of insecticides, spraying every other day (60–80 times per season), to control this pest (Francisco, 2007). Due to the large impact of *L. orbonalis* infestation, a genetically engineered eggplant was developed by the India-based Maharashtra Hybrid Seed Company (Mahyco). Mahyco used a *Bacillus thuringiensis cry1Ac* gene that had already been widely used in Bt cotton to make eggplant resistant to *L. orbonalis* infestation, presenting a solution to the pest problem as well as minimizing insecticide usage (Shelton et al., 2017). Bt eggplant was given government approval for planting in Bangladesh in 2013 and the technology was used by > 6500 growers in 2017 (Shelton et al., 2017).

In the Philippines, Bt eggplant was also developed and provided nearly complete control of EFSB in research trials (Hautea et al., 2016b) with a high potential to increase marketable yield, reduce costs and

Abbreviations: COI, cytochrome oxidase I; DNA, deoxyribonucleic acid; EFSB, eggplant fruit and shoot borer; GMYC, Generalized Mixed Yule Coalescent; PCR, polymerase chain reaction

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Table 1
Collection details of EFSB samples from different municipalities in the Philippines representing fifteen provinces.

Region	Province	Municipality	Date of collection/season[a]	Latitude	Longitude	Est. number of field collected samples
I	Ilocos Norte	Bacarra[b,c]	September 2017, WS	18.29	120.64	43
		Laoag[b]		18.19	120.66	33
		Batac[b,c]		18.01	120.57	35
	Pangasinan	Villasis[b,c]	April 2017, DS	15.90	120.58	90
		Asingan[b]		15.98	120.65	25
		Sta. Maria[b,c]	July 2017, WS	15.98	120.70	82
II	Isabela	Roxas[b,c]	August 2017, WS	17.10	121.61	29
		Aurora[b,c]		17.00	121.70	76
		Cabatuan[b,c]		16.93	121.64	58
	Cagayan	Sto. Niño[b,c]	September 2017, WS	17.90	121.59	28
		Solana[b,c]		17.59	121.59	26
III	Nueva Ecija	Quezon[b]	April 2017, DS	15.53	120.84	37
		General Tinio[b,c]		15.37	121.05	140
	Tarlac	Victoria[b,c]	February 2017, DS	15.58	120.66	200
IV-A	Laguna	Los Baños[b,c]	April 2017, DS	14.15	121.25	118
		San Pablo[b,c]		14.20	121.32	256
		Victoria[b,c]	August 2017, WS	14.04	121.34	38
	Quezon	Tiaong[b]	April 2017, DS	13.96	121.33	38
		Dolores[c]	July 2017, WS	14.01	121.35	135
		Sariaya[b,c]		13.84	121.47	87
		Candelaria[b,c]		13.91	121.41	89
	Batangas	Balete[b,c]	July 2016, WS	14.03	121.10	80
		Lipa[b,c]		13.99	121.20	80
		Batangas City[b,c]	September 2016, WS	13.69	121.07	80
V	Albay	Guinobatan[b,c]	May 2017, DS	13.20	123.57	174
		Tobaco City[b,c]		13.32	123.66	81
		Legaspi City[b,c]		13.18	123.73	76
VIII	Cebu	Cebu City[b,c]	April 2017, DS	10.37	123.79	24
X	Bukidnon	Malaybalay[b,c]	October 2017, WS	8.22	125.22	70
		Impasug-ong[b,c]		8.45	125.09	61
	Misamis Oriental	Tagaloan[b,c]		8.14	124.27	29
XI	Davao	Davao City[b,c]	August 2017, WS	7.21	125.47	29
XII	South Cotabato	General Santos[b,c]	October 2017, WS	6.22	125.13	46
		Tupi[b,c]		6.37	124.93	43
		Marbel[b,c]		6.54	124.93	83

[a] DS = dry season; WS = wet season.
[b] Sampled for *COI* gene sequence analysis.
[c] Sampled for morphometric analysis.

increase net profits of farmers (Francisco, 2007). However, there is still a knowledge gap on the identity of EFSB populations in the Philippines. EFSB was noted to be a new pest in the early 1970s, when it was observed to be causing farmers great losses due to its infestation (Navasero, 1983). However, it was unclear whether it was an introduced species via plant importation, or a previously unrecorded endemic species (Navasero, 1983). If EFSB is a previously unrecorded endemic species, a greater diversity of EFSB species is expected. In Africa, EFSB damaging Solanaceae crops was thought to be *L. orbonalis* until 2013, when a report stated that *L. orbonalis* only occurs in Asia (Gilligan and Pasoa, 2014). Mally et al. (2015) sought to delimit species of EFSB specimens from sub-Saharan Africa and were able to describe seven new *Leucinodes* species, which feed on *Solanum* species such as *S. aethiopicum*, *S. incanum*, and also *S. melongena*. *S. aethiopicum* and *S. incanum* occur in the Philippines although as ornamentals planted in undisturbed areas (Hautea et al., 2016a; Taylo and Hautea, 2016). The presence of these crop wild relatives of eggplant that are native to Africa in the Philippines may indicate with it the presence of African *Leucinodes* species as EFSB larvae may be brought in to the country in plants and fruits due to their internal feeding. It may be possible that the transport of ornamental African *Solanum* species to the Philippines brought with it African *Leucinodes*.

Investigations of EFSB in the Philippines stated that its description best fit the characteristics of *L. orbonalis* samples obtained from India (Navasero, 1983). Moreover, a recent study has confirmed *L. orbonalis* in the Philippines (Chang et al., 2014), though, the sampling location was only conducted from a market area, and not in agricultural provinces. Aside from these, no other formal studies have been conducted on the identification of this pest in the different regions of the Philippines and, to date, studies conducted in the Philippines on EFSB resulted in mostly unpublished reports on its pest biology (life history, host range, ovipositional behavior) and pest management. In line with the possible release of Bt eggplant, a genetic survey of Philippine EFSB populations is required in order to prepare large scale integrated pest management (IPM) efforts and develop insect resistance management (IRM) strategies.

The male genital structure is the most commonly used subject for Lepidopteran species identification because it is highly specialized and modified (Sapin et al., 2013). Through the use of morphometric analysis, a form of study which uses multivariate statistical methods in quantifying the size and shape of organism through part/organ measurements, relationships can be concluded Klingenberg, 2002). On the other hand, genes from the mitochondrial genome have been widely used to study the population structure of animal species. This is due to the characteristics of mitochondrial DNA, such as maternal inheritance, absence of recombination, and a neutral mutation rate that could approximately indicate species divergence (Galtier et al., 2009). Particularly, the mitochondrial cytochrome *c* oxidase I (*COI*) gene has been established as a core for a bio-identification or DNA barcoding system in animals (Hebert et al., 2003). *COI* sequences are also appropriate for assessing intraspecific variation due to its high degree of polymorphism (Hu et al., 2008). Thus, it is a useful tool as a phylogenetic marker that may reflect evolutionary patterns within and among species (Galtier et al., 2009). It has been used as a barcode to delimit species of *Leucinodes* in Africa (Mally et al., 2015), and as a phylogenetic marker in fruit fly (Boykin et al., 2014; Hu et al., 2008; Schutze et al., 2012; Shi