GENETIC DIVERSITY AND SUSCEPTIBILITY OF THE SELECTED VISAYAS POPULATIONS OF BROWN PLANTHOPPER, *Nilaparvata lugens* (Stål) (HEMIPTERA: DELPHACIDAE) TO *Beauveria bassiana* (Bals.-Criv.) Vuill.

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ABSTRACT

The brown planthopper (BPH), *Nilaparvata lugens* (Stål), is a notorious insect pest of rice due to its ability to transmit viruses such as rice grassy stunt virus and rice ragged stunt virus. Its direct feeding in rice can cause hopperburn, which is described as wilting of the crop, causing a decline in yield. Investigating the genetic diversity of BPH populations in the Philippines is crucial for improving pest control strategies. Hence, this study aims to determine the genetic diversity of the different BPH populations collected from various provinces in the Visayas region of the Philippines and determine their susceptibility to an entomopathogenic fungus, *Beauveria bassiana* (Bals.-Criv.) Vuill. The study was conducted from December 2022 to December 2023 using the BPH collected from different localities in Cebu, Bohol, and Negros Oriental. A total of fourteen sequences amplifying the *cytochrome C oxidase I* gene were analyzed. The nucleotide and haplotype diversities of all the sequences analyzed were 0.00276 and 0.780, respectively. Based on the molecular analysis, five distinct haplotypes were observed with moderate genetic differentiation (*Fst* = 0.08680). Analyses of molecular variance showed that 91.32% of variation was due to the difference within populations. The different populations of BPH exhibited similar degrees of susceptibility to *B. bassiana* at $1x10^8$ conidia/mL with up to 60% adult mortality and an LT50 of 4.08-4.26 days under laboratory conditions. The result may suggest that the little to moderate genetic difference among the BPH populations used in the study does not significantly affect their susceptibility to *B. bassiana*.

Keywords: *Nilaparvata lugens*, genetic diversity, *cytochrome c oxidase I*, entomopathogenic fungi, *Beauveria bassiana*

INTRODUCTION

Rice (*Oryza sativa* L.) is considered a staple food that provides more than 20% of the calories consumed worldwide (Sharif et al., 2014). Globally, the Philippines ranks eight among the world's top rice producers and sixth among the world's top rice consumers (USDA, 2023). Due to insufficient local production (Mamiit et al., 2021), the Philippines is currently the largest global importer of rice (USDA, 2023). One of the major constraints in rice production is insect pests because they can cause yield losses (Mondal et al., 2017). Brown planthopper (BPH), *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae), is one of the most important key insect pests of rice (Sanchez, et al., 1980; Ghaffar et al., 2011; Hereward et al., 2020). This insect transmits viral diseases such as rice grassy stunt virus and rice ragged stunt virus (Cabauatan et al., 2009). Its direct feeding causes hopperburn, which often resembles senescence that includes wilting, chlorosis, and stunted growth of the crop (Backus et al., 2005). Annually, its damage has caused an estimated loss of more than $300 million in rice production losses in Asia (Min et al., 2014).

Insecticide application is the most widely used and most effective control for managing BPH (Rahman & Jahan, 2006). However, misuse and overuse of these products have caused several drawbacks due to the ability of the BPH to develop insecticide resistance (Heong, 2009; Khoa et al., 2019). Furthermore, excessive use of insecticides would coincidentally kill the natural enemies, thereby reducing the presence of natural control (Bottrell & Schoenly, 2012). Due to the disadvantages of chemical control, the use of biological control has been regarded as one of the most promising technologies for sustainable agriculture (Tracy, 2014). Entomopathogenic fungi (EPF) are biological control agents that infect and often kill insects and other arthropods through cuticle penetration (Mantzoukas et al., 2022). *Beauveria bassiana* (Bals.-Criv.) Vuill. is a widely known species of EPF that is currently being utilized as a biological control agent against several arthropods (de Faria & Wraight, 2007). It is distributed worldwide (Zimmermann, 2007) with an extensive host range (Ownley et al., 2004) and can control insect pests without harming non-target organisms (Skinner et al., 2014; Mascarin & Jaronski, 2016; Dannon et al., 2020).

BPH populations vary in their physiological and ecological characteristics (Nagata & Matsuda, 1980). The genetic differences between BPH populations were observed by Mun et al. (1999). Identifying the differences among local BPH populations can be analyzed using mitochondrial genome sequences

(Matsumoto et al., 2013), which has become a widely used molecular marker to further understand the genetic structure (Roderick, 1996) and phylogenetic relationships in insects (Simon et al., 1994). One of the well-known mitochondrial genes, the gene encoding for the component I of *cytochrome oxidase (COI)* (Mun et al., 1999), has additional properties that make it particularly suited as a molecular marker for evolutionary studies (Lunt et al., 1996). *COI* is frequently used as a molecular marker since no other gene can be found in several taxonomic groups (Deagle et al., 2014). Besides its universality, this gene region was shown to be highly conserved within species while also being sufficiently variable between species, allowing reliable species identification (Pentinsaari et al., 2016).

Laquinta et al. (2019) have preliminary results in understanding the genetic structure of BPH in the different localities in Luzon. They used the *COI* gene region and reported the occurrence of polymorphism and numerous haplotypes in the Luzon population. In the study of Mun et al. (1999), the *COI* region of the BPH individuals collected from 11 localities in Asian countries was analyzed and reported the presence of three distinct haplotypes, and these haplotypes were all present in the sampled populations from the Philippines. Mashhoor et al. (2015) used the *COI* region to determine the genetic structure and phylogenetic status of BPH in Kerala, India. They reported similarities between BPH isolated in Kerala, India, with the population isolated from Andhra Pradesh and some populations isolated from Japan and China. In Indonesia, Winnie et al. (2020) combined the *COI* and *COII* regions to analyze six populations of BPH isolated from Java, Indonesia, where it showed that the populations have high haplotype diversity but low nucleotide diversity.

The ability of the insect to adapt to their local environment, resulting in the occurrence of variations within and among local populations, could limit the effectiveness of the current pest management practice against BPH. Hence, understanding the genetic diversity of the BPH population shall help design more efficient pest management practices against BPH. Here, we analyze the genetic diversity of the selected Visayas BPH populations and determine the susceptibility of these BPH populations to *B. bassiana.*

MATERIALS AND METHODS

Insect Samples

BPH samples were collected from June to July 2022 from the different fields located in the three provinces in Visayas, Philippines, namely, Asturias, Cebu (10.55546 °N, 123.72809 °E); Sierra Bullones, Bohol (coordinates not recorded); and Caticugan, Siaton, Negros Oriental (9.07914 °N, 123.02309 °E).

Amplification of *COI* Gene of Brown Planthopper

The DNA of an individual BPH adult was isolated following the manufacturer's protocol for the Animal Tissue Genomic DNA Purification Kit (Promega Wizard®, Madison, Wisconsin, USA) with some modifications, wherein the Proteinase K was incubated upon the addition of RNase for 30 mins at 37°C; cold isopropanol was used; and all centrifugation steps during DNA precipitation and rehydration were increased to 10 mins. The quantity and quality of the isolated DNA samples were determined using a UV/VIS spectrophotometer (BioDrop™). The *cytochrome c oxidase subunit I* (*COI*) gene amplification was done with the use of the following set of primers: CI-J-2183 5'-CAA CAT TTA TTT TGA TTT TTT GG-3' and TL2-N-3014 5'-TCC AAT GCA CTA ATC TGC CAT ATT A-3' (Mun et al., 1999). The polymerase chain reaction was carried out using a 25 µL PCR reaction mixture, which contained 12.5 µL of 2x Taq Master Mix (Vivantis, www.vivantistechnologies.com), 1 µL each of forward and reverse primers with a concentration of 10 pmol/µL, 8.5 µL of nuclease-free water, and 2 µL of 50 ng DNA template. The thermal profile used for amplification was 35 cycles of 94˚C for 1 min, a denaturation step of 48˚C for 30 s, and an annealing step of 72˚C for 45 sec (Mun et al., 1999). The amplified *COI* gene fragments were resolved in 1.5% agarose gel, stained with SYBR Safe DNA gel stain (Invitrogen, Carlsbad, California, USA), and the molecular size of the amplicons was estimated using the VC 100bp Plus DNA ladder (Vivantis, www.vivantistechnologies.com).

COI Gene Nucleotide Sequence Analyses

The amplified DNA samples were sent to Macrogen, Inc. (Seoul, South Korea) through the sequencing services of Kinovett Scientific Solutions Co. (Quezon City, Philippines). The obtained nucleotide sequences were aligned together with the *COI* gene sequence of *N. lugens* obtained from GenBank (Accession Number: NC_021748.1) using Clustal W (Thompson et al., 1994). The sequences were edited and trimmed into 698 base pairs using BioEdit Sequence

Alignment Editor Version 7 (Hall, 1999). The translation of the nucleotide sequences corresponding to 227 amino acid residues was carried out using ExPASy (https://web.expasy.org/translate/) (Gasteiger et al., 2003). The genetic diversity parameters, including the number of polymorphic sites, the number of haplotypes, transitions, transversion, nucleotide diversity, haplotype diversity, and nucleotide composition were estimated using DnaSP Version 6 (Rozas et al., 2017).

The pairwise *p* distances, pairwise *Fst,* and analyses of molecular variance (AMOVA) were determined using Arlequin Version 3 (Excoffier et al., 2005). The *COI* haplotypes of *N. lugens* were compared to the existing *COI* sequences of *N. lugens* in GenBank using the BLASTn program (http://blast.ncbi.nlm.nih.gov). The TCS haplotype networks were constructed using the TCS Network (Clement et al., 2000) to examine the relationships between the haplotypes.

Rearing of Brown Planthopper and Mass Production of *Beauveria bassiana*

The BPH populations were reared separately in cages at the National Crop Protection Center, College of Agriculture and Food Science, University of the Philippines Los Baños (NCPC-CAFS, UPLB), College, Laguna, Philippines. The BPH nymphs and adults collected from the three provinces in Visayas, Philippines, were fed with a susceptible variety of rice seedlings, Taichung Native 1 (TN1), grown in pots supplied in each cage, wherein 30-day-old rice seedlings planted in pots are placed inside a wire mesh cage. Adult BPH was used for biological assays of the entomopathogenic fungus. The entomopathogenic fungus, *B. bassiana,* was sourced from the Mycology Laboratory, NCPC-CAFS, UPLB, which was originally isolated from rice bug (*Leptocorisa oratorius* (Fabricius)). The fungus was subcultured in Potato Dextrose Agar (PDA) until pure cultures were obtained.

Mortality Assay against *N. lugens*

The conidial suspension with a concentration of $1x10^8$ conidia/mL was prepared by diluting the stock solution in 0.1% Tween 80 solution. The three BPH populations were exposed to the conidial suspension of *B. bassiana.* Rice seedlings were washed in running water and sterilized in rinses of 0.5% sodium hypochlorite and sterile distilled water. Afterwhich, *B. bassiana* suspension and 0.1% Tween 80 solution were sprayed on rice seedlings in fungal-treated and control set-ups, respectively. Set-ups were placed inside sterile test tubes. Ten adult BPH were introduced to each test tube, and each test tube represents one replicate. A total of six replicates were done in each BPH population. Surface-sterilized fresh rice leaves were introduced after 48 hours.

Data Analyses

The mortality of the BPH was recorded daily. Following Lacey and Brooks (1997), the cadavers were sterilized using 0.5% sodium hypochlorite for 30 seconds and washed twice in sterile distilled water. After that, the cadavers were placed in sterilized Petri dishes with moistened filter paper and stored at room temperature.

The mortality of the BPH was corrected using Abbott's Formula (Abbott, 1925). The data were checked for normality using Shapiro-Wilk test, upon confirming normality a one-way analysis of variance (ANOVA) was done for the percent mean cumulative mortality of the different BPH populations. All the statistical analyses were done using GraphPad Prism (Version 10.1.1).

RESULTS AND DISCUSSION

Genetic diversity analysis of *N. lugens* populations

The *COI* gene regions were successfully amplified from the three populations of BPH collected from Visayas, Philippines, namely, Asturias, Cebu; Sierra Bullones, Bohol; and Caticugan, Siaton, Negros Oriental (Figure 1). The amplicons were approximately 900 bp, which agrees with the reported *COI* gene region of BPH by Mun et al. (1999).

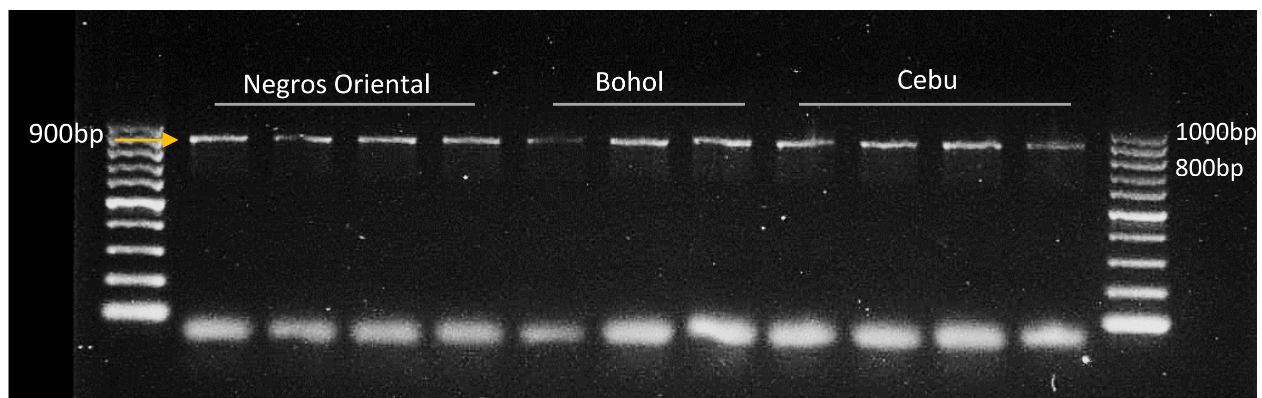

Figure 1. DNA fragments amplified from brown planthopper, *Nilaparvata lugens* (Stål), containing *cytochrome c oxidase I* (*COI*) gene sequence resolved in 1.5% agarose gel stained with SYBR® Safe DNA gel stain (Invitrogen, Thermo Fisher Scientific, Carlsbad, California, USA). The molecular weights of the PCR products were estimated using a 100 bp plus ladder (Vivantis www.vivantis.com). The DNA templates used were isolated from BPH collected from the Visayas provinces, namely Bohol, Cebu, and Negros Oriental.

The genetic diversity of BPH was analyzed based on the nucleotide sequences of the *COI* gene. Five polymorphic sites were identified at positions 103, 301, 436, 664, and 671, all of which were parsimony-informative sites (Figure 2). Polymorphisms at positions 301 and 671 were translations, while polymorphisms at positions 103, 436, and 664 were transversions. Upon translation using ExPASy, the variations were found to produce silent mutations at amino acids 35, 101, and 146 and missense mutations at amino acids 221 and 223 (Figure 3). The comparison of the diversity indices of the BPH populations is shown in Table 1. The nucleotide and haplotype diversities of all *COI* gene sequences analyzed were 0.00276 ± 0.00047 and 0.780 ± 0.085, respectively. The haplotype diversities of the different populations had a range of 0.500 to 0.800 (mean = 0.633), whereas the nucleotide diversities ranged from 0.00143 to 0.00430 (mean = 0.00248). Notably, haplotype diversity was relatively higher than nucleotide diversity, suggesting small differences between BPH haplotypes. This observation is consistent with recent population expansion from a low effective population size (Guru-Pirasanna-Pandi et al., 2022).

Table 1. Comparison of the molecular diversity indices of the brown planthopper, *Nilaparvata lugens* (Stål), collected from rice fields in three provinces (Bohol, Cebu, and Negros Oriental) in Visayas, Philippines.

INDICES	**COLLECTION SITES**			
	BOHOL	CEBU	NEGROS ORIENTAL	ALL
No. of Samples	5	4	5	14
Molecular diversity				
• **No. of Polymorphic**	2	2	5	5
• **No. of Transitions**	1	1	2	2
• **No. of Transversions**	1	1	3	3
• **No. of Substitutions**	2	2	5	5
Haplotype information (number of samples)	Haplotype 1 (3) Haplotype 2 (2)	Haplotype 1 (3) Haplotype 3 (1)	Haplotype 3 (2) Haplotype 4 (1) Haplotype 5 (2)	
Haplotype Diversity	0.600 ± 0.175	0.500 ± 0.265	0.800 ± 0.164	0.780 ± 0.085
Nucleotide Diversity	0.00172 ± 0.00050	0.00143 ± 0.00076	0.00430 ± 0.00097	0.00276 ± 0.00047
Nucleotide Composition				
• **C**	16.05	16.01	15.99	16.03
• **T**	37.45	37.39	37.42	37.43
• **A**	32.84	32.95	32.87	32.84
• **G**	13.67	13.65	13.72	13.70

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Haplotype-1  AATTTCTCAT ATTATTATAC AAGAAAGAGG AAAAAGGGAA ACTTTCGGAT CTATTGGAAT  60
Haplotype-2  AATTTCTCAT ATTATTATAC AAGAAAGAGG AAAAAGGGAA ACTTTCGGAT CTATTGGAAT  60
Haplotype-3  AATTTCTCAT ATTATTATAC AAGAAAGAGG AAAAAGGGAA ACTTTCGGAT CTATTGGAAT  60
Haplotype-4  AATTTCTCAT ATTATTATAC AAGAAAGAGG AAAAAGGGAA ACTTTCGGAT CTATTGGAAT  60
Haplotype-5  AATTTCTCAT ATTATTATAC AAGAAAGAGG AAAAAGGGAA ACTTTCGGAT CTATTGGAAT  60

Haplotype-1  AATTTATGCA ATAATTGCAA TTGGAATTTT AGGTTTTATT GTTTGAGCTC ACCATATATT  120
Haplotype-2  AATTTATGCA ATAATTGCAA TTGGAATTTT AGGTTTTATT GTTTGAGCTC ACCATATATT  120
Haplotype-3  AATTTATGCA ATAATTGCAA TTGGAATTTT AGGTTTTATT GTTTGAGCTC ACCATATATT  120
Haplotype-4  AATTTATGCA ATAATTGCAA TTGGAATTTT AGGTTTTATT GTTTGAGCTC ACCATATATT  120
Haplotype-5  AATTTATGCA ATAATTGCAA TTGGAATTTT AGGTTTTATT GTATGAGCTC ACCATATATT  120

Haplotype-1  TACTGTAGGT ATAGATATTG ATACCCGAGC CTATTTTACG TCAGCTACTA TAATTATTGC  180
Haplotype-2  TACTGTAGGT ATAGATATTG ATACCCGAGC CTATTTTACG TCAGCTACTA TAATTATTGC  180
Haplotype-3  TACTGTAGGT ATAGATATTG ATACCCGAGC CTATTTTACG TCAGCTACTA TAATTATTGC  180
Haplotype-4  TACTGTAGGT ATAGATATTG ATACCCGAGC CTATTTTACG TCAGCTACTA TAATTATTGC  180
Haplotype-5  TACTGTAGGT ATAGATATTG ATACCCGAGC CTATTTTACG TCAGCTACTA TAATTATTGC  180

Haplotype-1  GGTCCCCACC GGAATCAAAA TTTTTAGATG AATCGCAACA ATTTACGGTT CCAAAATGAA  240
Haplotype-2  GGTCCCCACC GGAATCAAAA TTTTTAGATG AATCGCAACA ATTTACGGTT CCAAAATGAA  240
Haplotype-3  GGTCCCCACC GGAATCAAAA TTTTTAGATG AATCGCAACA ATTTACGGTT CCAAAATGAA  240
Haplotype-4  GGTCCCCACC GGAATCAAAA TTTTTAGATG AATCGCAACA ATTTACGGTT CCAAAATGAA  240
Haplotype-5  GGTCCCCACC GGAATCAAAA TTTTTAGATG AATCGCAACA ATTTACGGTT CCAAAATGAA  240

Haplotype-1  CTTTTCCCCC CAAATAATTT GATCATTAGG ATTCATTTTA CTTTTTACTA TTGGAGGATT  300
Haplotype-2  CTTTTCCCCC CAAATAATTT GATCATTAGG ATTCATTTTA CTTTTTACTA TTGGAGGATT  300
Haplotype-3  CTTTTCCCCC CAAATAATTT GATCATTAGG ATTCATTTTA CTTTTTACTA TTGGAGGATT  300
Haplotype-4  CTTTTCCCCC CAAATAATTT GATCATTAGG ATTCATTTTA CTTTTTACTA TTGGAGGATT  300
Haplotype-5  CTTTTCCCCC CAAATAATTT GATCATTAGG ATTCATTTTA CTTTTTACTA TTGGAGGATT  300

Haplotype-1  AACAGGTGTA ATATTATCAA ATTCCTCAAT TGATATTATT CTACATGATA CCTATTATGT  360
Haplotype-2  AACAGGTGTA ATATTATCAA ATTCCTCAAT TGATATTATT CTACATGATA CCTATTATGT  360
Haplotype-3  GACAGGTGTA ATATTATCAA ATTCCTCAAT TGATATTATT CTACATGATA CCTATTATGT  360
Haplotype-4  AACAGGTGTA ATATTATCAA ATTCCTCAAT TGATATTATT CTACATGATA CCTATTATGT  360
Haplotype-5  AACAGGTGTA ATATTATCAA ATTCCTCAAT TGATATTATT CTACATGATA CCTATTATGT  360

Haplotype-1  AGTGGCTCAT TTTCACTATG TCCTTTCCAT GGGAGCAGTA TTCACCATTA TCGCTAGATT  420
Haplotype-2  AGTGGCTCAT TTTCACTATG TCCTTTCCAT GGGAGCAGTA TTCACCATTA TCGCTAGATT  420
Haplotype-3  AGTGGCTCAT TTTCACTATG TCCTTTCCAT GGGAGCAGTA TTCACCATTA TCGCTAGATT  420
Haplotype-4  AGTGGCTCAT TTTCACTATG TCCTTTCCAT GGGAGCAGTA TTCACCATTA TCGCTAGATT  420
Haplotype-5  AGTGGCTCAT TTTCACTATG TCCTTTCCAT GGGAGCAGTA TTCACCATTA TCGCTAGATT  420

Haplotype-1  TATCCATTGA TACCCCTTAT TTACAGGTAG AAACATAAAT AATAAATGAC TAAAAATTCA  480
Haplotype-2  TATCCATTGA TACCCCTTAT TTACAGGTAG AAACATAAAT AATAAATGAC TAAAAATTCA  480
Haplotype-3  TATCCATTGA TACCCATTAT TTACAGGTAG AAACATAAAT AATAAATGAC TAAAAATTCA  480
Haplotype-4  TATCCATTGA TACCCCTTAT TTACAGGTAG AAACATAAAT AATAAATGAC TAAAAATTCA  480
Haplotype-5  TATCCATTGA TACCCCTTAT TTACAGGTAG AAACATAAAT AATAAATGAC TAAAAATTCA  480

Haplotype-1  ATTTTATTCC ATATTTCTAG GAGTAAATTT AACATTTTTT CCCCAACATT TTTTAGGATT  540
Haplotype-2  ATTTTATTCC ATATTTCTAG GAGTAAATTT AACATTTTTT CCCCAACATT TTTTAGGATT  540
Haplotype-3  ATTTTATTCC ATATTTCTAG GAGTAAATTT AACATTTTTT CCCCAACATT TTTTAGGATT  540
Haplotype-4  ATTTTATTCC ATATTTCTAG GAGTAAATTT AACATTTTTT CCCCAACATT TTTTAGGATT  540
Haplotype-5  ATTTTATTCC ATATTTCTAG GAGTAAATTT AACATTTTTT CCCCAACATT TTTTAGGATT  540

Haplotype-1  AACTGGTATA CCACGACGAT ACTCTGACTA TCCAGATATA TACACCCTGT GAAACCTTTT  600
Haplotype-2  AACTGGTATA CCACGACGAT ACTCTGACTA TCCAGATATA TACACCCTGT GAAACCTTTT  600
Haplotype-3  AACTGGTATA CCACGACGAT ACTCTGACTA TCCAGATATA TACACCCTGT GAAACCTTTT  600
Haplotype-4  AACTGGTATA CCACGACGAT ACTCTGACTA TCCAGATATA TACACCCTGT GAAACCTTTT  600
Haplotype-5  AACTGGTATA CCACGACGAT ACTCTGACTA TCCAGATATA TACACCCTGT GAAACCTTTT  600

Haplotype-1  TTCTTCTATG GGTTCATTCA TTTCCTTAAT TAGAATTTTA ATATTAATGT TTATTATATG  660
Haplotype-2  TTCTTCTATG GGTTCATTCA TTTCCTTAAT TAGAATTTTA ATATTAATGT TTATTATATG  660
Haplotype-3  TTCTTCTATG GGTTCATTCA TTTCCTTAAT TAGAATTTTA ATATTAATGT TTATTATATG  660
Haplotype-4  TTCTTCTATG GGTTCATTCA TTTCCTTAAT TAGAATTTTA ATATTAATGT TTATTATATG  660
Haplotype-5  TTCTTCTATG GGTTCATTCA TTTCCTTAAT TAGAATTTTA ATATTAATGT TTATTATATG  660

Haplotype-1  AGAAAGATTA AGATTTAAAC GAAAAATGGT GTTTAAAA  698
Haplotype-2  AGATAGATTA GGATTTAAAC GAAAAATGGT GTTTAAAA  698
Haplotype-3  AGAAAGATTA AGATTTAAAC GAAAAATGGT GTTTAAAA  698
Haplotype-4  AGATAGATTA AGATTTAAAC GAAAAATGGT GTTTAAAA  698
Haplotype-5  AGATAGATTA GGATTTAAAC GAAAAATGGT GTTTAAAA  698
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Figure 2. Sequence alignment of the five *COI* haplotypes of the brown planthopper, *Nilaparvata lugens* (Stål), collected from rice fields in Bohol, Cebu, and Negros Oriental, Visayas, Philippines.

COI haplotypes can exhibit variability in the biological and ecological traits of an insect. This was demonstrated in the study of Camus et al. (2015), wherein the variation in mitochondrial haplotypes affects the sex and life stages of the insect. Furthermore, the mitochondrial genotype was considered to have a

linkage with aging, fecundity, starvation stress, and thermal tolerance. There are also evolutionary studies that documented the consequences that arise from the variation in the mitochondria (Ballard & Melvin, 2010). These variations that cause mutations could occur in every population. The effects of these mutations may be classified into three types, namely deleterious, neutral, and advantageous. Deleterious mutations decrease the fitness of the host, which may cause their quick elimination in the environment (Loewe & Hill, 2010). Meanwhile, neutral mutations are not affected by selections because of their small effect, and advantageous mutations increase the fitness of the host, allowing them a higher chance of survival (Eyre-Walker & Keightley, 2007; Loewe & Hill, 2010). The resulting mutants remain to strive in the environment depending on the intensity of genetic drift and purifying selection (Webster et al., 2023; Kimura et al., 1963). Thus, variations in the mitochondrial genome of BPH allow the development of diverse phenotypes that can confer an advantage to a specific environment, enabling the species to better adapt in response to the changing environment.

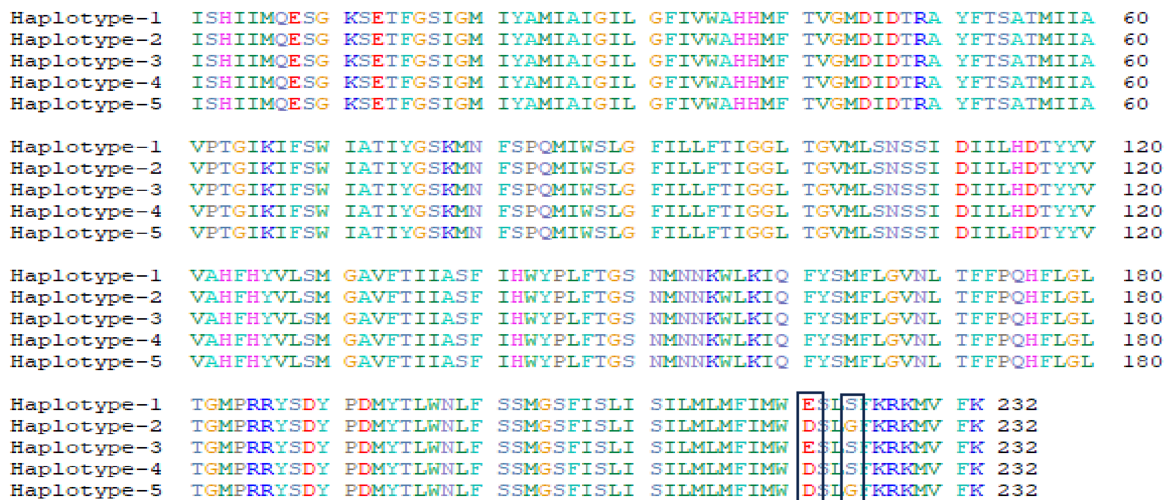

Figure 3. Multiple amino acid sequence alignment of the mitochondrial DNA *cytochrome oxidase I* gene of the brown planthopper, *Nilaparvata lugens* (Stål) collected from Bohol, Cebu, and Negros Oriental, Visayas, Philippines.

Understanding and considering the genetic diversity in pest populations is important in creating an effective and more efficient pest management strategy. The occurrence of biotypes in BPH is defined by the differences in their non-morphological traits as an effect of the variations in their genetic makeup (Jena & Kim, 2010). Different BPH biotypes show variations in virulence (Claridge & Den Hollander, 1980), resistance development (Myint et al., 2009), and host

preference for feeding or oviposition (Jena & Kim, 2010). Hence, the existing local BPH management could be affected, and perhaps some management strategies could not be effective due to the occurrence of biotypes that already adapted to the management strategies in that locality.

The pairwise fixation index (*Fst*) values, which measure the population differentiation of the BPH population were determined (Table 2). The results showed that *Fst* values between populations Cebu and Bohol (*Fst*=0.14931, P=0.32324) and Cebu and Negros Oriental (*Fst*=0.10087, P=0.30078) had shown moderate genetic differentiation, while Bohol and Negros Oriental (*Fst*=0.04454, P=0.42969) population had shown little genetic differentiation. However, all the compared populations were found to be non-significant. The non-significance of the *Fst* values may indicate that there is no genetic differentiation between the BPH populations. However, due to the small number of samples used in the study, it is suggested to increase the sample size and conduct a wider sample collection.

Table 2. Pairwise genetic *p*-distances (upper right) and pairwise *Fst* (lower left among populations of brown planthopper, *Nilaparvata lugens* (Stål) collected from Bohol, Cebu, and Negros Oriental, Visayas, Philippines.

PROVINCES	BOHOL	CEBU	NEGROS ORIENTAL
Bohol		0.32324	0.42969
Cebu	0.14931		0.30078
Negros Oriental	0.04454	0.10087	

Levels of genetic differentiation of *Fst* values: between 0 to 0.05 – little differentiation; between 0.05 to 0.15 – moderate differentiation; between 0.15 to 0.25 – great differentiation; 1 – complete isolation; P < 0.05.

The AMOVA is a statistical method in population genetics that quantifies the molecular variation within species (Huang et al., 2021). Table 3 shows the AMOVA of the three different populations of BPH, wherein the Tamura-Nei model was used in the molecular distance with 1000 permutations for the level of significance. The results showed that the contribution of genetic variation within the BPH population was 91.32%, which means great variation is accounted to the difference of BPH within the three localities. On the other hand, the contribution of genetic variation among populations was 8.68%. Therefore, *COI*-based genetic differentiation of the BPH populations studied originated from intra-populations. The genetic differentiation (0.08680), which was measured by the fixation index (*Fst*) as calculated by Arlequin (Excoffier et al., 2005), showed a moderate degree of genetic differentiation among BPH samples from the three localities.

Table 3. Analysis of molecular variance of the brown planthopper, *Nilaparvata lugens* (Stål), collected from Bohol, Cebu, and Negros Oriental, Visayas, Philippines.

SOURCE OF VARIATION	D.F.	SUM OF SQUARES	VARIANCE OF COMPONENTS	PERCENTAGE OF VARIATION
Among populations	2	2.607	0.08595 Va	8.68
Within populations	11	9.947	0.90431 Vb	91.32
Total	13	12.554	0.99026	

Significance tests based on 1023 permutations ($p > 0.05$)
Fixation Index *Fst* = 0.08680

Analysis of the *COI* nucleotide sequence of the fourteen individuals from the three provinces in Central Visayas, Philippines revealed a total of five distinct haplotypes (Table 1 and Figure 4). Haplotype 1 was the most common haplotype shared by two populations (Cebu and Bohol), representing 42.86% of the total *COI* gene sequenced. The second most abundant was Haplotype 3, shared by Negros Oriental and Cebu with 21.43% of the total *COI* gene sequenced. Haplotype 2, with 14.29% of the total *COI* gene sequenced, is unique to Bohol. Haplotype 5, with 14.29% of the total *COI* gene sequenced, is unique to Negros Oriental. Haplotype 4 was considered a singleton haplotype found in Negros Oriental.

The *COI* gene sequences of the five haplotypes were compared with the reported *COI* gene sequences in the GenBank, and all the samples were identified as *N. lugens,* as their nucleotide sequences were >99% to 100% identical to *N. lugens* with 100% query cover. The unrooted network of the five *COI* haplotypes found in three different provinces in Visayas, Philippines, is shown in Figure 5. The size of each circle is proportional to the number of samples with the observed haplotype, while each color represents the portion of samples collected from a specific location. The lines present across the branches represent the nucleotide that differs between the haplotypes. Haplotype 1 differs from haplotypes 4 and 3 by 1 nucleotide and 2 nucleotides, respectively. While Haplotypes 2 and 5, which were not directly connected to Haplotype 1, differed in two to three nucleotides. The major haplotype of BPH in Central Visayas is Haplotype 1, which is considered to be the original or ancestral haplotype in the region (Guru-Pirasanna-Pandi, 2022).

Figure 4. Geographic distribution of the five mitochondrial DNA *cytochrome oxidase I* haplotypes observed from brown planthopper, *Nilaparvata lugens* (Stål) collected in rice fields in Cebu, Bohol, Negros Oriental, Visayas, Philippines.

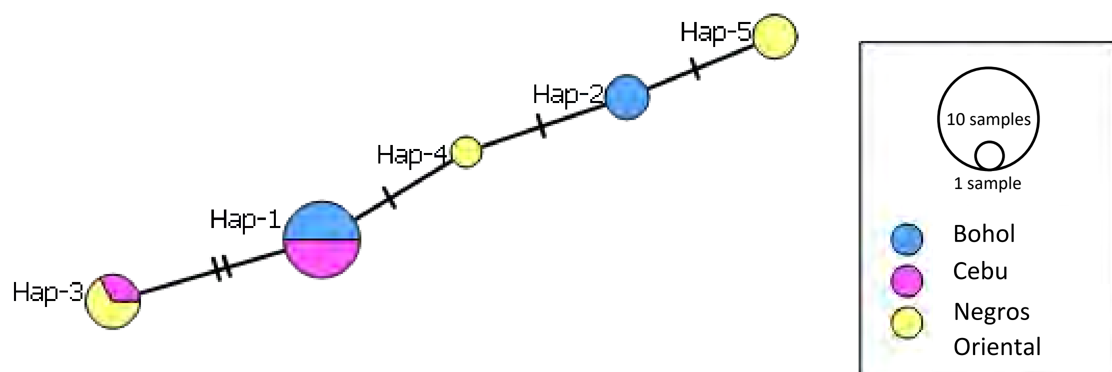

Figure 5. The haplotype network of the brown planthopper, *Nilaparvata lugens* (Stål), using the sequences of mitochondrial *cytochrome oxidase I.*

Evolutionary changes in the genetic makeup of the insect are induced by several selective pressures in its environment. This includes abiotic and biotic factors that affect the survivability of the insect. If the induced changes bring functional attributes to the pests, the variation can be distributed to insect populations (Karthika et al., 2017). Thus, the presence of the different haplotypes in the three populations may be the result of the insect's adaptation to the environment due to differences in control management in BPH.

Pathogenicity of *B. bassiana* to *N. lugens*

B. bassiana with a fungal concentration of $1x10^8$ conidia/mL caused successful infection against BPH populations. The progression of the fungal infection in the three different populations of BPH is shown in Figure 6. The infection of *B. bassiana* to BPH populations started at 24 hrs after treatment with an increasing trend of disease progression of up to 60% mortality at 72 hrs after treatment. The mortality of all the set-ups treated with *B. bassiana* was higher than 50% after 72 hours of post-infection. Thus, this finding aligns with the study of Atta et al. (2020) wherein *B. bassiana* at $1x10^8$ conidia/mL sprayed to rice stem pieces caused more than 50% mortality of BPH.

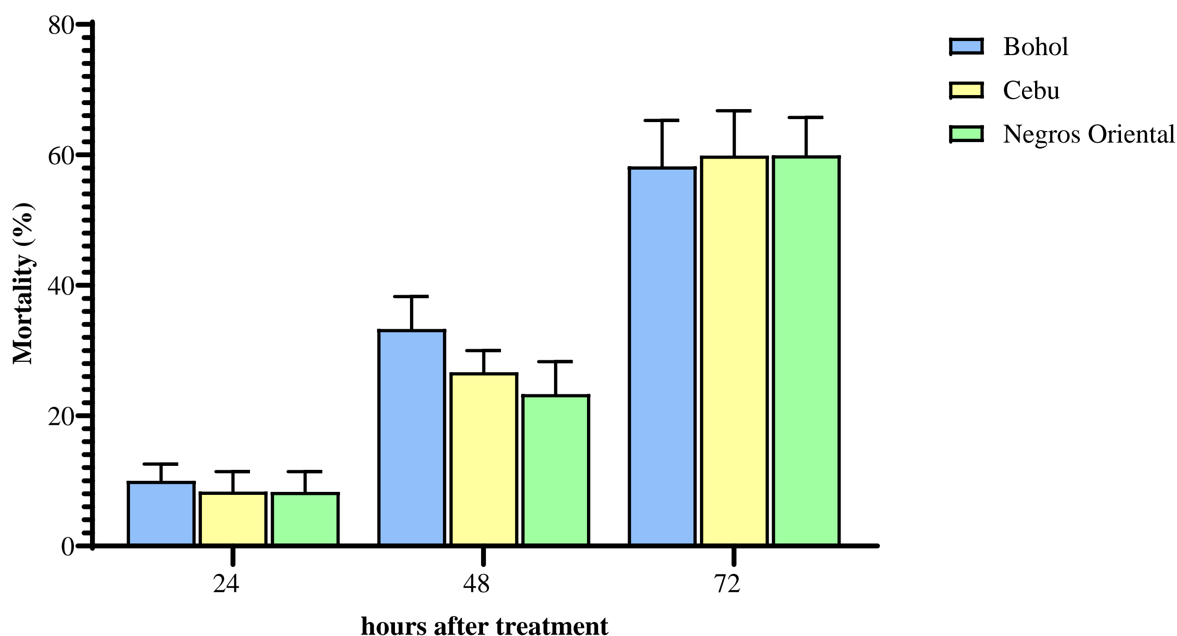

Figure 6. Cumulative corrected percent mortality in Bohol, Cebu, and Negros Oriental populations of adult brown planthopper, *Nilaparvata lugens* (Stål) after exposure to *Beauveria bassiana* (Bals.-Criv) Vuill. with a conidial concentration of $1x10^8$ conidia/mL.

Upon performing one-way ANOVA, there were no significant differences in the mortality of the treated BPH between populations (p = 0.9785; F = 0.02172). Table 4 summarizes the mean lethal time due to exposure of BPH to *B. bassiana.* The values calculated range from 4.08 to 4.26 days, with no significant difference in the mean lethal time of the BPH challenged with *B. bassiana.* This finding suggests that BPH populations were equally susceptible to *B. bassiana*. In addition, the mycosis was confirmed in BPH cadavers by observing the white fungal growth and a stiff body (Figure 7). The stiffness of the insect body is due to the fluid adsorption of the fungus (Skinner et al., 2014).

Table 4. Calculated mean lethal time values of the laboratory bioassays of *Beauveria bassiana* (Bals.-Criv) against the three different populations of *Nilaparvata lugens* (Stål) collected from Bohol, Cebu, and Negros Oriental, Visayas, Philippines.

CALCULATED MEAN LETHAL TIME (DAYS ± SE)		
Bohol	Cebu	Negros Oriental
4.17 ± 0.19	4.08 ± 0.20	4.26 ± 0.19

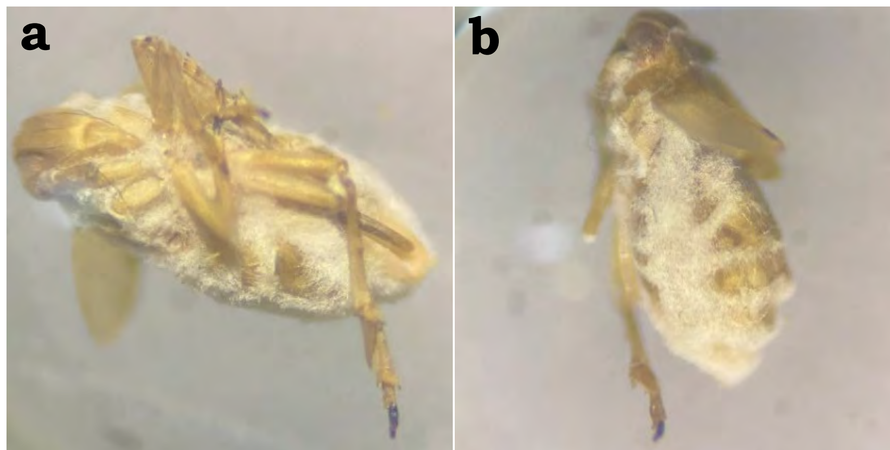

Figure 7. The cadavers of adult brown planthopper, *Nilaparvata lugens* (Stål), showing mycosis at ventral (a) and dorsolateral (b) sides after successful infection of *Beauveria bassiana* (Bals.-Criv.) at a conidial concentration of $1x10^8$ conidia/mL.

The results showed that *B. bassiana* isolated from a rice bug, *L. oratorius* (Fabricus), was able to cross-infect adult BPH. This conforms with the study of Uma Devi et al. (2008), which reported that *B. bassiana* is a generalist and can be used as a wide-ranged mycoinsectide after infecting nine different insect hosts. In the Philippines, *B. bassiana* were able to infect fall armyworm, *Spodoptera frugiperda* (Noctuidae), and caused lethal infection in the insect's

larval instars ranging from 23.64% to 97.42% (Montecalvo and Navasero, 2021). The results of the study with regards to the lethal effect of the *B. bassiana* isolated from *L. oratorius* infecting *N. lugens* shows the promising potential of the fungus as a biological control agent for BPH populations in Visayas, Philippines. *B. bassiana* could be incorporated into integrated pest management due to its several advantages. Using EPF is beneficial as they target specific pests and reduce the risk of insecticide resistance development.

The BPH populations with high genetic diversity were hypothesized to exhibit lower mortality when subjected to *B. bassiana.* Insect populations with higher levels of genetic diversity are generally more capable of adapting to environmental changes than those with lower genetic diversity (Birader, 2023) due to the insects' behavioral adaptations and immune capacity (Zhang et al., 2019). However, our findings have shown no significant differences in mortality of the three different BPH populations challenged with *B. bassiana* even the occurrence of genetic variations. Perhaps the occurrence of moderate genetic differentiation among the BPH populations does not affect their susceptibility to *B. bassiana.* Although some studies have shown that immunity of an insect population is reflected in its population genetics, for instance, in the study of Whitehorn et al. (2011), which showed the relationship between the genetic diversity of the moss carder bee (*Bombus muscorum*) and its immunity towards parasites. It was found that *B. muscorum* populations with low genetic diversity had a high occurrence of the insect's gut parasite. Similarly, Ebert et al. (2007) observed that the low genetic diversity of *Daphnia magna* increases the probability of parasitic infection. However, Ekroth et al. (2019) concluded that the disparity in successful pathogen infection between high genetic diversity and low genetic diversity populations was more pronounced in field investigations than in laboratory studies. Hence, field experiments should be conducted to verify the virulence of *B. bassiana* and the possible varying susceptibility of the BPH populations in Central Visayas, Philippines, to this beneficial fungus.

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