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The Brown Planthopper (*Nilaparvata lugens* Stal.) Attack and Its Genetic Diversity on Rice in Bali, Indonesia

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Population dynamics and damage intensity of brown planthopper (BPH) and *Nilaparvata lugens* (Stal.) provide information about the economic threshold status of *N. lugens* in Bali. This study enriched information on the genetic diversity of *N. lugens* isolates from eastern Indonesia, particularly Bali. It is the first report of *N. lugens* biotype Y in Indonesia. In addition, the latest information in this study is that rice varieties Situbagendit and Inpari 32, which were previously resistant to BPH, are now found to be susceptible to BPH. This information is critical as a basis for controlling *N. lugens* in Indonesia

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The Brown Planthopper (*Nilaparvata lugens* Stal.) Attack and Its Genetic Diversity on Rice in Bali, Indonesia

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Abstract. The brown planthopper is an important pest on rice crops in Indonesia. The genetic diversity of BPH isolates in western Indonesia has been extensively reported, whereas eastern Indonesia isolates have not been reported. This research aims to analyze genetic diversity and evaluate the BPH attack's intensity on Bali rice plants. The research method used was an observation of attack percentage, population dynamics, attack intensity, and genetic diversity of BPH in 9 districts in Bali (Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana). Molecular identification was carried out on *N. lugens* DNA in the mtCOI fragment. BPH attacks of >50% were found in the districts of Gianyar, Bangli, Jembrana, and Badung. The BPH population was primarily found in Ciherang and IR-64 varieties of rice in the Badung Regency, with 43.67 BPH per rice hill. In general, rice varieties grown in all observation locations were susceptible to BPH, such as Ciherang, IR-64, Inpari 32, and Situbagendit. In the Ciherang and IR-64 varieties, the highest attack intensity average value reached 30%. The sequence of *N. lugens* isolate from Bali Jembrana showed the highest nucleotide and amino acid homology with *N. lugens* isolate FSD-034 from Pakistan (MK301229) biotype Y of 99.5 -99.74% and 100%, respectively. This study found *N. lugens* biotype Y in rice plants for the first time in Indonesia. This study reported that Rice varieties Situbagendit and Inpari 32, previously resistant to BPH, are reported as susceptible to BPH.

Keywords: susceptible variety, Situbagendit, Inpari 32, genetic diversity, attack intensity

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Abbreviations (if any): The BPH, Rice Plants

Running title: The Brown Planthopper (*Nilaparvata lugens* Stal.) Attack and Its Genetic Diversity on Rice in Bali, Indonesia

INTRODUCTION

The brown planthopper (BPH) (*Nilaparvata lugens* Stal, Hemiptera: Delphacidae) is the most destructive rice pest in Indonesia. Repeated outbreaks of BPH in Indonesia are caused by continuous rice cultivation, extensive use, and over-application of insecticides (Baehaki and Mejaya 2015). This pest is vascular monophagous in rice (Cheng et al. 2013; Ferrater et al. 2015). Feeding by nymphs and imago at the base of the plant causes rapid wilting and drying of the plant (Bottrell and Schoenly 2012; Cheng et al. 2013; Bao and Zhang 2019). In addition, BPH is also a vector of Rice grassy stunt virus and Rice ragged stunt virus (Bao and Zhang 2019). At high population levels of *N. lugens* can cause significant losses in rice production (Cheng et al. 2013; Zheng et al. 2013; Bao and Zhang 2019).

The BPH cannot tolerate winter in northern Asia, including Japan, Korea, and northern China (He et al. 2012; Fu et al. 2012; Fu et al. 2014). The population originally came from subtropical and tropical areas by flying long distances during the summer (Fu et al. 2014; Hu et al. 2014). The intensification of rice production triggered the BPH outbreak in Tropical Asia during the green revolution era in the 1970s and 1980s (Bottrell and Schoenly 2012). Until now, *N. lugens* is the main problem causing rice harvest failure in several countries. Inaccurate identification and prolonged identification of *N. lugens* are obstacles to its field management strategy.

Traditionally, BPH has been identified at the species level by morphological features using anatomical characteristics, namely, wings, front, and external genitalia (Dupo and Barrion 2009). Accurate identification requires extensive expertise and experience and yet sometimes can lead to errors. Morphological identification by an entomologist can reduce the potential for errors. Practical morphological identification is only possible when dealing with small sample sizes and well-preserved specimens. Therefore, it is crucial to utilize a new identification method that is accurate, fast, time-saving, and suitable for large numbers of specimens.

Molecular techniques with high reproducibility and fast results offer an excellent alternative to traditional morphological classification. Several mitochondrial and nuclear genes have been used as genetic markers to differentiate related species. These include the mitochondrial cytochrome c oxidase subunit 1 (CO1) gene, nuclear 12S-16S-18S

ribosomal RNA genes, and ITS1 and ITS2 internal transcription spacers (Fukunaga et al. 2000; Brengues et al. 2014; Gomez-Polo et al. 2014; Wang et al. 2016; Liu et al. 2018). ITS1 and ITS2 are nonfunctional spacers that separate the 18S-5.8S and 5.8S-28S rRNA genes, respectively (Ji et al. 2003; Liu et al. 2018). As ITS sequences have low intra-species variation but high variation between species, they are helpful for species classification and phylogenetic analysis for morphologically similar organisms, both in prokaryotes and eukaryotes (Liu et al. 2009). Finally, from the molecular identification of the combined mitochondrial COI-COII and ten microsatellite marker loci (Winnie et al. 2020).

The genetic diversity of *N. lugens* has been reported in several countries such as China, South Korea, Pakistan, India, and Malaysia (Jing et al. 2012; Zheng et al. 2021; Anant et al. 2021; Latif et al. 2012). The genetic diversity of *N. lugens* in Indonesia is widely reported in western Indonesia (Java Island) (Winnie et al. 2020; Chaerani et al. 2021). Reports on the genetic diversity of *N. lugens* in eastern Indonesia have not been found. Therefore, this study aims to analyze genetic diversity and determine the intensity of BPH attacks on rice plants in eastern Indonesia, especially Bali.

MATERIALS AND METHODS

Brown Planthopper Sampling from Rice Dwarf Disease Endemic Areas

Samples were taken from nine locations in Bali Province (Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana). The brown planthopper samples taken from rice plants were nymphs and imagos. Nymphs and imagos were used for total DNA extraction. After arriving at the laboratory, the nymphs and imago were stored dry at -20ºC.

Observation of BPH Attack Symptoms and Quantity of BPH Population/rice hill

Observation of symptoms of BPH attack was carried out by observing symptoms of damage to rice plants due to BPH attack. The abundance of the BPH/rice hill population was obtained by counting all nymphs and imagoes obtained. Data on the population per cluster from 20 samples at each observation location were then averaged.

BPH Attack Percentage

The percentage of BPH attacks is calculated using the following formula:

$$P = \frac{a}{b} x\ 100\%$$

Note:
P = Attack percentage (%)
a = Number of rice hills affected by BPH
b = Number of rice hills observed

Damage Intensity

Determination of scoring on symptoms of rice damage due to BPH attack is based on Table 1. The intensity of damage due to a BPH attack is determined using the formula:

$$I = \sum_{i=1}^{i} \frac{Ni\ x\ Vi}{N\ x\ Z} x\ 100\%$$

Note:
I = Damage intensity
Ni = The number of affected rice hills on the score i
Vi = Score i
N = The number of rice hills observed
Z = Highest score

Total DNA Extraction from Brown Planthopper

Total DNA extraction of brown planthopper was obtained from one individual imago or one individual nymph based on the modified method of Goodwin et al. (1994). One individual imago was put into a microtube and then added with 100 µl of CTAB extraction buffer (2% CTAB, 1.4 M NaCl, 100 mM Tris-HCI, 20 mM EDTA, and 1% PVP (-40 °C)). Next, 1 µl of proteinase K was added, then the insects were crushed using a micro-pistil, vortexed, and incubated in a water bath of 65°C for 3 minutes. After that, the tube was added with 100 µl CI (chloroform: isoamyl alcohol) in a ratio of 24:1. The tube was then vortexed for 3 minutes and centrifuged at 10,000 rpm for 15 minutes. The supernatant formed was transferred to a new microtube (60 µl) and then added with 3 M NaOAc (pH 5.2), as much as 1/10 of the total volume of

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the supernatant. Isopropanol was added up to 2/3 of the total volume of the supernatant, then incubated at -20°C for one night. The tube was centrifuged at 10,000 rpm for 10 min, and the supernatant was discarded. The pellets were washed with 100 µl of 80% ethanol (cold) and centrifuged at 8000 rpm for 5 minutes. In the final step, the supernatant was removed, and the pellet was dried for approximately 1 hour. It was then added with a solution of 20 µl TE and stored at -20°C until used.

Amplification of mtCOI Fragments Using the PCR Method

PCR reactants were manufactured with a total volume of 25 µl consisting of 12.5 µl Go Tag Green Master Mix (Promega, US) and 9.5 µl ddH2O. DNA amplification of the mtCOI fragment was carried out using a pair of universal primers mtCOI LCO 1490 (3'-GGTCAACAAATCATAAAGATATTGG-5') and HCO 2198 (5'TAAACTTCA GGGTGACCA AAAAATCA-3') (Folmer et al. 1994) each 1 µl, and 1 µl DNA template. PCR reactions were carried out with a Perkin Elmer 480 Thermocycler (Applied Biosystem, US). The PCR reaction was initiated by initial denaturation for 5 min at 94°C. The PCR was continued for 35 cycles in the following order: 94°C for 1 minute, 52°C for 35 seconds, 72°C for 1 minute 30 seconds, and a final extension of 72°C for 7 minutes. The PCR results were then analyzed in 1% agarose gel. The DNA fragments of mtCOI were visualized using a UV transilluminator after being immersed in a 2% ethidium bromide solution for 15 minutes and photographed with a digital camera. The result of amplification by PCR technique was in the form of mtCOI DNA fragments with a size of ± 710 base pairs (pb).

Analysis of DNA Sequence Results

Nucleotide Sequencing DNA fragment purification and mtCOI nucleotide sequencing were performed at PT. 1st Base, Malaysia. The results were then registered in the NCBI gene bank (http://www.ncbi.nlm.nih.gov). Analysis of mtCOI DNA sequence data ChromasPro program was used to combine forward and reverse nucleotide sequences to obtain the mtCOI gene (ChromasPro version 2.01. 2006). The Bioedit program was used to compare mtCOI fragments between samples (Multiple alignments) (Hall 1999). The phylogenetic relationship was built by comparing the mtCOI sample fragments from the brown planthopper from Indonesia with the mtCOI fragments already stored in the NCBI GenBank (http://www.ncbi.nlm.nih.gov). The criteria for retrieving mtCOI fragments at GenBank were fragments with a nucleotide base length of ± 800 bp (Boykin et al. 2007) (Table 1). The phylogenetic tree was constructed using the PAUP 4.0b10 program (Swofford 2002) with the maximum parsimony cladistic quantitative method. The cladogram was compiled using the Heuristic method. The cladogram used results from the strick consensus with the statistical bootstrap test to obtain a 100% probability.

RESULTS AND DISCUSSION

The brown planthopper causes direct and indirect damage to rice plants. Direct damage was in the form of stunted and uneven growth of rice plants (Figure 1A and 1B), yellow plants (Figure 1C), and hopperburn caused by fluid in rice plant cells sucked by BPH nymphs, brachiptera (Fig. short wings), and macroptera (long wings) (Figures 1E and 1F). Indirect damage was caused by BPH, which acts as a vector of grass dwarf virus and empty dwarf virus, causing stunted rice plants (Figure 1D). Besides Bali or other parts of Indonesia, BPH attacks on rice crops were also reported in China, where hopperburn affected 60% of all examined crops (Hu et al. 2014).

A percentage of BPH attacks of more than 50% was found in Gianyar, Bangli, Jembrana, and Badung Regencies (Table 2). The BPH population was primarily found in Ciherang and IR-64 varieties of rice in the Badung Regency, with 43.67 BPH per rice hill (Table 2). Baehaki and Mejaya (2015) added that the economic threshold could be measured through the number or population of pests and planting age. BPH is said to have reached the economic threshold when the population of this pest was found in the field, as many as nine BPH per rice hill when the rice age was less than 40 DAP or 18 BPH when the rice was more than 40 DAP (Baehaki and Mejaya 2015). In general, rice varieties grown in all observation locations in Bali were BPH susceptible varieties, such as Ciherang, IR-64, Inpari 32, and Situbagendit.

The dynamics of BPH development in the field can be influenced by several factors, including host plant factors and natural enemies (Horgan et al. 2015; Kobayashi 2016). The host plant factors that affect the BPH population are related to the age of the rice plant. When the observations were made, the rice plants were still in the vegetative phase, aged 4-6 WAP. According to Jing et al. (2014), naturally, BPH usually comes to young rice fields, and insects usually come in the first two weeks after planting. Thus, the brown planthopper in rice cultivation might be the first generation of planthoppers that have not yet reproduced because one BPH life cycle takes between 3-4 weeks (IRRI 2009).

BPH observations in Denpasar, Tabanan, Karangasem, and Klungkung cities were dominated by macroptera imago (Table 2). According to Horgan et al. (2017), the planthopper that first came to the plantation was the macroptera planthopper as a winged immigrant planthopper. Meanwhile, in Badung, Gianyar, Buleleng, Bangli, and Jembrana regencies, nymphal BPH was dominated by BPH, and several individuals were in the imago phase of brachiptera and macroptera. The dominance of the nymph phase caused the population of BPH in Badung, Gianyar, Buleleng, Bangli, and Jembrana districts to be the highest when compared to the cities of Denpasar, Tabanan, Karangasem, and Klungkung. The

presence of the brachiptera planthopper might be contributed to the increase in the nymph population (Baehaki and Mejaya 2015). According to Horgan et al. (2015), rapid population growth usually occurs in groups with many young individuals.

The average intensity of BPH attack on Ciherang and IR-64 varieties of rice was higher than in other varieties. In the Ciherang and IR-64 varieties of rice, the average value of the highest attack intensity was 30% (Figure 2). It is because farmers grow rice varieties Ciherang and IR-64 from year to year without any replacement of other varieties. Furthermore, rice varieties Ciherang and IR-64 became very susceptible to BPH attacks. In addition, BPH is a pest that begins to attack rice plants from a young age, even when the rice is still in the nursery.

According to Sawada et al. (1993), fluctuations in BPH pest attacks are more influenced by the growth phase of the rice plant that is the host in the field. BPH pests are often found when rice plants are in the vegetative and generative stages (Bottrell and Schoenly 2012). Horgan et al. (2017) added that BPH pests could damage rice plants at all stages of growth and act as vectors for grass and dwarf viruses. BPH attack was higher when rice was in the vegetative phase than in the generative phase. It happens because the pests attack the young rice stalks. Considering the type of mouth of BPH, which is included in the suction, BPH can suck the liquid from the rice stems and cause the plant leaves to turn yellow (Anant et al. 2021). According to Choi et al. (2019), during the vegetative phase, food availability in the form of nitrogen is abundant in rice plants. Rice plants need nitrogen to form plant organs. Food is one of the factors that affect the life of insects. Qiu et al. (2004) continued that the N element absorbed by plants also serves as a source of nutrition for BPH. If food is available with good quality (suitable for pests), then the insect pest population will increase, and vice versa (Qiu et al. 2004).

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The mtCOI DNA band was only successfully amplified from the total DNA extraction of one imago or nymph and not more than one BPH imago. The mtCOI fragment that was successfully amplified corresponds to a size of ±710 bp in all samples from nine districts in Bali, namely Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana (Figure 3). Nucleotide and amino acid sequence analysis showed high homology with *N. lugens* sequences in the database at GenBank, 94.2 – 99.7% and 95.8 - 100%, respectively (Table 3). *N. lugens* sequences from Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana showed the highest nucleotide, and amino acid homology with *N. lugens* isolate FSD-034 from Pakistan (MK301229) biotype Y, respectively. 99.5 -99.74% and 100% (Table 3). The results of the molecular detection of *N. lugens* using the PCR method in Bali, Indonesia, are the first reports of the molecular character of *N. lugens* in Indonesia.

Samples from Indonesia formed a group with *N. lugens* biotype Y fragment mtCOI from Pakistan, India, South Korea, and China (Figure 4). This study found *N. lugens* biotype Y in rice plants for the first time in Indonesia. The Indonesian sample did not form separate groups according to the proximity of the district locations but formed a polytomy cladogram (Figure 4). This polytomy cladogram shows that the *N. lugens* between regencies (Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana) were observed to have the same ancestry. These results indicate high locomotion ability with genetic mixing between *N. lugens* in Bali isolates. Similar conditions were also demonstrated in *N. lugens* among Asian isolates using mitochondrial sequences showing genetic mixing. It can also be correlated with the theory of long-distance migration of *N. lugens*, which migrates from the tropics (northern Vietnam) in April-May to temperate regions (China, Korea, and Japan) in June-July as shown based on meteorological studies (Otuka et al. 2008). The population of *N. lugens* is a long-distance migratory flight from the tropics to temperate Asia before modern pesticides are widely used in tropical rice. Due to the infrequent use of insecticides prior to the 1960s in the tropics, factors other than insecticides may have triggered long-wing movements to form *N. lugens* populations (Bottrell and Schoenly 2012).

In previous studies in Indonesia, BPH biotypes 1, 2, 3, and 4 have been found. Chen et al. (2011) reported that the brown planthopper is a highly adaptive insect because it can form new biotypes. In early 1975 the IR-26 rice variety from IRRI Philippines was introduced. The IR-26 variety was unique because it contained a Bph1 resistant gene to anticipate fluctuations in the brown planthopper population. However, in 1976 there was a great population explosion in several rice production centers due to changes in the brown planthopper population from biotype 1 to biotype 2. As an anticipatory measure against brown planthopper biotype 2, in 1980, the IR-42 rice variety (containing the bph2 resistant gene) was introduced from IRRI Philippines. Unfortunately, in 1981 there was another explosion in the brown planthopper population in Simalungun, North Sumatra, and several other areas due to changes in the brown planthopper population from biotype 2 to biotype 3. To deal with the brown planthopper biotype 3, rice variety IR-56 was introduced (containing the gene bph3 resistance) in 1983 and IR-64 (containing the bph1+ resistance gene) in 1986. The introduction process continues. In 1991, the IR-74 variety (containing the bph3 resistant gene) was introduced. In 2006, the resistance gene IR-64 was broken because the brown planthopper population changed to biotype 4. The stability of the biotype zero brown planthoppers persisted for 41 years before becoming brown planthopper biotype 1. The change of brown planthopper biotype 1 to biotype 2 only took 4 years, and the change of biotype 2 brown planthopper to biotype 3 within 5 years. Until 2005, the brown planthopper biotype 3 was still dominated by biotype 3, and in 2006 the biotype 4 brown planthopper began to develop. The long existence of the biotype 3 brown planthopper was caused by the development of the IR-64 (bph1+) variety over a long period. IR-64 is a resistant variety (durable resistance) that can withstand changes in brown planthoppers to a more virulent biotype.

The continuous cultivation of IR-64 rice varieties by farmers in Bali led to the emergence of a new biotype BPH, namely Y. Insects of biotype Y originated from biotype 1 by eating YHY15 resistant varieties for more than two years for 33 generations (Jing et al. 2012). Rice varieties YHY15 carry the Bph15 resistance gene (Jing et al. 2012).

This study shows great potential in the population of *N. lugens* to adapt to previously resistant rice varieties. This study reported that rice varieties Situbagendit and Inpari 32, previously resistant to BPH, were susceptible to BPH. This research can provide information to farmers not to continuously plant susceptible varieties, which could cause BPH epidemics in the field, as well as the emergence of new, more virulent BPH biotypes. Thus a new control strategy based on a forecasting system can be developed for the regional management of this insect.

CONCLUTIONS

N. lugens that attacks rice plants in Bali (Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana) belongs to biotype Y. Symptoms of damage to rice plants are most severe in Badung Regency. Apart from Ciherang and IR-64 varieties, Situbagendit and Inpari 32 varieties are susceptible to BPH attack.

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TABLES LIST

Table 1. The damage score of rice plants due to BPH attack

Score	Appearance	Description
0	Healthy	No planthopper was found in any rice hill.
1	Very light damage	The rice hills occupied by the planthoppers did not show dead midribs, few exuviae, and the rice stalks had not yet overgrown with Dematium and Cladosporium fungi that followed the brown planthopper attack.
3	Slightly damaged	The rice hills occupied by the planthoppers have shown dead midribs, many exuviae, and the rice stems are overgrown with Dematium and Cladosporium fungi that follow the brown planthopper attack.
5	Heavily damaged	Rice hills inhabited by planthoppers showed damage marked by many dead midribs, many exuviae, stunted and black-looking tillers, and overgrown with Dematium and Cladosporium fungi.
7	Partially dead	Some of the stems in the rice hills die, or the rice hills withers due to planthoppers attack.
9	Hopperburn	Rice hills die from hopperburn

Sumber: Baehaki (2012)

Table 2. Population and symptoms of BPH attack on rice plants in Bali

Location	**Rice varieties**	**Rice plant age (DAP)**	**BPH attack percentage (%)**	**BPH population abundance (individues/rice hills)**
Denpasar City	Situbagendit, Inpari 32	35	35.43	7.41
Badung	Ciherang, IR-64	42	73.61	43.67
Gianyar	Ciherang, Inpari 32	45	52.26	12.49
Tabanan	Inpari 32	41	37.94	9.26
Buleleng	Ciherang, IR-64	33	46.82	11.28
Karangasem	Situbagendit	30	32.73	7.92
Klungkung	Inpari 32	43	35.89	8.53
Bangli	Ciherang, IR-64	42	52.80	14.83
Jembrana	Ciherang, Inpari 32	36	57.32	11.95

Note: DAP= day after planting

Table 3. Nucleotide (nt) and amino acid (aa) homology of *N. lugens* in rice from Bali, Indonesia, compared with *N. lugens* from other countries in GenBank

Isolate	Origin of isolate	Biotype	Accession number	Homology nt (aa) (%) *N. lugens*_IDN_								
				Denpasar	Badung	Gianyar	Tabanan	Buleleng	Karangasem	Klungkung	Bangli	Jembrana
FSD-034	PAK	Y	MK301229	99.5 (100)	99.6 (100)	99.5 (100)	99.5 (100)	99.6 (100)	99.5 (100)	99.7 (100)	99.5 (100)	99.6 (100)
HZZ55	IND	Y	MK032794	99.4 (100)	99.5 (100)	99.4 (100)	99.5 (100)	99.4 (100)	99.5 (100)	99.6 (100)	99.5 (100)	99.6 (100)
SAEVG_Morph0111	IND	Y	MN520923	99.4 (100)	99.5 (100)	99.4 (100)	99.5 (100)	99.4 (100)	99.5 (100)	99.5 (100)	99.5 (100)	99.6 (100)
KBPH	KOR	Y	MK590088	99.3 (100)	99.5 (100)	99.4 (100)	99.4 (100)	99.3 (100)	99.4 (100)	99.4 (100)	99.4 (100)	99.5 (100)
KOREA_BPH	KOR	Y	LC461184	99.3 (100)	99.5 (100)	99.4 (100)	99.4 (100)	99.3 (100)	99.4 (100)	99.4 (100)	99.4 (100)	99.5 (100)
WUHAN-Y	CHN	Y	KC333653	99.3 (100)	99.5 (100)	99.4 (100)	99.4 (100)	99.3 (100)	99.4 (100)	99.4 (100)	99.3 (100)	99.4 (100)
WUHAN-3	CHN	3	JN563997	97.8 (98.1)	97.2 (97.8)	97.5 (98.9)	97.5 (98.9)	97.4 (97.9)	97.8 (98.1)	97.5 (98.9)	97.2 (98.8)	97.6 (98.0)
WUHAN-2	CHN	2	JN563996	96.3 (97.5)	96.3 (97.5)	96.4 (97.5)	96.3 (97.5)	96.2 (97.4)	96.4 (97.5)	96.3 (97.5)	96.2 (97.4)	96.3 (97.5)
WUHAN-1	CHN	1	JN563995	95.3 (96.7)	95.4 (96.7)	95.3 (96.7)	95.4 (96.7)	95.6 (96.8)	95.6 (96.8)	95.3 (96.7)	95.4 (96.7)	95.3 (96.7)
GX	CHN	1	LC461186	95.3 (96.7)	95.3 (96.7)	95.3 (96.7)	95.4 (96.7)	95.5 (96.8)	95.5 (96.8)	95.3 (96.7)	95.3 (96.7)	95.3 (96.7)
Gangavathi	IND	1	OL451531	95.3 (96.7)	95.3 (96.7)	95.3 (96.7)	95.3 (96.7)	95.5 (96.8)	95.5 (96.8)	95.3 (96.7)	95.3 (96.7)	95.3 (96.7)
WUHAN-L	CHN	L	KC333654	94.2 (95.8)	94.4 (96.2)	94.2 (95.8)	94.4 (96.2)	94.3 (96.0)	94.4 (96.2)	94.2 (95.8)	94.4 (96.2)	94.4 (96.2)
N. bakeri	CHN	-	JX266790	84.6 (85.6)	85.2 (86.1)	84.8 (85.9)	84.8 (85.9)	84.6 (85.6)	85.2 (86.1)	84.8 (85.9)	85.2 (86.1)	85.2 (86.1)
Sogatella furcifera	CHN	-	HM160123	75.6 (76.9)	75.6 (76.9)	76.2 (77.8)	77.6 (78.4)	77.4 (78.4)	76.8 (77.8)	75.6 (76.9)	76.8 (77.8)	77.6 (78.4)

Notes: nt (nucleotide), aa (amino acid), IDN (Indonesia), PAK (Pakistan), IND (India), KOR (South Korea), CHN (China), *N. bakeri* and *Sogatella furcifera* from China was used as outgroups

FIGURES LIST

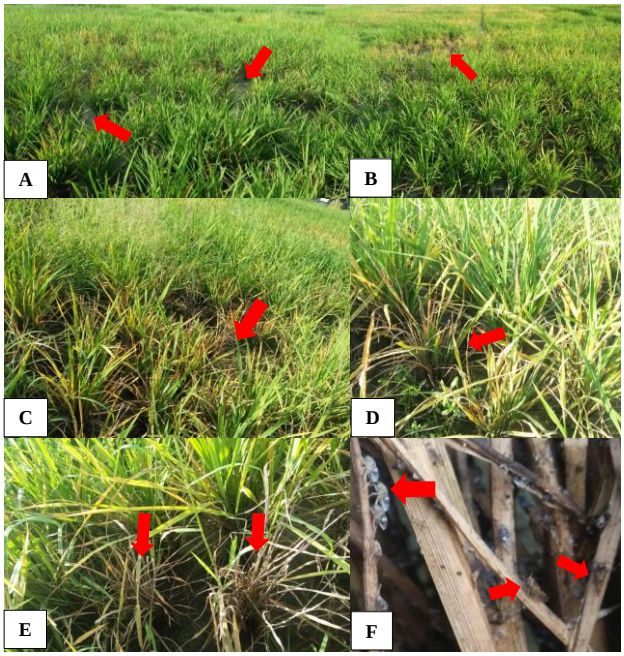

Figure 1. Symptoms of BPH attack on rice plants in Bali: A. rice plant growth is stunted; B. uneven plant growth (spots); C. yellow plant; D. dwarf rice plants; E. plants die like burning (hopperburn); F. BPH brachiptera and macroptera were found on rice stalks.

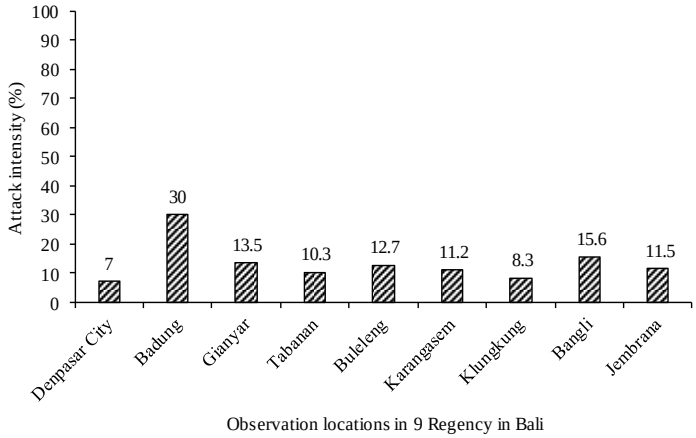

Figure 2. The attack intensity of *N. lugens* on rice in Bali Province

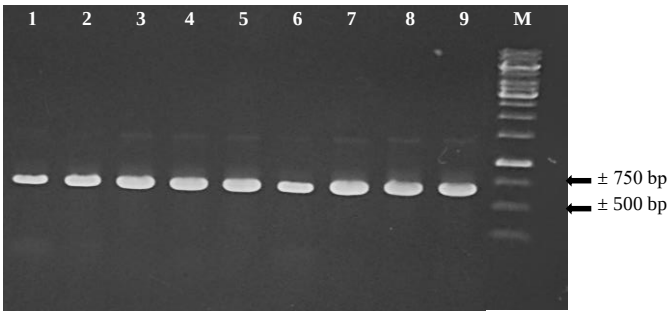

Figure 3. DNA amplification of *N. lugens* in rice plants in Bali using primers LCO 1490/HCO 2198. 1. Denpasar City, 2. Bagung, 3. Gianyar, 4. Tabanan, 5. Buleleng, 6. Karangasemt, 7. Klungkung, 8. Bangli, 9. Jembrana, and M. DNA marker 1 kb (Thermo Scientific)

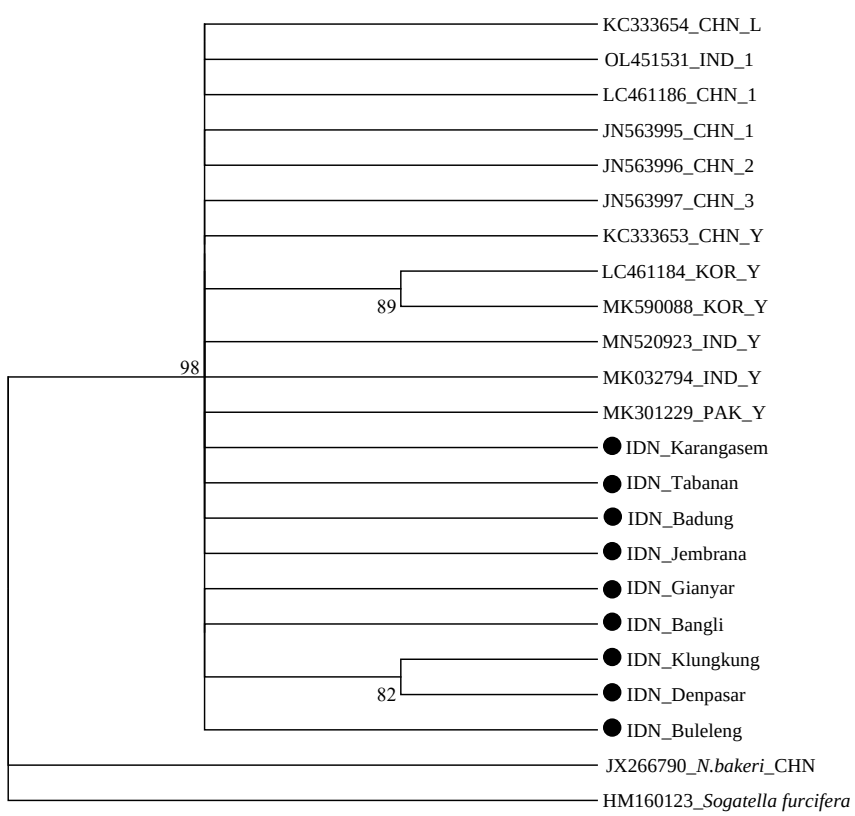

Figure 4. The cladogram of the mtCOI fragment of *N. lugens* from eastern Indonesia, Bali (Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana) was compared with mtCOI fragments from several regions of the world that had been deposited on the NCBI website. *N. bakeri* and *Sogatella furcifera* from China were used as outgroups. The numbers on the branching cladograms represent bootstrap values with 100% probability. IDN (Indonesia), PAK (Pakistan), IND (India), KOR (South Korea), and CHN (China), isolates marked with black dots are Bali isolates.

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The Brown Planthopper (*Nilaparvata lugens* Stal.) Attack and Its Genetic Diversity on Rice in Bali, Indonesia

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Population dynamics and damage intensity of brown planthopper (BPH) and *Nilaparvata lugens* (Stal.) provide information about the economic threshold status of *N. lugens* in Bali. This study enriched information on the genetic diversity of *N. lugens* isolates from eastern Indonesia, particularly Bali. It is the first report of *N. lugens* biotype Y in Indonesia. In addition, the latest information in this study is that rice varieties Situbagendit and Inpari 32, which were previously resistant to BPH, are now found to be susceptible to BPH. This information is critical as a basis for controlling *N. lugens* in Indonesia

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Listihani Listihani

The Brown Planthopper (*Nilaparvata lugens*) Attack and Its Genetic Diversity on Rice in Bali, Indonesia

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Abstract. The brown planthopper is an important pest on rice crops in Indonesia. The genetic diversity of BPH isolates in western Indonesia has been extensively reported, whereas eastern Indonesia isolates have not been reported. The research method used was an observation of attack percentage, population dynamics, attack intensity, and genetic diversity of BPH in 9 districts in Bali. Molecular identification was carried out on *N. lugens* DNA in the mtCOI fragment. BPH attacks of >50% were found in the districts of Gianyar, Bangli, Jembrana, and Badung. The BPH population was primarily found in Ciherang and IR-64 varieties of rice in the Badung Regency, with 43.67 BPH per rice hill. In general, rice varieties grown in all observation locations were susceptible to BPH, such as Ciherang, IR-64, Inpari 32, and Situbagendit. In the Ciherang and IR-64 varieties, the highest attack intensity average value reached 30%. The sequence of *N. lugens* isolate from Bali Jembrana showed the highest nucleotide and amino acid homology with *N. lugens* isolate FSD-034 from Pakistan (MK301229) biotype Y of 99.5 -99.74% and 100%, respectively. This study found *N. lugens* biotype Y in rice plants for the first time in Indonesia. This study reported that Rice varieties Situbagendit and Inpari 32, previously resistant to BPH, are reported as susceptible to BPH.

Keywords: susceptible variety, Situbagendit, Inpari 32, genetic diversity, attack intensity

Abbreviations (if any): The BPH, Rice Plants

Running title: The Brown Planthopper (*Nilaparvata lugens*.) Attack and Its Genetic Diversity on Rice in Bali, Indonesia

INTRODUCTION

The brown planthopper (BPH) (*Nilaparvata lugens*) is the **most** destructive rice pest in Indonesia. Repeated outbreaks of BPH in Indonesia are caused by continuous rice cultivation, extensive use, and over-application of insecticides (Baehaki and Mejaya 2015). This pest is vascular monophagous in rice (Cheng et al. 2013; Ferrater et al. 2015). Feeding by nymphs and imago at the base of the plant causes rapid wilting and drying of the plant (Bottrell and Schoenly 2012; Cheng et al. 2013; Bao and Zhang 2019). In addition, BPH is also a vector of Rice grassy stunt virus and Rice ragged stunt virus (Bao and Zhang 2019). At high population levels of *N. lugens* can cause significant losses in rice production (Cheng et al. 2013; Zheng et al. 2013; Bao and Zhang 2019).

The BPH cannot tolerate winter in northern Asia, including Japan, Korea, and northern China (He et al. 2012; Fu et al. 2012; Fu et al. 2014). The population originally came from subtropical and tropical areas by flying long distances during the summer (Fu et al. 2014; Hu et al. 2014). The intensification of rice production triggered the BPH outbreak in Tropical Asia during the green revolution era in the 1970s and 1980s (Bottrell and Schoenly 2012). *N. lugens* is the main problem causing rice harvest failure in several countries. Inaccurate identification and prolonged identification of *N. lugens* are obstacles to its field management strategy.

Traditionally, BPH has been identified at the species level by morphological features using anatomical characteristics, namely, wings, front, and external genitalia (Dupo and Barrion 2009). Accurate identification requires extensive expertise and experience and yet sometimes can lead to errors. Morphological identification by an entomologist can reduce the potential for errors. Practical morphological identification is only possible when dealing with small sample sizes and well-preserved specimens. It is crucial to utilize a new identification method that is accurate, fast, time-saving, and suitable for large numbers of specimens.

Molecular techniques with high reproducibility and fast results offer an excellent alternative to traditional morphological classification. Several mitochondrial and nuclear genes have been used as genetic markers to differentiate related species. These include the mitochondrial cytochrome c oxidase subunit 1 (CO1) gene, nuclear 12S-16S-18S ribosomal RNA genes (Fukunaga et al. 2000; Brengues et al. 2014; Gomez-Polo et al. 2014; Wang et al. 2016; Liu et al.

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2018). As ITS sequences have low intra-species variation but high variation between species, they are helpful for species classification and phylogenetic analysis for morphologically similar organisms, both in prokaryotes and eukaryotes (Liu et al. 2009). Finally, from the molecular identification of the combined mitochondrial COI-COII and ten microsatellite marker loci (Winnie et al. 2020)..

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MATERIALS AND METHODS

Brown Planthopper Sampling from Rice Dwarf Disease Endemic Areas

Samples were taken from nine locations in Bali Province (Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana). The brown planthopper samples taken from rice plants were nymphs and imagos. Nymphs and imagos were used for total DNA extraction. After arriving at the laboratory, the nymphs and imago were stored dry at -20ºC.

Observation of BPH Attack Symptoms and Quantity of BPH Population/rice hill

Observation of symptoms of BPH attack was carried out by observing symptoms of damage to rice plants due to BPH attack. The abundance of the BPH/rice hill population was obtained by counting all nymphs and imagoes obtained. Data on the population per cluster from 20 samples at each observation location were then averaged.

BPH Attack Percentage

The percentage of BPH attacks is calculated using the following formula:

$$P = \frac{a}{b} x\ 100\%$$

Note:
P = Attack percentage (%)
a = Number of rice hills affected by BPH
b = Number of rice hills observed

Damage Intensity

Determination of scoring on symptoms of rice damage due to BPH attack is based on Table 1. The intensity of damage due to a BPH attack is determined using the formula:

$$I = \sum_{i=1}^{i} \frac{Ni\ x\ Vi}{N\ x\ Z} x\ 100\%$$

Note:
I = Damage intensity
Ni = The number of affected rice hills on the score i
Vi = Score i
N = The number of rice hills observed
Z = Highest score

Total DNA Extraction from Brown Planthopper

Total DNA extraction of brown planthopper was obtained from one individual imago or one individual nymph. One individual imago was put into a microtube and then added with 100 µl of CTAB extraction buffer (2% CTAB, 1.4 M NaCl, 100 mM Tris-HCI, 20 mM EDTA, and 1% PVP (-40 °C)). Next, 1 µl of proteinase K was added, then the insects were crushed using a micro-pistil, vortexed, and incubated in a water bath of 65°C. After that, the tube was added with 100 µl CI (chloroform: isoamyl alcohol) in a ratio of 24:1. The tube was then vortexed for 3 minutes and centrifuged at 10,000 rpm for 15 minutes. The supernatant formed was transferred to a new microtube (60 µl) and then added with 3 M NaOAc (pH 5.2), as much as 1/10 of the total volume of the supernatant. Isopropanol was added up to 2/3 of the total volume of the supernatant, then incubated at -20°C for one night. The tube was centrifuged at 10,000 rpm for 10 min, and the supernatant was discarded. The pellets were washed with 100 µl of 80% ethanol (cold) and centrifuged at 8000 rpm for 5 minutes. In the final step, the supernatant was removed, and the pellet was dried for approximately 1 hour. It was then added with a solution of 20 µl TE and stored at -20°C until used.

Amplification of mtCOI Fragments Using the PCR Method

PCR reactants were manufactured with a total volume of 25 µl consisting of 12.5 µl Go Tag Green Master Mix and 9.5 µl ddH2O. DNA amplification of the mtCOI fragment was carried out using a pair of universal primers mtCOI LCO 1490 (3'-GGTCAACAAATCATAAAGATATTGG-5') and HCO 2198 (5'TAAACTTCA GGGTGACCA AAAAATCA-3') (Folmer et al. 1994) each 1 µl, and 1 µl DNA template. PCR reactions were carried out with a Perkin Elmer 480 Thermocycler. The PCR reaction was initiated by initial denaturation for 5 min at 94°C. The PCR was continued for 35 cycles in the following order: 94°C for 1 minute, 52°C for 35 seconds, 72°C for 1 minute 30 seconds, and a final extension of 72°C for 7 minutes. The PCR results were then analyzed in 1% agarose gel. The DNA fragments of mtCOI were visualized using a UV transilluminator after being immersed in a 2% ethidium bromide solution for 15 minutes and photographed with a digital camera.

Analysis of DNA Sequence Results

Nucleotide Sequencing DNA fragment purification and mtCOI nucleotide sequencing were performed at PT. 1st Base, Malaysia. The results were then registered in the NCBI gene bank (http://www.ncbi.nlm.nih.gov). Analysis of mtCOI DNA sequence data ChromasPro program was used to combine forward and reverse nucleotide sequences to obtain the mtCOI gene. The Bioedit program was used to compare mtCOI fragments between samples (Multiple alignments) (Hall 1999). The phylogenetic relationship was built by comparing the mtCOI sample fragments from the brown planthopper from Indonesia with the mtCOI fragments already stored in the NCBI GenBank (http://www.ncbi.nlm.nih.gov). The criteria for retrieving mtCOI fragments at GenBank were fragments with a nucleotide base length of ± 800 bp (Boykin et al. 2007) (Table 1). The phylogenetic tree was constructed using the PAUP 4.0b10 program (Swofford 2002) with the maximum parsimony cladistic quantitative method. The cladogram was compiled using the Heuristic method. The cladogram used results from the strick consensus with the statistical bootstrap test to obtain a 100% probability.

RESULTS AND DISCUSSION

The brown planthopper causes direct and indirect damage to rice plants. Direct damage was in the form of stunted and uneven growth of rice plants (Figure 1A and 1B), yellow plants (Figure 1C), and hopperburn caused by fluid in rice plant cells sucked by BPH nymphs, brachiptera (Fig. short wings), and macroptera (long wings) (Figures 1E and 1F). Indirect damage was caused by BPH, which acts as a vector of grass dwarf virus and empty dwarf virus, causing stunted rice plants (Figure 1D). Besides Bali or other parts of Indonesia, BPH attacks on rice crops were also reported in China, where hopperburn affected 60% of all examined crops.

A percentage of BPH attacks of more than 50% was found in Gianyar, Bangli, Jembrana, and Badung Regencies (Table 2). The BPH population was primarily found in Ciherang and IR-64 varieties of rice in the Badung Regency, with 43.67 BPH per rice hill (Table 2). Baehaki and Mejaya (2015) added that the economic threshold could be measured through the number or population of pests and planting age. BPH is said to have reached the economic threshold when the population of this pest was found in the field, as many as nine BPH per rice hill when the rice age was less than 40 DAP or 18 BPH when the rice was more than 40 DAP (Baehaki and Mejaya 2015). In general, rice varieties grown in all observation locations in Bali were BPH susceptible varieties, such as Ciherang, IR-64, Inpari 32, and Situbagendit.

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The dynamics of BPH development in the field can be influenced by several factors, including host plant factors and natural enemies (Horgan et al. 2015; Kobayashi 2016). The host plant factors that affect the BPH population are related to the age of the rice plant. When the observations were made, the rice plants were still in the vegetative phase. According to Jing et al. (2014), naturally, BPH usually comes to young rice fields, and insects usually come in the first two weeks after planting. Thus, the brown planthopper in rice cultivation might be the first generation of planthoppers that have not yet reproduced because one BPH life cycle takes between 3-4 weeks (IRRI 2009).

BPH observations in Denpasar, Tabanan, Karangasem, and Klungkung cities were dominated by macroptera imago. According to Horgan et al. (2017), the planthopper that first came to the plantation was the macroptera planthopper as a winged immigrant planthopper. In Badung, Gianyar, Buleleng, Bangli, and Jembrana regencies, nymphal BPH was dominated by BPH, and several individuals were in the imago phase of brachiptera and macroptera. The dominance of the nymph phase caused the population of BPH in Badung, Gianyar, Buleleng, Bangli, and Jembrana districts to be the highest when compared to the cities of Denpasar, Tabanan, Karangasem, and Klungkung. The presence of the brachiptera planthopper might be contributed to the increase in the nymph population (Baehaki and Mejaya 2015).

The average intensity of BPH attack on Ciherang and IR-64 varieties of rice was higher than in other varieties. In the Ciherang and IR-64 varieties of rice, the average value of the highest attack intensity was 30% (Figure 2). It is because farmers grow rice varieties Ciherang and IR-64 from year to year without any replacement of other varieties. Furthermore, rice varieties Ciherang and IR-64 became very susceptible to BPH attacks. In addition, BPH is a pest that begins to attack rice plants from a young age, even when the rice is still in the nursery.

According to Sawada et al. (1993), fluctuations in BPH pest attacks are more influenced by the growth phase of the rice plant that is the host in the field. BPH pests are often found when rice plants are in the vegetative and generative stages (Bottrell and Schoenly 2012). Horgan et al. (2017) added that BPH pests could damage rice plants at all stages of growth and act as vectors for grass and dwarf viruses. BPH attack was higher when rice was in the vegetative phase than in the generative phase. It happens because the pests attack the young rice stalks. Considering the type of mouth of BPH, which is included in the suction, BPH can suck the liquid from the rice stems and cause the plant leaves to turn yellow (Anant et al. 2021). According to Choi et al. (2019), during the vegetative phase, food availability in the form of nitrogen is abundant in rice plants. Rice plants need nitrogen to form plant organs. Food is one of the factors that affect the life of insects. Qiu et al. (2004) continued that the N element absorbed by plants also serves as a source of nutrition for BPH. If food is available with good quality, then the insect pest population will increase, and vice versa (Qiu et al. 2004).

The mtCOI DNA band was only successfully amplified from the total DNA extraction of one imago or nymph and not more than one BPH imago. The mtCOI fragment that was successfully amplified corresponds in all samples from nine districts in Bali, namely Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana (Figure 3). Nucleotide and amino acid sequence analysis showed high homology with *N. lugens* sequences in the database at GenBank, 94.2 – 99.7% and 95.8 - 100% (Table 3). *N. lugens* sequences from Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana showed the highest nucleotide, and amino acid homology with *N. lugens* isolate FSD-034 from Pakistan (MK301229) biotype Y, respectively. 99.5 -99.74% and 100%, respectively (Table 3). The results of the molecular detection of *N. lugens* using the PCR method in Bali, Indonesia, are the first reports of the molecular character of *N. lugens* in Indonesia.

Samples from Indonesia formed a group with *N. lugens* biotype Y fragment mtCOI from Pakistan, India, South Korea, and China (Figure 4). This study found *N. lugens* biotype Y in rice plants for the first time in Indonesia. The Indonesian sample did not form separate groups according to the proximity of the district locations but formed a polytomy cladogram (Figure 4). This polytomy cladogram shows that the *N. lugens* between regencies (Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana) were observed to have the same ancestry. These results indicate high locomotion ability with genetic mixing between *N. lugens* in Bali isolates. Similar conditions were also demonstrated in *N. lugens* among Asian isolates using mitochondrial sequences showing genetic mixing. It can also be correlated with the theory of long-distance migration of *N. lugens*, which migrates from the tropics (northern Vietnam) in April-May to temperate regions (China, Korea, and Japan) in June-July as shown based on meteorological studies (Otuka et al. 2008).

In previous studies in Indonesia, BPH biotypes 1, 2, 3, and 4 have been found. Chen et al. (2011) reported that the brown planthopper is a highly adaptive insect. In early 1975 the IR-26 rice variety from IRRI Philippines was introduced. The IR-26 variety was unique because it contained a Bph1 resistant gene to anticipate fluctuations in the brown planthopper population. However, in 1976 there was a great population explosion in several rice production centers due to changes in the brown planthopper population from biotype 1 to biotype 2. As an anticipatory measure against brown planthopper biotype 2, in 1980, the IR-42 rice variety (containing the bph2 resistant gene) was introduced from IRRI Philippines. Unfortunately, in 1981 there was another explosion in the brown planthopper population in Simalungun, North Sumatra, and several other areas due to changes in the brown planthopper population from biotype 2 to biotype 3. To deal with the brown planthopper biotype 3, rice variety IR-56 was introduced (containing the gene bph3 resistance) in 1983 and IR-64 (containing the bph1+ resistance gene) in 1986. The introduction process continues. In 1991, the IR-74 variety (containing the bph3 resistant gene) was introduced. In 2006, the resistance gene IR-64 was broken because the brown planthopper population changed to biotype 4. The stability of the biotype zero brown planthoppers persisted for 41 years before becoming brown planthopper biotype 1. The change of brown planthopper biotype 1 to biotype 2 only took 4

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years, and the change of biotype 2 brown planthopper to biotype 3 within 5 years. Until 2005, the brown planthopper biotype 3 was still dominated by biotype 3, and in 2006 the biotype 4 brown planthopper began to develop.

The continuous cultivation of IR-64 rice varieties by farmers in Bali led to the emergence of a new biotype BPH, namely Y. Insects of biotype Y originated from biotype 1 by eating YHY15 resistant varieties for more than two years for 33 generations (Jing et al. 2012). Rice varieties YHY15 carry the Bph15 resistance gene (Jing et al. 2012).

This study shows great potential in the population of *N. lugens* to adapt to previously resistant rice varieties. This study reported that rice varieties Situbagendit and Inpari 32, previously resistant to BPH, were susceptible to BPH. This research can provide information to farmers not to continuously plant susceptible varieties, which could cause BPH epidemics in the field, as well as the emergence of new, more virulent BPH biotypes. Thus a new control strategy based on a forecasting system can be developed for the regional management of this insect.

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CONCLUTIONS

N. lugens that attacks rice plants in Bali (Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana) belongs to biotype Y. Symptoms of damage to rice plants are most severe in Badung Regency. Apart from Ciherang and IR-64 varieties, Situbagendit and Inpari 32 varieties are susceptible to BPH attack.

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TABLES LIST

Table 1. The damage score of rice plants due to BPH attack

Score	Appearance	Description
0	Healthy	No planthopper was found in any rice hill.
1	Very light damage	The rice hills occupied by the planthoppers did not show dead midribs, few exuviae, and the rice stalks had not yet overgrown with Dematium and Cladosporium fungi that followed the brown planthopper attack.
3	Slightly damaged	The rice hills occupied by the planthoppers have shown dead midribs, many exuviae, and the rice stems are overgrown with Dematium and Cladosporium fungi that follow the brown planthopper attack.
5	Heavily damaged	Rice hills inhabited by planthoppers showed damage marked by many dead midribs, many exuviae, stunted and black-looking tillers, and overgrown with Dematium and Cladosporium fungi.
7	Partially dead	Some of the stems in the rice hill die, or the rice hill withers due to planthoppers attack.
9	Hopperburn	Rice hills die from hopperburn

Sumber: Baehaki (2012)

Table 2. Population and symptoms of BPH attack on rice plants in Bali

Location	**Rice varieties**	**Rice plant age (DAP)**	**BPH attack percentage (%)**	**BPH population abundance (individues/rice hills)**
Denpasar City	Situbagendit, Inpari 32	35	35.43	7.41
Badung	Ciherang, IR-64	42	73.61	43.67
Gianyar	Ciherang, Inpari 32	45	52.26	12.49
Tabanan	Inpari 32	41	37.94	9.26
Buleleng	Ciherang, IR-64	33	46.82	11.28
Karangasem	Situbagendit	30	32.73	7.92
Klungkung	Inpari 32	43	35.89	8.53
Bangli	Ciherang, IR-64	42	52.80	14.83
Jembrana	Ciherang, Inpari 32	36	57.32	11.95

Note: DAP= day after planting

Table 3. Nucleotide (nt) and amino acid (aa) homology of *N. lugens* in rice from Bali, Indonesia, compared with *N. lugens* from other countries in GenBank

Isolate	Origin of isolate	Biotype	Accession number	Homology nt (aa) (%) *N. lugens*_IDN_								
				Denpasar	Badung	Gianyar	Tabanan	Buleleng	Karangasem	Klungkung	Bangli	Jembrana
FSD-034	PAK	Y	MK301229	99.5 (100)	99.6 (100)	99.5 (100)	99.5 (100)	99.6 (100)	99.5 (100)	99.7 (100)	99.5 (100)	99.6 (100)
HZZ55	IND	Y	MK032794	99.4 (100)	99.5 (100)	99.4 (100)	99.5 (100)	99.4 (100)	99.5 (100)	99.6 (100)	99.5 (100)	99.6 (100)
SAEVG_Morph0111	IND	Y	MN520923	99.4 (100)	99.5 (100)	99.4 (100)	99.5 (100)	99.4 (100)	99.5 (100)	99.5 (100)	99.5 (100)	99.6 (100)
KBPH	KOR	Y	MK590088	99.3 (100)	99.5 (100)	99.4 (100)	99.4 (100)	99.3 (100)	99.4 (100)	99.4 (100)	99.4 (100)	99.5 (100)
KOREA_BPH	KOR	Y	LC461184	99.3 (100)	99.5 (100)	99.4 (100)	99.4 (100)	99.3 (100)	99.4 (100)	99.4 (100)	99.4 (100)	99.5 (100)
WUHAN-Y	CHN	Y	KC333653	99.3 (100)	99.5 (100)	99.4 (100)	99.4 (100)	99.3 (100)	99.4 (100)	99.4 (100)	99.3 (100)	99.4 (100)
WUHAN-3	CHN	3	JN563997	97.8 (98.1)	97.2 (97.8)	97.5 (98.9)	97.5 (98.9)	97.4 (97.9)	97.8 (98.1)	97.5 (98.9)	97.2 (98.8)	97.6 (98.0)
WUHAN-2	CHN	2	JN563996	96.3 (97.5)	96.3 (97.5)	96.4 (97.5)	96.3 (97.5)	96.2 (97.4)	96.4 (97.5)	96.3 (97.5)	96.2 (97.4)	96.3 (97.5)
WUHAN-1	CHN	1	JN563995	95.3 (96.7)	95.4 (96.7)	95.3 (96.7)	95.4 (96.7)	95.6 (96.8)	95.6 (96.8)	95.3 (96.7)	95.4 (96.7)	95.3 (96.7)
GX	CHN	1	LC461186	95.3 (96.7)	95.3 (96.7)	95.3 (96.7)	95.4 (96.7)	95.5 (96.8)	95.5 (96.8)	95.3 (96.7)	95.3 (96.7)	95.3 (96.7)
Gangavathi	IND	1	OL451531	95.3 (96.7)	95.3 (96.7)	95.3 (96.7)	95.3 (96.7)	95.5 (96.8)	95.5 (96.8)	95.3 (96.7)	95.3 (96.7)	95.3 (96.7)
WUHAN-L	CHN	L	KC333654	94.2 (95.8)	94.4 (96.2)	94.2 (95.8)	94.4 (96.2)	94.3 (96.0)	94.4 (96.2)	94.2 (95.8)	94.4 (96.2)	94.4 (96.2)
N. bakeri	CHN	-	JX266790	84.6 (85.6)	85.2 (86.1)	84.8 (85.9)	84.8 (85.9)	84.6 (85.6)	85.2 (86.1)	84.8 (85.9)	85.2 (86.1)	85.2 (86.1)
Sogatella furcifera	CHN	-	HM160123	75.6 (76.9)	75.6 (76.9)	76.2 (77.8)	77.6 (78.4)	77.4 (78.4)	76.8 (77.8)	75.6 (76.9)	76.8 (77.8)	77.6 (78.4)

Notes: nt (nucleotide), aa (amino acid), IDN (Indonesia), PAK (Pakistan), IND (India), KOR (South Korea), CHN (China), *N. bakeri* and *Sogatella furcifera* from China was used as outgroups

FIGURES LIST

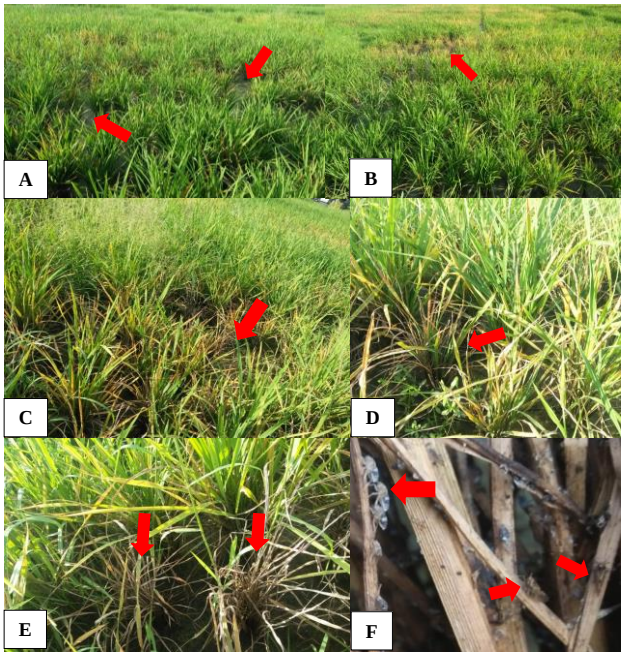

Figure 1. Symptoms of BPH attack on rice plants in Bali: A. rice plant growth is stunted; B. uneven plant growth (spots); C. yellow plant; D. dwarf rice plants; E. plants die like burning (hopperburn); F. BPH brachiptera and macroptera were found on rice stalks.

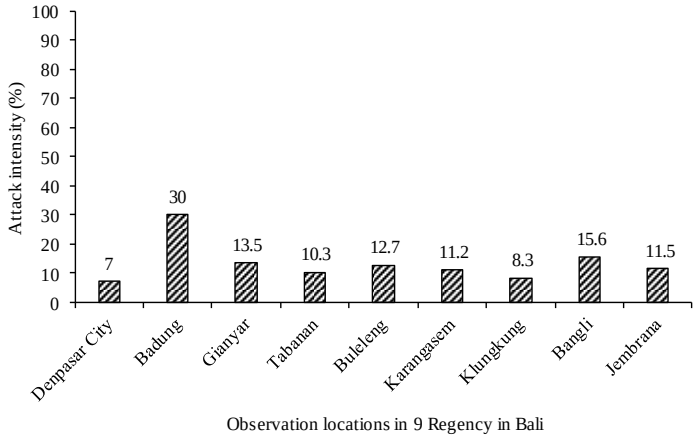

Figure 2. The attack intensity of *N. lugens* on rice in Bali Province

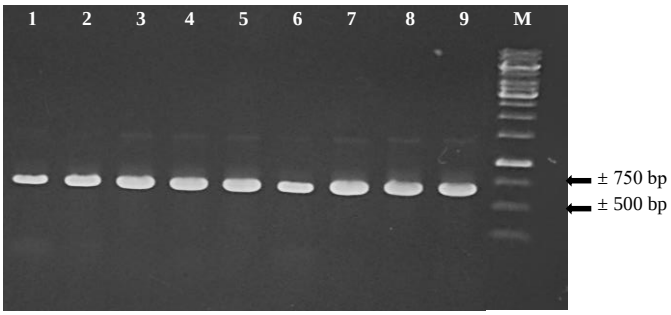

Figure 3. DNA amplification of *N. lugens* in rice plants in Bali using primers LCO 1490/HCO 2198. 1. Denpasar City, 2. Bagung, 3. Gianyar, 4. Tabanan, 5. Buleleng, 6. Karangasemt, 7. Klungkung, 8. Bangli, 9. Jembrana, and M. DNA marker 1 kb (Thermo Scientific)

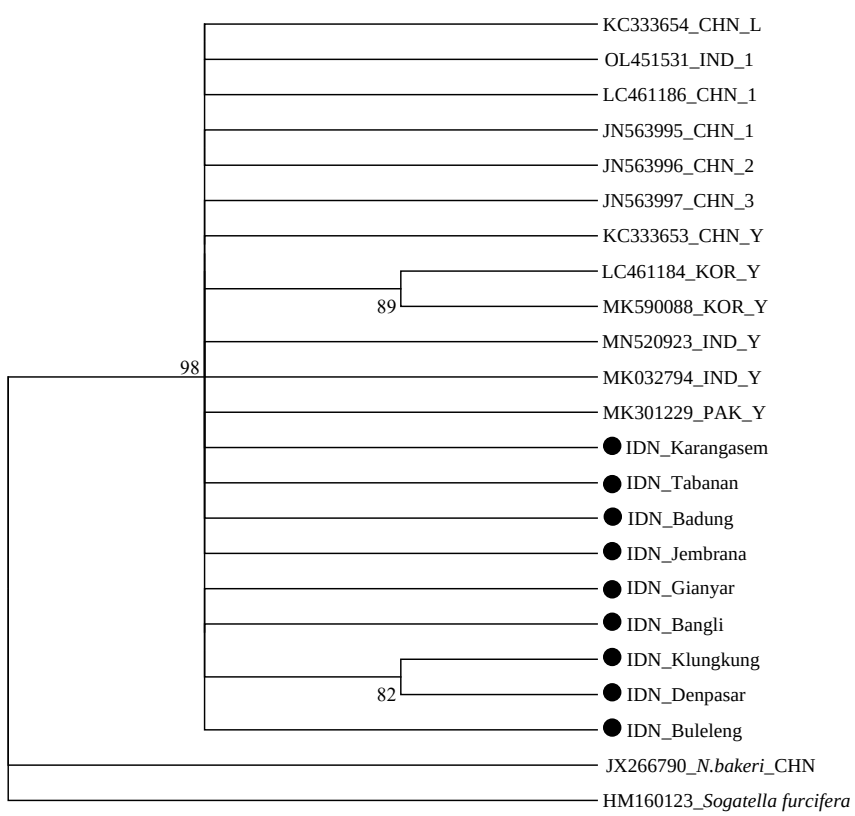

Figure 4. The cladogram of the mtCOI fragment of *N. lugens* from eastern Indonesia, Bali (Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana) was compared with mtCOI fragments from several regions of the world that had been deposited on the NCBI website. *N. bakeri* and *Sogatella furcifera* from China were used as outgroups. The numbers on the branching cladograms represent bootstrap values with 100% probability. IDN (Indonesia), PAK (Pakistan), IND (India), KOR (South Korea), and CHN (China), isolates marked with black dots are Bali isolates.

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Population dynamics and damage intensity of brown planthopper (BPH) and *Nilaparvata lugens* (Stal.) provide information about the economic threshold status of *N. lugens* in Bali. This study enriched information on the genetic diversity of *N. lugens* isolates from eastern Indonesia, particularly Bali. It is the first report of *N. lugens* biotype Y in Indonesia. In addition, the latest information in this study is that rice varieties Situbagendit and Inpari 32, which were previously resistant to BPH, are now found to be susceptible to BPH. This information is critical as a basis for controlling *N. lugens* in Indonesia

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Listihani Listihani

The Brown Planthopper (*Nilaparvata lugens* Stal.) Attack and Its Genetic Diversity on Rice in Bali, Indonesia

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Abstract. The brown planthopper is an important pest on rice crops in Indonesia. The genetic diversity of BPH isolates in western Indonesia has been extensively reported, whereas eastern Indonesia isolates have not been reported. This research aims to analyze genetic diversity and evaluate the BPH attack's intensity on Bali rice plants. The research method used was an observation of attack percentage, population dynamics, attack intensity, and genetic diversity of BPH in 9 districts in Bali (Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana). Molecular identification was carried out on *N. lugens* DNA in the mtCOI fragment. BPH attacks of >50% were found in the districts of Gianyar, Bangli, Jembrana, and Badung. The BPH population was primarily found in Ciherang and IR-64 varieties of rice in the Badung Regency, with 43.67 BPH per rice hill. In general, rice varieties grown in all observation locations were susceptible to BPH, such as Ciherang, IR-64, Inpari 32, and Situbagendit. In the Ciherang and IR-64 varieties, the highest attack intensity average value reached 30%. The sequence of *N. lugens* isolate from Bali Jembrana showed the highest nucleotide and amino acid homology with *N. lugens* isolate FSD-034 from Pakistan (MK301229) biotype Y of 99.5 -99.74% and 100%, respectively. This study found *N. lugens* biotype Y in rice plants for the first time in Indonesia. This study reported that Rice varieties Situbagendit and Inpari 32, previously resistant to BPH, are reported as susceptible to BPH.

Keywords: attack intensity, genetic diversity, Inpari 32, Situbagendit, susceptible variety

Abbreviations (if any): The BPH, Rice Plants

Running title: The Brown Planthopper (*Nilaparvata lugens* Stal.) Attack and Its Genetic Diversity on Rice in Bali, Indonesia

INTRODUCTION

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The brown planthopper (BPH) (*Nilaparvata lugens* Stal, Hemiptera: Delphacidae) is the most destructive rice pest in Indonesia. Repeated outbreaks of BPH in Indonesia are caused by continuous rice cultivation, extensive use, and over-application of insecticides (Baehaki and Mejaya 2015). This pest is vascular monophagous in rice (Cheng et al. 2013; Ferrater et al. 2015). Feeding by nymphs and imago at the base of the plant causes rapid wilting and drying of the plant (Bottrell and Schoenly 2012; Cheng et al. 2013; Bao and Zhang 2019). In addition, BPH is also a vector of *Rice grassy stunt virus* and *Rice ragged stunt virus* (Bao and Zhang 2019). At high population levels of *N. lugens* can cause significant losses in rice production (Cheng et al. 2013; Zheng et al. 2013; Bao and Zhang 2019).

The BPH cannot tolerate winter in northern Asia, including Japan, Korea, and northern China (He et al. 2012; Fu et al. 2012; Fu et al. 2014). The population originally came from subtropical and tropical areas by flying long distances during the summer (Fu et al. 2014; Hu et al. 2014). BPH infestation in temperate climates originated from annual migrations from tropical Asia and China (He et al. 2012). During autumn, BPH re-migrates (north-to-south) and BPH populations have been studied in China and India (Bottrell and Schoenly 2012). Such return migration may help explain how long-distance migration is maintained in the winter.

The intensification of rice production triggered the BPH outbreak in Tropical Asia during the green revolution era in the 1970s and 1980s (Bottrell and Schoenly 2012). Until now, *N. lugens* is the main problem causing rice harvest failure in several countries. Inaccurate identification and prolonged identification of *N. lugens* are obstacles to its field management strategy.

Traditionally, BPH has been identified at the species level by morphological features using anatomical characteristics, namely, wings, front, and external genitalia (Dupo and Barrion 2009). Accurate identification requires extensive expertise and experience and yet sometimes can lead to errors. Morphological identification by an entomologist can reduce the potential for errors. Practical morphological identification is only possible when dealing with small sample

sizes and well-preserved specimens. Therefore, it is crucial to utilize a new identification method that is accurate, fast, time-saving, and suitable for large numbers of specimens.

Molecular techniques with high reproducibility and fast results offer an excellent alternative to traditional morphological classification. Several mitochondrial and nuclear genes have been used as genetic markers to differentiate related species. These include the mitochondrial cytochrome c oxidase subunit 1 (CO1) gene, nuclear 12S-16S-18S ribosomal RNA genes, and ITS1 and ITS2 internal transcription spacers (Fukunaga et al. 2000; Brengues et al. 2014; Gomez-Polo et al. 2014; Wang et al. 2016; Liu et al. 2018). ITS1 and ITS2 are nonfunctional spacers that separate the 18S-5.8S and 5.8S-28S rRNA genes, respectively (Ji et al. 2003; Liu et al. 2018). As ITS sequences have low intra-species variation but high variation between species, they are helpful for species classification and phylogenetic analysis for morphologically similar organisms, both in prokaryotes and eukaryotes (Liu et al. 2009). Finally, from the molecular identification of the combined mitochondrial COI-COII and ten microsatellite marker loci (Winnie et al. 2020).

The genetic diversity of *N. lugens* has been reported in several countries such as China, South Korea, Pakistan, India, and Malaysia (Jing et al. 2012; Zheng et al. 2021; Anant et al. 2021; Latif et al. 2012). The genetic diversity of *N. lugens* in Indonesia is widely reported in western Indonesia (Java Island) (Winnie et al. 2020; Chaerani et al. 2021). Reports on the genetic diversity of *N. lugens* in eastern Indonesia have not been found. Therefore, this study aims to analyze genetic diversity and determine the intensity of BPH attacks on rice plants in eastern Indonesia, especially Bali.

MATERIALS AND METHODS

Brown Planthopper Sampling from Rice Dwarf Disease Endemic Areas

Samples were taken from nine locations in Bali Province (Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana). The brown planthopper samples taken from rice plants were nymphs and imagos. Nymphs and imagos were used for total DNA extraction. After arriving at the laboratory, the nymphs and imago were stored dry at -20ºC.

Observation of BPH Attack Symptoms and Quantity of BPH Population/rice hill

Observation of symptoms of BPH attack was carried out by observing symptoms of damage to rice plants due to BPH attack. The abundance of the BPH/rice hill population was obtained by counting all nymphs and imagoes obtained. Data on the population per cluster from 20 samples at each observation location were then averaged.

BPH Attack Percentage

The percentage of BPH attacks is calculated using the following formula:

$$P = \frac{a}{b} \, x \, 100\%$$

Note:
P = Attack percentage (%)
a = Number of rice hills affected by BPH
b = Number of rice hills observed

Damage Intensity

Determination of scoring on symptoms of rice damage due to BPH attack is based on Table 1. The intensity of damage due to a BPH attack is determined using the formula (Erdiansyah and Damanhuri 2018):

$$I = \sum_{i=1}^{i} \frac{Ni \, x \, Vi}{N \, x \, Z} \, x \, 100\%$$

Note:
I = Damage intensity
Ni = The number of affected rice hills on the score i
Vi = Score i
N = The number of rice hills observed
Z = Highest score

Total DNA Extraction from Brown Planthopper

Total DNA extraction of brown planthopper was obtained from one individual imago or one individual nymph based on the modified method of Goodwin et al. (1994). One individual imago was put into a microtube and then added

with 100 µl of CTAB extraction buffer (2% CTAB, 1.4 M NaCl, 100 mM Tris-HCl, 20 mM EDTA, and 1% PVP (-40 °C)). Next, 1 µl of proteinase K was added, then the insects were crushed using a micro-pistil, vortexed, and incubated in a water bath of 65°C for 3 minutes. After that, the tube was added with 100 µl CI (chloroform: isoamyl alcohol) in a ratio of 24:1. The tube was then vortexed for 3 minutes and centrifuged at 10,000 rpm for 15 minutes. The supernatant formed was transferred to a new microtube (60 µl) and then added with 3 M NaOAc (pH 5.2), as much as 1/10 of the total volume of the supernatant. Isopropanol was added up to 2/3 of the total volume of the supernatant, then incubated at -20°C for one night. The tube was centrifuged at 10,000 rpm for 10 min, and the supernatant was discarded. The pellets were washed with 100 µl of 80% ethanol (cold) and centrifuged at 8000 rpm for 5 minutes. In the final step, the supernatant was removed, and the pellet was dried for approximately 1 hour. It was then added with a solution of 20 µl TE and stored at -20°C until used.

Amplification of mtCOI Fragments Using the PCR Method

PCR reactants were manufactured with a total volume of 25 µl consisting of 12.5 µl Go Tag Green Master Mix (Promega, US) and 9.5 µl ddH2O. DNA amplification of the mtCOI fragment was carried out using a pair of universal primers mtCOI LCO 1490 (3'-GGTCAACAAATCATAAAGATATTGG-5') and HCO 2198 (5'TAAACTTCA GGGTGACCA AAAAATCA-3') (Folmer et al. 1994) each 1 µl, and 1 µl DNA template. PCR reactions were carried out with a Perkin Elmer 480 Thermocycler (Applied Biosystem, US). The PCR reaction was initiated by initial denaturation for 5 min at 94°C. The PCR was continued for 35 cycles in the following order: 94°C for 1 minute, 52°C for 35 seconds, 72°C for 1 minute 30 seconds, and a final extension of 72°C for 7 minutes. The PCR results were then analyzed in 1% agarose gel. The DNA fragments of mtCOI were visualized using a UV transilluminator after being immersed in a 2% ethidium bromide solution for 15 minutes and photographed with a digital camera. The result of amplification by PCR technique was in the form of mtCOI DNA fragments with a size of ± 710 base pairs (pb).

Analysis of DNA Sequence Results

Nucleotide Sequencing DNA fragment purification and mtCOI nucleotide sequencing were performed at PT. 1st Base, Malaysia. The results were then registered in the NCBI gene bank (http://www.ncbi.nlm.nih.gov). Analysis of mtCOI DNA sequence data ChromasPro program was used to combine forward and reverse nucleotide sequences to obtain the mtCOI gene (ChromasPro version 2.01. 2006). The Bioedit program was used to compare mtCOI fragments between samples (Multiple alignments) (Hall 1999). The phylogenetic relationship was built by comparing the mtCOI sample fragments from the brown planthopper from Indonesia with the mtCOI fragments already stored in the NCBI GenBank (http://www.ncbi.nlm.nih.gov). The criteria for retrieving mtCOI fragments at GenBank were fragments with a nucleotide base length of ± 800 bp (Boykin et al. 2007) (Table 1). The phylogenetic tree was constructed using the PAUP 4.0b10 program (Swofford 2002) with the maximum parsimony cladistic quantitative method. The cladogram was compiled using the Heuristic method. The cladogram used results from the strick consensus with the statistical bootstrap test to obtain a 100% probability.

RESULTS AND DISCUSSION

The brown planthopper causes direct and indirect damage to rice plants. Direct damage was in the form of stunted and uneven growth of rice plants (Figure 1A and 1B), yellow plants (Figure 1C), and hopperburn caused by fluid in rice plant cells sucked by BPH nymphs, brachiptera (Fig. short wings), and macroptera (long wings) (Figures 1E and 1F). Indirect damage was caused by BPH, which acts as a vector of grass dwarf virus and empty dwarf virus, causing stunted rice plants (Figure 1D). Besides Bali or other parts of Indonesia, BPH attacks on rice crops were also reported in China, where hopperburn affected 60% of all examined crops (Hu et al. 2014).

A percentage of BPH attacks of more than 50% was found in Gianyar, Bangli, Jembrana, and Badung Regencies (Table 2). The BPH population was primarily found in Ciherang and IR-64 varieties of rice in the Badung Regency, with 43.67 BPH per rice hill (Table 2). Baehaki and Mejaya (2015) added that the economic threshold could be measured through the number or population of pests and planting age. BPH is said to have reached the economic threshold when the population of this pest was found in the field, as many as nine BPH per rice hill when the rice age was less than 40 DAP or 18 BPH when the rice was more than 40 DAP (Baehaki and Mejaya 2015). In general, rice varieties grown in all observation locations in Bali were BPH susceptible varieties, such as Ciherang, IR-64, Inpari 32, and Situbagendit.

The dynamics of BPH development in the field can be influenced by several factors, including host plant factors and natural enemies (Horgan et al. 2015; Kobayashi 2016). The host plant factors that affect the BPH population are related to the age of the rice plant. When the observations were made, the rice plants were still in the vegetative phase, aged 4-6 WAP. According to Jing et al. (2014), naturally, BPH usually comes to young rice fields, and insects usually come in the first two weeks after planting. Thus, the brown planthopper in rice cultivation might be the first generation of planthoppers that have not yet reproduced because one BPH life cycle takes between 3-4 weeks (IRRI 2009).

BPH observations in Denpasar, Tabanan, Karangasem, and Klungkung cities were dominated by macroptera imago (Table 2). According to Horgan et al. (2017), the planthopper that first came to the plantation was the macroptera

planthopper as a winged immigrant planthopper. Meanwhile, in Badung, Gianyar, Buleleng, Bangli, and Jembrana regencies, nymphal BPH was dominated by BPH, and several individuals were in the imago phase of brachiptera and macroptera. The dominance of the nymph phase caused the population of BPH in Badung, Gianyar, Buleleng, Bangli, and Jembrana districts to be the highest when compared to the cities of Denpasar, Tabanan, Karangasem, and Klungkung. The presence of the brachiptera planthopper might be contributed to the increase in the nymph population (Baehaki and Mejaya 2015). According to Horgan et al. (2015), rapid population growth usually occurs in groups with many young individuals.

The average intensity of BPH attack on Ciherang and IR-64 varieties of rice was higher than in other varieties. In the Ciherang and IR-64 varieties of rice, the average value of the highest attack intensity was 30% (Figure 2). It is because farmers grow rice varieties Ciherang and IR-64 from year to year without any replacement of other varieties. Furthermore, rice varieties Ciherang and IR-64 became very susceptible to BPH attacks. In addition, BPH is a pest that begins to attack rice plants from a young age, even when the rice is still in the nursery.

According to Sawada et al. (1993), fluctuations in BPH pest attacks are more influenced by the growth phase of the rice plant that is the host in the field. BPH pests are often found when rice plants are in the vegetative and generative stages (Bottrell and Schoenly 2012). Horgan et al. (2017) added that BPH pests could damage rice plants at all stages of growth and act as vectors for grass and dwarf viruses. BPH attack was higher when rice was in the vegetative phase than in the generative phase (Horgan et al. 2015). It happens because the pests attack the young rice stalks. Considering the type of mouth of BPH, which is included in the suction, BPH can suck the liquid from the rice stems and cause the plant leaves to turn yellow (Anant et al. 2021). According to Choi et al. (2019), during the vegetative phase, food availability in the form of nitrogen is abundant in rice plants. Rice plants need nitrogen to form plant organs. Food is one of the factors that affect the life of insects. Qiu et al. (2004) continued that the N element absorbed by plants also serves as a source of nutrition for BPH. If food is available with good quality (suitable for pests), then the insect pest population will increase, and vice versa (Qiu et al. 2004).

The mtCOI DNA band was only successfully amplified from the total DNA extraction of one imago or nymph and not more than one BPH imago. The mtCOI fragment that was successfully amplified corresponds to a size of ±710 bp in all samples from nine districts in Bali, namely Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana (Figure 3). Nucleotide and amino acid sequence analysis showed high homology with *N. lugens* sequences in the database at GenBank, 94.2 – 99.7% and 95.8 - 100%, respectively (Table 3). *N. lugens* sequences from Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana showed the highest nucleotide, and amino acid homology with *N. lugens* isolate FSD-034 from Pakistan (MK301229) biotype Y, respectively. 99.5 -99.74% and 100% (Table 3). The results of the molecular detection of *N. lugens* using the PCR method in Bali, Indonesia, are the first reports of the molecular character of *N. lugens* in Indonesia.

Samples from Indonesia formed a group with *N. lugens* biotype Y fragment mtCOI from Pakistan, India, South Korea, and China (Figure 4). This study found *N. lugens* biotype Y in rice plants for the first time in Indonesia. The Indonesian sample did not form separate groups according to the proximity of the district locations but formed a polytomy cladogram (Figure 4). This polytomy cladogram shows that the *N. lugens* between regencies (Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana) were observed to have the same ancestry. These results indicate high locomotion ability with genetic mixing between *N. lugens* in Bali isolates. Similar conditions were also demonstrated in *N. lugens* among Asian isolates using mitochondrial sequences showing genetic mixing. It can also be correlated with the theory of long-distance migration of *N. lugens*, which migrates from the tropics (northern Vietnam) in April-May to temperate regions (China, Korea, and Japan) in June-July as shown based on meteorological studies (Otuka et al. 2008). The population of *N. lugens* is a long-distance migratory flight from the tropics to temperate Asia before modern pesticides are widely used in tropical rice. Due to the infrequent use of insecticides prior to the 1960s in the tropics, factors other than insecticides may have triggered long-wing movements to form *N. lugens* populations (Bottrell and Schoenly 2012).

In previous studies in Indonesia, BPH biotypes 1, 2, 3, and 4 have been found. Chen et al. (2011) reported that the brown planthopper is a highly adaptive insect because it can form new biotypes. In early 1975 the IR-26 rice variety from IRRI Philippines was introduced. The IR-26 variety was unique because it contained a Bph1 resistant gene to anticipate fluctuations in the brown planthopper population. However, in 1976 there was a great population explosion in several rice production centers due to changes in the brown planthopper population from biotype 1 to biotype 2. As an anticipatory measure against brown planthopper biotype 2, in 1980, the IR-42 rice variety (containing the bph2 resistant gene) was introduced from IRRI Philippines. Unfortunately, in 1981 there was another explosion in the brown planthopper population in Simalungun, North Sumatra, and several other areas due to changes in the brown planthopper population from biotype 2 to biotype 3. To deal with the brown planthopper biotype 3, rice variety IR-56 was introduced (containing the gene bph3 resistance) in 1983 and IR-64 (containing the bph1+ resistance gene) in 1986. The introduction process continues. In 1991, the IR-74 variety (containing the bph3 resistant gene) was introduced. In 2006, the resistance gene IR-64 was broken because the brown planthopper population changed to biotype 4. The stability of the biotype zero brown planthoppers persisted for 41 years before becoming brown planthopper biotype 1. The change of brown planthopper biotype 1 to biotype 2 only took 4 years, and the change of biotype 2 brown planthopper to biotype 3 within 5 years. Until 2005, the brown planthopper biotype 3 was still dominated by biotype 3, and in 2006 the biotype 4 brown planthopper began to develop. The long existence of the biotype 3 brown planthopper was caused by the development of the IR-64

(bph1+) variety over a long period. IR-64 is a resistant variety (durable resistance) that can withstand changes in brown planthoppers to a more virulent biotype.

The continuous cultivation of IR-64 rice varieties by farmers in Bali led to the emergence of a new biotype BPH, namely Y. Insects of biotype Y originated from biotype 1 by eating YHY15 resistant varieties for more than two years for 33 generations (Jing et al. 2012). Rice varieties YHY15 carry the Bph15 resistance gene (Jing et al. 2012).

This study shows great potential in the population of *N. lugens* to adapt to previously resistant rice varieties. This study reported that rice varieties Situbagendit and Inpari 32, previously resistant to BPH, were susceptible to BPH. This research can provide information to farmers not to continuously plant susceptible varieties, which could cause BPH epidemics in the field, as well as the emergence of new, more virulent BPH biotypes. Thus a new control strategy based on a forecasting system can be developed for the regional management of this insect.

CONCLUTIONS

N. lugens that attacks rice plants in Bali (Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana) belongs to biotype Y. Symptoms of damage to rice plants are most severe in Badung Regency. Apart from Ciherang and IR-64 varieties, Situbagendit and Inpari 32 varieties are susceptible to BPH attack.

ACKNOWLEDGEMENTS

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REFERENCES

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TABLES LIST

Table 1. The damage score of rice plants due to BPH attack

Score	Appearance	Description
0	Healthy	No planthopper was found in any rice hill.
1	Very light damage	The rice hills occupied by the planthoppers did not show dead midribs, few exuviae, and the rice stalks had not yet overgrown with Dematium and Cladosporium fungi that followed the brown planthopper attack.
3	Slightly damaged	The rice hills occupied by the planthoppers have shown dead midribs, many exuviae, and the rice stems are overgrown with Dematium and Cladosporium fungi that follow the brown planthopper attack.
5	Heavily damaged	Rice hills inhabited by planthoppers showed damage marked by many dead midribs, many exuviae, stunted and black-looking tillers, and overgrown with Dematium and Cladosporium fungi.
7	Partially dead	Some of the stems in the rice hill die, or the rice hill withers due to planthoppers attack.
9	Hopperburn	Rice hills die from hopperburn

Sumber: Baehaki (2012)

Table 2. Population and symptoms of BPH attack on rice plants in Bali

Location	**Rice varieties**	**Rice plant age (DAP)**	**BPH attack percentage (%)**	**BPH population abundance (individues/rice hills)**
Denpasar City	Situbagendit, Inpari 32	35	35.43	7.41
Badung	Ciherang, IR-64	42	73.61	43.67
Gianyar	Ciherang, Inpari 32	45	52.26	12.49
Tabanan	Inpari 32	41	37.94	9.26
Buleleng	Ciherang, IR-64	33	46.82	11.28
Karangasem	Situbagendit	30	32.73	7.92
Klungkung	Inpari 32	43	35.89	8.53
Bangli	Ciherang, IR-64	42	52.80	14.83
Jembrana	Ciherang, Inpari 32	36	57.32	11.95

Note: DAP= day after planting

Table 3. Nucleotide (nt) and amino acid (aa) homology of *N. lugens* in rice from Bali, Indonesia, compared with *N. lugens* from other countries in GenBank

Isolate	Origin of isolate	Biotype	Accession number	Homology nt (aa) (%) *N. lugens*_IDN_								
				Denpasar	Badung	Gianyar	Tabanan	Buleleng	Karangasem	Klungkung	Bangli	Jembrana
FSD-034	PAK	Y	MK301229	99.5 (100)	99.6 (100)	99.5 (100)	99.5 (100)	99.6 (100)	99.5 (100)	99.7 (100)	99.5 (100)	99.6 (100)
HZZ55	IND	Y	MK032794	99.4 (100)	99.5 (100)	99.4 (100)	99.5 (100)	99.4 (100)	99.5 (100)	99.6 (100)	99.5 (100)	99.6 (100)
SAEVG_Morph0111	IND	Y	MN520923	99.4 (100)	99.5 (100)	99.4 (100)	99.5 (100)	99.4 (100)	99.5 (100)	99.5 (100)	99.5 (100)	99.6 (100)
KBPH	KOR	Y	MK590088	99.3 (100)	99.5 (100)	99.4 (100)	99.4 (100)	99.3 (100)	99.4 (100)	99.4 (100)	99.4 (100)	99.5 (100)
KOREA_BPH	KOR	Y	LC461184	99.3 (100)	99.5 (100)	99.4 (100)	99.4 (100)	99.3 (100)	99.4 (100)	99.4 (100)	99.4 (100)	99.5 (100)
WUHAN-Y	CHN	Y	KC333653	99.3 (100)	99.5 (100)	99.4 (100)	99.4 (100)	99.3 (100)	99.4 (100)	99.4 (100)	99.3 (100)	99.4 (100)
WUHAN-3	CHN	3	JN563997	97.8 (98.1)	97.2 (97.8)	97.5 (98.9)	97.5 (98.9)	97.4 (97.9)	97.8 (98.1)	97.5 (98.9)	97.2 (98.8)	97.6 (98.0)
WUHAN-2	CHN	2	JN563996	96.3 (97.5)	96.3 (97.5)	96.4 (97.5)	96.3 (97.5)	96.2 (97.4)	96.4 (97.5)	96.3 (97.5)	96.2 (97.4)	96.3 (97.5)
WUHAN-1	CHN	1	JN563995	95.3 (96.7)	95.4 (96.7)	95.3 (96.7)	95.4 (96.7)	95.6 (96.8)	95.6 (96.8)	95.3 (96.7)	95.4 (96.7)	95.3 (96.7)
GX	CHN	1	LC461186	95.3 (96.7)	95.3 (96.7)	95.3 (96.7)	95.4 (96.7)	95.5 (96.8)	95.5 (96.8)	95.3 (96.7)	95.3 (96.7)	95.3 (96.7)
Gangavathi	IND	1	OL451531	95.3 (96.7)	95.3 (96.7)	95.3 (96.7)	95.3 (96.7)	95.5 (96.8)	95.5 (96.8)	95.3 (96.7)	95.3 (96.7)	95.3 (96.7)
WUHAN-L	CHN	L	KC333654	94.2 (95.8)	94.4 (96.2)	94.2 (95.8)	94.4 (96.2)	94.3 (96.0)	94.4 (96.2)	94.2 (95.8)	94.4 (96.2)	94.4 (96.2)
N. bakeri	CHN	-	JX266790	84.6 (85.6)	85.2 (86.1)	84.8 (85.9)	84.8 (85.9)	84.6 (85.6)	85.2 (86.1)	84.8 (85.9)	85.2 (86.1)	85.2 (86.1)
Sogatella furcifera	CHN	-	HM160123	75.6 (76.9)	75.6 (76.9)	76.2 (77.8)	77.6 (78.4)	77.4 (78.4)	76.8 (77.8)	75.6 (76.9)	76.8 (77.8)	77.6 (78.4)

Notes: nt (nucleotide), aa (amino acid), IDN (Indonesia), PAK (Pakistan), IND (India), KOR (South Korea), CHN (China), *N. bakeri* and *Sogatella furcifera* from China was used as outgroups

FIGURES LIST

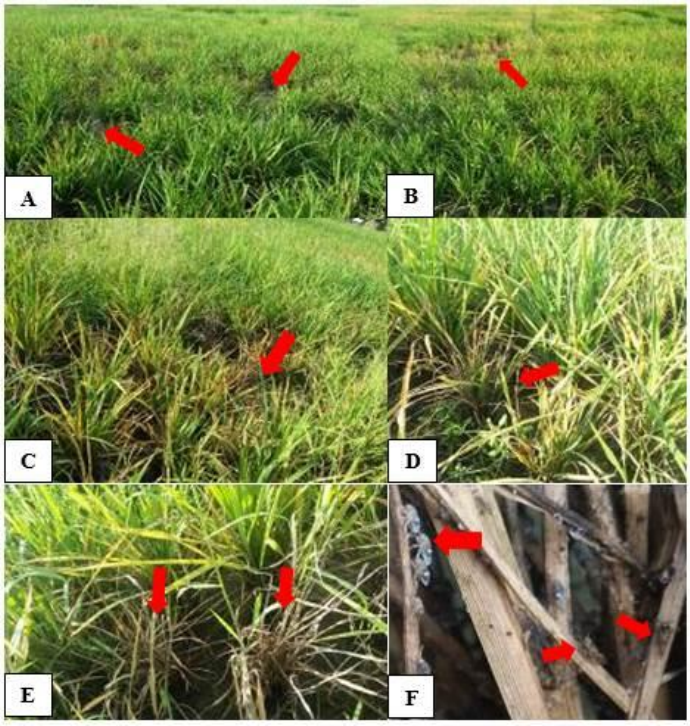

Figure 1. Symptoms of BPH attack on rice plants in Bali: A. rice plant growth is stunted; B. uneven plant growth (spots); C. yellow plant; D. dwarf rice plants; E. plants die like burning (hopperburn); F. BPH brachiptera and macroptera were found on rice stalks.

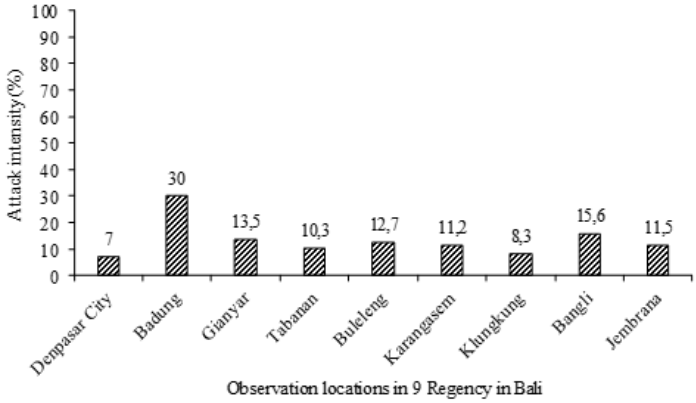

Figure 2. The attack intensity of *N. lugens* on rice in Bali Province

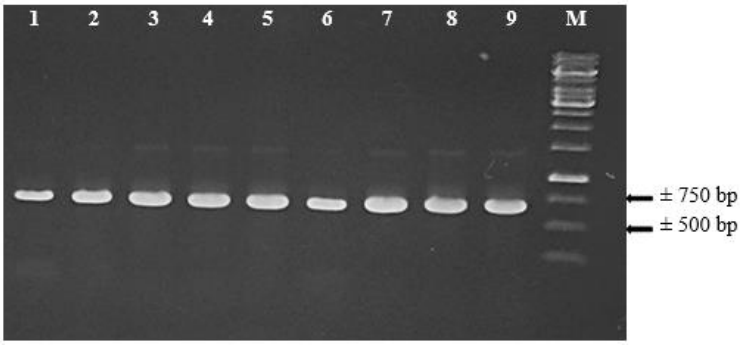

Figure 3. DNA amplification of *N. lugens* in rice plants in Bali using primers LCO 1490/HCO 2198. 1. Denpasar City, 2. Bagung, 3. Gianyar, 4. Tabanan, 5. Buleleng, 6. Karangasemt, 7. Klungkung, 8. Bangli, 9. Jembrana, and M. DNA marker 1 kb (Thermo Scientific)

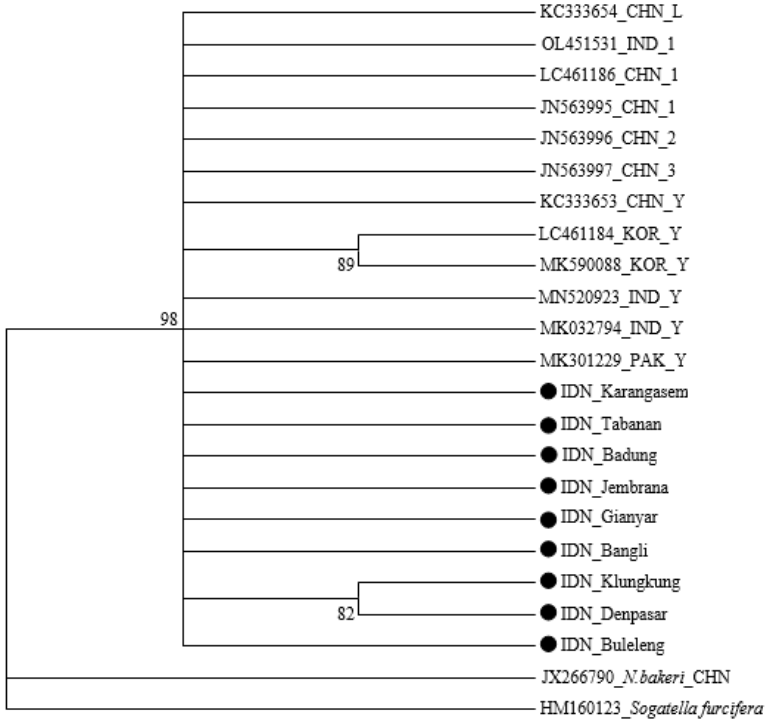

Figure 4. The cladogram of the mtCOI fragment of *N. lugens* from eastern Indonesia, Bali (Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana) was compared with mtCOI fragments from several regions of the world that had been deposited on the NCBI website. *N. bakeri* and *Sogatella furcifera* from China were used as outgroups. The numbers on the branching cladograms represent bootstrap values with 100% probability. IDN (Indonesia), PAK (Pakistan), IND (India), KOR (South Korea), and CHN (China), isolates marked with black dots are Bali isolates.

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The brown planthopper (*Nilaparvata lugens* Stal.) attack and its genetic diversity on rice in Bali, Indonesia

Abstract. The brown planthopper (*Nilaparvata lugens* Stal.) is an important pest on rice crops in Indonesia. The genetic diversity of BPH isolates in western Indonesia has been extensively reported, whereas eastern Indonesia isolates have not been reported. This research aims to analyze genetic diversity and evaluate the BPH attack's intensity on Bali rice plants. The research method used was an observation of attack percentage, population dynamics, attack intensity, and genetic diversity of BPH in 9 districts in Bali (Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana). Molecular identification was carried out on *N. lugens* DNA in the mtCOI fragment. BPH attacks of >50% were found in the districts of Gianyar, Bangli, Jembrana, and Badung. The BPH population was primarily found in Ciherang and IR-64 varieties of rice in the Badung Regency, with 43.67 BPH per rice hill. In general, rice varieties grown in all observation locations were susceptible to BPH, such as Ciherang, IR-64, Inpari 32, and Situbagendit. In the Ciherang and IR-64 varieties, the highest attack intensity average value reached 30%. The sequence of *N. lugens* isolate from Bali Jembrana showed the highest nucleotide and amino acid homology with *N. lugens* isolate FSD-034 from Pakistan (MK301229) biotype Y of 99.5 -99.74% and 100%, respectively. This study found *N. lugens* biotype Y in rice plants for the first time in Indonesia. This study reported that Rice varieties Situbagendit and Inpari 32, previously resistant to BPH, are reported as susceptible to BPH.

Keywords: attack intensity, genetic diversity, Inpari 32, Situbagendit, susceptible variety

Running title: The Brown Planthopper Attack and Its Genetic Diversity

INTRODUCTION

The brown planthopper (BPH) (*Nilaparvata lugens* Stal, Hemiptera: Delphacidae) is the most destructive rice pest in Indonesia. Repeated outbreaks of BPH in Indonesia are caused by continuous rice cultivation, extensive use, and over-application of insecticides (Baehaki 2012). This pest is vascular monophagous in rice (Cheng et al. 2013; Ferrater et al. 2013; Triwidodo 2020). Feeding by nymphs and imago at the base of the plant causes rapid wilting and drying of the plant (Bottrell and Schoenly 2012; Cheng et al. 2013; Bao and Zhang 2019). In addition, BPH is also a vector of *Rice grassy stunt virus* and *Rice ragged stunt virus* (Bao and Zhang 2019). At high population levels of *N. lugens* can cause significant losses in rice production (Cheng et al. 2013; Zheng et al. 2013; Bao and Zhang 2019).

The BPH cannot tolerate winter in northern Asia, including Japan, Korea, and northern China (He et al. 2012; Fu et al. 2012; Fu et al. 2014). The population originally came from subtropical and tropical areas by flying long distances during the summer (Fu et al. 2014; Hu et al. 2014). BPH infestation in temperate climates originated from annual migrations from tropical Asia and China (He et al. 2012). During autumn, BPH re-migrates (north-to-south) and BPH populations have been studied in China and India (Bottrell and Schoenly 2012). Such return migration may help explain how long-distance migration is maintained in the winter.

The intensification of rice production triggered the BPH outbreak in Tropical Asia during the green revolution era in the 1970s and 1980s (Bottrell and Schoenly 2012). Until now, *N. lugens* is the main problem causing rice harvest failure in several countries. Inaccurate identification and prolonged identification of *N. lugens* are obstacles to its field management strategy.

Traditionally, BPH has been identified at the species level by morphological features using anatomical characteristics, namely, wings, front, and external genitalia (Lv et al. 2015). Accurate identification requires extensive expertise and experience and yet sometimes can lead to errors. Morphological identification by an entomologist can reduce the potential for errors. Practical morphological identification is only possible when dealing with small sample sizes and well-preserved specimens. Therefore, it is crucial to utilize a new identification method that is accurate, fast, time-saving, and suitable for large numbers of specimens.

Molecular techniques with high reproducibility and fast results offer an excellent alternative to traditional morphological classification. Several mitochondrial and nuclear genes have been used as genetic markers to differentiate related species. These include the mitochondrial cytochrome c oxidase subunit 1 (CO1) gene, nuclear 12S-16S-18S ribosomal RNA genes, and ITS1 and ITS2 internal transcription spacers (Brengues et al. 2014; Gomez-Polo et al. 2014; Yu et al. 2014; Wang et al. 2016; Zheng et al. 2021). ITS1 and ITS2 are nonfunctional spacers that separate the 18S-5.8S

and 5.8S-28S rRNA genes, respectively (Wang et al. 2016; Zheng et al. 2021). As ITS sequences have low intra-species variation but high variation between species, they are helpful for species classification and phylogenetic analysis for morphologically similar organisms, both in prokaryotes and eukaryotes (Zheng et al. 2021). Finally, from the molecular identification of the combined mitochondrial COI-COII and ten microsatellite marker loci (Winnie et al. 2020).

The genetic diversity of *N. lugens* has been reported in several countries such as China, South Korea, Pakistan, India, and Malaysia (Jing et al. 2012; Latif et al. 2012; Anant et al. 2021; Zheng et al. 2021). The genetic diversity of *N. lugens* in Indonesia is widely reported in western Indonesia (Java Island) (Winnie et al. 2020; Chaerani et al. 2021). Reports on the genetic diversity of *N. lugens* in eastern Indonesia have not been found. Therefore, this study aims to analyze genetic diversity and determine the intensity of BPH attacks on rice plants in eastern Indonesia, especially Bali.

MATERIALS AND METHODS

Brown Planthopper Sampling from Rice Dwarf Disease Endemic Areas

Samples were taken from nine locations at the rice cultivation center in Bali Province (Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana). The brown planthopper samples taken from rice plants were nymphs and imagos. Nymphs and imagos were used for total DNA extraction. After arriving at the laboratory, the nymphs and imago were stored dry at -20ºC.

Observation of BPH Attack Symptoms and Quantity of BPH Population/rice hill

Observation of symptoms of BPH attack was carried out by observing symptoms of damage to rice plants due to BPH attack. The abundance of the BPH/rice hill population was obtained by counting all nymphs and imagoes obtained. Data on the population per cluster from 20 samples or 20 rice hills at each observation fields were then averaged. For each location, 3 fields of rice cultivation center were taken which were used for observation

BPH Attack Percentage

The percentage of BPH attacks is calculated using the following formula:

$$P = \frac{a}{b} x\ 100\%$$

Note:
P = Attack percentage (%)
a = Number of rice hills affected by BPH
b = Number of rice hills observed

Damage Intensity

Determination of scoring on symptoms of rice damage due to BPH attack is based on Table 1. The intensity of damage due to a BPH attack is determined using the formula (Erdiansyah and Damanhuri 2018):

$$I = \sum_{i=1}^{i} \frac{Ni\ x\ Vi}{N\ x\ Z} x\ 100\%$$

Note:
I = Damage intensity
Ni = The number of affected rice hills on the score i
Vi = Score i
N = The number of rice hills observed
Z = Highest score

Total DNA Extraction from Brown Planthopper

Total DNA extraction of brown planthopper was obtained from one individual imago or one individual nymph based on the modified method of Goodwin et al. (1994). One individual imago was put into a microtube and then added to 100 µl of CTAB (Cethyl Trimethyl Ammonium Bromida) extraction buffer (2% CTAB, 1.4 M NaCl, 100 mM Tris-HCI, 20 mM EDTA (Ethylenediaminetetraacetic acid), and 1% PVP (-40 °C)). Next, 1 µl of proteinase K was added, then the insects were crushed using a micro-pistil, vortexed, and incubated in a water bath of 65°C for 3 minutes. After that, the tube was added with 100 µl CI (chloroform: isoamyl alcohol) in a ratio of 24:1. The tube was then vortexed for 3 minutes and centrifuged at 10,000 rpm for 15 minutes. The supernatant formed was transferred to a new microtube (60 µl) and then added with 3 M NaOAc (pH 5.2), as much as 1/10 of the total volume of the supernatant. Isopropanol was added up to 2/3 of the total volume of the supernatant, then incubated at -20°C for one night. The tube was centrifuged at 10,000 rpm for 10 min, and the supernatant was discarded. The pellets were washed with 100 µl of 80% ethanol (cold) and centrifuged at

8000 rpm for 5 minutes. In the final step, the supernatant was removed, and the pellet was dried for approximately 1 hour. It was then added to a solution of 20 µl TE and stored at -20°C until used.

Amplification of mtCOI Fragments Using the PCR Method

PCR reactants were manufactured with a total volume of 25 µl consisting of 12.5 µl Go Tag Green Master Mix (Promega, US) and 9.5 µl ddH2O. DNA amplification of the mtCOI fragment was carried out using a pair of universal primers mtCOI LCO 1490 (3'-GGTCAACAAATCATAAAGATATTGG-5') and HCO 2198 (5'TAAACTTCA GGGTGACCA AAAAATCA-3') (Folmer et al. 1994) each 1 µl, and 1 µl DNA template. PCR reactions were carried out with a Perkin Elmer 480 Thermocycler (Applied Biosystem, US). The PCR reaction was initiated by initial denaturation for 5 min at 94°C. The PCR was continued for 35 cycles in the following order: 94°C for 1 minute, 52°C for 35 seconds, 72°C for 1 minute 30 seconds, and a final extension of 72°C for 7 minutes. The PCR results were then analyzed in 1% agarose gel. The DNA fragments of mtCOI were visualized using a UV transilluminator after being immersed in a 2% ethidium bromide solution for 15 minutes and photographed with a digital camera. The result of amplification by PCR technique was in the form of mtCOI DNA fragments with a size of ± 710 base pairs (pb).

Analysis of DNA Sequence Results

Nucleotide Sequencing DNA fragment purification and mtCOI nucleotide sequencing were performed at PT. 1st Base, Malaysia. The results were then registered in the NCBI gene bank (http://www.ncbi.nlm.nih.gov). Analysis of mtCOI DNA sequence data ChromasPro program was used to combine forward and reverse nucleotide sequences to obtain the mtCOI gene (ChromasPro version 2.01. 2006). The Bioedit program was used to compare mtCOI fragments between samples (Multiple alignments). The phylogenetic relationship was built by comparing the mtCOI sample fragments from the brown planthopper from Indonesia with the mtCOI fragments already stored in the NCBI GenBank (http://www.ncbi.nlm.nih.gov). The criteria for retrieving mtCOI fragments at GenBank were fragments with a nucleotide base length of ± 710 bp (Boykin et al. 2007) (Table 3; Figure 3). The phylogenetic tree was constructed using the PAUP 4.0b10 program with the maximum parsimony cladistic quantitative method. The cladogram was compiled using the Heuristic method. The cladogram used results from the strick consensus with the statistical bootstrap test to obtain a 100% probability.

RESULTS AND DISCUSSION

The brown planthopper causes direct and indirect damage to rice plants. Direct damage was in the form of stunted and uneven growth of rice plants (Figure 1A and 1B), yellow plants (Figure 1C), and hopperburn caused by fluid in rice plant cells sucked by BPH nymphs, brachiptera (Fig. short wings), and macroptera (long wings) (Figures 1E and 1F). Indirect damage was caused by BPH, which acts as a vector of *Rice grassy stunt virus* and *Rice ragged stunt virus*, causing stunted rice plants (Figure 1D). Besides Bali or other parts of Indonesia, BPH attacks on rice crops were also reported in China, where hopperburn affected 60% of all examined crops (Hu et al. 2014). Transmission of the stunt virus by the brown planthoppers occurs persistently (Horgan et al. 2015). Virus infection causes damage to plants because viruses use plant proteins for replication, resulting in loss of crop production (Listihani et al. 2020; Damayanti et al. 2022; Listihani et al. 2022; Pandawani et al. 2022; Selangga and Listihani 2022; Selangga et al. 2022). Therefore, infection with RGSV and RRSV in rice plants causes rice to lack nutrients to the point of stunting.

A percentage of BPH attacks of more than 50% was found in Gianyar, Bangli, Jembrana, and Badung Regencies (Table 2). The BPH population was primarily found in Ciherang and IR-64 varieties of rice in the Badung Regency, with 43.67 BPH per rice hill (Table 2). Baehaki (2012) added that the economic threshold could be measured through the number or population of pests and planting age. BPH is said to have reached the economic threshold when the population of this pest was found in the field, as many as nine BPH per rice hill when the rice age was less than 40 DAP or 18 BPH when the rice was more than 40 DAP (Baehaki 2012). In general, rice varieties grown in all observation locations in Bali were BPH susceptible varieties, such as Ciherang, IR-64, Inpari 32, and Situbagendit.

The dynamics of BPH development in the field can be influenced by several factors, including host plant factors and natural enemies (Ferrater et al. 2015; Horgan et al. 2015; Kobayashi 2016). The host plant factors that affect the BPH population are related to the age of the rice plant. When the observations were made, the rice plants were still in the vegetative phase, aged 4-6 WAP. According to Jing et al. (2014), naturally, BPH usually comes to young rice fields, and insects usually come in the first two weeks after planting. Thus, the brown planthopper in rice cultivation might be the first generation of planthoppers that have not yet reproduced because one BPH life cycle takes between 3-4 weeks (IRRI 2009).

BPH observations in Denpasar, Tabanan, Karangasem, and Klungkung cities were dominated by macroptera imago (Table 2). According to Horgan et al. (2017), the planthopper that first came to the plantation was the macroptera planthopper as a winged immigrant planthopper. Meanwhile, in Badung, Gianyar, Buleleng, Bangli, and Jembrana regencies, nymphal BPH was dominated by BPH, and several individuals were in the imago phase of brachiptera and macroptera. The dominance of the nymph phase caused the population of BPH in Badung, Gianyar, Buleleng, Bangli, and Jembrana districts to be the highest when compared to the cities of Denpasar, Tabanan, Karangasem, and Klungkung. The

presence of the brachiptera planthopper might be contributed to the increase in the nymph population (Baehaki 2012). Rapid population growth usually occurs in groups with many young individuals (Horgan et al. 2015; Triwidodo and Listihani 2020).

The average intensity of BPH attack on Ciherang and IR-64 varieties of rice was higher than in other varieties. In the Ciherang and IR-64 varieties of rice, the average value of the highest attack intensity was 30% (Figure 2). It is because farmers grow rice varieties Ciherang and IR-64 from year to year without any replacement of other varieties. Furthermore, rice varieties Ciherang and IR-64 became very susceptible to BPH attacks. In addition, BPH is a pest that begins to attack rice plants from a young age, even when the rice is still in the nursery.

According to Vu et al. (2014), fluctuations in BPH pest attacks are more influenced by the growth phase of the rice plant that is the host in the field. BPH pests are often found when rice plants are in the vegetative and generative stages (Bottrell and Schoenly 2012). Horgan et al. (2017) added that BPH pests could damage rice plants at all stages of growth and act as vectors for grass and dwarf viruses. BPH attack was higher when rice was in the vegetative phase than in the generative phase (Horgan et al. 2015). It happens because the pests attack the young rice stalks. Considering the type of mouth of BPH, which is included in the suction, BPH can suck the liquid from the rice stems and cause the plant leaves to turn yellow (Anant et al. 2021). According to Choi et al. (2019) and Sutrawati et al. (2021), during the vegetative phase, food availability in the form of nitrogen is abundant in rice plants. Rice plants need nitrogen to form plant organs. Food is one of the factors that affect the life of insects. Horgan (2018) continued that the N element absorbed by plants also serves as a source of nutrition for BPH. If food is available with good quality (suitable for pests), then the insect pest population will increase, and vice versa (Horgan 2018; Triwidodo and Listihani 2020).

The mtCOI DNA band was successfully amplified from the total DNA extraction of one imago or nymph of BPH. The mtCOI fragment that was successfully amplified corresponds to a size of ±710 bp in all samples from nine districts in Bali, namely Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana (Figure 3). Nucleotide and amino acid sequence analysis showed high homology with *N. lugens* sequences in the database at GenBank, 94.2 – 99.7% and 95.8 - 100%, respectively (Table 3). *N. lugens* sequences from Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana showed the highest nucleotide, and amino acid homology with *N. lugens* isolate FSD-034 from Pakistan (MK301229) biotype Y, respectively. 99.5 -99.74% and 100% (Table 3). The results of the molecular detection of *N. lugens* using the PCR method in Bali, Indonesia, are the first reports of the molecular character of *N. lugens* in Indonesia.

Samples from Indonesia formed a group with *N. lugens* biotype Y fragment mtCOI from Pakistan, India, South Korea, and China (Figure 4). This study found *N. lugens* biotype Y in rice plants for the first time in Indonesia. The Indonesian sample did not form separate groups according to the proximity of the district locations but formed a polytomy cladogram (Figure 4). This polytomy cladogram shows that the *N. lugens* between regencies (Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana) were observed to have the same ancestry. These results indicate high locomotion ability with genetic mixing between *N. lugens* in Bali isolates. Similar conditions were also demonstrated in *N. lugens* among Asian isolates using mitochondrial sequences showing genetic mixing. It can also be correlated with the theory of long-distance migration of *N. lugens*, which migrates from the tropics (northern Vietnam) in April-May to temperate regions (China, Korea, and Japan) in June-July as shown based on meteorological studies (Otuka et al. 2008). The population of *N. lugens* is a long-distance migratory flight from the tropics to temperate Asia before modern pesticides are widely used in tropical rice. Due to the infrequent use of insecticides prior to the 1960s in the tropics, factors other than insecticides may have triggered long-wing movements to form *N. lugens* populations (Bottrell and Schoenly 2012).

In previous studies in Indonesia, BPH biotypes 1, 2, 3, and 4 have been found. Kobayashi et al. (2014) reported that the brown planthopper is a highly adaptive insect because it can form new biotypes. In early 1975 the IR-26 rice variety from IRRI Philippines was introduced. The IR-26 variety was unique because it contained a Bph1 resistant gene to anticipate fluctuations in the brown planthopper population. However, in 1976 there was a great population explosion in several rice production centers due to changes in the brown planthopper population from biotype 1 to biotype 2. As an anticipatory measure against brown planthopper biotype 2, in 1980, the IR-42 rice variety (containing the bph2 resistant gene) was introduced from IRRI Philippines. Unfortunately, in 1981 there was another explosion in the brown planthopper population in Simalungun, North Sumatra, and several other areas due to changes in the brown planthopper population from biotype 2 to biotype 3. To deal with the brown planthopper biotype 3, rice variety IR-56 was introduced (containing the gene bph3 resistance) in 1983 and IR-64 (containing the bph1+ resistance gene) in 1986. The introduction process continues. In 1991, the IR-74 variety (containing the bph3 resistant gene) was introduced. In 2006, the resistance gene IR-64 was broken because the brown planthopper population changed to biotype 4 (Baehaki 2012). The stability of the biotype zero brown planthoppers persisted for 41 years before becoming brown planthopper biotype 1. The change of brown planthopper biotype 1 to biotype 2 only took 4 years, and the change of biotype 2 brown planthopper to biotype 3 within 5 years. Until 2005, the brown planthopper biotype 3 was still dominated by biotype 3, and in 2006 the biotype 4 brown planthopper began to develop. The long existence of the biotype 3 brown planthopper was caused by the development of the IR-64 (bph1+) variety over a long period. IR-64 is a resistant variety (durable resistance) that can withstand changes in brown planthoppers to a more virulent biotype.

The continuous cultivation of IR-64 rice varieties by farmers in Bali led to the emergence of a new biotype BPH, namely Y. Insects of biotype Y originated from biotype 1 by eating YHY15 resistant varieties for more than two years for 33 generations (Jing et al. 2012). Rice varieties YHY15 carry the Bph15 resistance gene (Jing et al. 2012).

This study shows great potential in the population of *N. lugens* to adapt to previously resistant rice varieties. This study reported that rice varieties Situbagendit and Inpari 32, previously resistant to BPH, were susceptible to BPH. This research can provide information to farmers not to continuously plant susceptible varieties, which could cause BPH epidemics in the field, as well as the emergence of new, more virulent BPH biotypes. Thus a new control strategy based on a forecasting system can be developed for the regional management of this insect.

In conclusion, *N. lugens* that attacks rice plants in Bali (Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana) belongs to biotype Y. Symptoms of damage to rice plants are most severe in Badung Regency. Apart from Ciherang and IR-64 varieties, Situbagendit and Inpari 32 varieties are susceptible to BPH attack.

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TABLES LIST

Table 1. The damage score of rice plants due to BPH attack

Score	Appearance	Description
0	Healthy	No planthopper was found in any rice hill.
1	Very light damage	The rice hills occupied by the planthoppers did not show dead midribs, few exuviae, and the rice stalks had not yet overgrown with *Colletotrichum dematium* and Cladosporium fungi that followed the brown planthopper attack.
3	Slightly damaged	The rice hills occupied by the planthoppers have shown dead midribs, many exuviae, and the rice stems are overgrown with *Colletotrichum dematium* and Cladosporium fungi that follow the brown planthopper attack.
5	Heavily damaged	Rice hills inhabited by planthoppers showed damage marked by many dead midribs, many exuviae, stunted and black-looking tillers, and overgrown with *Colletotrichum dematium* and Cladosporium fungi.
7	Partially dead	Some of the stems in the rice hill die, or the rice hill withers due to planthoppers attack.
9	Hopperburn	Rice hills die from hopperburn

Sumber: Baehaki (2012)

Table 2. Population and symptoms of BPH attack on rice plants in Bali

Location	**Rice varieties**	**Rice plant age (DAP)**	**BPH attack percentage (%)**	**BPH population abundance (individues/rice hills)**
Denpasar City	Situbagendit, Inpari 32	35	35.43	7.41
Badung	Ciherang, IR-64	42	73.61	43.67
Gianyar	Ciherang, Inpari 32	45	52.26	12.49
Tabanan	Inpari 32	41	37.94	9.26
Buleleng	Ciherang, IR-64	33	46.82	11.28
Karangasem	Situbagendit	30	32.73	7.92
Klungkung	Inpari 32	43	35.89	8.53
Bangli	Ciherang, IR-64	42	52.80	14.83
Jembrana	Ciherang, Inpari 32	36	57.32	11.95

Note: DAP= day after planting

Table 3. Nucleotide (nt) and amino acid (aa) homology of *N. lugens* in rice from Bali, Indonesia, compared with *N. lugens* from other countries in GenBank

Isolate	Origin of isolate	Biotype	Accession number	Homology nt (aa) (%) *N. lugens*_IDN_								
				Denpasar	Badung	Gianyar	Tabanan	Buleleng	Karangasem	Klungkung	Bangli	Jembrana
FSD-034	PAK	Y	MK301229	99.5 (100)	99.6 (100)	99.5 (100)	99.5 (100)	99.6 (100)	99.5 (100)	99.7 (100)	99.5 (100)	99.6 (100)
HZZ55	IND	Y	MK032794	99.4 (100)	99.5 (100)	99.4 (100)	99.5 (100)	99.4 (100)	99.5 (100)	99.6 (100)	99.5 (100)	99.6 (100)
SAEVG_Morph0111	IND	Y	MN520923	99.4 (100)	99.5 (100)	99.4 (100)	99.5 (100)	99.4 (100)	99.5 (100)	99.5 (100)	99.5 (100)	99.6 (100)
KBPH	KOR	Y	MK590088	99.3 (100)	99.5 (100)	99.4 (100)	99.4 (100)	99.3 (100)	99.4 (100)	99.4 (100)	99.4 (100)	99.5 (100)
KOREA_BPH	KOR	Y	LC461184	99.3 (100)	99.5 (100)	99.4 (100)	99.4 (100)	99.3 (100)	99.4 (100)	99.4 (100)	99.4 (100)	99.5 (100)
WUHAN-Y	CHN	Y	KC333653	99.3 (100)	99.5 (100)	99.4 (100)	99.4 (100)	99.3 (100)	99.4 (100)	99.4 (100)	99.3 (100)	99.4 (100)
WUHAN-3	CHN	3	JN563997	97.8 (98.1)	97.2 (97.8)	97.5 (98.9)	97.5 (98.9)	97.4 (97.9)	97.8 (98.1)	97.5 (98.9)	97.2 (98.8)	97.6 (98.0)
WUHAN-2	CHN	2	JN563996	96.3 (97.5)	96.3 (97.5)	96.4 (97.5)	96.3 (97.5)	96.2 (97.4)	96.4 (97.5)	96.3 (97.5)	96.2 (97.4)	96.3 (97.5)
WUHAN-1	CHN	1	JN563995	95.3 (96.7)	95.4 (96.7)	95.3 (96.7)	95.4 (96.7)	95.6 (96.8)	95.6 (96.8)	95.3 (96.7)	95.4 (96.7)	95.3 (96.7)
GX	CHN	1	LC461186	95.3 (96.7)	95.3 (96.7)	95.3 (96.7)	95.4 (96.7)	95.5 (96.8)	95.5 (96.8)	95.3 (96.7)	95.3 (96.7)	95.3 (96.7)
Gangavathi	IND	1	OL451531	95.3 (96.7)	95.3 (96.7)	95.3 (96.7)	95.3 (96.7)	95.5 (96.8)	95.5 (96.8)	95.3 (96.7)	95.3 (96.7)	95.3 (96.7)
WUHAN-L	CHN	L	KC333654	94.2 (95.8)	94.4 (96.2)	94.2 (95.8)	94.4 (96.2)	94.3 (96.0)	94.4 (96.2)	94.2 (95.8)	94.4 (96.2)	94.4 (96.2)
N. bakeri	CHN	-	JX266790	84.6 (85.6)	85.2 (86.1)	84.8 (85.9)	84.8 (85.9)	84.6 (85.6)	85.2 (86.1)	84.8 (85.9)	85.2 (86.1)	85.2 (86.1)
Sogatella furcifera	CHN	-	HM160123	75.6 (76.9)	75.6 (76.9)	76.2 (77.8)	77.6 (78.4)	77.4 (78.4)	76.8 (77.8)	75.6 (76.9)	76.8 (77.8)	77.6 (78.4)

Notes: nt (nucleotide), aa (amino acid), IDN (Indonesia), PAK (Pakistan), IND (India), KOR (South Korea), CHN (China), *N. bakeri* and *Sogatella furcifera* from China was used as outgroups

FIGURES LIST

Figure 1. Symptoms of BPH attack on rice plants in Bali: A. rice plant growth is stunted; B. uneven plant growth (spots); C. yellow plant; D. dwarf rice plants; E. plants die like burning (hopperburn); F. BPH brachiptera and macroptera were found on rice stalks.

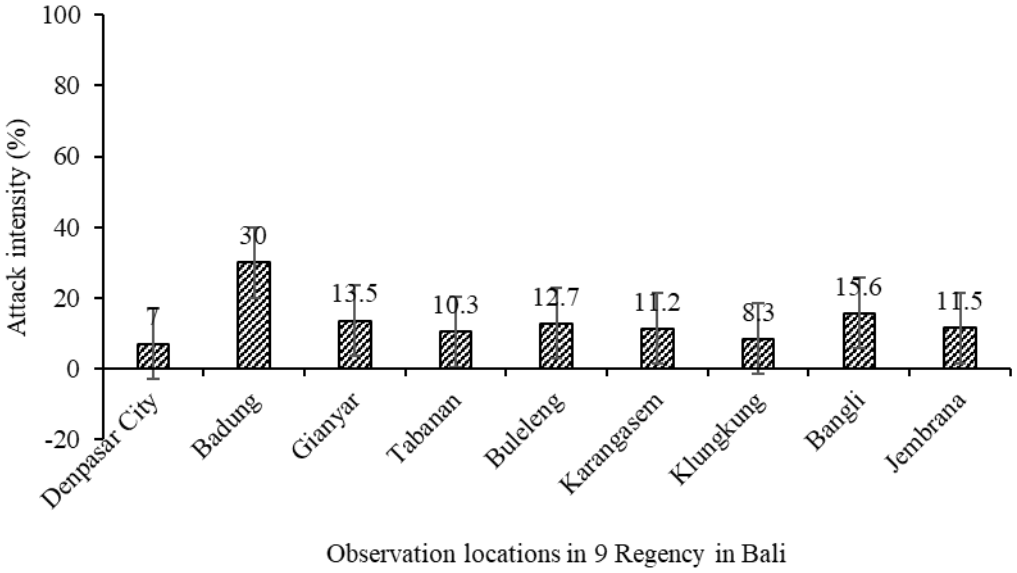

Figure 2. The attack intensity of *N. lugens* on rice in Bali Province

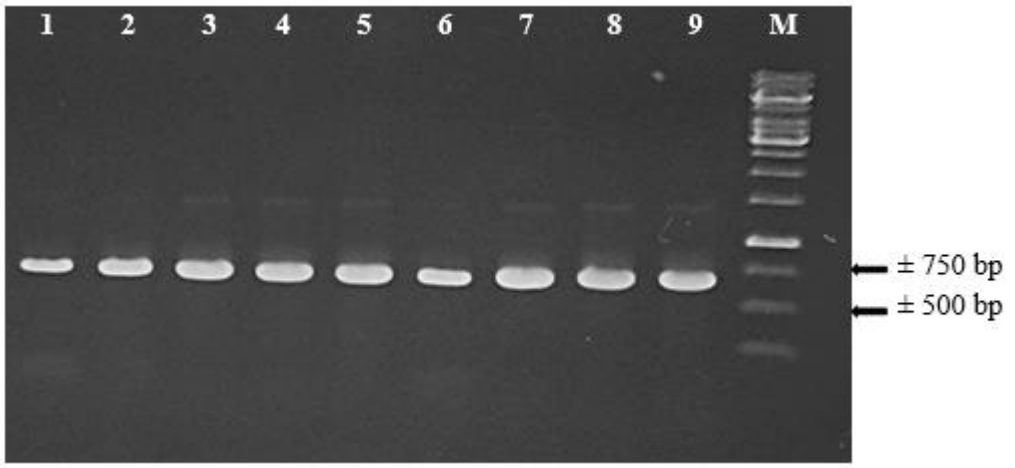

Figure 3. DNA amplification of *N. lugens* in rice plants in Bali using primers LCO 1490/HCO 2198. 1. Denpasar City, 2. Bagung, 3. Gianyar, 4. Tabanan, 5. Buleleng, 6. Karangasemt, 7. Klungkung, 8. Bangli, 9. Jembrana, and M. DNA marker 1 kb (Thermo Scientific)

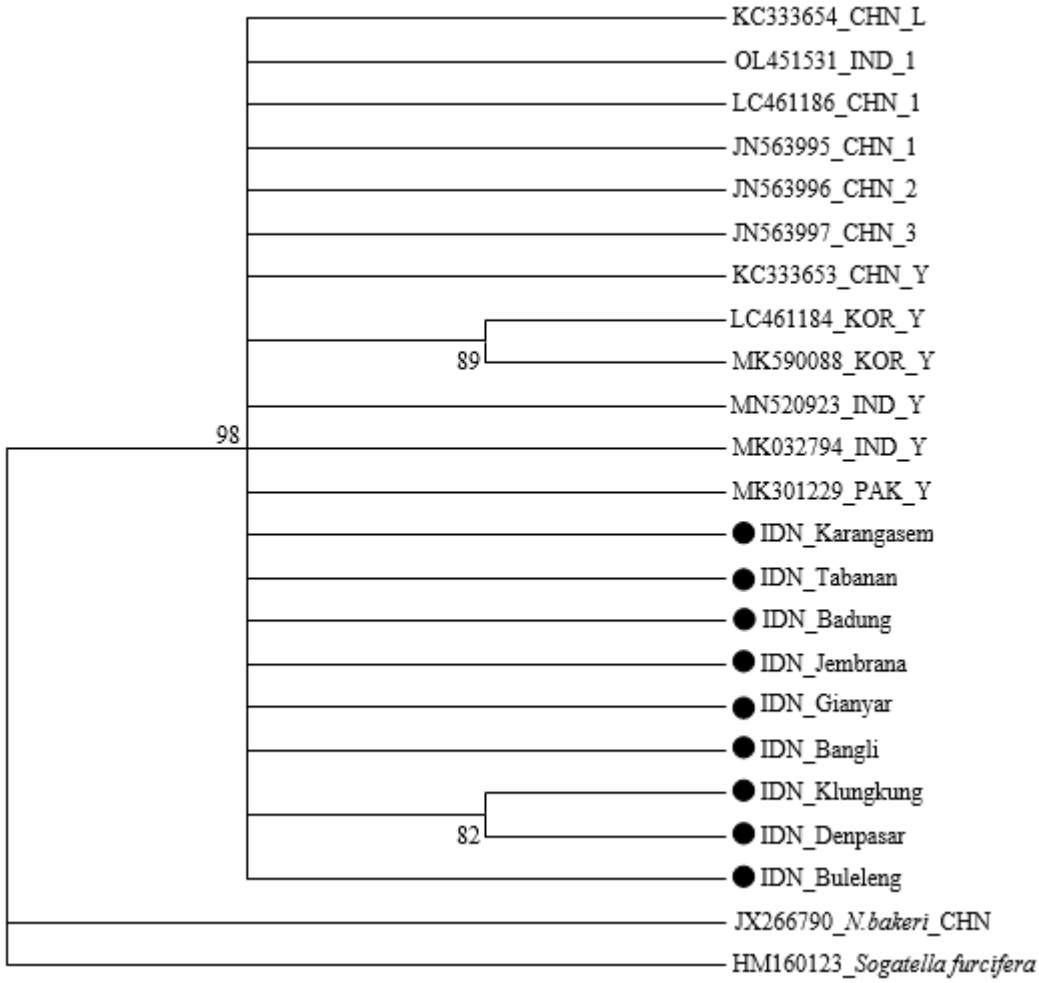

Figure 4. The cladogram of the mtCOI fragment of *N. lugens* from eastern Indonesia, Bali (Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana) was compared with mtCOI fragments from several regions of the world that had been deposited on the NCBI website. *N. bakeri* and *Sogatella furcifera* from China were used as outgroups. The numbers on the branching cladograms represent bootstrap values with 100% probability. IDN (Indonesia), PAK (Pakistan), IND (India), KOR (South Korea), and CHN (China), isolates marked with black dots are Bali isolates.

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The brown planthopper (*Nilaparvata lugens*) attack and its genetic diversity on rice in Bali, Indonesia

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Abstract. *Listihani L, Ariati PEP, Yuniti IGAD, Selangga DGW. 2022. The brown planthopper (Nilaparvata lugens) attack and its genetic diversity on rice in Bali, Indonesia. Biodiversitas 23: 4696-4704.* The brown planthopper (*Nilaparvata lugens*) is an important pest on rice crops in Indonesia. The genetic diversity of BPH isolates in western Indonesia has been extensively reported, whereas eastern Indonesia isolates have not been reported. This research aims to analyze genetic diversity and evaluate the BPH attack's intensity on Bali rice plants. The research method used was an observation of attack percentage, population dynamics, attack intensity, and genetic diversity of BPH in 9 districts in Bali (Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana). Molecular identification was carried out on *N. lugens* DNA in the mtCOI fragment. BPH attacks of >50% were found in the districts of Gianyar, Bangli, Jembrana, and Badung. The BPH population was primarily found in Ciherang and IR-64 varieties of rice in the Badung Regency, with 43.67 BPH per rice hill. In general, rice varieties grown in all observation locations were susceptible to BPH, such as Ciherang, IR-64, Inpari 32, and Situbagendit. In the Ciherang and IR-64 varieties, the highest attack intensity average value reached 30%. The sequence of *N. lugens* isolate from Bali Jembrana showed the highest nucleotide and amino acid homology with *N. lugens* isolate FSD-034 from Pakistan (MK301229) biotype Y of 99.5 -99.74% and 100%, respectively. This study found *N. lugens* biotype Y in rice plants for the first time in Indonesia. This study reported that Rice varieties Situbagendit and Inpari 32, previously resistant to BPH, are reported as susceptible to BPH.

Keywords: Attack intensity, genetic diversity, Inpari 32, Situbagendit, susceptible variety

INTRODUCTION

The brown planthopper (BPH) (*Nilaparvata lugens*, Hemiptera: Delphacidae) is the most destructive rice pest in Indonesia. Repeated outbreaks of BPH in Indonesia are caused by continuous rice cultivation, extensive use, and over-application of insecticides (Baehaki 2012). This pest is vascular monophagous in rice (Cheng et al. 2013; Ferrater et al. 2013; Triwidodo 2020). Feeding by nymphs and imago at the base of the plant causes rapid wilting and drying of the plant (Bottrell and Schoenly 2012; Cheng et al. 2013; Bao and Zhang 2019). In addition, BPH is also a vector of Rice grassy stunt virus and Rice ragged stunt virus (Bao and Zhang 2019). High population levels of *N. lugens* can cause significant losses in rice production (Cheng et al. 2013; Zheng et al. 2013; Bao and Zhang 2019).

The BPH cannot tolerate winter in northern Asia, including Japan, Korea, and northern China (Fu et al. 2012; He et al. 2012; Fu et al. 2014). The population originally came from subtropical and tropical areas by flying long distances during the summer (Fu et al. 2014; Hu et al. 2014). BPH infestation in temperate climates originated from annual migrations from tropical Asia and China (He et al. 2012). During autumn, BPH re-migrates (north-to-south) and BPH populations have been studied in China and India (Bottrell and Schoenly 2012). Such return migration may help explain how long-distance migration is maintained in the winter.

The intensification of rice production triggered the BPH outbreak in Tropical Asia during the green revolution era in the 1970s and 1980s (Bottrell and Schoenly 2012). Until now, *N. lugens* is the main problem causing rice harvest failure in several countries. Inaccurate identification and prolonged identification of *N. lugens* are obstacles to its field management strategy.

Traditionally, BPH has been identified at the species level by morphological features using anatomical characteristics, namely, wings, front, and external genitalia (Lv et al. 2015). Accurate identification requires extensive expertise and experience and yet sometimes can lead to errors. Morphological identification by an entomologist can reduce the potential for errors. Practical morphological identification is only possible when dealing with small sample sizes and well-preserved specimens. Therefore, it is crucial to utilize a new identification method that is accurate, fast, time-saving, and suitable for large numbers of specimens.

Molecular techniques with high reproducibility and fast results offer an excellent alternative to traditional morphological classification. Several mitochondrial and nuclear genes have been used as genetic markers to differentiate related species. These include the mitochondrial cytochrome c oxidase subunit 1 (CO1) gene,

nuclear 12S-16S-18S ribosomal RNA genes, and ITS1 and ITS2 internal transcription spacers (Brengues et al. 2014; Gomez-Polo et al. 2014; Yu et al. 2014; Wang et al. 2016; Zheng et al. 2021). ITS1 and ITS2 are nonfunctional spacers that separate the 18S-5.8S and 5.8S-28S rRNA genes, respectively (Wang et al. 2016; Zheng et al. 2021). As ITS sequences have low intra-species variation but high variation between species, they are helpful for species classification and phylogenetic analysis for morphologically similar organisms, both in prokaryotes and eukaryotes (Zheng et al. 2021). Finally, from the molecular identification of the combined mitochondrial COI-COII and ten microsatellite marker loci (Winnie et al. 2020).

The genetic diversity of *N. lugens* has been reported in several countries, such as China, South Korea, Pakistan, India, and Malaysia (Jing et al. 2012; Latif et al. 2012; Anant et al. 2021; Zheng et al. 2021). The genetic diversity of *N. lugens* in Indonesia is widely reported in western Indonesia (Java Island) (Winnie et al. 2020; Chaerani et al. 2021). Reports on the genetic diversity of *N. lugens* in eastern Indonesia have not been found. Therefore, this study aims to analyze genetic diversity and determine the intensity of BPH attacks on rice plants in eastern Indonesia, especially Bali.

MATERIALS AND METHODS

Brown planthopper sampling from rice dwarf disease endemic areas

Samples were taken from nine locations at the rice cultivation center in Bali Province (Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana). The brown planthopper samples taken from rice plants were nymphs and imagos. Nymphs and imagos were used for total DNA extraction. After arriving at the laboratory, the nymphs and imago were stored dry at -20ºC.

Observation of BPH attack symptoms and quantity of BPH population/rice hills

Observation of symptoms of BPH attack was carried out by observing symptoms of damage to rice plants due to BPH attack. The abundance of the BPH/rice hills population was obtained by counting all nymphs and imagoes obtained. Data on the population per cluster from 20 samples or 20 rice hills at each observation fields were then averaged. For each location, 3 fields of rice cultivation center were taken, which were used for observation.

BPH attack percentage

The percentage of BPH attacks is calculated using the following formula:

$$P = \frac{a}{b} x\ 100\%$$

Where:
P : Attack percentage (%)
a : Number of rice hills affected by BPH
b : Number of rice hills observed

Damage intensity

Determination of scoring on symptoms of rice damage due to BPH attack is based on Table 1. The intensity of damage due to a BPH attack is determined using the formula (Erdiansyah and Damanhuri 2018):

$$I = \sum_{i=1}^{i} \frac{Ni\ x\ Vi}{N\ x\ Z} x\ 100\%$$

Where:
I : Damage intensity
Ni : The number of affected rice hills on the score i
Vi : Score i
N : The number of rice hills observed
Z : Highest score

Total DNA extraction from brown planthopper

Total DNA extraction of brown planthopper was obtained from one individual imago or one individual nymph based on the modified method of Goodwin et al. (1994). One individual imago was put into a microtube and then added to 100 µL of CTAB (Cethyl Trimethyl Ammonium Bromida) extraction buffer (2% CTAB, 1.4 M NaCl, 100 mM Tris-HCI, 20 mM EDTA (Ethylenediaminetetraacetic acid), and 1% PVP (-40°C). Next, 1 µL of proteinase K was added, then the insects were crushed using a micro-pistil, vortexed, and incubated in a water bath of 65°C for 3 minutes.

Table 1. The damage score of rice plants due to BPH attack (Baehaki 2012)

Score	Appearance	Description
0	Healthy	No planthopper was found in any rice hill
1	Very light damage	The rice hills occupied by the planthoppers did not show dead midribs, few exuviae, and the rice stalks had not yet overgrown with *Colletotrichum dematium* and Cladosporium fungi that followed the brown planthopper attack
3	Slightly damaged	The rice hills occupied by the planthoppers have shown dead midribs, many exuviae, and the rice stems are overgrown with *Colletotrichum dematium* and Cladosporium fungi that follow the brown planthopper attack
5	Heavily damaged	Rice hills inhabited by planthoppers showed damage marked by many dead midribs, many exuviae, stunted and black-looking tillers, and overgrown with *Colletotrichum dematium* and Cladosporium fungi
7	Partially dead	Some of the stems in the rice hill die, or the rice hill withers due to planthoppers attack
9	Hopperburn	Rice hills die from hopperburn

After that, the tube was added with 100 µL CI (chloroform: isoamyl alcohol) in a ratio of 24:1. The tube was then vortexed for 3 minutes and centrifuged at 10,000 rpm for 15 minutes. The supernatant formed was transferred to a new microtube (60 µL) and then added with 3 M NaOAc (pH 5.2), as much as 1/10 of the total volume of the supernatant. Isopropanol was added up to 2/3 of the total volume of the supernatant, then incubated at -20°C for one night. The tube was centrifuged at 10,000 rpm for 10 min, and the supernatant was discarded. The pellets were washed with 100 µL of 80% ethanol (cold) and centrifuged at 8000 rpm for 5 minutes. In the final step, the supernatant was removed, and the pellet was dried for approximately 1 hour. It was then added to a solution of 20 µL TE and stored at -20°C until used.

Amplification of mtCOI fragments using the PCR method

PCR reactants were manufactured with a total volume of 25 µL consisting of 12.5 µL Go Tag Green Master Mix (Promega, US) and 9.5 µL ddH_2O. DNA amplification of the mtCOI fragment was carried out using a pair of universal primers mtCOI LCO 1490 (3'-GGTCAACAAATCATAAAGATATTGG-5') and HCO 2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al. 1994) each 1 µL, and 1 µL DNA template. PCR reactions were carried out with a Perkin Elmer 480 Thermocycler (Applied Biosystem, US). The PCR reaction was initiated by initial denaturation for 5 min at 94°C. The PCR was continued for 35 cycles in the following order: 94°C for 1 minute, 52°C for 35 seconds, 72°C for 1 minute 30 seconds, and a final extension of 72°C for 7 minutes. The PCR results were then analyzed in 1% agarose gel. The DNA fragments of mtCOI were visualized using a UV transilluminator after being immersed in a 2% ethidium bromide solution for 15 minutes and photographed with a digital camera. The result of amplification by PCR technique was in the form of mtCOI DNA fragments with a size of ±710 base pairs (bp).

Analysis of DNA sequence results

Nucleotide sequencing DNA fragment purification and mtCOI nucleotide sequencing were performed at PT. 1st Base, Malaysia. The results were then registered in the NCBI gene bank (http://www.ncbi.nlm.nih.gov). Analysis of mtCOI DNA sequence data ChromasPro program was used to combine forward and reverse nucleotide sequences to obtain the mtCOI gene (ChromasPro version 2.01. 2006). The Bioedit program was used to compare mtCOI fragments between samples (Multiple alignments). The phylogenetic relationship was built by comparing the mtCOI sample fragments from the brown planthopper from Indonesia with the mtCOI fragments already stored in the NCBI GenBank (http://www.ncbi.nlm.nih.gov). The criteria for retrieving mtCOI fragments at GenBank were fragments with a nucleotide base length of ±710 bp (Boykin et al. 2007) (Table 3, Figure 3). The phylogenetic tree was constructed using the PAUP 4.0b10 program with the maximum parsimony cladistic quantitative method. The cladogram was compiled using the Heuristic method. The cladogram used results from the strict consensus with the statistical bootstrap test to obtain a 100% probability.

RESULTS AND DISCUSSION

The brown planthopper causes direct and indirect damage to rice plants. Direct damage was in the form of stunted and uneven growth of rice plants (Figure 1A and 1B), yellow plants (Figure 1C), and hopperburn caused by fluid in rice plant cells sucked by BPH nymphs, brachyptera (short wings), and macroptera (long wings) (Figures 1E and 1F). Indirect damage was caused by BPH, which acts as a vector of Rice grassy stunt virus and Rice ragged stunt virus, causing stunted rice plants (Figure 1D). Besides Bali or other parts of Indonesia, BPH attacks on rice crops were also reported in China, where hopperburn affected 60% of all examined crops (Hu et al. 2014). Transmission of the stunt virus by the brown planthoppers occurs persistently (Horgan et al. 2015). Virus infection causes damage to plants because viruses use plant proteins for replication, resulting in loss of crop production (Listihani et al. 2020; Damayanti et al. 2022; Listihani et al. 2022; Pandawani et al. 2022; Selangga and Listihani 2022; Selangga et al. 2022). Therefore, infection with RGSV and RRSV in rice plants causes the rice to lack nutrients to the point of stunting.

A percentage of BPH attacks of more than 50% was found in Gianyar, Bangli, Jembrana, and Badung Regencies (Table 2). The BPH population was primarily found in Ciherang and IR-64 varieties of rice in the Badung Regency, with 43.67 BPH per rice hill. Baehaki (2012) added that the economic threshold could be measured through the number or population of pests and planting age. BPH is said to have reached the economic threshold when the population of this pest was found in the field, as many as nine BPH per rice hill when the rice age was less than 40 DAP or 18 BPH when the rice was more than 40 DAP (Baehaki 2012). In general, rice varieties grown in all observation locations in Bali were BPH susceptible varieties, such as Ciherang, IR-64, Inpari 32, and Situbagendit.

The dynamics of BPH development in the field can be influenced by several factors, including host plant factors and natural enemies (Ferrater et al. 2015; Horgan et al. 2015; Kobayashi 2016). The host plant factors that affect the BPH population are related to the age of the rice plant. When the observations were made, the rice plants were still in the vegetative phase, aged 4-6 WAP. According to Jing et al. (2014), naturally, BPH usually comes to young rice fields, and insects usually come in the first two weeks after planting. Thus, the brown planthopper in rice cultivation might be the first generation of planthoppers that have not yet reproduced because one BPH life cycle takes between 3-4 weeks (IRRI 2009).

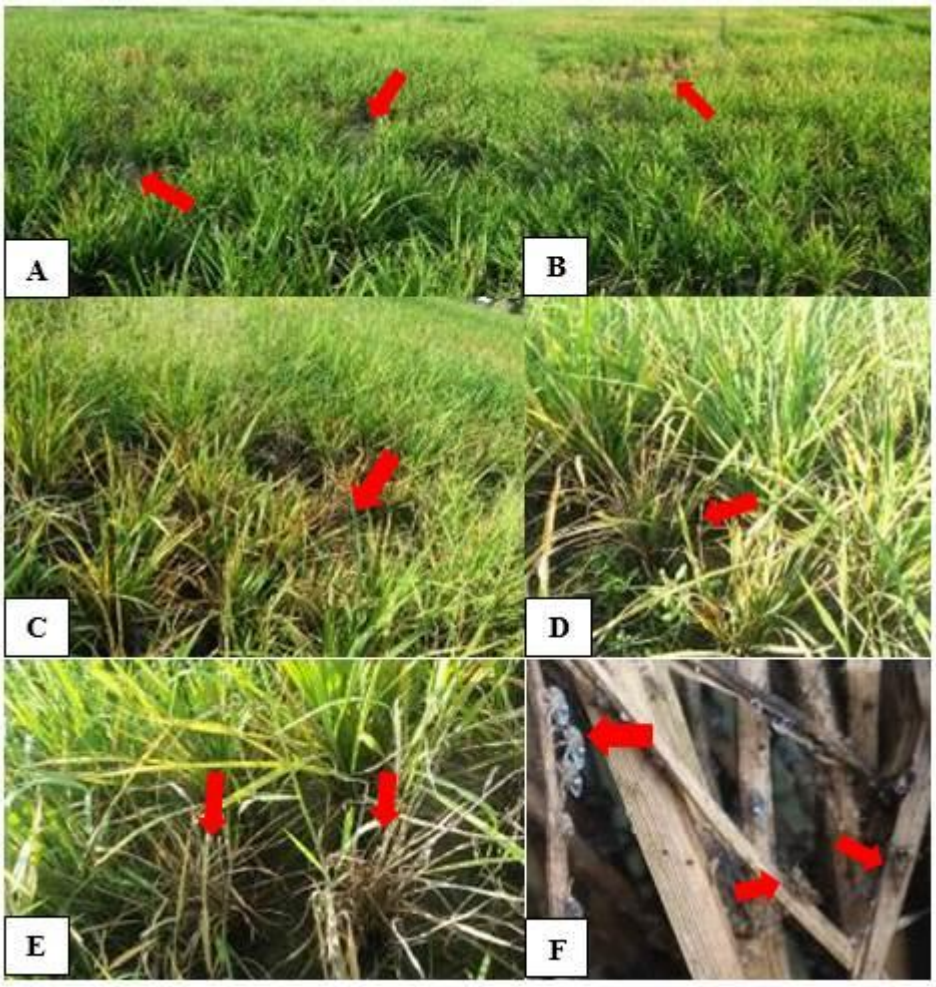

Figure 1. Symptoms of BPH attack on rice plants in Bali. A. Rice plant growth is stunted; B. Uneven plant growth (spots); C. Yellow plant; D. Dwarf rice plants; E. Plants die like burning (hopperburn); F. BPH brachiptera and macroptera were found on rice stalks

Table 2. Population and symptoms of BPH attack on rice plants in Bali, Indonesia

Location	Rice varieties	Rice plant age (DAP)	BPH attack percentage (%)	BPH population abundance (individues/rice hills)
Denpasar City	Situbagendit, Inpari 32	35	35.43	7.41
Badung	Ciherang, IR-64	42	73.61	43.67
Gianyar	Ciherang, Inpari 32	45	52.26	12.49
Tabanan	Inpari 32	41	37.94	9.26
Buleleng	Ciherang, IR-64	33	46.82	11.28
Karangasem	Situbagendit	30	32.73	7.92
Klungkung	Inpari 32	43	35.89	8.53
Bangli	Ciherang, IR-64	42	52.80	14.83
Jembrana	Ciherang, Inpari 32	36	57.32	11.95

Note: DAP: day after planting

BPH observations in Denpasar, Tabanan, Karangasem, and Klungkung districts were dominated by macroptera imago (Table 2). According to Horgan et al. (2017), the planthopper that first came to the plantation was the macroptera planthopper as a winged immigrant planthopper. Meanwhile, in Badung, Gianyar, Buleleng, Bangli, and Jembrana districts, nymphs BPH was dominated by BPH, and several individuals were in the imago phase of brachyptera and macroptera. The dominance of the nymph phase caused the population of BPH in Badung, Gianyar, Buleleng, Bangli, and Jembrana districts to be the highest when compared to the districts of Denpasar, Tabanan, Karangasem, and Klungkung. The presence of the brachyptera planthopper might be contributed to the increase in the nymph population (Baehaki 2012). Rapid population growth usually occurs in groups with many young individuals (Horgan et al. 2015; Triwidodo and Listihani 2020).

The average intensity of BPH attack on Ciherang and IR-64 varieties of rice was higher than in other varieties. In the Ciherang and IR-64 varieties of rice, the average value of the highest attack intensity was 30% (Figure 2). It is because farmers grow rice varieties Ciherang and IR-64 from year to year without any replacement of other varieties. Furthermore, rice varieties Ciherang and IR-64 became very susceptible to BPH attacks. In addition, BPH is a pest that begins to attack rice plants from a young age, even when the rice is still in the nursery.

According to Vu et al. (2014), fluctuations in BPH pest attacks are more influenced by the growth phase of the rice plant that is the host in the field. BPH pests are often found when rice plants are in the vegetative and generative stages (Bottrell and Schoenly 2012). Horgan et al. (2017) added that BPH pests could damage rice plants at all stages of growth and act as vectors for grass and dwarf viruses. BPH attack was higher when rice was in the vegetative phase than in the generative phase (Horgan et al. 2015). It happens because the pests attack the young rice stalks. Considering the type of mouth of BPH, which is included in the suction, BPH can suck the liquid from the rice stems and cause the plant leaves to turn yellow (Anant et al. 2021). According to Choi et al. (2019) and Sutrawati et al. (2021), during the vegetative phase, food availability in the form of nitrogen is abundant in rice plants. Rice plants need nitrogen to form plant organs. Food is one of the factors that affect the life of insects. Horgan (2018) continued that the N element absorbed by plants also serves as a source of nutrition for BPH. If food is available with good quality (suitable for pests), then the insect pest population will increase, and vice versa (Horgan 2018; Triwidodo and Listihani 2020).

The mtCOI DNA band was successfully amplified from the total DNA extraction of one imago or nymph of BPH. The mtCOI fragment that was successfully amplified corresponds to a size of ±710 bp in all samples from nine districts in Bali, namely Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana (Figure 3). Nucleotide and amino acid sequence analysis showed high homology with *N. lugens* sequences in the database at GenBank, 94.2-99.7% and 95.8-100%, respectively (Table 3). *Nilaparvata lugens* sequences from Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana showed the highest nucleotide, and amino acid homology with *N. lugens* isolate FSD-034 from Pakistan (MK301229) biotype Y, respectively. 99.5-99.74% and 100% (Table 3). The results of the molecular detection of *N. lugens* using the PCR method in Bali, Indonesia, are the first reports of the molecular character of *N. lugens* in Indonesia.

Samples from Indonesia formed a group with *N. lugens* biotype Y fragment mtCOI from Pakistan, India, South Korea, and China (Figure 4). This study found *N. lugens* biotype Y in rice plants for the first time in Indonesia. The Indonesian sample did not form separate groups according to the proximity of the district locations but formed a polytomy cladogram (Figure 4). This polytomy cladogram shows that the *N. lugens* between regencies (Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana) were observed to have the same ancestry. These results indicate high locomotion ability with genetic mixing between *N. lugens* in Bali isolates. Similar conditions were also demonstrated in *N. lugens* among Asian isolates using mitochondrial sequences showing genetic mixing. It can also be correlated with the theory of long-distance migration of *N. lugens*, which migrates from the tropics (northern Vietnam) in April-May to temperate regions (China, Korea, and Japan) in June-July as shown based on meteorological studies (Otuka et al. 2008). The population of *N. lugens* is a long-distance migratory flight from the tropics to temperate Asia before modern pesticides are widely used in tropical rice. Due to the infrequent use of insecticides prior to the 1960s in the tropics, factors other than insecticides may have triggered long-wing movements to form *N. lugens* populations (Bottrell and Schoenly 2012).

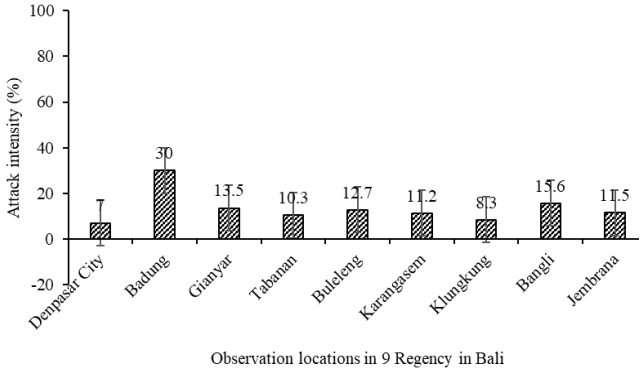

Figure 2. The attack intensity of *Nilaparvata lugens* on rice in Bali Province, Indonesia

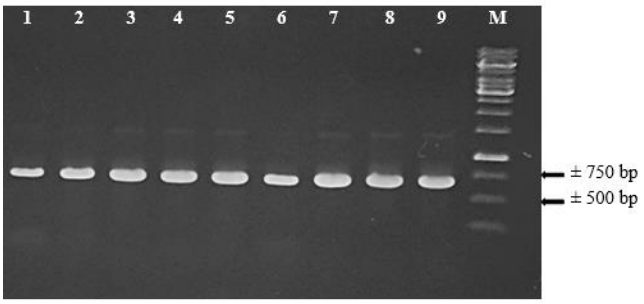

Figure 3. DNA amplification of *Nilaparvata lugens* in rice plants in Bali using primers LCO 1490/HCO 2198. 1. Denpasar City; 2. Bagung; 3. Gianyar; 4. Tabanan; 5. Buleleng; 6. Karangasemt; 7. Klungkung; 8. Bangli; 9. Jembrana and M. DNA marker 1 kb (Thermo Scientific)

Table 3. Nucleotide (nt) and amino acid (aa) homology of *Nilaparvata lugens* in rice from Bali, Indonesia, compared with *Nilaparvata lugens* from other countries in GenBank

Isolate	Origin of isolate	Biotype	Accession number	Homology nt (aa) (%) *Nilaparvata lugens*_IDN_								
				Denpasar	Badung	Gianyar	Tabanan	Buleleng	Karangasem	Klungkung	Bangli	Jembrana
FSD-034	PAK	Y	MK301229	99.5 (100)	99.6 (100)	99.5 (100)	99.5 (100)	99.6 (100)	99.5 (100)	99.7 (100)	99.5 (100)	99.6 (100)
HZZ55	IND	Y	MK032794	99.4 (100)	99.5 (100)	99.4 (100)	99.5 (100)	99.4 (100)	99.5 (100)	99.6 (100)	99.5 (100)	99.6 (100)
SAEVG_Morph0111	IND	Y	MN520923	99.4 (100)	99.5 (100)	99.4 (100)	99.5 (100)	99.4 (100)	99.5 (100)	99.5 (100)	99.5 (100)	99.6 (100)
KBPH	KOR	Y	MK590088	99.3 (100)	99.5 (100)	99.4 (100)	99.4 (100)	99.3 (100)	99.4 (100)	99.4 (100)	99.4 (100)	99.5 (100)
KOREA_BPH	KOR	Y	LC461184	99.3 (100)	99.5 (100)	99.4 (100)	99.4 (100)	99.3 (100)	99.4 (100)	99.4 (100)	99.4 (100)	99.5 (100)
WUHAN-Y	CHN	Y	KC333653	99.3 (100)	99.5 (100)	99.4 (100)	99.4 (100)	99.3 (100)	99.4 (100)	99.4 (100)	99.3 (100)	99.4 (100)
WUHAN-3	CHN	3	JN563997	97.8 (98.1)	97.2 (97.8)	97.5 (98.9)	97.5 (98.9)	97.4 (97.9)	97.8 (98.1)	97.5 (98.9)	97.2 (98.8)	97.6 (98.0)
WUHAN-2	CHN	2	JN563996	96.3 (97.5)	96.3 (97.5)	96.4 (97.5)	96.3 (97.5)	96.2 (97.4)	96.4 (97.5)	96.3 (97.5)	96.2 (97.4)	96.3 (97.5)
WUHAN-1	CHN	1	JN563995	95.3 (96.7)	95.4 (96.7)	95.3 (96.7)	95.4 (96.7)	95.6 (96.8)	95.6 (96.8)	95.3 (96.7)	95.4 (96.7)	95.3 (96.7)
GX	CHN	1	LC461186	95.3 (96.7)	95.3 (96.7)	95.3 (96.7)	95.4 (96.7)	95.5 (96.8)	95.5 (96.8)	95.3 (96.7)	95.3 (96.7)	95.3 (96.7)
Gangavathi	IND	1	OL451531	95.3 (96.7)	95.3 (96.7)	95.3 (96.7)	95.3 (96.7)	95.5 (96.8)	95.5 (96.8)	95.3 (96.7)	95.3 (96.7)	95.3 (96.7)
WUHAN-L	CHN	L	KC333654	94.2 (95.8)	94.4 (96.2)	94.2 (95.8)	94.4 (96.2)	94.3 (96.0)	94.4 (96.2)	94.2 (95.8)	94.4 (96.2)	94.4 (96.2)
Nilaparvata bakeri	CHN	-	JX266790	84.6 (85.6)	85.2 (86.1)	84.8 (85.9)	84.8 (85.9)	84.6 (85.6)	85.2 (86.1)	84.8 (85.9)	85.2 (86.1)	85.2 (86.1)
Sogatella furcifera	CHN	-	HM160123	75.6 (76.9)	75.6 (76.9)	76.2 (77.8)	77.6 (78.4)	77.4 (78.4)	76.8 (77.8)	75.6 (76.9)	76.8 (77.8)	77.6 (78.4)

Notes: nt (nucleotide); aa (amino acid); IDN (Indonesia); PAK (Pakistan); IND (India); KOR (South Korea); CHN (China); *Nilaparvata bakeri* and *Sogatella furcifera* from China was used as outgroups

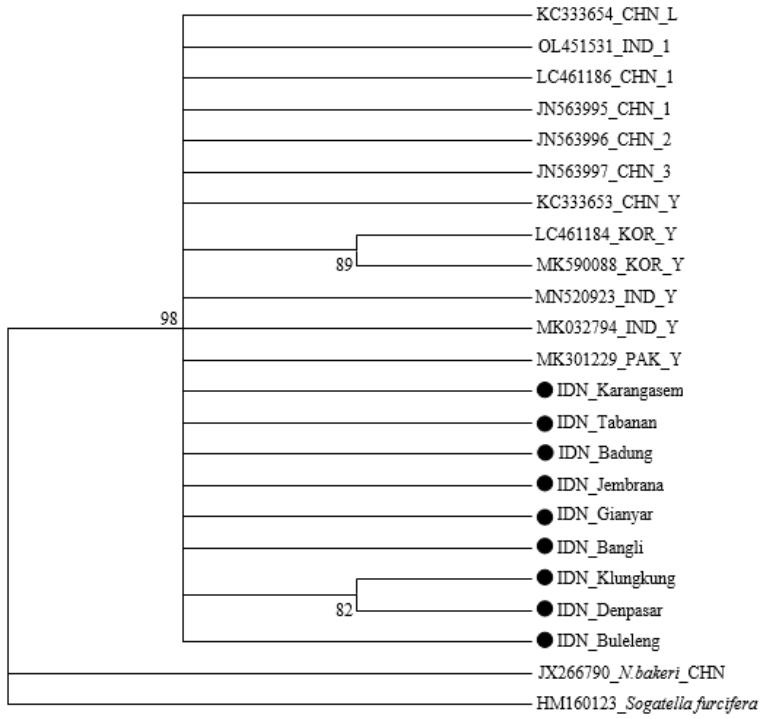

Figure 4. The cladogram of the mtCOI fragment of *Nilaparvata lugens* from eastern Indonesia, Bali (Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana) was compared with mtCOI fragments from several regions of the world that had been deposited on the NCBI website. *Nilaparvata bakeri* and *Sogatella furcifera* from China were used as outgroups. The numbers on the branching cladograms represent bootstrap values with 100% probability. IDN (Indonesia), PAK (Pakistan), IND (India), KOR (South Korea), and CHN (China), isolates marked with black dots are Bali isolates

In previous studies in Indonesia, BPH biotypes 1, 2, 3, and 4 have been found. Kobayashi et al. (2014) reported that the brown planthopper is a highly adaptive insect because it can form new biotypes. In early 1975 the IR-26 rice variety from IRRI Philippines was introduced. The IR-26 variety was unique because it contained a Bph1 resistant gene to anticipate fluctuations in the brown planthopper population. However, in 1976 there was a great population explosion in several rice production centers due to changes in the brown planthopper population from biotype 1 to biotype 2. As an anticipatory measure against brown planthopper biotype 2, in 1980, the IR-42 rice variety (containing the bph2 resistant gene) was introduced from IRRI Philippines. Unfortunately, in 1981 there was another explosion in the brown planthopper population in Simalungun, North Sumatra, and several other areas due to changes in the brown planthopper population from biotype 2 to biotype 3. To deal with the brown planthopper biotype 3, rice variety IR-56 was introduced (containing the gene bph3 resistance) in 1983 and IR-64 (containing the bph1+ resistance gene) in 1986. The introduction process continues. In 1991, the IR-74 variety (containing the bph3 resistant gene) was introduced. In 2006, the resistance gene IR-64 was broken because the brown planthopper population changed to biotype 4 (Baehaki 2012). The stability of the biotype zero brown planthoppers persisted for 41 years before becoming brown planthopper biotype 1. The change of brown planthopper biotype 1 to biotype 2 only took 4 years, and the change of biotype 2 brown planthopper to biotype 3 within 5 years. Until 2005, the brown planthopper biotype 3 was still dominated by biotype 3, and in 2006 the biotype 4 brown planthopper began to develop. The long existence of the biotype 3 brown planthopper was caused by the development of the IR-64 (bph1+) variety over a long period. IR-64 is a resistant variety (durable resistance) that can withstand changes in brown planthoppers to a more virulent biotype.

The continuous cultivation of IR-64 rice varieties by farmers in Bali led to the emergence of a new biotype BPH, namely Y. Insects of biotype Y originated from biotype 1 by eating YHY15 resistant varieties for more than two years for 33 generations (Jing et al. 2012). Rice varieties YHY15 carry the Bph15 resistance gene (Jing et al. 2012).

This study shows great potential in the population of *N. lugens* to adapt to previously resistant rice varieties. This study reported that rice varieties Situbagendit and Inpari 32, previously resistant to BPH, were susceptible to BPH. This research can provide information to farmers not to continuously plant susceptible varieties, which could cause BPH epidemics in the field, as well as the emergence of new, more virulent BPH biotypes. Thus a new control strategy based on a forecasting system can be developed for the regional management of this insect.

In conclusion, *N. lugens* that attack rice plants in Bali (Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana) belongs to biotype Y. Symptoms of damage to rice plants are most severe in Badung Regency. Apart from Ciherang and IR-64

varieties, Situbagendit and Inpari 32 varieties are susceptible to BPH attack.

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The brown planthopper (*Nilaparvata lugens* Stal.) attack and its genetic diversity on rice in Bali, Indonesia

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Abstract. *Listihani L, Ariati PEP, Yuniti IGAD, Selangga DGW. 2022. The brown planthopper (*Nilaparvata lugens *Stal.) attack and its genetic diversity on rice in Bali, Indonesia. Biodiversitas 23: xxxx.* The brown planthopper (*Nilaparvata lugens* Stal.) is an important pest on rice crops in Indonesia.. The genetic diversity of BPH isolates in western Indonesia has been extensively reported, whereas eastern Indonesia isolates have not been reported. This research aims to analyze genetic diversity and evaluate the BPH attack's intensity on Bali rice plants. The research method used was an observation of attack percentage, population dynamics, attack intensity, and genetic diversity of BPH in 9 districts in Bali (Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana). Molecular identification was carried out on *N. lugens* DNA in the mtCOI fragment. BPH attacks of >50% were found in the districts of Gianyar, Bangli, Jembrana, and Badung. The BPH population was primarily found in Ciherang and IR-64 varieties of rice in the Badung Regency, with 43.67 BPH per rice hill. In general, rice varieties grown in all observation locations were susceptible to BPH, such as Ciherang, IR-64, Inpari 32, and Situbagendit. In the Ciherang and IR-64 varieties, the highest attack intensity average value reached 30%. The sequence of *N. lugens* isolate from Bali Jembrana showed the highest nucleotide and amino acid homology with *N. lugens* isolate FSD-034 from Pakistan (MK301229) biotype Y of 99.5 -99.74% and 100%, respectively. This study found *N. lugens* biotype Y in rice plants for the first time in Indonesia. This study reported that Rice varieties Situbagendit and Inpari 32, previously resistant to BPH, are reported as susceptible to BPH.

Keywords: attack intensity, genetic diversity, Inpari 32, Situbagendit, susceptible variety

INTRODUCTION

The brown planthopper (BPH) (*Nilaparvata lugens* Stal, Hemiptera: Delphacidae) is the most destructive rice pest in Indonesia. Repeated outbreaks of BPH in Indonesia are caused by continuous rice cultivation, extensive use, and over-application of insecticides (Baehaki 2012). This pest is vascular monophagous in rice (Cheng et al. 2013; Ferrater et al. 2013; Triwidodo 2020). Feeding by nymphs and imago at the base of the plant causes rapid wilting and drying of the plant (Bottrell and Schoenly 2012; Cheng et al. 2013; Bao and Zhang 2019). In addition, BPH is also a vector of *Rice grassy stunt virus* and *Rice ragged stunt virus* (Bao and Zhang 2019). At high population levels of *N. lugens* can cause significant losses in rice production (Cheng et al. 2013; Zheng et al. 2013; Bao and Zhang 2019).

The BPH cannot tolerate winter in northern Asia, including Japan, Korea, and northern China (He et al. 2012; Fu et al. 2012; Fu et al. 2014). The population originally came from subtropical and tropical areas by flying long distances during the summer (Fu et al. 2014; Hu et al. 2014). BPH infestation in temperate climates originated from annual migrations from tropical Asia and China (He et al. 2012). During autumn, BPH re-migrates (north-to-south) and BPH populations have been studied in China and India (Bottrell and Schoenly 2012). Such return migration may help explain how long-distance migration is maintained in the winter.

The intensification of rice production triggered the BPH outbreak in Tropical Asia during the green revolution era in the 1970s and 1980s (Bottrell and Schoenly 2012). Until now, *N. lugens* is the main problem causing rice harvest failure in several countries. Inaccurate identification and prolonged identification of *N. lugens* are obstacles to its field management strategy.

Traditionally, BPH has been identified at the species level by morphological features using anatomical characteristics, namely, wings, front, and external genitalia (Lv et al. 2015). Accurate identification requires extensive expertise and experience and yet sometimes can lead to errors. Morphological identification by an entomologist can reduce the potential for errors. Practical morphological identification is only possible when dealing with small sample sizes and well-preserved specimens. Therefore, it is crucial to utilize a new identification method that is accurate, fast, time-saving, and suitable for large numbers of specimens.

Molecular techniques with high reproducibility and fast results offer an excellent alternative to traditional morphological classification. Several mitochondrial and nuclear genes have been used as genetic markers to

differentiate related species. These include the mitochondrial cytochrome c oxidase subunit 1 (CO1) gene, nuclear 12S-16S-18S ribosomal RNA genes, and ITS1 and ITS2 internal transcription spacers (Brengues et al. 2014; Gomez-Polo et al. 2014; Yu et al. 2014; Wang et al. 2016; Zheng et al. 2021). ITS1 and ITS2 are nonfunctional spacers that separate the 18S-5.8S and 5.8S-28S rRNA genes, respectively (Wang et al. 2016; Zheng et al. 2021). As ITS sequences have low intra-species variation but high variation between species, they are helpful for species classification and phylogenetic analysis for morphologically similar organisms, both in prokaryotes and eukaryotes (Zheng et al. 2021). Finally, from the molecular identification of the combined mitochondrial COI-COII and ten microsatellite marker loci (Winnie et al. 2020).

The genetic diversity of *N. lugens* has been reported in several countries such as China, South Korea, Pakistan, India, and Malaysia (Jing et al. 2012; Latif et al. 2012; Anant et al. 2021; Zheng et al. 2021). The genetic diversity of *N. lugens* in Indonesia is widely reported in western Indonesia (Java Island) (Winnie et al. 2020; Chaerani et al. 2021). Reports on the genetic diversity of *N. lugens* in eastern Indonesia have not been found. Therefore, this study aims to analyze genetic diversity and determine the intensity of BPH attacks on rice plants in eastern Indonesia, especially Bali.

MATERIALS AND METHODS

Brown Planthopper Sampling from Rice Dwarf Disease Endemic Areas

Samples were taken from nine locations at the rice cultivation center in Bali Province (Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana). The brown planthopper samples taken from rice plants were nymphs and imagos. Nymphs and imagos were used for total DNA extraction. After arriving at the laboratory, the nymphs and imago were stored dry at -20ºC.

Observation of BPH Attack Symptoms and Quantity of BPH Population/rice hills

Observation of symptoms of BPH attack was carried out by observing symptoms of damage to rice plants due to BPH attack. The abundance of the BPH/rice hills population was obtained by counting all nymphs and imagoes obtained. Data on the population per cluster from 20 samples or 20 rice hills at each observation fields were then averaged. For each location, 3 fields of rice cultivation center were taken which were used for observation

BPH Attack Percentage

The percentage of BPH attacks is calculated using the following formula:

$$P = \frac{a}{b} x\ 100\%$$

Note:
P = Attack percentage (%)
a = Number of rice hills affected by BPH
b = Number of rice hills observed

Damage Intensity

Determination of scoring on symptoms of rice damage due to BPH attack is based on Table 1. The intensity of damage due to a BPH attack is determined using the formula (Erdiansyah and Damanhuri 2018):

$$I = \sum_{i=1}^{i} \frac{Ni\ x\ Vi}{N\ x\ Z} x\ 100\%$$

Note:
I = Damage intensity
Ni = The number of affected rice hills on the score i
Vi = Score i
N = The number of rice hills observed
Z = Highest score

Total DNA Extraction from Brown Planthopper

Total DNA extraction of brown planthopper was obtained from one individual imago or one individual nymph based on the modified method of Goodwin et al. (1994). One individual imago was put into a microtube and then added to 100 µl of CTAB (Cethyl Trimethyl Ammonium Bromida) extraction buffer (2% CTAB, 1.4 M NaCl, 100 mM Tris-HCI, 20 mM EDTA (Ethylenediaminetetraacetic acid), and 1% PVP (-40 °C)). Next, 1 µl of proteinase K was added, then the insects were crushed using a micro-pistil, vortexed, and incubated in a water bath of 65°C for 3 minutes. After that, the tube was added with 100 µl CI (chloroform: isoamyl alcohol) in a ratio of 24:1. The tube was then vortexed for 3 minutes and centrifuged at 10,000 rpm for 15 minutes. The supernatant formed was transferred to a new microtube (60 µl) and then added with 3 M NaOAc (pH 5.2), as much as 1/10 of the total volume of the supernatant. Isopropanol was added up to 2/3 of the total volume of the supernatant, then incubated at -20°C for one night. The tube was centrifuged at 10,000 rpm for 10 min, and the supernatant was discarded. The pellets were washed with 100 µl of 80% ethanol (cold) and centrifuged at 8000 rpm for 5 minutes. In the final step, the supernatant was removed, and the pellet was dried for approximately 1 hour. It was then added to a solution of 20 µl TE and stored at -20°C until used.

Amplification of mtCOI Fragments Using the PCR Method

PCR reactants were manufactured with a total volume of 25 µl consisting of 12.5 µl Go Tag Green Master Mix (Promega, US) and 9.5 µl ddH_2O. DNA amplification of the mtCOI fragment was carried out using a pair of universal primers mtCOI LCO 1490 (3'-GGTCAACAAATCATAAAGATATTGG-5') and HCO 2198 (5'-TAAACTTCA–GGGTGACCA–AAAAATCA-3') (Folmer et al. 1994) each 1 µl, and 1 µl DNA template. PCR reactions were carried out with a Perkin Elmer 480 Thermocycler (Applied Biosystem, US). The PCR reaction was initiated by initial denaturation for 5 min at 94°C. The

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PCR was continued for 35 cycles in the following order: 94°C for 1 minute, 52°C for 35 seconds, 72°C for 1 minute 30 seconds, and a final extension of 72°C for 7 minutes. The PCR results were then analyzed in 1% agarose gel. The DNA fragments of mtCOI were visualized using a UV transilluminator after being immersed in a 2% ethidium bromide solution for 15 minutes and photographed with a digital camera. The result of amplification by PCR technique was in the form of mtCOI DNA fragments with a size of ± 710 base pairs (~~p~~bp).

Analysis of DNA Sequence Results

Nucleotide ~~s~~Sequencing DNA fragment purification and mtCOI nucleotide sequencing were performed at PT. 1st Base, Malaysia. The results were then registered in the NCBI gene bank (http://www.ncbi.nlm.nih.gov). Analysis of mtCOI DNA sequence data ChromasPro program was used to combine forward and reverse nucleotide sequences to obtain the mtCOI gene (ChromasPro version 2.01. 2006). The Bioedit program was used to compare mtCOI fragments between samples (Multiple alignments). The phylogenetic relationship was built by comparing the mtCOI sample fragments from the brown planthopper from Indonesia with the mtCOI fragments already stored in the NCBI GenBank (http://www.ncbi.nlm.nih.gov). The criteria for retrieving mtCOI fragments at GenBank were fragments with a nucleotide base length of ± 710 bp (Boykin et al. 2007) (Table 3; Figure 3). The phylogenetic tree was constructed using the PAUP 4.0b10 program with the maximum parsimony cladistic quantitative method. The cladogram was compiled using the Heuristic method. The cladogram used results from the strick consensus with the statistical bootstrap test to obtain a 100% probability.

RESULTS AND DISCUSSION

The brown planthopper causes direct and indirect damage to rice plants. Direct damage was in the form of stunted and uneven growth of rice plants (Figure 1A and 1B), yellow plants (Figure 1C), and hopperburn caused by fluid in rice plant cells sucked by BPH nymphs, brachiptera (~~Fig.~~ short wings), and macroptera (long wings) (Figures 1E and 1F). Indirect damage was caused by BPH, which acts as a vector of *Rice grassy stunt virus* and *Rice ragged stunt virus*, causing stunted rice plants (Figure 1D). Besides Bali or other parts of Indonesia, BPH attacks on rice crops were also reported in China, where hopperburn affected 60% of all examined crops (Hu et al. 2014). Transmission of the stunt virus by the brown planthoppers occurs persistently (Horgan et al. 2015). Virus infection causes damage to plants because viruses use plant proteins for replication, resulting in loss of crop production (Listihani et al. 2020; Damayanti et al. 2022; Listihani et al. 2022; Pandawani et al. 2022; Selangga and Listihani 2022; Selangga et al. 2022). Therefore, infection with RGSV and RRSV in rice plants causes rice to lack nutrients to the point of stunting.

A percentage of BPH attacks of more than 50% was found in Gianyar, Bangli, Jembrana, and Badung Regencies (Table 2). The BPH population was primarily found in Ciherang and IR-64 varieties of rice in the Badung Regency, with 43.67 BPH per rice hills (Table 2). Baehaki (2012) added that the economic threshold could be measured through the number or population of pests and planting age. BPH is said to have reached the economic threshold when the population of this pest was found in the field, as many as nine BPH per rice hill when the rice age was less than 40 DAP or 18 BPH when the rice was more than 40 DAP (Baehaki 2012). In general, rice varieties grown in all observation locations in Bali were BPH susceptible varieties, such as Ciherang, IR-64, Inpari 32, and Situbagendit.

The dynamics of BPH development in the field can be influenced by several factors, including host plant factors and natural enemies (Ferrater et al. 2015; Horgan et al. 2015; Kobayashi 2016). The host plant factors that affect the BPH population are related to the age of the rice plant. When the observations were made, the rice plants were still in the vegetative phase, aged 4-6 WAP. According to Jing et al. (2014), naturally, BPH usually comes to young rice fields, and insects usually come in the first two weeks after planting. Thus, the brown planthopper in rice cultivation might be the first generation of planthoppers that have not yet reproduced because one BPH life cycle takes between 3-4 weeks (IRRI 2009).

BPH observations in Denpasar, Tabanan, Karangasem, and Klungkung ~~cities~~ districts were dominated by macroptera imago (Table 2). According to Horgan et al. (2017), the planthopper that first came to the plantation was the macroptera planthopper as a winged immigrant planthopper. Meanwhile, in Badung, Gianyar, Buleleng, Bangli, and Jembrana districts~~regencies~~, nymph~~sal~~ BPH was dominated by BPH, and several individuals were in the imago phase of brachiptera and macroptera. The dominance of the nymph phase caused the population of BPH in Badung, Gianyar, Buleleng, Bangli, and Jembrana districts to be the highest when compared to the districts~~cities~~ of Denpasar, Tabanan, Karangasem, and Klungkung. The presence of the brachiptera planthopper might be contributed to the increase in the nymph population (Baehaki 2012). Rapid population growth usually occurs in groups with many young individuals (Horgan et al. 2015; Triwidodo and Listihani 2020).

The average intensity of BPH attack on Ciherang and IR-64 varieties of rice was higher than in other varieties. In the Ciherang and IR-64 varieties of rice, the average value of the highest attack intensity was 30% (Figure 2). It is because farmers grow rice varieties Ciherang and IR-64 from year to year without any replacement of other varieties. Furthermore, rice varieties Ciherang and IR-64 became very susceptible to BPH attacks. In addition, BPH is a pest that begins to attack rice plants from a young age, even when the rice is still in the nursery.

According to Vu et al. (2014), fluctuations in BPH pest attacks are more influenced by the growth phase of the rice plant that is the host in the field. BPH pests are often found when rice plants are in the vegetative and generative stages (Bottrell and Schoenly 2012). Horgan et al. (2017) added that BPH pests could damage rice plants at all stages of

growth and act as vectors for grass and dwarf viruses. BPH attack was higher when rice was in the vegetative phase than in the generative phase (Horgan et al. 2015). It happens because the pests attack the young rice stalks. Considering the type of mouth of BPH, which is included in the suction, BPH can suck the liquid from the rice stems and cause the plant leaves to turn yellow (Anant et al. 2021). According to Choi et al. (2019) and Sutrawati et al. (2021), during the vegetative phase, food availability in the form of nitrogen is abundant in rice plants. Rice plants need nitrogen to form plant organs. Food is one of the factors that affect the life of insects. Horgan (2018) continued that the N element absorbed by plants also serves as a source of nutrition for BPH. If food is available with good quality (suitable for pests), then the insect pest population will increase, and vice versa (Horgan 2018; Triwidodo and Listihani 2020).

The mtCOI DNA band was successfully amplified from the total DNA extraction of one imago or nymph of BPH. The mtCOI fragment that was successfully amplified corresponds to a size of ±710 bp in all samples from nine districts in Bali, namely Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana (Figure 3). Nucleotide and amino acid sequence analysis showed high homology with *N. lugens* sequences in the database at GenBank, 94.2 – 99.7% and 95.8 - 100%, respectively (Table 3). *N. lugens* sequences from Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana showed the highest nucleotide, and amino acid homology with *N. lugens* isolate FSD-034 from Pakistan (MK301229) biotype Y, respectively. 99.5 -99.74% and 100% (Table 3). The results of the molecular detection of *N. lugens* using the PCR method in Bali, Indonesia, are the first reports of the molecular character of *N. lugens* in Indonesia.

Samples from Indonesia formed a group with *N. lugens* biotype Y fragment mtCOI from Pakistan, India, South Korea, and China (Figure 4). This study found *N. lugens* biotype Y in rice plants for the first time in Indonesia. The Indonesian sample did not form separate groups according to the proximity of the district locations but formed a polytomy cladogram (Figure 4). This polytomy cladogram shows that the *N. lugens* between regencies (Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana) were observed to have the same ancestry. These results indicate high locomotion ability with genetic mixing between *N. lugens* in Bali isolates. Similar conditions were also demonstrated in *N. lugens* among Asian isolates using mitochondrial sequences showing genetic mixing. It can also be correlated with the theory of long-distance migration of *N. lugens*, which migrates from the tropics (northern Vietnam) in April-May to temperate regions (China, Korea, and Japan) in June-July as shown based on meteorological studies (Otuka et al. 2008). The population of *N. lugens* is a long-distance migratory flight from the tropics to temperate Asia before modern pesticides are widely used in tropical rice. Due to the infrequent use of insecticides prior to the 1960s in the tropics, factors other than insecticides may have triggered long-wing movements to form *N. lugens* populations (Bottrell and Schoenly 2012).

In previous studies in Indonesia, BPH biotypes 1, 2, 3, and 4 have been found. Kobayashi et al. (2014) reported that the brown planthopper is a highly adaptive insect because it can form new biotypes. In early 1975 the IR-26 rice variety from IRRI Philippines was introduced. The IR-26 variety was unique because it contained a Bph1 resistant gene to anticipate fluctuations in the brown planthopper population. However, in 1976 there was a great population explosion in several rice production centers due to changes in the brown planthopper population from biotype 1 to biotype 2. As an anticipatory measure against brown planthopper biotype 2, in 1980, the IR-42 rice variety (containing the bph2 resistant gene) was introduced from IRRI Philippines. Unfortunately, in 1981 there was another explosion in the brown planthopper population in Simalungun, North Sumatra, and several other areas due to changes in the brown planthopper population from biotype 2 to biotype 3. To deal with the brown planthopper biotype 3, rice variety IR-56 was introduced (containing the gene bph3 resistance) in 1983 and IR-64 (containing the bph1+ resistance gene) in 1986. The introduction process continues. In 1991, the IR-74 variety (containing the bph3 resistant gene) was introduced. In 2006, the resistance gene IR-64 was broken because the brown planthopper population changed to biotype 4 (Baehaki 2012). The stability of the biotype zero brown planthoppers persisted for 41 years before becoming brown planthopper biotype 1. The change of brown planthopper biotype 1 to biotype 2 only took 4 years, and the change of biotype 2 brown planthopper to biotype 3 within 5 years. Until 2005, the brown planthopper biotype 3 was still dominated by biotype 3, and in 2006 the biotype 4 brown planthopper began to develop. The long existence of the biotype 3 brown planthopper was caused by the development of the IR-64 (bph1+) variety over a long period. IR-64 is a resistant variety (durable resistance) that can withstand changes in brown planthoppers to a more virulent biotype.

The continuous cultivation of IR-64 rice varieties by farmers in Bali led to the emergence of a new biotype BPH, namely Y. Insects of biotype Y originated from biotype 1 by eating YHY15 resistant varieties for more than two years for 33 generations (Jing et al. 2012). Rice varieties YHY15 carry the Bph15 resistance gene (Jing et al. 2012).

This study shows great potential in the population of *N. lugens* to adapt to previously resistant rice varieties. This study reported that rice varieties Situbagendit and Inpari 32, previously resistant to BPH, were susceptible to BPH. This research can provide information to farmers not to continuously plant susceptible varieties, which could cause BPH epidemics in the field, as well as the emergence of new, more virulent BPH biotypes. Thus a new control strategy based on a forecasting system can be developed for the regional management of this insect.

In conclusion, *N. lugens* that attacks rice plants in Bali (Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana) belongs to biotype Y. Symptoms of damage to rice plants are most severe in Badung Regency. Apart from Ciherang and IR-64

varieties, Situbagendit and Inpari 32 varieties are susceptible to BPH attack.

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Table 1. The damage score of rice plants due to BPH attack

Score	Appearance	Description
0	Healthy	No planthopper was found in any rice hill.
1	Very light damage	The rice hills occupied by the planthoppers did not show dead midribs, few exuviae, and the rice stalks had not yet overgrown with *Colletotrichum dematium* and Cladosporium fungi that followed the brown planthopper attack.
3	Slightly damaged	The rice hills occupied by the planthoppers have shown dead midribs, many exuviae, and the rice stems are overgrown with *Colletotrichum dematium* and Cladosporium fungi that follow the brown planthopper attack.
5	Heavily damaged	Rice hills inhabited by planthoppers showed damage marked by many dead midribs, many exuviae, stunted and black-looking tillers, and overgrown with *Colletotrichum dematium* and Cladosporium fungi.
7	Partially dead	Some of the stems in the rice hill die, or the rice hill withers due to planthoppers attack.
9	Hopperburn	Rice hills die from hopperburn

Sumber: Baehaki (2012)

Table 2. Population and symptoms of BPH attack on rice plants in Bali

Location	Rice varieties	Rice plant age (DAP)	BPH attack percentage (%)	BPH population abundance (individues/rice hills)
Denpasar City	Situbagendit, Inpari 32	35	35.43	7.41
Badung	Ciherang, IR-64	42	73.61	43.67
Gianyar	Ciherang, Inpari 32	45	52.26	12.49
Tabanan	Inpari 32	41	37.94	9.26
Buleleng	Ciherang, IR-64	33	46.82	11.28
Karangasem	Situbagendit	30	32.73	7.92
Klungkung	Inpari 32	43	35.89	8.53
Bangli	Ciherang, IR-64	42	52.80	14.83
Jembrana	Ciherang, Inpari 32	36	57.32	11.95

Note: DAP= day after planting

Table 3. Nucleotide (nt) and amino acid (aa) homology of *N. lugens* in rice from Bali, Indonesia, compared with *N. lugens* from other countries in GenBank

Isolate	Origin of isolate	Biotype	Accession number	Homology nt (aa) (%) *N. lugens*_IDN_								
				Denpasar	Badung	Gianyar	Tabanan	Buleleng	Karangasem	Klungkung	Bangli	Jembrana
FSD-034	PAK	Y	MK301229	99.5 (100)	99.6 (100)	99.5 (100)	99.5 (100)	99.6 (100)	99.5 (100)	99.7 (100)	99.5 (100)	99.6 (100)
HZZ55	IND	Y	MK032794	99.4 (100)	99.5 (100)	99.4 (100)	99.5 (100)	99.4 (100)	99.5 (100)	99.6 (100)	99.5 (100)	99.6 (100)
SAEVG_Morph0111	IND	Y	MN520923	99.4 (100)	99.5 (100)	99.4 (100)	99.5 (100)	99.4 (100)	99.5 (100)	99.5 (100)	99.5 (100)	99.6 (100)
KBPH	KOR	Y	MK590088	99.3 (100)	99.5 (100)	99.4 (100)	99.4 (100)	99.3 (100)	99.4 (100)	99.4 (100)	99.4 (100)	99.5 (100)
KOREA_BPH	KOR	Y	LC461184	99.3 (100)	99.5 (100)	99.4 (100)	99.4 (100)	99.3 (100)	99.4 (100)	99.4 (100)	99.4 (100)	99.5 (100)
WUHAN-Y	CHN	Y	KC333653	99.3 (100)	99.5 (100)	99.4 (100)	99.4 (100)	99.3 (100)	99.4 (100)	99.4 (100)	99.3 (100)	99.4 (100)
WUHAN-3	CHN	3	JN563997	97.8 (98.1)	97.2 (97.8)	97.5 (98.9)	97.5 (98.9)	97.4 (97.9)	97.8 (98.1)	97.5 (98.9)	97.2 (98.8)	97.6 (98.0)
WUHAN-2	CHN	2	JN563996	96.3 (97.5)	96.3 (97.5)	96.4 (97.5)	96.3 (97.5)	96.2 (97.4)	96.4 (97.5)	96.3 (97.5)	96.2 (97.4)	96.3 (97.5)
WUHAN-1	CHN	1	JN563995	95.3 (96.7)	95.4 (96.7)	95.3 (96.7)	95.4 (96.7)	95.6 (96.8)	95.6 (96.8)	95.3 (96.7)	95.4 (96.7)	95.3 (96.7)
GX	CHN	1	LC461186	95.3 (96.7)	95.3 (96.7)	95.3 (96.7)	95.4 (96.7)	95.5 (96.8)	95.5 (96.8)	95.3 (96.7)	95.3 (96.7)	95.3 (96.7)
Gangavathi	IND	1	OL451531	95.3 (96.7)	95.3 (96.7)	95.3 (96.7)	95.3 (96.7)	95.5 (96.8)	95.5 (96.8)	95.3 (96.7)	95.3 (96.7)	95.3 (96.7)
WUHAN-L	CHN	L	KC333654	94.2 (95.8)	94.4 (96.2)	94.2 (95.8)	94.4 (96.2)	94.3 (96.0)	94.4 (96.2)	94.2 (95.8)	94.4 (96.2)	94.4 (96.2)
N. bakeri	CHN	-	JX266790	84.6 (85.6)	85.2 (86.1)	84.8 (85.9)	84.8 (85.9)	84.6 (85.6)	85.2 (86.1)	84.8 (85.9)	85.2 (86.1)	85.2 (86.1)
Sogatella furcifera	CHN	-	HM160123	75.6 (76.9)	75.6 (76.9)	76.2 (77.8)	77.6 (78.4)	77.4 (78.4)	76.8 (77.8)	75.6 (76.9)	76.8 (77.8)	77.6 (78.4)

Notes: nt (nucleotide), aa (amino acid), IDN (Indonesia), PAK (Pakistan), IND (India), KOR (South Korea), CHN (China), *N. bakeri* and *Sogatella furcifera* from China was used as outgroups

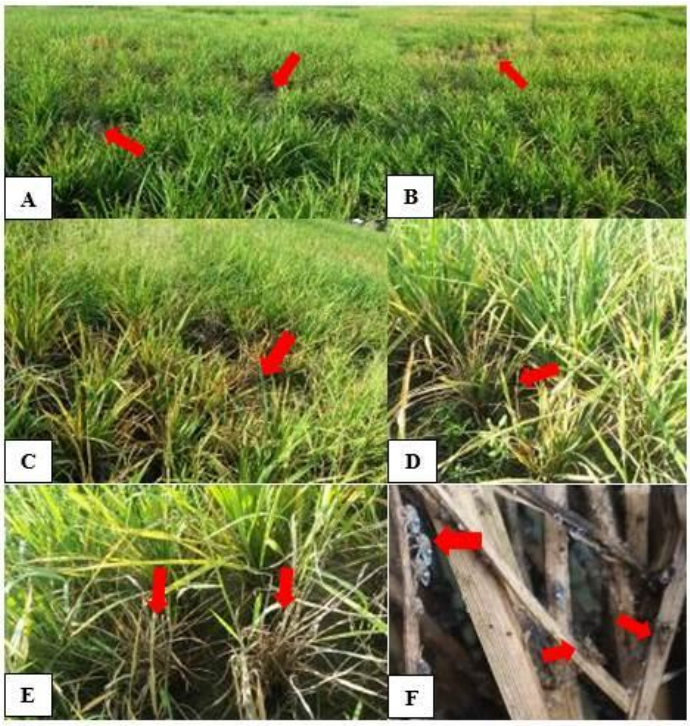

Figure 1. Symptoms of BPH attack on rice plants in Bali: A. rice plant growth is stunted; B. uneven plant growth (spots); C. yellow plant; D. dwarf rice plants; E. plants die like burning (hopperburn); F. BPH brachiptera and macroptera were found on rice stalks

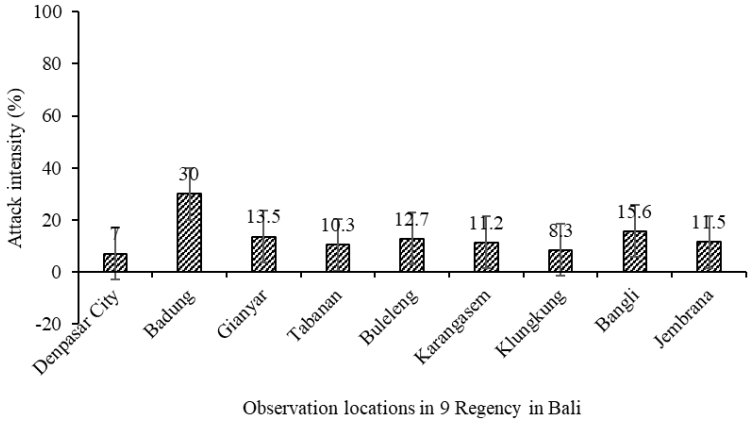

Figure 2. The attack intensity of *N. lugens* on rice in Bali Province

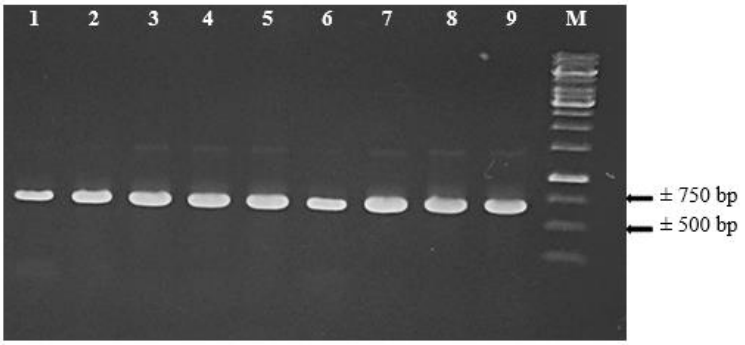

Figure 3. DNA amplification of *N. lugens* in rice plants in Bali using primers LCO 1490/HCO 2198. 1. Denpasar City, 2. Bagung, 3. Gianyar, 4. Tabanan, 5. Buleleng, 6. Karangasemt, 7. Klungkung, 8. Bangli, 9. Jembrana, and M. DNA marker 1 kb (Thermo Scientific)

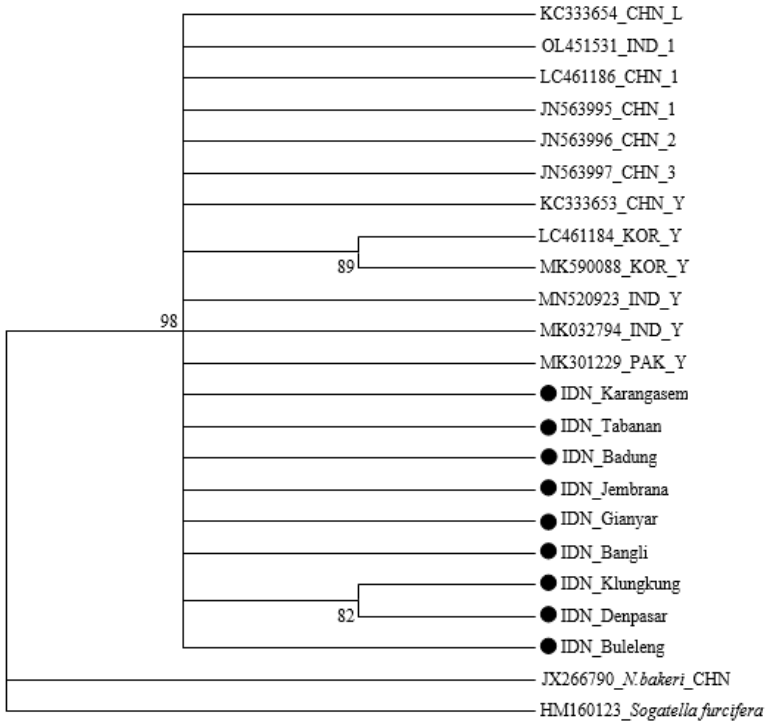

Figure 4. The cladogram of the mtCOI fragment of *N. lugens* from eastern Indonesia, Bali (Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana) was compared with mtCOI fragments from several regions of the world that had been deposited on the NCBI website. *N. bakeri* and *Sogatella furcifera* from China were used as outgroups. The numbers on the branching cladograms represent bootstrap values with 100% probability. IDN (Indonesia), PAK (Pakistan), IND (India), KOR (South Korea), and CHN (China), isolates marked with black dots are Bali isolates.