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**COVERING LETTER**

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I am here with enclosed a research article,

**Title:**

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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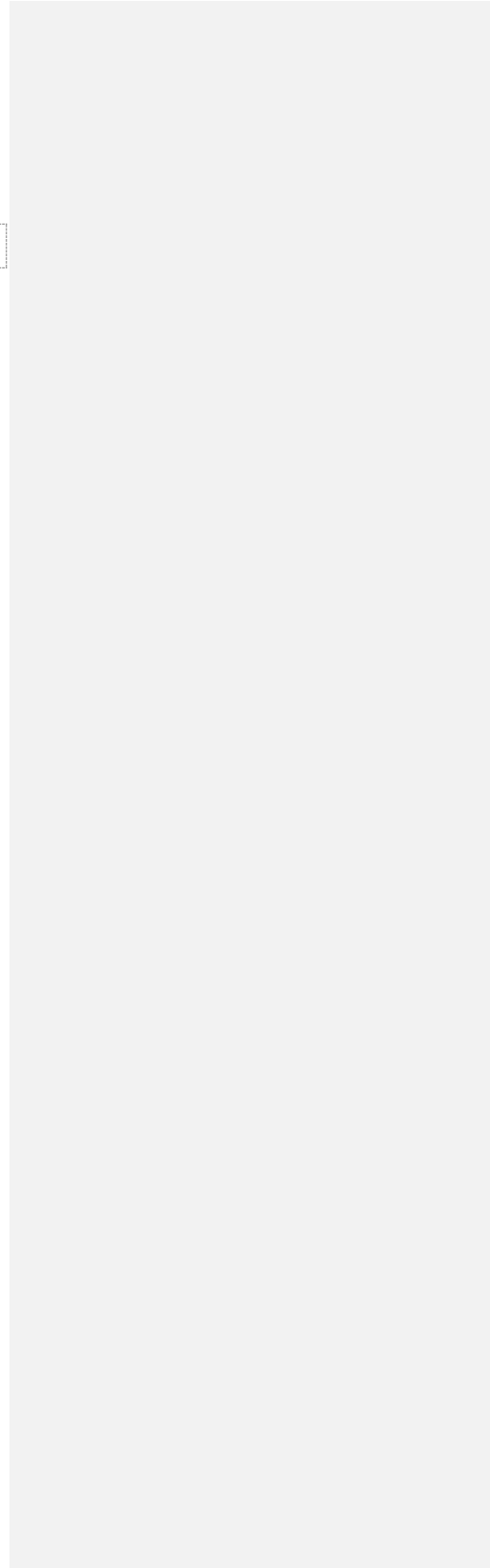
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Listihani Listihani



# 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:** 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

80 ribosomal RNA genes, and ITS1 and ITS2 internal transcription spacers (Fukunaga et al. 2000; Brengues et al. 2014;  
81 Gomez-Polo et al. 2014; Wang et al. 2016; Liu et al. 2018). ITS1 and ITS2 are nonfunctional spacers that separate the  
82 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  
83 variation but high variation between species, they are helpful for species classification and phylogenetic analysis for  
84 morphologically similar organisms, both in prokaryotes and eukaryotes (Liu et al. 2009). Finally, from the molecular  
85 identification of the combined mitochondrial COI-COII and ten microsatellite marker loci (Winnie et al. 2020).

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

## 91 MATERIALS AND METHODS

### 92 Brown Planthopper Sampling from Rice Dwarf Disease Endemic Areas

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

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

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

### 103 BPH Attack Percentage

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

$$105 P = \frac{a}{b} \times 100\%$$

106 Note:

107 P = Attack percentage (%)

108 a = Number of rice hills affected by BPH

109 b = Number of rice hills observed

### 111 Damage Intensity

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

$$114 I = \sum_{i=1}^i \frac{N_i \times V_i}{N \times Z} \times 100\%$$

115

116

117

118 Note:

119 I = Damage intensity

120 N<sub>i</sub> = The number of affected rice hills on the score i

121 V<sub>i</sub> = Score i

122 N = The number of rice hills observed

123 Z = Highest score

### 125 Total DNA Extraction from Brown Planthopper

126 Total DNA extraction of brown planthopper was obtained from one individual imago or one individual nymph  
127 based on the modified method of Goodwin et al. (1994). One individual imago was put into a microtube and then added  
128 with 100 µl of CTAB extraction buffer (2% CTAB, 1.4 M NaCl, 100 mM Tris-HCl, 20 mM EDTA, and 1% PVP (-40  
129 °C)). Next, 1 µl of proteinase K was added, then the insects were crushed using a micro-pistil, vortexed, and incubated in a  
130 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  
131 24:1. The tube was then vortexed for 3 minutes and centrifuged at 10,000 rpm for 15 minutes. The supernatant formed was  
132 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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133 the supernatant. Isopropanol was added up to 2/3 of the total volume of the supernatant, then incubated at -20°C for one  
134 night. The tube was centrifuged at 10,000 rpm for 10 min, and the supernatant was discarded. The pellets were washed  
135 with 100 µl of 80% ethanol (cold) and centrifuged at 8000 rpm for 5 minutes. In the final step, the supernatant was  
136 removed, and the pellet was dried for approximately 1 hour. It was then added with a solution of 20 µl TE and stored at -  
137 20°C until used.

#### 139 **Amplification of mtCOI Fragments Using the PCR Method**

140 PCR reactants were manufactured with a total volume of 25 µl consisting of 12.5 µl Go Tag Green Master Mix  
141 (Promega, US) and 9.5 µl ddH<sub>2</sub>O. DNA amplification of the mtCOI fragment was carried out using a pair of universal  
142 primers mtCOI LCO 1490 (3'-GGTCAACAAATCATAAAGATATTGG-5') and HCO 2198 (5'TAAACTCA  
143 GGGTGACCA AAAAATCA-3') (Folmer et al. 1994) each 1 µl, and 1 µl DNA template. PCR reactions were carried out  
144 with a Perkin Elmer 480 Thermocycler (Applied Biosystem, US). The PCR reaction was initiated by initial denaturation  
145 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,  
146 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%  
147 agarose gel. The DNA fragments of mtCOI were visualized using a UV transilluminator after being immersed in a 2%  
148 ethidium bromide solution for 15 minutes and photographed with a digital camera. The result of amplification by PCR  
149 technique was in the form of mtCOI DNA fragments with a size of ± 710 base pairs (pb).

#### 151 **Analysis of DNA Sequence Results**

152 Nucleotide Sequencing DNA fragment purification and mtCOI nucleotide sequencing were performed at PT. 1st  
153 Base, Malaysia. The results were then registered in the NCBI gene bank (<http://www.ncbi.nlm.nih.gov>). Analysis of  
154 mtCOI DNA sequence data ChromasPro program was used to combine forward and reverse nucleotide sequences to obtain  
155 the mtCOI gene (ChromasPro version 2.01, 2006). The Bioedit program was used to compare mtCOI fragments between  
156 samples (Multiple alignments) (Hall 1999). The phylogenetic relationship was built by comparing the mtCOI sample  
157 fragments from the brown planthopper from Indonesia with the mtCOI fragments already stored in the NCBI GenBank  
158 (<http://www.ncbi.nlm.nih.gov>). The criteria for retrieving mtCOI fragments at GenBank were fragments with a nucleotide  
159 base length of ± 800 bp (Boykin et al. 2007) (Table 1). The phylogenetic tree was constructed using the PAUP 4.0b10  
160 program (Swofford 2002) with the maximum parsimony cladistic quantitative method. The cladogram was compiled using  
161 the Heuristic method. The cladogram used results from the strict consensus with the statistical bootstrap test to obtain a  
162 100% probability.

## 163 **RESULTS AND DISCUSSION**

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

170 A percentage of BPH attacks of more than 50% was found in Gianyar, Bangli, Jembrana, and Badung Regencies  
171 (Table 2). The BPH population was primarily found in Ciherang and IR-64 varieties of rice in the Badung Regency, with  
172 43.67 BPH per rice hill (Table 2). Baehaki and Mejaya (2015) added that the economic threshold could be measured  
173 through the number or population of pests and planting age. BPH is said to have reached the economic threshold when the  
174 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  
175 18 BPH when the rice was more than 40 DAP (Baehaki and Mejaya 2015). In general, rice varieties grown in all  
176 observation locations in Bali were BPH susceptible varieties, such as Ciherang, IR-64, Inpari 32, and Situbagendit.

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

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

189 presence of the brachiptera planthopper might be contributed to the increase in the nymph population (Baehaki and Mejaya  
190 2015). According to Horgan et al. (2015), rapid population growth usually occurs in groups with many young individuals.

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

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

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

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

229 In previous studies in Indonesia, BPH biotypes 1, 2, 3, and 4 have been found. Chen et al. (2011) reported that the  
230 brown planthopper is a highly adaptive insect because it can form new biotypes. In early 1975 the IR-26 rice variety from  
231 IRRRI Philippines was introduced. The IR-26 variety was unique because it contained a Bph1 resistant gene to anticipate  
232 fluctuations in the brown planthopper population. However, in 1976 there was a great population explosion in several rice  
233 production centers due to changes in the brown planthopper population from biotype 1 to biotype 2. As an anticipatory  
234 measure against brown planthopper biotype 2, in 1980, the IR-42 rice variety (containing the bph2 resistant gene) was  
235 introduced from IRRRI Philippines. Unfortunately, in 1981 there was another explosion in the brown planthopper  
236 population in Simalungun, North Sumatra, and several other areas due to changes in the brown planthopper population  
237 from biotype 2 to biotype 3. To deal with the brown planthopper biotype 3, rice variety IR-56 was introduced (containing  
238 the gene bph3 resistance) in 1983 and IR-64 (containing the bph1+ resistance gene) in 1986. The introduction process  
239 continues. In 1991, the IR-74 variety (containing the bph3 resistant gene) was introduced. In 2006, the resistance gene IR-  
240 64 was broken because the brown planthopper population changed to biotype 4. The stability of the biotype zero brown  
241 planthoppers persisted for 41 years before becoming brown planthopper biotype 1. The change of brown planthopper  
242 biotype 1 to biotype 2 only took 4 years, and the change of biotype 2 brown planthopper to biotype 3 within 5 years. Until  
243 2005, the brown planthopper biotype 3 was still dominated by biotype 3, and in 2006 the biotype 4 brown planthopper  
244 began to develop. The long existence of the biotype 3 brown planthopper was caused by the development of the IR-64  
245 (bph1+) variety over a long period. IR-64 is a resistant variety (durable resistance) that can withstand changes in brown  
246 planthoppers to a more virulent biotype.

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247 The continuous cultivation of IR-64 rice varieties by farmers in Bali led to the emergence of a new biotype BPH,  
248 namely Y. Insects of biotype Y originated from biotype 1 by eating YHY15 resistant varieties for more than two years for  
249 33 generations (Jing et al. 2012). Rice varieties YHY15 carry the Bph15 resistance gene (Jing et al. 2012).

250 This study shows great potential in the population of *N. lugens* to adapt to previously resistant rice varieties. This  
251 study reported that rice varieties Situbagendit and Inpari 32, previously resistant to BPH, were susceptible to BPH. This  
252 research can provide information to farmers not to continuously plant susceptible varieties, which could cause BPH  
253 epidemics in the field, as well as the emergence of new, more virulent BPH biotypes. Thus a new control strategy based on  
254 a forecasting system can be developed for the regional management of this insect.  
255

## 256 CONCLUSIONS

257 *N. lugens* that attacks rice plants in Bali (Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar  
258 City, Buleleng, and Jembrana) belongs to biotype Y. Symptoms of damage to rice plants are most severe in Badung  
259 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

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**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) (%) <i>N. lugens</i> 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)
<i>N. bakeri</i>	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)
<i>Sogatella furcifera</i>	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)

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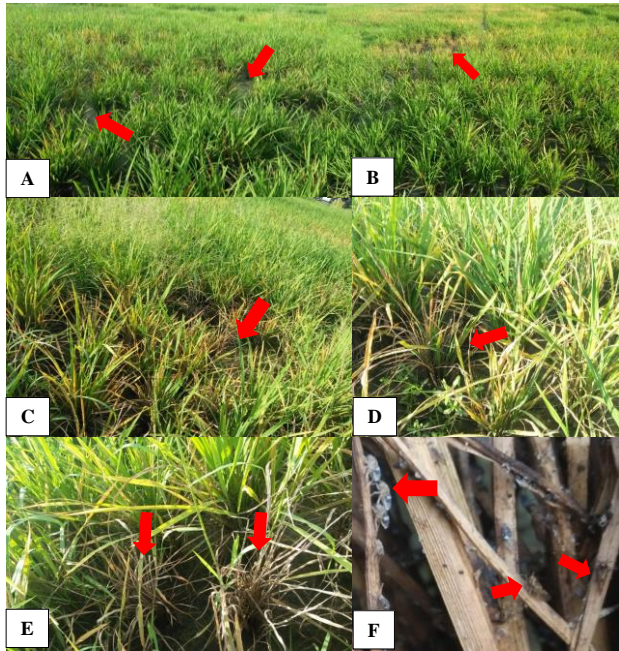
Notes: nt (nucleotide), aa (amino acid), IDN (Indonesia), PAK (Pakistan), IND (India), KOR (South Korea), CHN (China), *N. bakeri* and *Sogatella furcifera* from China was used as outgroups

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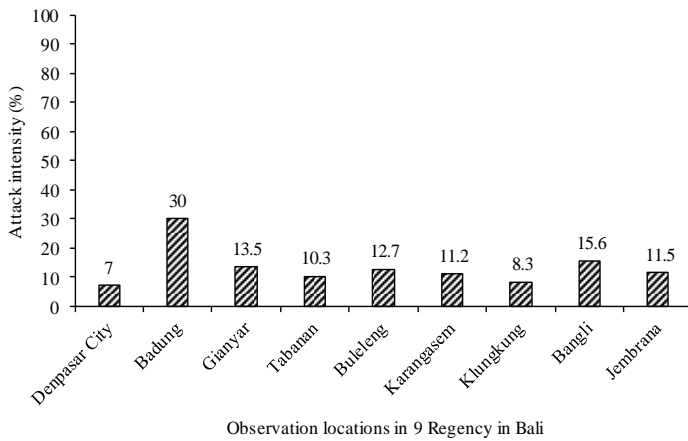
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7 **FIGURES LIST**

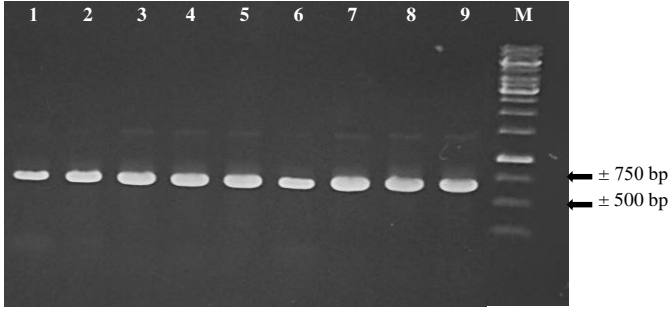


24 **Figure 1.** Symptoms of BPH attack on rice plants in Bali: A. rice plant growth is stunted; B. uneven plant growth (spots); C. yellow  
25 plant; D. dwarf rice plants; E. plants die like burning (hopperburn); F. BPH brachiptera and macroptera were found on rice stalks.

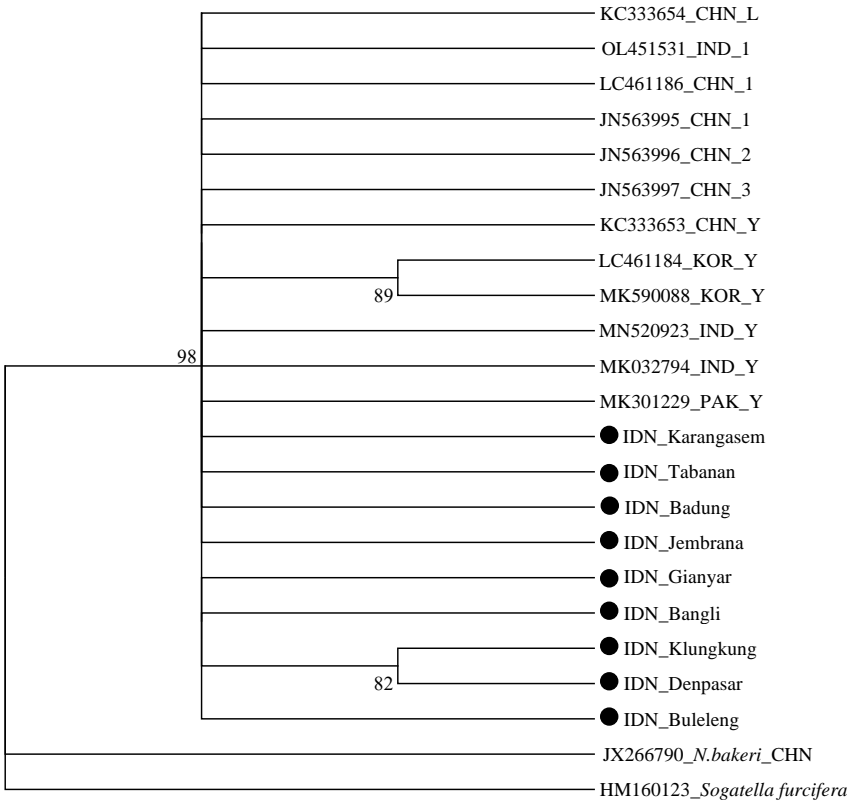


27 **Figure 2.** The attack intensity of *N. lugens* on rice in Bali Province

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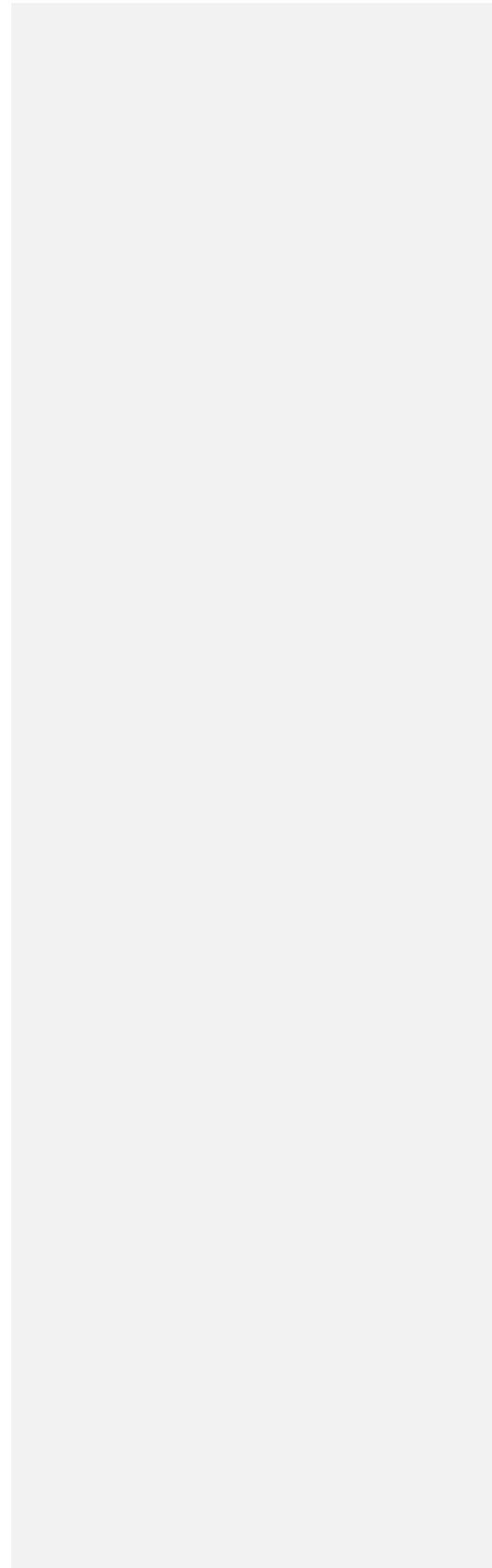


**Figure 3.** DNA amplification of *N. lugens* in rice plants in Bali using primers LCO 1490/HCO 2198. 1. Denpasar City, 2. Bagung, 3. Gianyar, 4. Tabanan, 5. Buleleng, 6. Karangasemt, 7. Klungkung, 8. Bangli, 9. Jembrana, and M. DNA marker 1 kb (Thermo Scientific)



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

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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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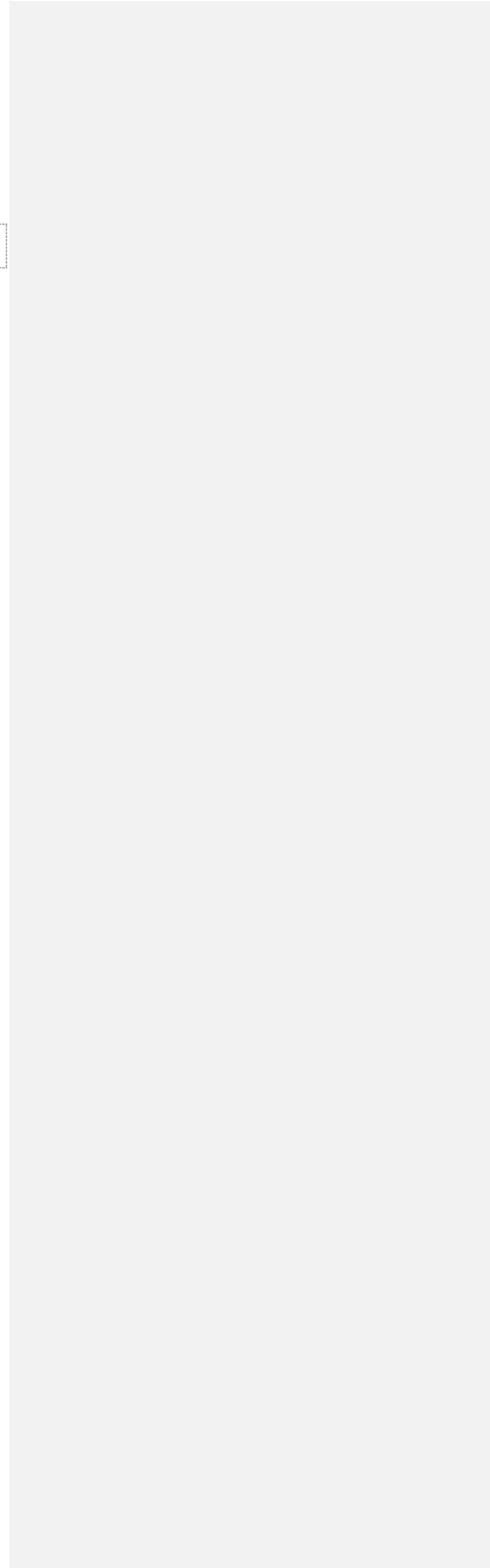
Denpasar July 2022



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Listihani Listihani



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

Commented [A1]: *Nilaparvata lugens* Stal.?

LISTIHANI LISTIHANI<sup>1\*</sup>, PUTU EKA PASMIDI ARIATI<sup>1</sup>, I GUSTI AYU DIAH YUNITI<sup>1</sup>, DEWA GEDE WIRYANGGA SELANGGA<sup>2</sup>

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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.

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**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*) Attack and Its Genetic Diversity on Rice in Bali, Indonesia

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## 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).

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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.

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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.

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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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**Commented [A12]:** please add to reference on the genetic diversity of *N. lugens*

## 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 -20°C.

### 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:

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

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:

$$I = \sum_{i=1}^i \frac{N_i \times V_i}{N \times Z} \times 100\%$$

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109

110

111

112 Note:  
113 I = Damage intensity  
114 Ni = The number of affected rice hills on the score i  
115 Vi = Score i  
116 N = The number of rice hills observed  
117 Z = Highest score  
118

#### 119 Total DNA Extraction from Brown Planthopper

120 Total DNA extraction of brown planthopper was obtained from one individual imago or one individual nymph.  
121 One individual imago was put into a microtube and then added with 100 µl of CTAB extraction buffer (2% CTAB, 1.4 M  
122 NaCl, 100 mM Tris-HCl, 20 mM EDTA, and 1% PVP (-40 °C)). Next, 1 µl of proteinase K was added, then the insects  
123 were crushed using a micro-pistil, vortexed, and incubated in a water bath of 65°C. After that, the tube was added with 100  
124 µl CI (chloroform: isoamyl alcohol) in a ratio of 24:1. The tube was then vortexed for 3 minutes and centrifuged at 10,000  
125 rpm for 15 minutes. The supernatant formed was transferred to a new microtube (60 µl) and then added with 3 M NaOAc  
126 (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  
127 the supernatant, then incubated at -20°C for one night. The tube was centrifuged at 10,000 rpm for 10 min, and the  
128 supernatant was discarded. The pellets were washed with 100 µl of 80% ethanol (cold) and centrifuged at 8000 rpm for 5  
129 minutes. In the final step, the supernatant was removed, and the pellet was dried for approximately 1 hour. It was then  
130 added with a solution of 20 µl TE and stored at -20°C until used.  
131

#### 132 Amplification of mtCOI Fragments Using the PCR Method

133 PCR reactants were manufactured with a total volume of 25 µl consisting of 12.5 µl Go Tag Green Master Mix  
134 and 9.5 µl ddH<sub>2</sub>O. DNA amplification of the mtCOI fragment was carried out using a pair of universal primers mtCOI  
135 LCO 1490 (3'-GGTCAACAAATCATAAAGATATTGG-5') and HCO 2198 (5'TAAACTTCA GGGTGACCA  
136 AAAAATCA-3') (Folmer et al. 1994) each 1 µl, and 1 µl DNA template. PCR reactions were carried out with a Perkin  
137 Elmer 480 Thermocycler. The PCR reaction was initiated by initial denaturation for 5 min at 94°C. The PCR was  
138 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  
139 a final extension of 72°C for 7 minutes. The PCR results were then analyzed in 1% agarose gel. The DNA fragments of  
140 mtCOI were visualized using a UV transilluminator after being immersed in a 2% ethidium bromide solution for 15  
141 minutes and photographed with a digital camera.  
142

#### 143 Analysis of DNA Sequence Results

144 Nucleotide Sequencing DNA fragment purification and mtCOI nucleotide sequencing were performed at PT. 1st  
145 Base, Malaysia. The results were then registered in the NCBI gene bank (<http://www.ncbi.nlm.nih.gov>). Analysis of  
146 mtCOI DNA sequence data ChromasPro program was used to combine forward and reverse nucleotide sequences to obtain  
147 the mtCOI gene. The Bioedit program was used to compare mtCOI fragments between samples (Multiple alignments)  
148 (Hall 1999). The phylogenetic relationship was built by comparing the mtCOI sample fragments from the brown  
149 planthopper from Indonesia with the mtCOI fragments already stored in the NCBI GenBank  
150 (<http://www.ncbi.nlm.nih.gov>). The criteria for retrieving mtCOI fragments at GenBank were fragments with a nucleotide  
151 base length of ± 800 bp (Boykin et al. 2007) (Table 1). The phylogenetic tree was constructed using the PAUP 4.0b10  
152 program (Swofford 2002) with the maximum parsimony cladistic quantitative method. The cladogram was compiled using  
153 the Heuristic method. The cladogram used results from the strict consensus with the statistical bootstrap test to obtain a  
154 100% probability.

## 155 RESULTS AND DISCUSSION

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

162 A percentage of BPH attacks of more than 50% was found in Gianyar, Bangli, Jembrana, and Badung Regencies  
163 (Table 2). The BPH population was primarily found in Ciherang and IR-64 varieties of rice in the Badung Regency, with  
164 43.67 BPH per rice hill (Table 2). Baehaki and Mejaya (2015) added that the economic threshold could be measured  
165 through the number or population of pests and planting age. BPH is said to have reached the economic threshold when the  
166 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  
167 18 BPH when the rice was more than 40 DAP (Baehaki and Mejaya 2015). In general, rice varieties grown in all  
168 observation locations in Bali were BPH susceptible varieties, such as Ciherang, IR-64, Inpari 32, and Situbagendit.

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

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

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

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

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

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

216 In previous studies in Indonesia, BPH biotypes 1, 2, 3, and 4 have been found. Chen et al. (2011) reported that the  
217 brown planthopper is a highly adaptive insect. In early 1975 the IR-26 rice variety from IRRI Philippines was introduced.  
218 The IR-26 variety was unique because it contained a Bph1 resistant gene to anticipate fluctuations in the brown  
219 planthopper population. However, in 1976 there was a great population explosion in several rice production centers due to  
220 changes in the brown planthopper population from biotype 1 to biotype 2. As an anticipatory measure against brown  
221 planthopper biotype 2, in 1980, the IR-42 rice variety (containing the bph2 resistant gene) was introduced from IRRI  
222 Philippines. Unfortunately, in 1981 there was another explosion in the brown planthopper population in Simalungun,  
223 North Sumatra, and several other areas due to changes in the brown planthopper population from biotype 2 to biotype 3.  
224 To deal with the brown planthopper biotype 3, rice variety IR-56 was introduced (containing the gene bph3 resistance) in  
225 1983 and IR-64 (containing the bph1+ resistance gene) in 1986. The introduction process continues. In 1991, the IR-74  
226 variety (containing the bph3 resistant gene) was introduced. In 2006, the resistance gene IR-64 was broken because the  
227 brown planthopper population changed to biotype 4. The stability of the biotype zero brown planthoppers persisted for 41  
228 years before becoming brown planthopper biotype 1. The change of brown planthopper biotype 1 to biotype 2 only took 4

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229 years, and the change of biotype 2 brown planthopper to biotype 3 within 5 years. Until 2005, the brown planthopper  
230 biotype 3 was still dominated by biotype 3, and in 2006 the biotype 4 brown planthopper began to develop.  
231 The continuous cultivation of IR-64 rice varieties by farmers in Bali led to the emergence of a new biotype BPH,  
232 namely Y. Insects of biotype Y originated from biotype 1 by eating YHY15 resistant varieties for more than two years for  
233 33 generations (Jing et al. 2012). Rice varieties YHY15 carry the Bph15 resistance gene (Jing et al. 2012).  
234 This study shows great potential in the population of *N. lugens* to adapt to previously resistant rice varieties. This  
235 study reported that rice varieties Situbagendit and Inpari 32, previously resistant to BPH, were susceptible to BPH. This  
236 research can provide information to farmers not to continuously plant susceptible varieties, which could cause BPH  
237 epidemics in the field, as well as the emergence of new, more virulent BPH biotypes. Thus a new control strategy based on  
238 a forecasting system can be developed for the regional management of this insect.  
239

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## 240 CONCLUTIONS

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

## 244 ACKNOWLEDGEMENTS

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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

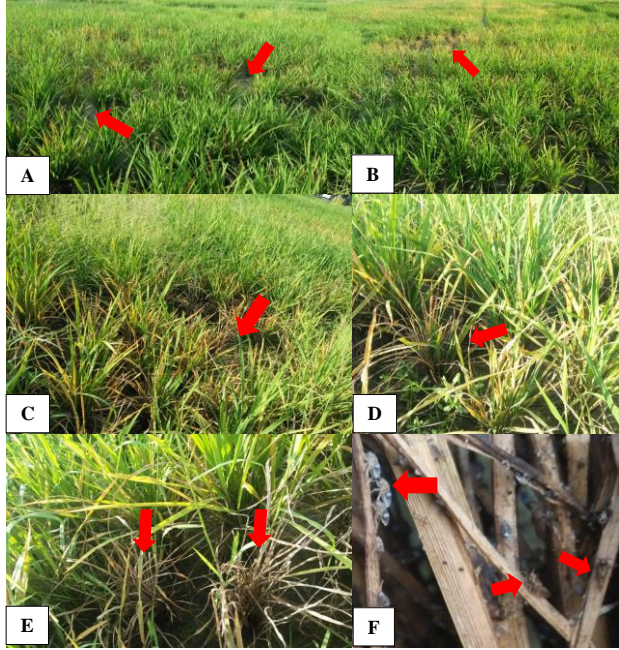
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**Table 3.** Nucleotide (nt) and amino acid (aa) homology of *N. lugens* in rice from Bali, Indonesia, compared with *N. lugens* from other countries in GenBank

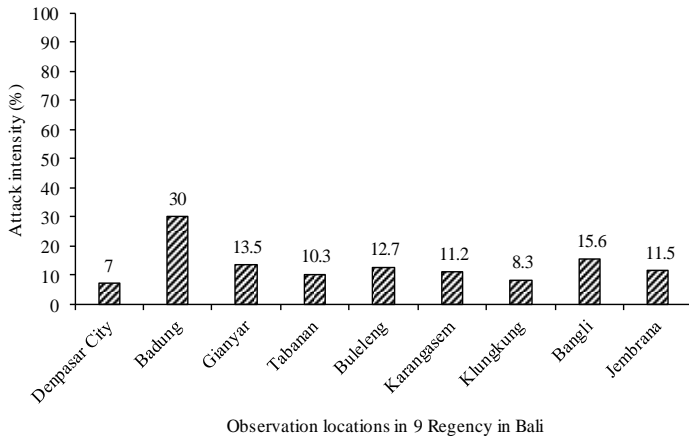
Isolate	Origin of isolate	Biotype	Accession number	Homology nt (aa) (%) <i>N. lugens</i> IDN_								
				Denpasar	Badung	Gianyar	Tabanan	Buleleng	Karangasem	Klungkung	Bangli	Jembrana
FSD-034	PAK	Y	MK301229	99.5 (100)	99.6 (100)	99.5 (100)	99.5 (100)	99.6 (100)	99.5 (100)	99.7 (100)	99.5 (100)	99.6 (100)
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)
<i>N. bakeri</i>	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)
<i>Sogatella furcifera</i>	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

7 **FIGURES LIST**

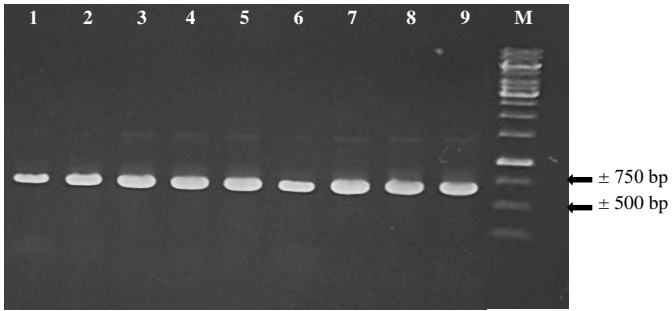


24 **Figure 1.** Symptoms of BPH attack on rice plants in Bali: A. rice plant growth is stunted; B. uneven plant growth (spots); C. yellow  
25 plant; D. dwarf rice plants; E. plants die like burning (hopperburn); F. BPH brachiptera and macroptera were found on rice stalks.

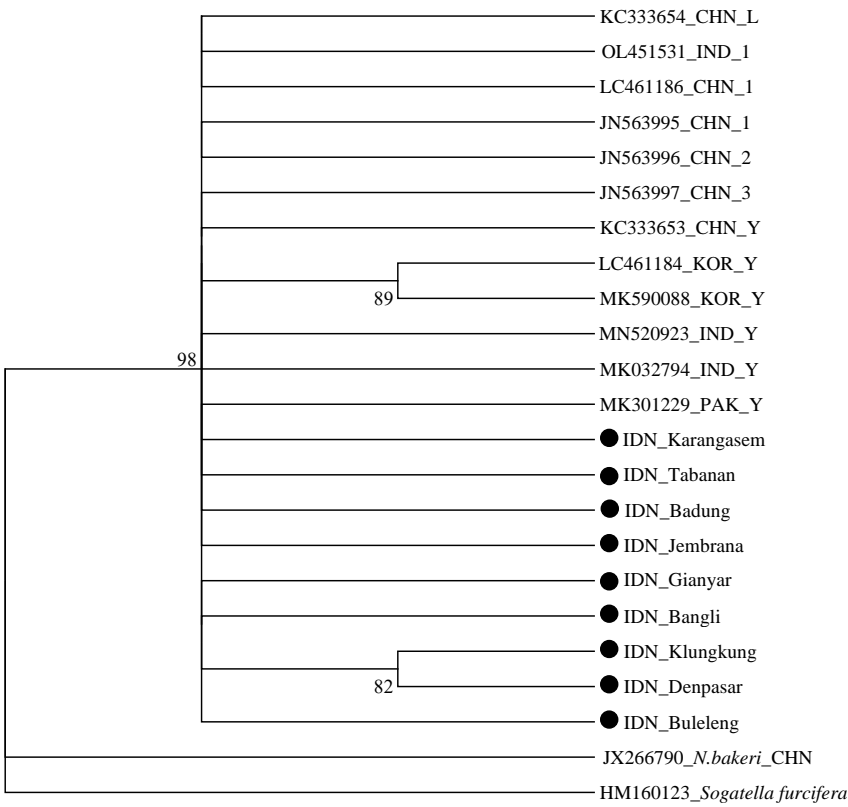


27 **Figure 2.** The attack intensity of *N. lugens* on rice in Bali Province

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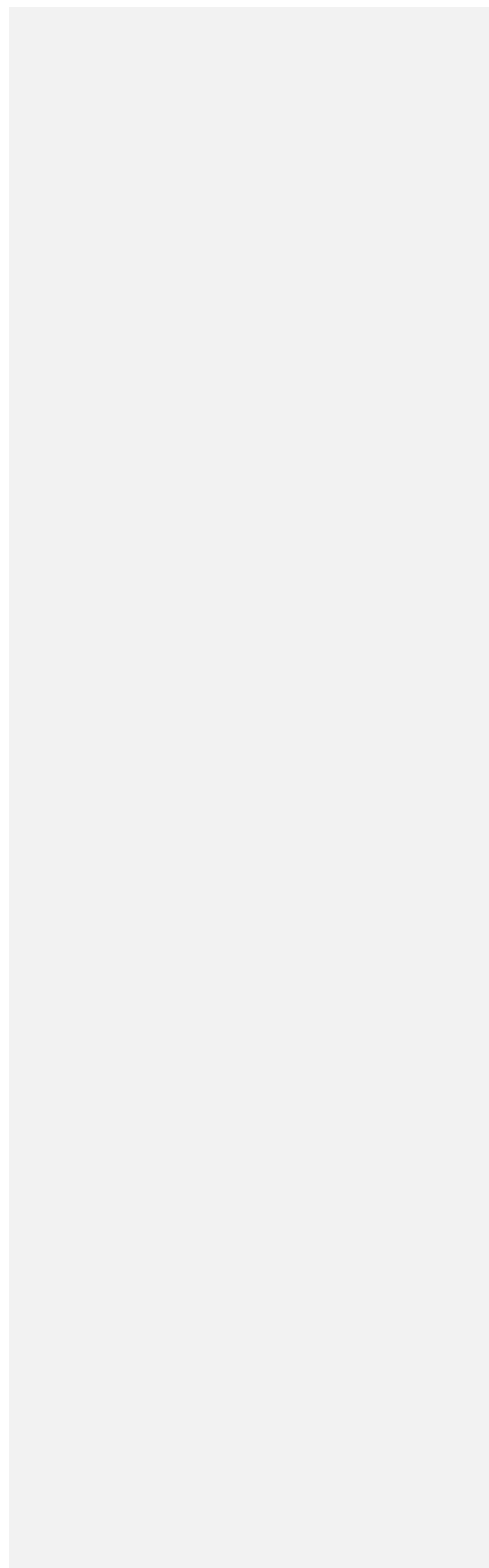


**Figure 3.** DNA amplification of *N. lugens* in rice plants in Bali using primers LCO 1490/HCO 2198. 1. Denpasar City, 2. Bagung, 3. Gianyar, 4. Tabanan, 5. Buleleng, 6. Karangasemt, 7. Klungkung, 8. Bangli, 9. Jembrana, and M. DNA marker 1 kb (Thermo Scientific)



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

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

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**Novelty:**

(state your claimed novelty of the findings versus current knowledge)

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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This manuscript has not been published and is not under consideration for publication to any other journal or any other type of publication (including web hosting) either by me or any of my co-authors.  
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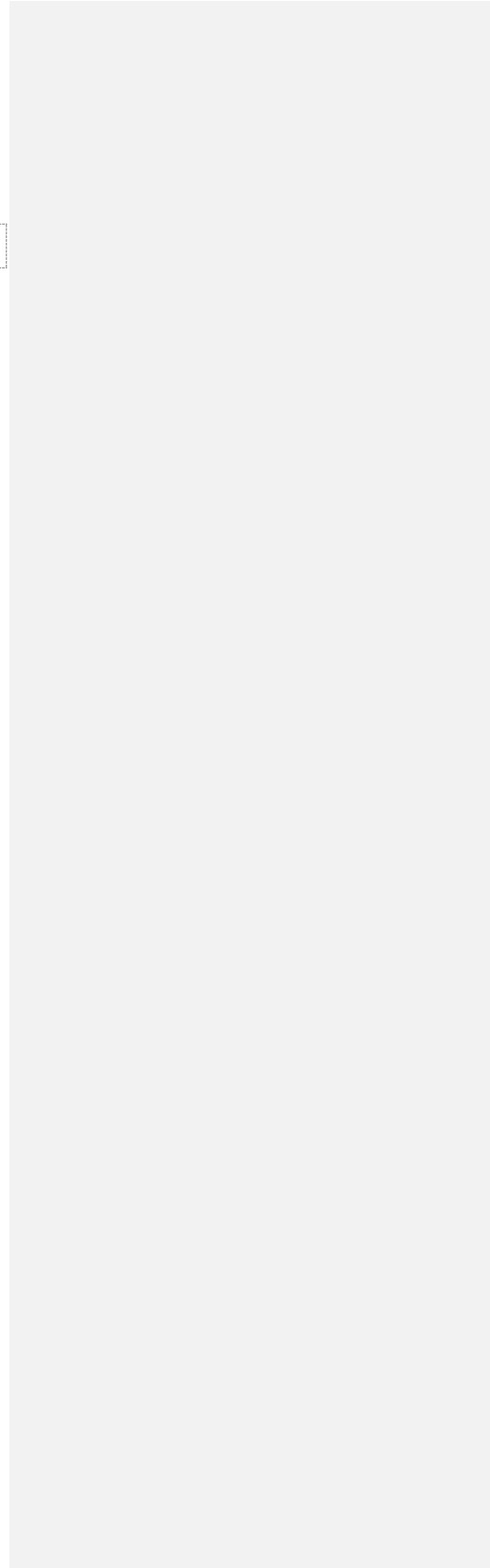
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Denpasar July 2022

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**Sincerely yours,**  
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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

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

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

81 Molecular techniques with high reproducibility and fast results offer an excellent alternative to traditional  
82 morphological classification. Several mitochondrial and nuclear genes have been used as genetic markers to differentiate  
83 related species. These include the mitochondrial cytochrome c oxidase subunit 1 (CO1) gene, nuclear 12S-16S-18S  
84 ribosomal RNA genes, and ITS1 and ITS2 internal transcription spacers (Fukunaga et al. 2000; Brengues et al. 2014;  
85 Gomez-Polo et al. 2014; Wang et al. 2016; Liu et al. 2018). ITS1 and ITS2 are nonfunctional spacers that separate the  
86 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  
87 variation but high variation between species, they are helpful for species classification and phylogenetic analysis for  
88 morphologically similar organisms, both in prokaryotes and eukaryotes (Liu et al. 2009). Finally, from the molecular  
89 identification of the combined mitochondrial COI-COII and ten microsatellite marker loci (Winnie et al. 2020).

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

## 95 MATERIALS AND METHODS

### 96 Brown Planthopper Sampling from Rice Dwarf Disease Endemic Areas

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

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

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

### 107 BPH Attack Percentage

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

$$109 P = \frac{a}{b} \times 100\%$$

110 Note:

111 P = Attack percentage (%)

112 a = Number of rice hills affected by BPH

113 b = Number of rice hills observed

### 115 Damage Intensity

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

$$118 I = \sum_{i=1}^i \frac{N_i \times V_i}{N \times Z} \times 100\%$$

122 Note:

123 I = Damage intensity

124 Ni = The number of affected rice hills on the score i

125 Vi = Score i

126 N = The number of rice hills observed

127 Z = Highest score

### 129 Total DNA Extraction from Brown Planthopper

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

132 with 100 µl of CTAB extraction buffer (2% CTAB, 1.4 M NaCl, 100 mM Tris-HCl, 20 mM EDTA, and 1% PVP (-40  
133 °C)). Next, 1 µl of proteinase K was added, then the insects were crushed using a micro-pistil, vortexed, and incubated in a  
134 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  
135 24:1. The tube was then vortexed for 3 minutes and centrifuged at 10,000 rpm for 15 minutes. The supernatant formed was  
136 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  
137 the supernatant. Isopropanol was added up to 2/3 of the total volume of the supernatant, then incubated at -20°C for one  
138 night. The tube was centrifuged at 10,000 rpm for 10 min, and the supernatant was discarded. The pellets were washed  
139 with 100 µl of 80% ethanol (cold) and centrifuged at 8000 rpm for 5 minutes. In the final step, the supernatant was  
140 removed, and the pellet was dried for approximately 1 hour. It was then added with a solution of 20 µl TE and stored at -  
141 20°C until used.

142

#### 143 **Amplification of mtCOI Fragments Using the PCR Method**

144 PCR reactants were manufactured with a total volume of 25 µl consisting of 12.5 µl Go Tag Green Master Mix  
145 (Promega, US) and 9.5 µl ddH<sub>2</sub>O. DNA amplification of the mtCOI fragment was carried out using a pair of universal  
146 primers mtCOI LCO 1490 (3'-GGTCAACAAATCATAAAGATATTGG-5') and HCO 2198 (5'TAAACTTCA  
147 GGGTGACCA AAAAATCA-3') (Folmer et al. 1994) each 1 µl, and 1 µl DNA template. PCR reactions were carried out  
148 with a Perkin Elmer 480 Thermocycler (Applied Biosystem, US). The PCR reaction was initiated by initial denaturation  
149 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,  
150 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%  
151 agarose gel. The DNA fragments of mtCOI were visualized using a UV transilluminator after being immersed in a 2%  
152 ethidium bromide solution for 15 minutes and photographed with a digital camera. The result of amplification by PCR  
153 technique was in the form of mtCOI DNA fragments with a size of ± 710 base pairs (pb).

154

#### 155 **Analysis of DNA Sequence Results**

156 Nucleotide Sequencing DNA fragment purification and mtCOI nucleotide sequencing were performed at PT. 1st  
157 Base, Malaysia. The results were then registered in the NCBI gene bank (<http://www.ncbi.nlm.nih.gov>). Analysis of  
158 mtCOI DNA sequence data ChromasPro program was used to combine forward and reverse nucleotide sequences to obtain  
159 the mtCOI gene (ChromasPro version 2.01, 2006). The Bioedit program was used to compare mtCOI fragments between  
160 samples (Multiple alignments) (Hall 1999). The phylogenetic relationship was built by comparing the mtCOI sample  
161 fragments from the brown planthopper from Indonesia with the mtCOI fragments already stored in the NCBI GenBank  
162 (<http://www.ncbi.nlm.nih.gov>). The criteria for retrieving mtCOI fragments at GenBank were fragments with a nucleotide  
163 base length of ± 800 bp (Boykin et al. 2007) (Table 1). The phylogenetic tree was constructed using the PAUP 4.0b10  
164 program (Swofford 2002) with the maximum parsimony cladistic quantitative method. The cladogram was compiled using  
165 the Heuristic method. The cladogram used results from the strict consensus with the statistical bootstrap test to obtain a  
166 100% probability.

167

## 167 **RESULTS AND DISCUSSION**

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

174 A percentage of BPH attacks of more than 50% was found in Gianyar, Bangli, Jembrana, and Badung Regencies  
175 (Table 2). The BPH population was primarily found in Ciherang and IR-64 varieties of rice in the Badung Regency, with  
176 43.67 BPH per rice hill (Table 2). Baehaki and Mejaya (2015) added that the economic threshold could be measured  
177 through the number or population of pests and planting age. BPH is said to have reached the economic threshold when the  
178 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  
179 18 BPH when the rice was more than 40 DAP (Baehaki and Mejaya 2015). In general, rice varieties grown in all  
180 observation locations in Bali were BPH susceptible varieties, such as Ciherang, IR-64, Inpari 32, and Situbagendit.

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

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

189 planthopper as a winged immigrant planthopper. Meanwhile, in Badung, Gianyar, Buleleng, Bangli, and Jembrana  
190 regencies, nymphal BPH was dominated by BPH, and several individuals were in the imago phase of brachiptera  
191 and macroptera. The dominance of the nymph phase caused the population of BPH in Badung, Gianyar, Buleleng, Bangli, and  
192 Jembrana districts to be the highest when compared to the cities of Denpasar, Tabanan, Karangasem, and Klungkung. The  
193 presence of the brachiptera planthopper might be contributed to the increase in the nymph population (Baehaki and Mejaya  
194 2015). According to Horgan et al. (2015), rapid population growth usually occurs in groups with many young individuals.

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

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

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

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

233 In previous studies in Indonesia, BPH biotypes 1, 2, 3, and 4 have been found. Chen et al. (2011) reported that the  
234 brown planthopper is a highly adaptive insect because it can form new biotypes. In early 1975 the IR-26 rice variety from  
235 IRRI Philippines was introduced. The IR-26 variety was unique because it contained a *Bph1* resistant gene to anticipate  
236 fluctuations in the brown planthopper population. However, in 1976 there was a great population explosion in several rice  
237 production centers due to changes in the brown planthopper population from biotype 1 to biotype 2. As an anticipatory  
238 measure against brown planthopper biotype 2, in 1980, the IR-42 rice variety (containing the *bph2* resistant gene) was  
239 introduced from IRRI Philippines. Unfortunately, in 1981 there was another explosion in the brown planthopper  
240 population in Simalungun, North Sumatra, and several other areas due to changes in the brown planthopper population  
241 from biotype 2 to biotype 3. To deal with the brown planthopper biotype 3, rice variety IR-56 was introduced (containing  
242 the gene *bph3* resistance) in 1983 and IR-64 (containing the *bph1+* resistance gene) in 1986. The introduction process  
243 continues. In 1991, the IR-74 variety (containing the *bph3* resistant gene) was introduced. In 2006, the resistance gene IR-  
244 64 was broken because the brown planthopper population changed to biotype 4. The stability of the biotype zero brown  
245 planthoppers persisted for 41 years before becoming brown planthopper biotype 1. The change of brown planthopper  
246 biotype 1 to biotype 2 only took 4 years, and the change of biotype 2 brown planthopper to biotype 3 within 5 years. Until  
247 2005, the brown planthopper biotype 3 was still dominated by biotype 3, and in 2006 the biotype 4 brown planthopper  
248 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.

## CONCLUSIONS

*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



1

2

**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) (%) <i>N. lugens</i> 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)
<i>N. bakeri</i>	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)
<i>Sogatella furcifera</i>	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)

3

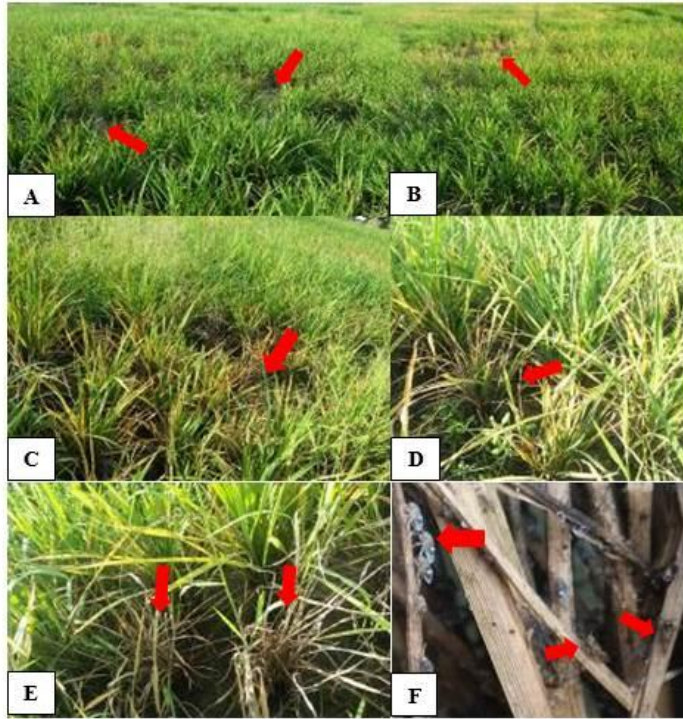
Notes: nt (nucleotide), aa (amino acid), IDN (Indonesia), PAK (Pakistan), IND (India), KOR (South Korea), CHN (China), *N. bakeri* and *Sogatella furcifera* from China was used as outgroups

4

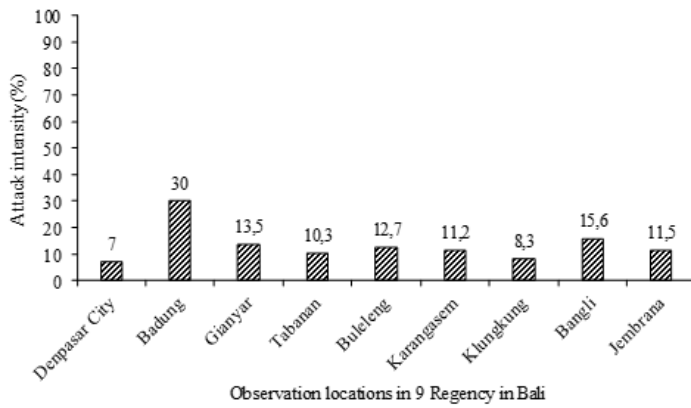
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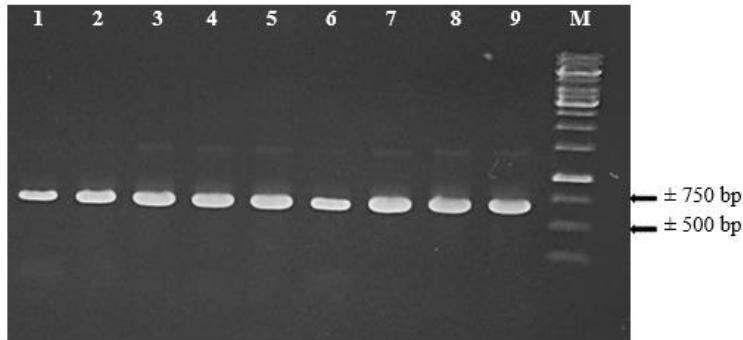
FIGURES LIST



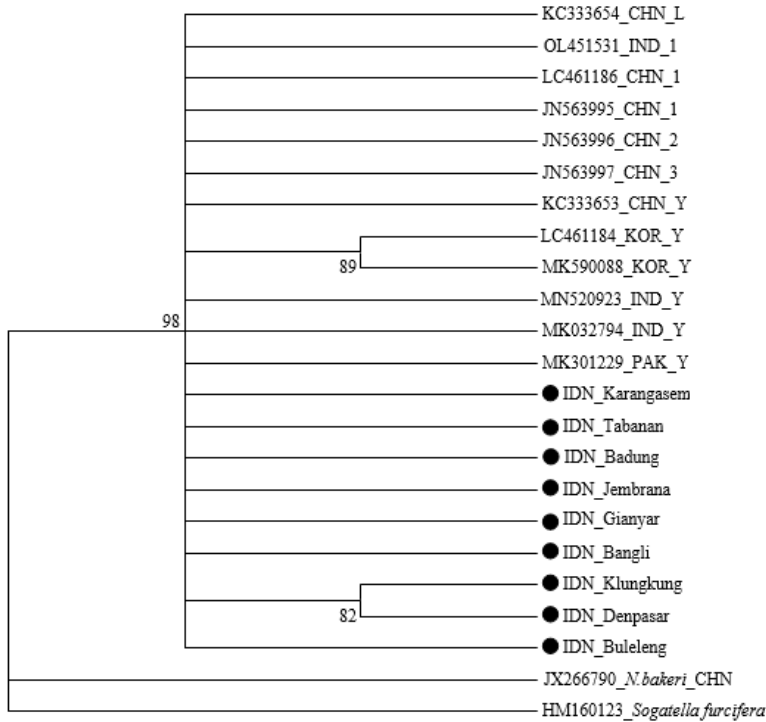
8  
9  
10 **Figure 1.** Symptoms of BPH attack on rice plants in Bali: A. rice plant growth is stunted; B. uneven plant growth (spots); C. yellow plant; D. dwarf rice plants; E. plants die like burning (hopperburn); F. BPH brachiptera and macroptera were found on rice stalks.



11  
12 **Figure 2.** The attack intensity of *N. lugens* on rice in Bali Province



15  
16 **Figure 3.** DNA amplification of *N. lugens* in rice plants in Bali using primers LCO 1490/HCO 2198. 1. Denpasar City, 2. Bagung, 3.  
17 Gianyar, 4. Tabanan, 5. Buleleng, 6. Karangasemt, 7. Klungkung, 8. Bangli, 9. Jembrana, and M. DNA marker 1 kb (Thermo Scientific)



18  
19 **Figure 4.** The cladogram of the mtCOI fragment of *N. lugens* from eastern Indonesia, Bali (Badung, Gianyar, Klungkung, Bangli,  
20 Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana) was compared with mtCOI fragments from several regions of the world  
21 that had been deposited on the NCBI website. *N. bakeri* and *Sogatella furcifera* from China were used as outgroups. The numbers on the  
22 branching cladograms represent bootstrap values with 100% probability. IDN (Indonesia), PAK (Pakistan), IND (India), KOR (South  
23 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 (Bregues 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

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

57 The genetic diversity of *N. lugens* has been reported in several countries such as China, South Korea, Pakistan, India,  
58 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  
59 Indonesia is widely reported in western Indonesia (Java Island) (Winnie et al. 2020; Chaerani et al. 2021). Reports on the  
60 genetic diversity of *N. lugens* in eastern Indonesia have not been found. Therefore, this study aims to analyze genetic  
61 diversity and determine the intensity of BPH attacks on rice plants in eastern Indonesia, especially Bali.

62

## MATERIALS AND METHODS

### 63 Brown Planthopper Sampling from Rice Dwarf Disease Endemic Areas

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

68

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

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

74

### 75 BPH Attack Percentage

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

$$77 \quad P = \frac{a}{b} \times 100\%$$

78 Note:

79 P = Attack percentage (%)

80 a = Number of rice hills affected by BPH

81 b = Number of rice hills observed

82

### 83 Damage Intensity

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

$$86 \quad I = \sum_{i=1}^i \frac{N_i \times V_i}{N \times Z} \times 100\%$$

87

88 Note:

89 I = Damage intensity

90 Ni = The number of affected rice hills on the score i

91 Vi = Score i

92 N = The number of rice hills observed

93 Z = Highest score

94

### 95 Total DNA Extraction from Brown Planthopper

96 Total DNA extraction of brown planthopper was obtained from one individual imago or one individual nymph based  
97 on the modified method of Goodwin et al. (1994). One individual imago was put into a microtube and then added to 100 µl  
98 of CTAB (Cethyl Trimethyl Ammonium Bromida) extraction buffer (2% CTAB, 1.4 M NaCl, 100 mM Tris-HCl, 20 mM  
99 EDTA (Ethylendiaminetetraacetic acid), and 1% PVP (-40 °C)). Next, 1 µl of proteinase K was added, then the insects  
100 were crushed using a micro-pistil, vortexed, and incubated in a water bath of 65°C for 3 minutes. After that, the tube was  
101 added with 100 µl CI (chloroform: isoamyl alcohol) in a ratio of 24:1. The tube was then vortexed for 3 minutes and  
102 centrifuged at 10,000 rpm for 15 minutes. The supernatant formed was transferred to a new microtube (60 µl) and then  
103 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  
104 of the total volume of the supernatant, then incubated at -20°C for one night. The tube was centrifuged at 10,000 rpm  
105 for 10 min, and the supernatant was discarded. The pellets were washed with 100 µl of 80% ethanol (cold) and centrifuged at

106 8000 rpm for 5 minutes. In the final step, the supernatant was removed, and the pellet was dried for approximately 1 hour.  
107 **It was then added to a solution** of 20 µl TE and stored at -20°C until used.  
108

#### 109 **Amplification of mtCOI Fragments Using the PCR Method**

110 PCR reactants were manufactured with a total volume of 25 µl consisting of 12.5 µl Go Tag Green Master Mix  
111 (Promega, US) and 9.5 µl ddH<sub>2</sub>O. DNA amplification of the mtCOI fragment was carried out using a pair of universal  
112 primers mtCOI LCO 1490 (3'-GGTCAACAAATCATAAAGATATTGG-5') and HCO 2198 (5'TAAACTTCA  
113 GGGTGACCA AAAAATCA-3') (Folmer et al. 1994) each 1 µl, and 1 µl DNA template. PCR reactions were carried out  
114 with a Perkin Elmer 480 Thermocycler (Applied Biosystem, US). The PCR reaction was initiated by initial denaturation  
115 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,  
116 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%  
117 agarose gel. The DNA fragments of mtCOI were visualized using a UV transilluminator after being immersed in a 2%  
118 ethidium bromide solution for 15 minutes and photographed with a digital camera. The result of amplification by PCR  
119 technique was in the form of mtCOI DNA fragments with a size of ± 710 base pairs (pb).  
120

#### 121 **Analysis of DNA Sequence Results**

122 Nucleotide Sequencing DNA fragment purification and mtCOI nucleotide sequencing were performed at PT. 1st Base,  
123 Malaysia. The results were then registered in the NCBI gene bank (<http://www.ncbi.nlm.nih.gov>). Analysis of mtCOI  
124 DNA sequence data ChromasPro program was used to combine forward and reverse nucleotide sequences to obtain the  
125 mtCOI gene (ChromasPro version 2.01. 2006). The Bioedit program was used to compare mtCOI fragments between  
126 samples (Multiple alignments). The phylogenetic relationship was built by comparing the mtCOI sample fragments from  
127 the brown planthopper from Indonesia with the mtCOI fragments already stored in the NCBI GenBank  
128 (<http://www.ncbi.nlm.nih.gov>). The criteria for retrieving mtCOI fragments at GenBank were fragments with a nucleotide  
129 base length of ± 710 bp (Boykin et al. 2007) (Table 3; Figure 3). The phylogenetic tree was constructed using the PAUP  
130 4.0b10 program with the maximum parsimony cladistic quantitative method. The cladogram was compiled using the  
131 Heuristic method. The cladogram used results from the strict consensus with the statistical bootstrap test to obtain a 100%  
132 probability.

133

## **RESULTS AND DISCUSSION**

134 The brown planthopper causes direct and indirect damage to rice plants. Direct damage was in the form of stunted and  
135 uneven growth of rice plants (Figure 1A and 1B), yellow plants (Figure 1C), and hopperburn caused by fluid in rice plant  
136 cells sucked by BPH nymphs, brachiptera (Fig. short wings), and macroptera (long wings) (Figures 1E and 1F). Indirect  
137 damage was caused by BPH, which acts as a vector of *Rice grassy stunt virus* and *Rice ragged stunt virus*, causing stunted  
138 rice plants (Figure 1D). Besides Bali or other parts of Indonesia, BPH attacks on rice crops were also reported in China,  
139 where hopperburn affected 60% of all examined crops (Hu et al. 2014). Transmission of the stunt virus by the brown  
140 planthoppers occurs persistently (Horgan et al. 2015). Virus infection causes damage to plants because viruses use plant  
141 proteins for replication, resulting in loss of crop production (Listihani et al. 2020; Damayanti et al. 2022; Listihani et al.  
142 2022; Pandawani et al. 2022; Selangga and Listihani 2022; Selangga et al. 2022). Therefore, infection with RGSV and  
143 RRSV in rice plants causes rice to lack nutrients to the point of stunting.

144 A percentage of BPH attacks of more than 50% was found in Gianyar, Bangli, Jembrana, and Badung Regencies  
145 (Table 2). The BPH population was primarily found in Ciherang and IR-64 varieties of rice in the Badung Regency, with  
146 43.67 BPH per rice hill (Table 2). Baehaki (2012) added that the economic threshold could be measured through the  
147 number or population of pests and planting age. BPH is said to have reached the economic threshold when the population  
148 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  
149 when the rice was more than 40 DAP (Baehaki 2012). In general, rice varieties grown in all observation locations in Bali  
150 were BPH susceptible varieties, such as Ciherang, IR-64, Inpari 32, and Situbagendit.

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

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

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

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

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

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

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

204 In previous studies in Indonesia, BPH biotypes 1, 2, 3, and 4 have been found. Kobayashi et al. (2014) reported that the  
205 brown planthopper is a highly adaptive insect because it can form new biotypes. In early 1975 the IR-26 rice variety from  
206 IRRI Philippines was introduced. The IR-26 variety was unique because it contained a Bph1 resistant gene to anticipate  
207 fluctuations in the brown planthopper population. However, in 1976 there was a great population explosion in several rice  
208 production centers due to changes in the brown planthopper population from biotype 1 to biotype 2. As an anticipatory  
209 measure against brown planthopper biotype 2, in 1980, the IR-42 rice variety (containing the bph2 resistant gene) was  
210 introduced from IRRI Philippines. Unfortunately, in 1981 there was another explosion in the brown planthopper  
211 population in Simalungun, North Sumatra, and several other areas due to changes in the brown planthopper population  
212 from biotype 2 to biotype 3. To deal with the brown planthopper biotype 3, rice variety IR-56 was introduced (containing  
213 the gene bph3 resistance) in 1983 and IR-64 (containing the bph1+ resistance gene) in 1986. The introduction process  
214 continues. In 1991, the IR-74 variety (containing the bph3 resistant gene) was introduced. In 2006, the resistance gene IR-  
215 64 was broken because the brown planthopper population changed to biotype 4 (Baehaki 2012). The stability of the  
216 biotype zero brown planthoppers persisted for 41 years before becoming brown planthopper biotype 1. The change of  
217 brown planthopper biotype 1 to biotype 2 only took 4 years, and the change of biotype 2 brown planthopper to biotype 3  
218 within 5 years. Until 2005, the brown planthopper biotype 3 was still dominated by biotype 3, and in 2006 the biotype 4  
219 brown planthopper began to develop. The long existence of the biotype 3 brown planthopper was caused by the  
220 development of the IR-64 (bph1+) variety over a long period. IR-64 is a resistant variety (durable resistance) that can  
221 withstand changes in brown planthoppers to a more virulent biotype.



222 The continuous cultivation of IR-64 rice varieties by farmers in Bali led to the emergence of a new biotype BPH,  
223 namely Y. Insects of biotype Y originated from biotype 1 by eating YHY15 resistant varieties for more than two years for  
224 33 generations (Jing et al. 2012). Rice varieties YHY15 carry the Bph15 resistance gene (Jing et al. 2012).

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

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

1  
2**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) (%) <i>N. lugens</i> 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)
<i>N. bakeri</i>	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)
<i>Sogatella furcifera</i>	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)

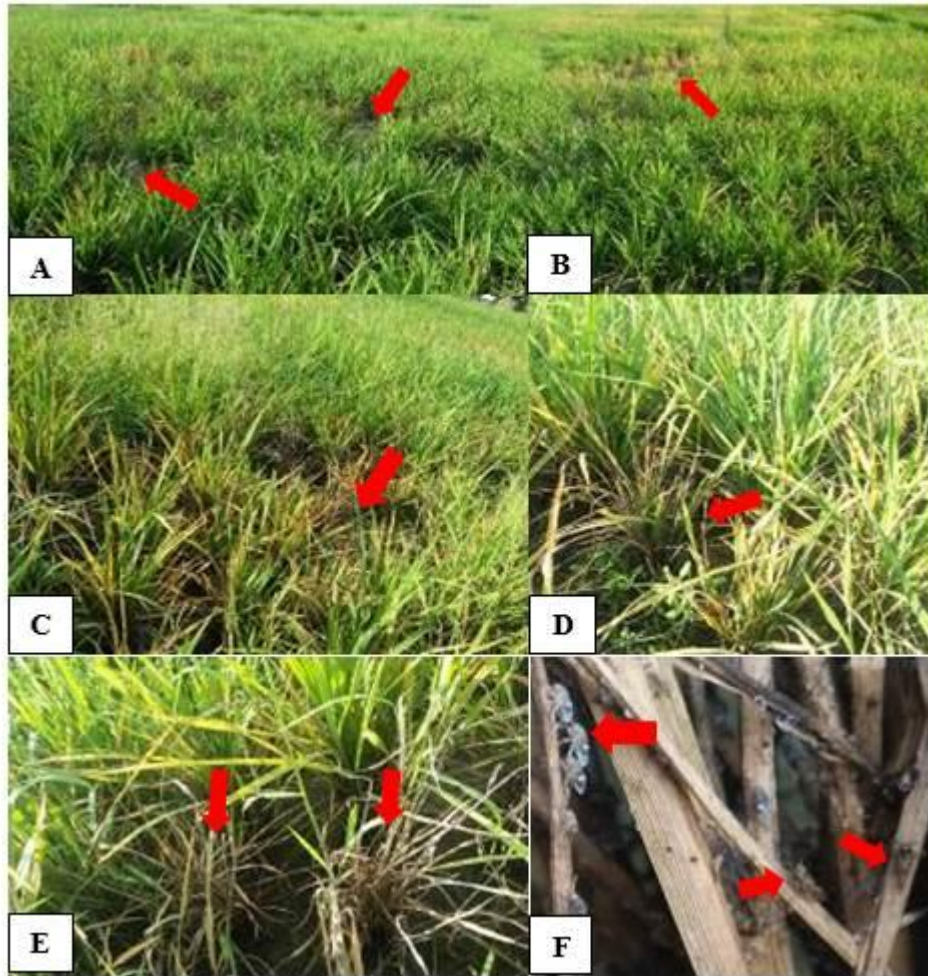
3 Notes: nt (nucleotide), aa (amino acid), IDN (Indonesia), PAK (Pakistan), IND (India), KOR (South Korea), CHN (China), *N. bakeri* and *Sogatella furcifera* from China was used as outgroups

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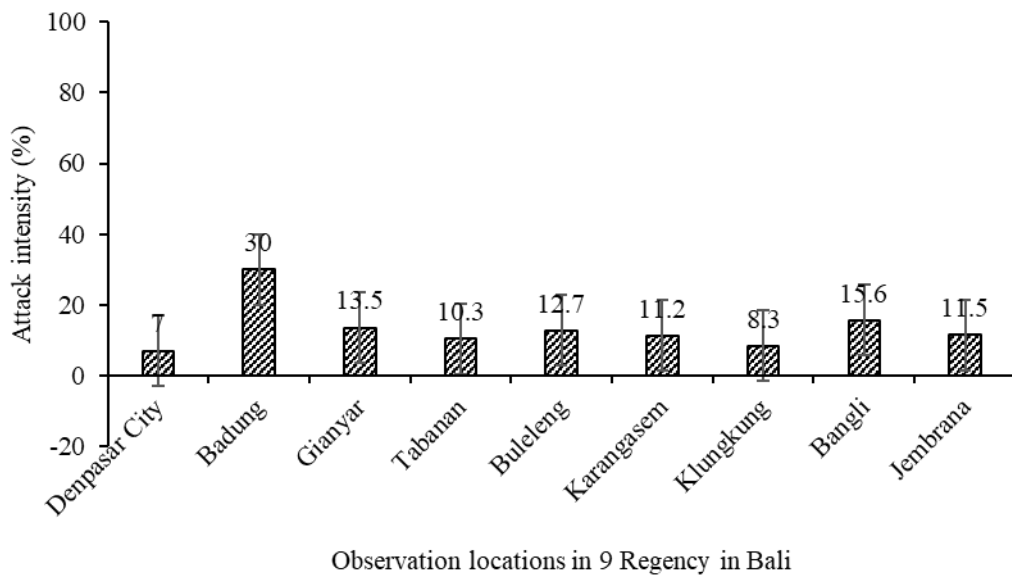
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FIGURES LIST



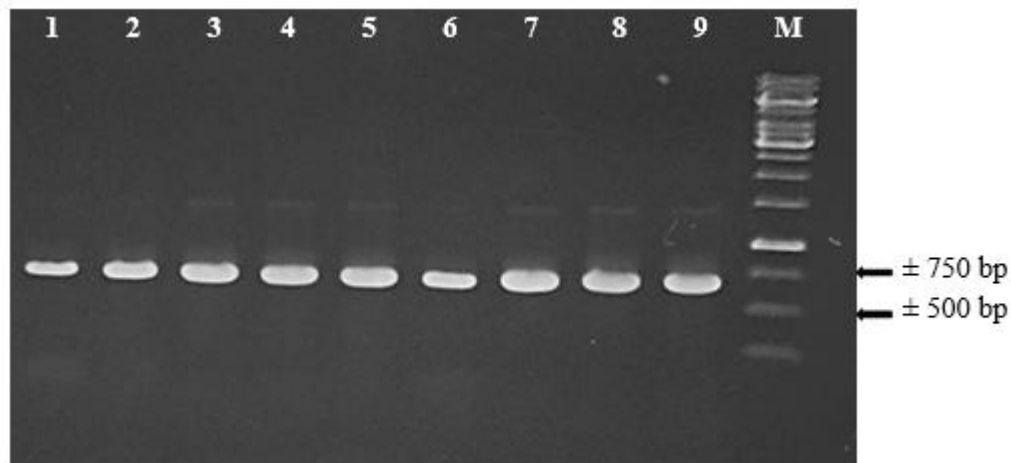
**Figure 1.** Symptoms of BPH attack on rice plants in Bali: A. rice plant growth is stunted; B. uneven plant growth (spots); C. yellow plant; D. dwarf rice plants; E. plants die like burning (hopperburn); F. BPH brachiptera and macroptera were found on rice stalks.

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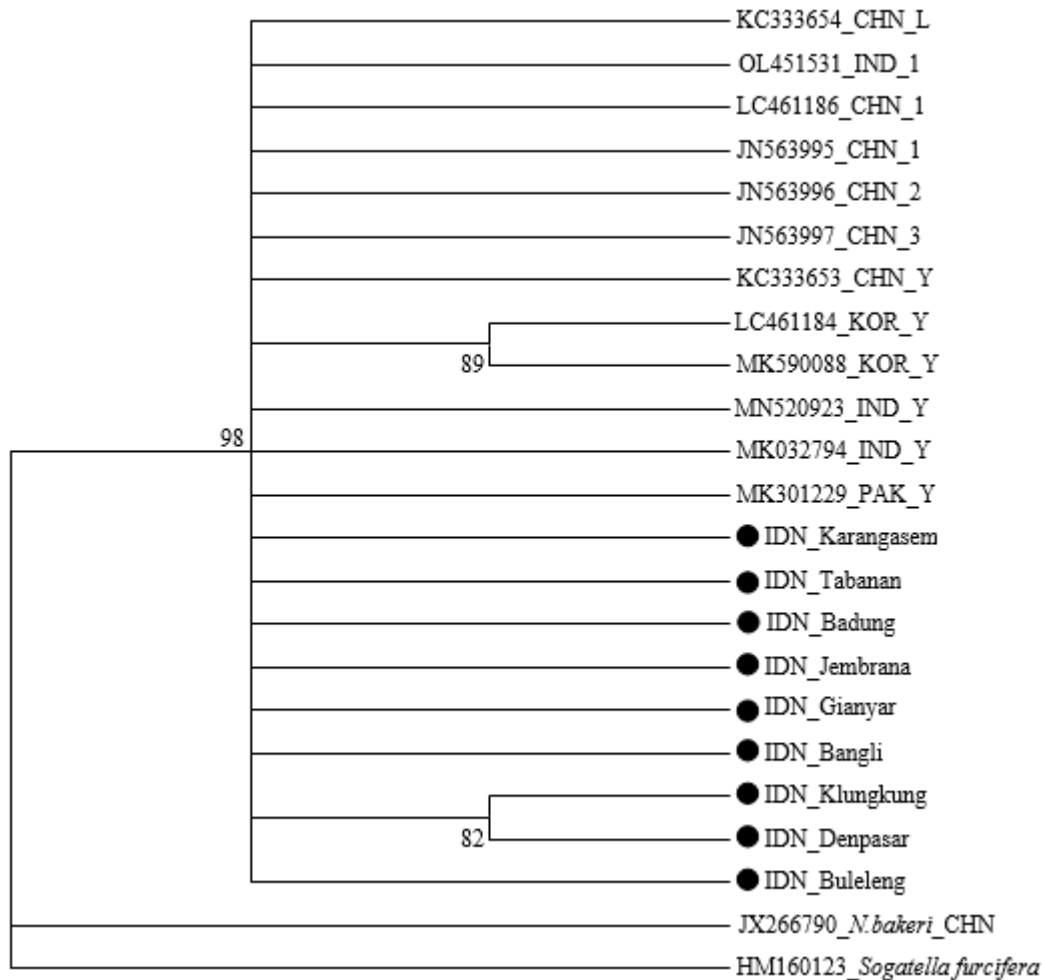


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**Figure 2.** The attack intensity of *N. lugens* on rice in Bali Province

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**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)

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**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.

# 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 (Bregues 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 -20°C.

### 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:

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

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):

$$I = \sum_{i=1}^i \frac{N_i \times V_i}{N \times Z} \times 100\%$$

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-HCl, 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 <i>Colletotrichum dematium</i> 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 <i>Colletotrichum dematium</i> 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 <i>Colletotrichum dematium</i> 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  $\mu$ L 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  $\mu$ L) 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  $\mu$ L 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  $\mu$ L 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  $\mu$ L consisting of 12.5  $\mu$ L Go Tag Green Master Mix (Promega, US) and 9.5  $\mu$ L ddH<sub>2</sub>O. 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'-TAAACTTCAGGGTGACCAAAAATCA-3') (Folmer et al. 1994) each 1  $\mu$ L, and 1  $\mu$ L 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  $\pm$ 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  $\pm$ 710 bp (Boykin et al. 2007) (Table 3, Figure 3). The phylogenetic tree was constructed using the PAUP 4.0b10 program with

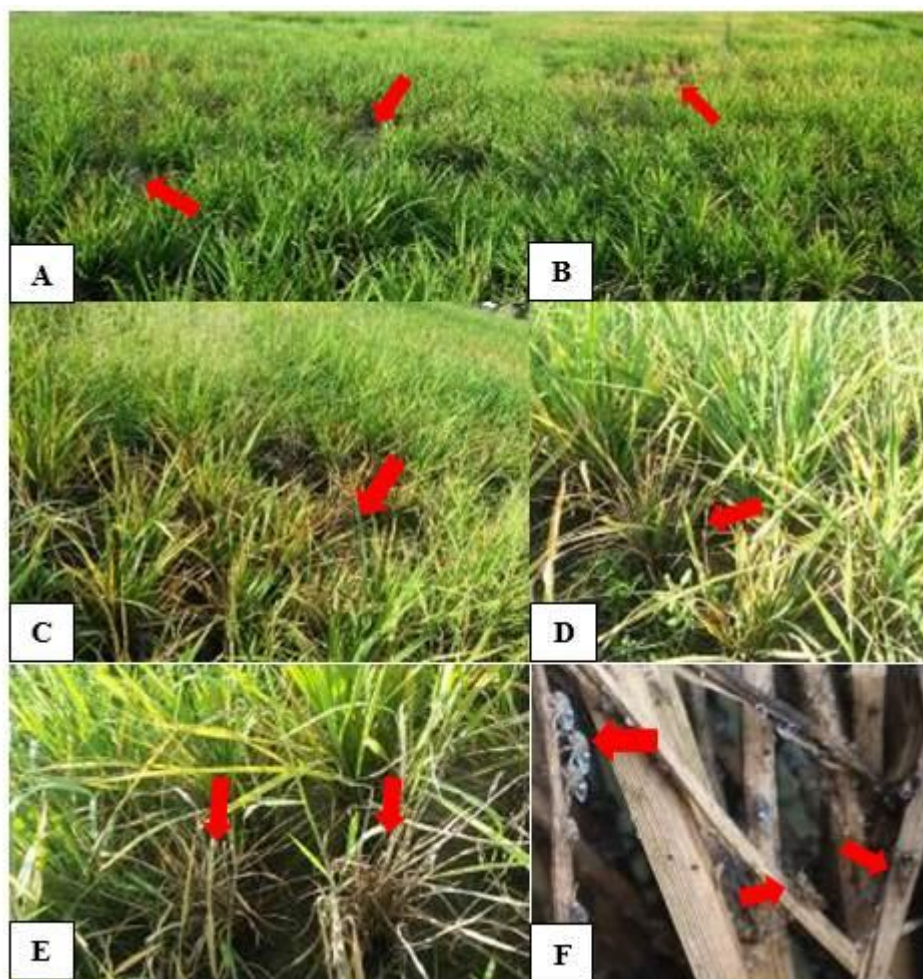
the maximum parsimony cladistic quantitative method. The cladogram was compiled using the Heuristic method. The cladogram used results from the strict consensus with the statistical bootstrap test to obtain a 100% probability.

## **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 brachyptera 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).

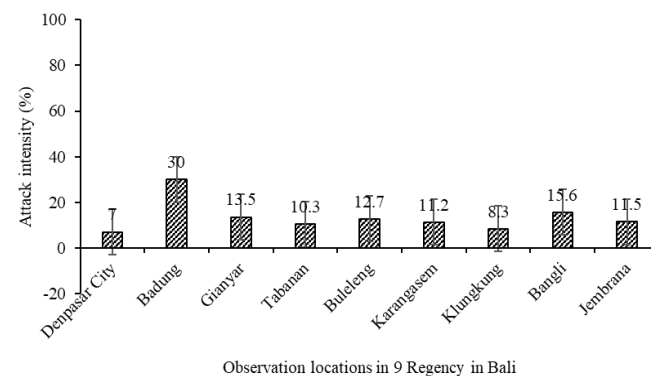
The average intensity of BPH attack on Ciherang and IR-64 varieties of rice was higher than in other varieties. In the Ciherang and IR-64 varieties of rice, the average value of the highest attack intensity was 30% (Figure 2). It is because farmers grow rice varieties Ciherang and IR-64 from year to 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).

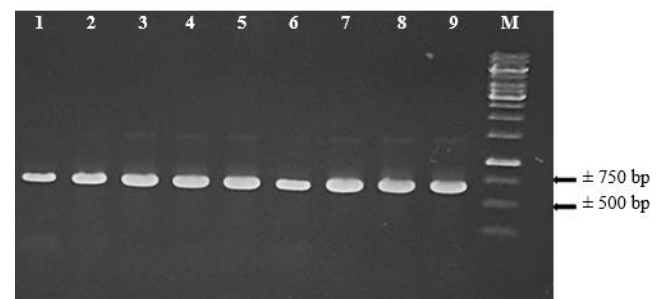
The mtCOI DNA band was successfully amplified from the total DNA extraction of one imago or nymph of BPH. The mtCOI fragment that was successfully amplified corresponds to a size of  $\pm 710$  bp in all samples from nine districts in Bali, namely Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, 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

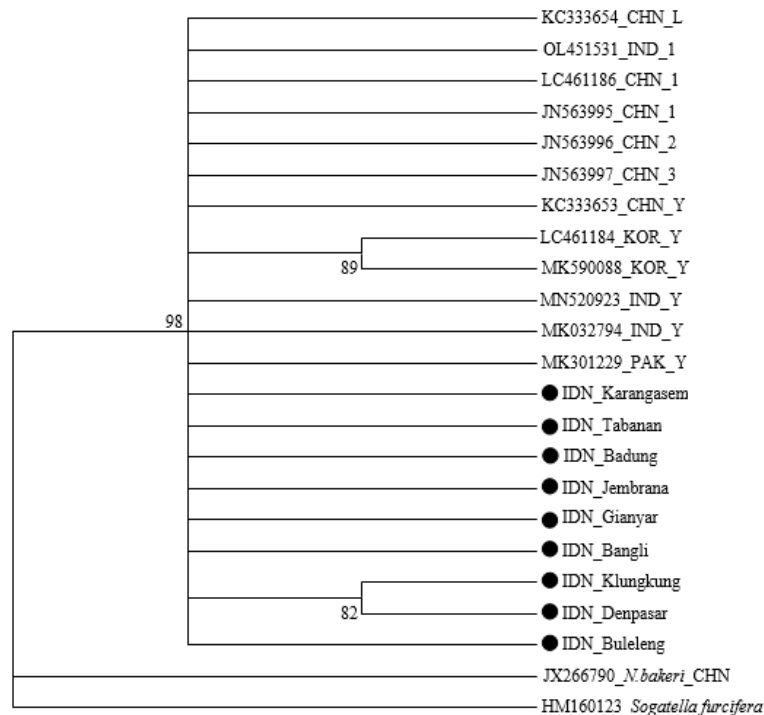


**Figure 3.** DNA amplification of *Nilaparvata lugens* in rice plants in Bali using primers LCO 1490/HCO 2198. 1. Denpasar City; 2. Bagung; 3. Gianyar; 4. Tabanan; 5. Buleleng; 6. Karangasem; 7. Klungkung; 8. Bangli; 9. Jembrana and M. DNA marker 1 kb (Thermo Scientific)

**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) (%) <i>Nilaparvata lugens</i> 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)
<i>Nilaparvata bakeri</i>	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)
<i>Sogatella furcifera</i>	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 (Bregues 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 -20°C.

### 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:

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

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):

$$I = \sum_{i=1}^i \frac{N_i \times V_i}{N \times Z} \times 100\%$$

Note:

I = Damage intensity

N<sub>i</sub> = The number of affected rice hills on the score i

V<sub>i</sub> = 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-HCl, 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-pistol, 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<sub>2</sub>O. DNA amplification of the mtCOI fragment was carried out using a pair of universal primers mtCOI LCO 1490 (3'-GGTCAACAATCATAAAGATATTGG-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  $\pm 710$  base pairs (pbp).

#### 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  $\pm 710$  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, 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~~, nymphs ~~and~~ 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  $\pm 710$  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 <i>Colletotrichum dematium</i> 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 <i>Colletotrichum dematium</i> 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 <i>Colletotrichum dematium</i> 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

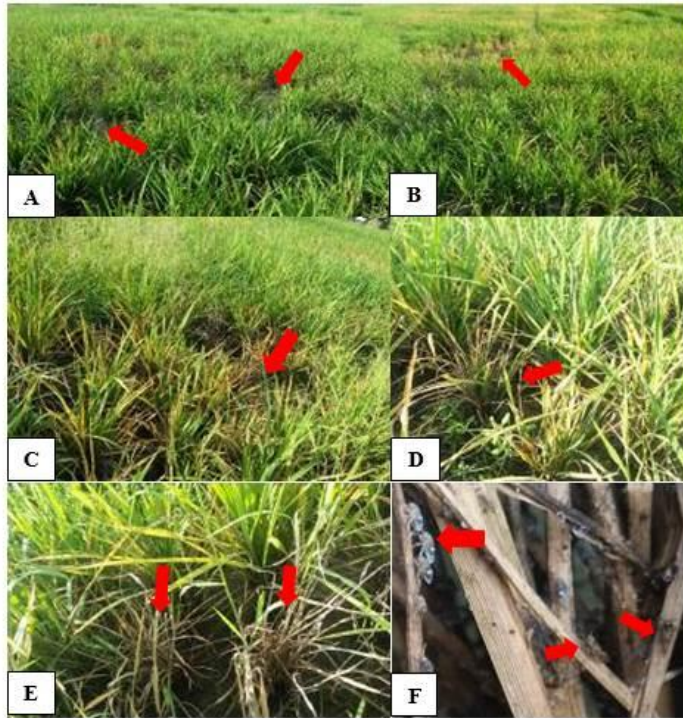
Note: DAP= day after planting

**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

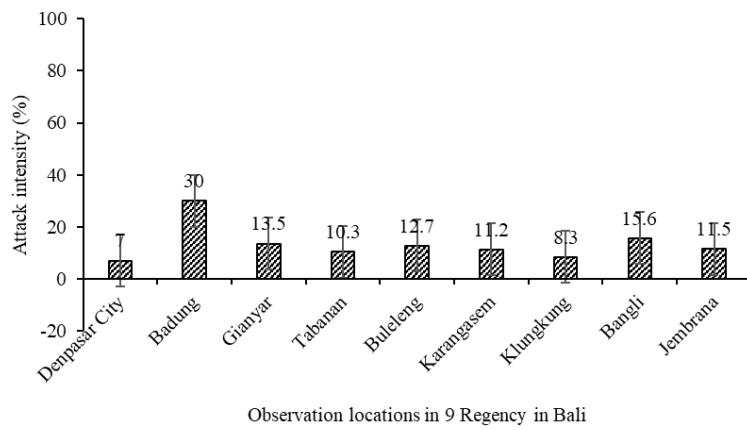
Isolate	Origin of isolate	Biotype	Accession number	Homology nt (aa) (%) <i>N. lugens</i> IDN								
				Denpasar	Badung	Gianyar	Tabanan	Buleleng	Karangasem	Klungkung	Bangli	Jembrana
FSD-034	PAK	Y	MK301229	99.5 (100)	99.6 (100)	99.5 (100)	99.5 (100)	99.6 (100)	99.5 (100)	99.7 (100)	99.5 (100)	99.6 (100)
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)
<i>N. bakeri</i>	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)
<i>Sogatella furcifera</i>	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

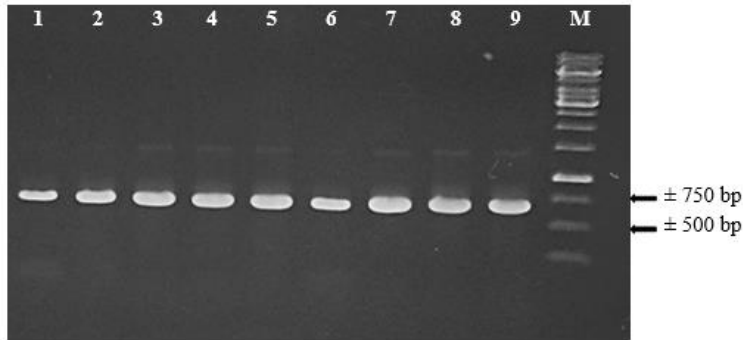




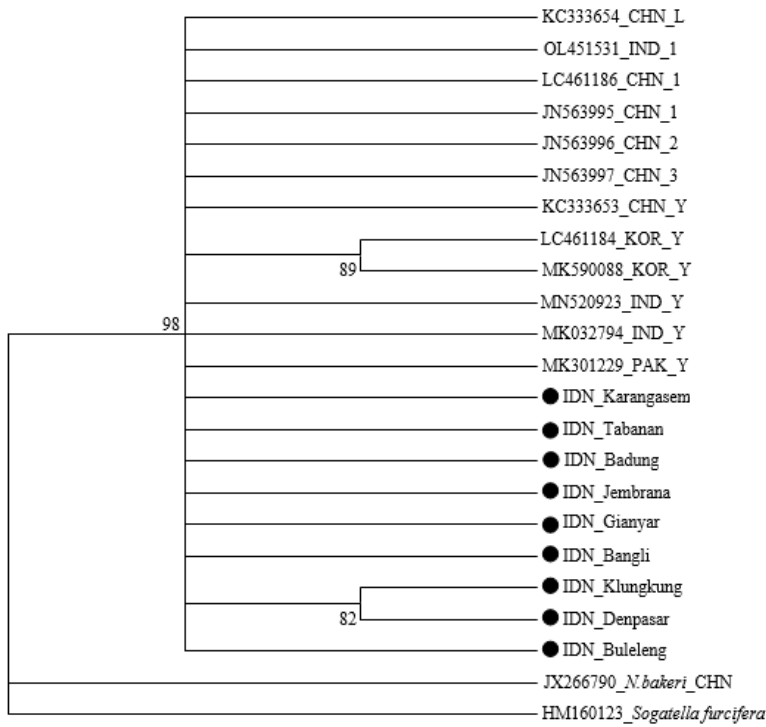
**Figure 1.** Symptoms of BPH attack on rice plants in Bali: A. rice plant growth is stunted; B. uneven plant growth (spots); C. yellow plant; D. dwarf rice plants; E. plants die like burning (hopperburn); F. BPH brachiptera and macroptera were found on rice stalks



**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.

