

1 **MOLECULAR CHARACTERIZATION OF *PHYTOPHTHORA PALMIVORA* RESPONSIBLE FOR**
2 **BUD ROT DISEASE OF OIL PALM IN COLOMBIA**

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11
12 **Abstract**

13
14 Bud rot disease is a damaging disease of oil palm in Colombia. The pathogen responsible for this disease is a
15 species of oomycetes, *Phytophthora palmivora* which is also the causal pathogen of several tropical crop
16 diseases such as fruit rot and stem canker of cocoa, rubber, durian and jackfruit. No outbreaks of bud rot have
17 been reported in oil palm in Malaysia or other Southeast Asian countries, despite this particular species being
18 present in the region. Analysis of the genomic sequences of several genetic markers; the internal transcribe
19 spacer regions (ITS) of the ribosomal RNA gene cluster, *beta-tubulin* gene, *translation elongation factor 1*
20 *alpha* gene (EF-1 α), *cytochrome c oxidase subunit I & II* (COXI and COXII) gene cluster along with amplified
21 fragment length polymorphism (AFLP) analyses have been carried out to investigate the genetic diversity and
22 variation of *P. palmivora* isolates from around the world and from different hosts in comparison to Colombian
23 oil palm isolates, as one of the steps in understanding why this species of oomycetes causes devastating damage
24 to oil palm in Latin America but not in other regions. Phylogenetic analyses of these regions showed that the
25 Colombian oil palm isolates were not separated from Malaysian isolates. AFLP analysis and a new marker
26 PPHPAV, targeting an unclassified hypothetical protein, was found to be able to differentiate Malaysian and
27 Colombian isolates and showed a clear clade separations. Despite this, pathogenicity studies did not show any
28 significant differences in the level of aggressiveness of different isolates against oil palm in glasshouse tests.

29 **Keywords:** bud rot disease, oil palm, oomycetes, *Phytophthora palmivora*,

30

31 **INTRODUCTION**

32 *Phytophthora palmivora* belongs to the genus *Phytophthora*, another member of which was responsible for the
33 potato famine in the middle of 19th century (Cooke and Anderson 2013) and is placed in the phylum of
34 oomycota (Pseudofungi), class of oomycetes and a member of the Pythiaceae family (Hawksworth et al. 1995).
35 Most species in the *Phytophthora* genus are plant pathogens responsible for some of the world's most
36 destructive diseases of crops and native vegetation (Brasier 1992; Ho 2018).

37 *Phytophthora palmivora* is one of the important *Phytophthora* species in tropical and sub-tropical
38 regions. This species attacks a wide range of plants and can cause diseases in different parts of the plant. It is
39 responsible for various diseases in many tropical perennial crops such as black pod and stem canker of cocoa
40 (*Theobroma cacao*) (Auwah and Frimpong 2002; Turner 1960) and jackfruit (*Artocarpus heterophyllus*) (Tri
41 et al. 2015), patch canker, black stripe, green pod rot of rubber (*Hevea brasiliensis*) (Chee 1975; Sdoodee
42 2004), and trunk canker, root rot, fruit rot of durian (*Durio zibethinus*) (Lim and Chan 1986; Pongpisutta and
43 Sangchote 2004) and canker, fruit and root rot in citrus (*Citrus* spp.) (Ahmed et al. 2013; Tashiro et al. 2012).
44 The pathogen is also responsible for various other diseases of cassava (*Manihot esculenta* Crantz) (Sankar et
45 al. 2013), macadamia (*Macadamia integrifolia*) (Aragaki and Uchida 1980), papaya (*Carica papaya*)
46 (Zentmyer 1988), pineapple (*Ananas comosus*), betel vine (*Piper betle*) (Maiti and Sen 1979; Turner 1969b),
47 olive (*Olea* spp.) (Chliyeh et al. 2013), orchid (Uchida and Aragaki 1991), cherry (*Prunus avium* L.)
48 (Türkölmez et al. 2015) and several palms species including coconut (Blaha et al. 1994; Ordoñez et al. 2016)
49 and ornamental palm *Washingtonia robusta* (Elliott 2006; Garofalo and McMillan 1999). The pathogen is
50 responsible for the bud rot and premature nutfall diseases of the coconut (*Cocos nucifera*) in the Philippines
51 (Concibido-Manohar 2004), Indonesia (Smith and Flood 2001) and India (Sharadraj and Mohanan 2014).

52 Recently, *P. palmivora* has also been found to be responsible for a devastating disease of oil palm in
53 Colombia and other oil palm producing countries in South America also known as bud rot disease (Torres et
54 al. 2016). Bud rot disease has been a serious problem in oil palm plantations in South America for more than
55 50 years with severe outbreaks in Colombia, Brazil, Ecuador, Panama and Surinam and some cases in Costa
56 Rica, Nicaragua, Honduras, Peru and Venezuela (Martínez et al. 2010). The disease is also known by various

57 other names such as as '*podricion del cogollo*' (PC) in Spanish speaking countries including Colombia, and
58 '*speerrot*' or '*lethal spear rot*' in Surinam. In Costa Rica, it is known as '*flecha seca*' (Henry et al. 2015). The
59 description of spear rot disease in Surinam by Van de Lande and Zadoks (1999) matched the description in
60 Martinez (2009) and Martínez et al. (2009). Kastelein et al. (1990) and Beuther et al. (1992) described oil palm
61 disease with similar symptoms such as fatal yellowing or '*Amarelecimento Fatal*' (AF) in Brazil. Nevertheless,
62 Boari (2008) believed that fatal yellowing (AF) in Brazil is different to bud rot disease of Colombia. Boari et
63 al. (2012) have listed several studies conducted on AF to understand the epidemiology of the disease but
64 whether these diseases are actually the same as bud rot is still under debate.

65 Symptoms of bud rot disease in oil palm are similar to the bud rot disease of coconut with the initial
66 symptom being chlorosis of the young unopened frond or spear leaf (Darus 2000; De Franqueville 2003;
67 Kastelein et al. 1990; Navia et al. 2014). The infected tissue becomes desiccated and dies, leaving necrotic
68 patches. Browning of internal tissues can be observed when the unopened spear is unfolded. If the infection
69 stops, the small desiccated, necrotic patches damage the frond formation creating various external symptoms
70 such as the '*shark bite*' look on the frond, desiccated and necrotic leaflets and loss of some part of the fronds
71 depending on the level of infection during the initial stages. If the infection continues, the whole spear leaf
72 might become infected, necrotic and turn brown, and then the infection continues to the other fronds in the
73 centre of the palm crown. In the advanced stage, the fronds snap, followed by collapse of the upper crown, but
74 the mature leaves (lower crown) remain green for several months, because they are not affected by the
75 pathogen, although the palms cease production. Usually at this stage, the basal tissue rots, as indicated by the
76 presence of dark brown tissue internally. The palm can recover if the infections have not yet gone too deep into
77 the apical meristem and the rotting stops as indicated by production of new leaves. The first new frond is
78 usually smaller, shorter, more erect and slightly more chlorotic than normal fronds and the growth is slower
79 causing the stunted appearance of the new crown. It has been suggested that bud rot disease should be classified
80 into two forms, and the form when the palm can recover is a non-lethal form. The bud rot found in the eastern
81 region of Colombia (Llanos) is believed to be the non-lethal form, whilst in the southwestern region it is the
82 lethal form. The lethal form is aggressive and can cause total destruction and palm death. Turner (1981)
83 suggested that the non-lethal form of bud rot should be called '*bud rot little leaf*', due to the formation of the
84 malformed fronds during recovery, and the lethal form as '*lethal bud rot*'. In the lethal form, the infection and

85 rotting does not stop and advances to the heart of the palm (*cogollo*) and eventually affects the leaf primordia
86 and apical meristem. If the apical meristem, which is the growing point of the palm, is destroyed, the palm will
87 not produce leaves and fruits, and eventually die. It is not known why there are lethal and non-lethal forms of
88 bud rot; current hypotheses are that it may be because of pathogenicity factors, physiology of the palm or other
89 biotic and abiotic reasons.

90 In Malaysia and South East Asia, the current status of bud rot disease incidence in oil palm plantations
91 is unclear. According to Albertazzi-Leandro et al. (2005) as cited by Turner (1981), symptoms similar to
92 ‘*pudrición del cogollo*’ are not new in Asia. Sharples (1928) and Bunting et al. (1934) have described the
93 disease based on observations of collapse of unopened spear leaves which might be the non-lethal form of bud
94 rot disease of oil palm. Until now, no reports of the lethal form and outbreaks of the disease have been reported
95 in Malaysia or other Southeast Asian countries despite the fact that *P. palmivora* is a common pathogen to this
96 region on other plant species. It is not known why this species causes devastating damage to oil palm in
97 Colombia and other Latin American countries. One possibility is that the *P. palmivora* pathogenic to oil palm
98 in Colombia is genetically distinct from *P. palmivora* in Malaysia, and one of the steps for addressing this
99 question is to identify the phylogenetic relationship and genetic variation of the *P. palmivora* species from both
100 regions and also other regions around the world.

101 The advances in molecular techniques, particularly PCR and DNA sequencing, have fuelled
102 bioinformatics studies of DNA data of organisms. DNA nucleotide sequence analysis has contributed to the
103 understanding of the phylogenetic and molecular diversity of organisms including in the *Phytophthora* genus
104 (Scibetta et al. 2012). Sequencing of specific target regions (single and multiple) has been widely used to study
105 the diversity of *Phytophthora* (Hu et al. 2013; Rahman et al. 2015), *Pythium* (Arcate et al. 2006) and other
106 microbes such as fungi (Korabecna 2007), phytoplasmas (Jović et al. 2011) and plants (Ritland et al. 1993).
107 Molecular analysis of DNA sequences by Crawford et al. (1996), Cooke and Duncan (1997), Cooke et al.
108 (2000) and Förster et al. (2000) have increased the understanding of the phylogenetic relationships between
109 *Phytophthora* species. Their work has been based mainly on the nucleotide sequence data of a single DNA
110 region, the rDNA internal transcribed spacer (ITS). Earlier work on analysis of sequences to investigate genetic
111 diversity, phylogenetics and genetic variation of *Phytophthora* and fungi were also based on this rDNA and
112 ITS region (Bruns et al. 1992); however, other regions and genes of nuclear or mitochondrial DNA have more

113 recently been explored extensively, such as *beta-tubulin* (β -*tubulin*), *translation elongation factor 1 alpha* (*EF-*
114 *1a*), *NADH dehydrogenase subunit I*, *cytochrome c oxidase subunit I* (*CoxI*) and *subunit II* (*CoxII*) either being
115 analyzed individually or as multi-locus/multi-gene combinations (Blair et al. 2008; Kroon et al. 2004; Martin
116 and Tooley 2003b; Villa et al. 2006). Phylogenetic analysis based on multiple genes has also been reported for
117 many fungal species such as *Fusarium* (Nalim et al. 2009) and *Corynespora* (Shimomoto et al. 2011).

118 Apart from the analysis of DNA sequences using selected regions as molecular markers, DNA
119 fingerprinting methods such as amplified fragment length polymorphism (AFLP) have also been widely used
120 to study genetic variation, phylogenetic relationships, population evolution, and diversity without knowing the
121 DNA sequences of the studied organism including oomycetes (Abu-El Samen et al. 2003; Ivors et al. 2004).
122 AFLP is a PCR-based fingerprinting technique that is similar to the random amplified polymorphic DNA
123 (RAPD) but offers higher stringency while retaining time efficiency (Mueller et al. 1996) and has proven useful
124 for investigating genetic variation among individuals (Mueller and Wolfenbarger 1999).

125 In this study, we have adopted DNA sequence analysis and the AFLP fingerprinting techniques to
126 molecularly characterize *P. palmivora* isolates from Colombia and Malaysia, particularly focusing on the study
127 of the genetic variations between these isolates.

128

129

130 **MATERIALS AND METHODS**

131

132 *Isolation of Phytophthora from oil palm, cocoa and durian*

133 *Phytophthora palmivora* isolate PPC280574 was isolated from infected young unopened spear leaves of oil
134 palm in Colombia. The isolation was carried out using a baiting technique using pear as described by Torres et
135 al. (2010). Isolation of *P. palmivora* from cocoa and durian in Malaysia was carried out using direct plating of
136 the diseased tissue onto P₁₀VP agar (CMA; 16 g, distilled water; 1000 ml, pentachloronitrobenzene (PCNB);
137 100 $\mu\text{g ml}^{-1}$, pimaricin; 10 $\mu\text{g ml}^{-1}$, vancomycin; 200 $\mu\text{g ml}^{-1}$ (Tsao and Ocana 1969). The plates were
138 incubated at 25°C +/- 2°C in the dark and examined daily under microscope for initial identification of
139 *Phytophthora* based on morphological characteristics described by Waterhouse (1963) and Gallegly and Hong
140 (2008). The outgrown culture was transferred onto fresh P₁₀VP agar before sub-culturing onto carrot agar as

141 described by Drenth and Sendall (2001)(CA; 15g agar, fresh carrot; 200 g, distilled water; 1000 ml). All
 142 cultures were maintained in the UK, where this work was undertaken due to restrictions on importing new
 143 isolates to Malaysia for biosafety reasons, on carrot agar with or without antibiotic supplements at temperatures
 144 of 25°C +/- 2°C.

145

146 *DNA isolation and PCR amplification*

147 Between 100-120 mg of *Phytophthora* mycelium was scraped from the surface of 7 to 10 day old colonies
 148 grown on carrot agar, placed into a sterile 2 ml screw-capped tube and homogenized using glass beads and a
 149 miller. DNA extractions of the cultures were then carried out using DNeasy Plant Mini Kit (Qiagen) following
 150 the manufacturer's protocol. Primers to amplify the regions of the internal transcribed spacer (ITS) regions,
 151 *translation elongation factor 1 alpha* gene (*EF-1α*), *beta-tubulin* gene (*β-tubulin*), *cytochrome oxidase II*
 152 (*CoxII*), *cytochrome oxidase I* (*CoxI*) genes of the mitochondrial DNA and PpHPAV marker from this study
 153 are detailed in Table 1. All oligonucleotides were synthesized by Sigma-Aldrich, UK. PCR amplification was
 154 performed in 25 µl volumes consisting of 12.5 µl of master mix (MangoTaq™ DNA Polymerase), 1 µl each of
 155 forward and reverse primers (10 pmol/µl), 9.5 µl sterile distilled water and 1 µl of template DNA.
 156 Amplification was set at 95°C for 2 min for initial denaturation, followed by 30 cycles of denaturation at 95°C
 157 for 1 min. Annealing was set for 1 min at 41°C for *CoxI*, 55°C (ITS, *CoxII*), and 64°C for *β-tubulin* and *EF-*
 158 *1α*), followed by the extension/elongation at 72°C for 1 min 30 sec and final extension at 72°C for 10 min. The
 159 amplicons were run in 1.2% agarose gels stained with ethidium bromide in Tris-borate-EDTA (TBE) buffer at
 160 100 volts for 25-40 min alongside with 1kb DNA marker ladder. The presence of single clear bands was
 161 checked for successful amplification using a gel imager. The amplified products were then purified with the
 162 QIAquick® PCR Purification Kit (QIAGEN), following manufacturer's instructions.

163

164

Table 1 Primers used for PCR amplification

Marker	Oligo-nucleotide	Sequence 5' to 3'	Reference
ITS regions	ITS1	TCC GTA GGTGAA CCTGCG G	White et al. (1990)
	ITS4	TCCTCCGCTTAT TGATATGC	Crawford et al. (1996)

<i>EF-1α</i>	EF1AF	TCACGATCGACATTGCCCTG	Kroon et al. (2004)
	EF1AR	ACGGCTCGAGGATGACCATG	
<i>CoxII</i>	FM82	TTGGCAATTAGGTTTTCAAGATCC	Martin and Tooley (2003b)
	FM78	ACAAATTTCACTACATTGTCC	
<i>CoxI</i>	OomCoxI _{Lev}	TCAWCWMGATGGCTTTTTTCAAC	Ginetti et al. (2014)
	up	RRHWACKTGACTDATRATACCAAA	Robideau et al. (2011)
	Fm85mod		
<i>β-tubulin</i>	BT5	GTATCATGTGCACGTACTCGG	Villa et al. (2006).
	BT6	CAAGAAAGCCTTACGACGGA	
PpHPAV	AV1F	AATGACGGCTTCTGCGTTTG	This study
	AV1R	GGCGTGACTACAGAGTGTCC	

165

166

167 *Cloning and sequencing*

168 Cloning of PCR amplicons was conducted using the cloning kit, pGem[®]-T Easy Vector System I (Promega)
169 using competent cells of *Escherichia coli* cells strain DH5 α and transformation was conducted using heat shock
170 treatment. PCR amplification of targeted using primer M13 forward (5'-GTAAAACGACGGCCAGT-3') and
171 M13 reverse (5'-CAGGAAACAGCTATGAC-3'). Purified amplicons were sent for sequencing at Eurofin
172 MWG Operon, UK. Sequencing data was checked, cleaned and edited using GAP4 software package (Staden-
173 package, USA) by removing the vector sequences, correcting the base errors and generating contig sequences
174 from forward and reverse sequences of each individual clone. For identification of the isolates, ITS sequences
175 were subjected to nucleotide-nucleotide searches with the Basic Local Alignment Search Tool - BLASTn
176 algorithm at the NCBI website (<http://www.ncbi.nlm.nih.gov/BLAST/>). The outputs from the BLAST searches
177 were sorted based on the maximum identity. Identification of each isolate was based on the maximum scoring
178 of identity value and query coverage.

179

180 *Sequence analysis and phylogenetic analyses*

181 Sequence alignments were performed using ClustalW (Thompson et al. 1994) using default settings. The
182 phylogenetic trees were constructed using the Maximum Likelihood method based on the Tamura-Nei model
183 (Tamura and Nei 1993); both were conducted using *MEGA 6.06* (Tamura et al. 2013) using the data from the
184 sequences obtained in this study, combined with additional sequences obtained from GenBank®, indicated by
185 the presence of accession numbers in the brackets. The evolutionary distances were compared nucleotide-by-
186 nucleotide using the nucleotide substitution model of Maximum Composite Likelihood with rate uniformity
187 and homogeneity pattern as implemented in *MEGA version 6.06* with bootstrap tests of 1000 replicates to
188 estimate error (Felsenstein 1985; Tamura et al. 2004). All alignment gaps and missing data were deleted before
189 the calculation using the complete-deletion option. Phylogenetic trees were constructed using individual
190 datasets of each marker. Nucleotide sequences of all markers were concatenated using *SequenceMatrix* (Vaidya
191 et al. 2011) but the dataset was limited with nucleotide data only available for all five markers of the ITS
192 regions, *EF-1 α* , *β -tubulin*, *CoxI* and *CoxII* genes. All external gaps were manually inspected and deleted before
193 alignment. Alignment and phylogenetic analyses of concatenated datasets were carried out with the same
194 method as individual datasets.

195

196 *Amplified fragment length polymorphism (AFLP)*

197 Digestion of 400-500 ng genomic DNA was done with 10 U *EcoRI* and 5 U *MseI* in 2x *EcoRI* restriction
198 enzyme buffer Tango™ in a total volume of 25 μ l at 37°C for 3 hours. Enzymes were deactivated at 65°C for
199 5 min. *EcoRI* adapters (5'-CTCGTAGACTGCGTACC-3' and 5'-AATTGGTACGCAGTCTAC-3') and *MseI*
200 adapters (5'-GACGATGAGTCCTGAG-3' and 5'-TACTCAGGACTCAT-3') were prepared by mixing 20 μ l
201 each of forward and reverse adapters (100 pmol/ μ l) with 160 μ l distilled sterile water and then incubated at
202 65°C for 10 min and allowed to cool slowly to room temperature. Ligation was carried out by adding 1 μ l 10
203 pmol/ μ l *EcoRI* adapter, 1 μ l 10 pmol/ μ l *MseI* adapter, 1 μ l 1U/ μ l T4 DNA ligase enzyme, 8 μ l 10x T4 DNA
204 ligase buffer and 11 μ l of sterile distilled water to the ligation mixture tube and incubated at 4°C overnight.
205 The digestion-ligation solution was diluted with TBE buffer at 1:10 ratio and kept at -20°C. Pre-amplification
206 was carried out using 5 μ l of diluted ligation mixture, 1 μ l 10 pmol/ μ l *EcoRI*-universal primer (5'-
207 CGTAGACTGCGTACCAATTC-3') and 1 μ l 10 pmol/ μ l *MseI*-universal primer (5'-

208 GACGATGAGTCCTGAGTAA-3'), 18 µl sterile distilled water and Illustra™ puReTaq Ready-To-Go™ PCR
209 Beads (GE Healthcare, UK). Amplification conditions were 94°C for 1 min followed by 10 cycles at 94°C for
210 40 sec, 65°C for 1 min and 72°C for 1 min and then 25 additional cycles at 94°C for 40 sec, 56°C for 1 min
211 and 72°C for 1 min. Selective amplification was done using a mixture of 5 µl of diluted pre-amplification
212 product (1:20 dilution), 18 µl of sterile distilled water and Illustra™ puReTaq Ready-To-Go™ PCR Beads
213 (GE Healthcare, UK) together with combination of 1 µl 10 pmol/µl EcoRI selective primer with different
214 nucleotide tails (n) (5'-CGTAGACTGCGTACCAATTC-n-3') labelled with WellRED® fluorescence, D3 or
215 D4 dye and 1 µl 10 pmol/µl MseI selective primers with different nucleotide tails (n) (5'-
216 GACGATGAGTCCTGAGTAA-n-3') and each assay were carried out in two replicates. Based on previous
217 primers selection, three informative primers were used for analysis: *EcoRI* - A / *MseI*-AG, *EcoRI* - AC / *MseI*-
218 AG and *EcoRI* - TA / *MseI*-AG. Amplification products were separated using 2% w/v agarose gels run at 120
219 volts for 60 min and sent for automated capillary electrophoresis using the CEQ™ 8000 System. Data from
220 the CEQ Genetic Analysis System was exported to *MS Excel* and manually examined, cleaned up and edited
221 before being transformed to binary coding. The absence of a peak/band is denoted with '0' and the presence of
222 a band is denoted with '1'. Monomorphic fragment peaks were not scored. The phylogenetic analysis of the
223 AFLP data was done using *FreeTree* software using UPGMA (Hampl et al. 2001; Pavlicek et al. 1999). The
224 distance matrix was calculated using Nei and Li distance (Nei and Li 1979). Resampling was done by
225 bootstrapping with 1000 replicates. The phylogenetic tree derived from *FreeTree* was viewed using *Treeview*
226 and *MEGA 6.0.6*.

227

228 *Pathogenicity evaluations*

229 Zoospore production: Zoospore inoculum was prepared as described by Dick et al. (2014) and Chee (1975)
230 with modification. The isolates were grown on carrot agar at 25°C +/- 2°C for 7 days. Old stock cultures were
231 reactivated by using the fruit bait technique prior to culturing. Four to five mycelial plugs cut from the actively
232 growing region of agar culture plates were immersed in sterilized carrot juice in a 90 mm Petri dish and
233 incubated at room temperature with illumination for 7-10 days. The zoospore release was induced by
234 incubating the culture in the dark at 4°C for approximately 20 to 45 min followed by exposure to room
235 temperature (25°C to 28°C). The zoospore suspension was then collected in a sterile beaker. The concentration

236 of zoospores was determined microscopically using a *Neubauer* haemocytometer. Zoospore suspensions were
237 used within two hours of preparation. Detached-leaf assay: The assay was conducted using mature leaves,
238 green unopened spear leaves and white unopened spear leaves taken from 12 month old oil palms grown in the
239 glasshouse. The leaves were cut into small piece about 14 cm in length. The white unopened spears were
240 divided into two parts, the upper older (greenish) part and lower younger (whitish) part. The leaf/spear pieces
241 were washed with tap water and surface sterilized using 2% v/v sodium hypochlorite (NaOCl) by dipping the
242 whole leaf into the solution for 60 seconds followed by rinsing with sterile distilled water twice and then left
243 to completely dry on clean tissue towels. Each end of the piece was cut approximately 0.5 cm from the margin.
244 The spear leaves were pricked/wounded twice using a sterile sharp pointed blade (no. 11) approximately 4 cm
245 from the end on both sides. The clean 5 mm x 5 mm cotton plugs were put on top of the wounded sites. One
246 hundred microliters (μ l) of zoospore suspension (10,000 zoospore/ml) was dropped onto the cotton pad.
247 Distilled water was used in control assays. The inoculation was also done using mycelial plugs. The chambers
248 were covered and incubated at room temperature with illumination for 7 days. Presence of lesions was observed
249 and diseased leaf tissue samples were cut into small pieces, soaked in 3% KOH for five minutes and observed
250 microscopically using a compound microscope.

251

252 Nursery Inoculation: Oil palm germinated seeds (*Dura x Pisifera*) were sown into trays filled with a mixture
253 of soil (Levington F2 Seed & Modular Compost) and perlite at the ratio of 8:1 in a glasshouse at 28°C (day)
254 and 22°C (night) with a photoperiod of 14-16h. After 3 months, the seedlings were transferred into larger pots
255 (5 litre) filled with a soil mixture of sand based soil (John Innes No.3), perlite and vermiculite with the ratio of
256 8:1:1. The seedlings were watered every day during summer and on alternate days during winter and fed with
257 liquid fertilizer (10% solution) containing N, P, K in the ratio of 4:2:2 and trace elements. The humidity in the
258 glasshouse was maintained by wetting the floor of the glasshouse every morning. Inoculation was carried out
259 based on the methods by Sarria et al. (2016) with modification where three point wounding was introduced
260 prior to inoculation at the base of the seedlings using a sterile hyperdermic needle (21G \times 1 1/2" /0.8 \times 40 mm).
261 Volume of inoculum was at 2 ml zoospore suspension (10^3 - 10^4 spore/ml). The whole plant was covered with
262 a clean plastic bag for 30 days to retain humidity. Inoculations were carried out in the same glasshouse where
263 the seedlings were grown and the conditions were maintained throughout the experiment and were done at

264 least in triplicate. The inoculated seedlings were observed for any development of lesion or any physical
265 symptoms of bud rot disease as described by Sarria et al. (2016). Diseased samples were collected and re-
266 isolated by direct plating onto selective media and confirmed by microscopic evaluation.

267

268 **RESULTS**

269 *Oomycete Isolates*

270 Thirty one isolates of *P. palmivora* from different hosts and geographical origins worldwide, eleven isolates of
271 other *Phytophthora* species and a *Pythium* spp. were analysed in this study. Details of isolates collected and
272 used in this study are as presented in Table 2. Isolates P19537 and P19538 identified as *P. palmivora* by
273 WOGRC were re-identified as *P. parasitica* and isolate CBS358.59 from the CBS-KNAW, were re-identified
274 as *P. colocasiae* in this study.

275

276 *DNA amplifications*

277 PCR amplification of ITS regions of *Phytophthora* and *Pythium* produced approximately 900 bp PCR
278 fragment. Fragments amplified for *CoxII* and *CoxI* genes using primer pairs FM82/FM78 and
279 OomCoxI Levup/Fm85mod were 600 bp and 800 bp respectively. Primer pair BT5/BT6 amplified the beta
280 tubulin region of 750 bp. For primer EF1AF/EF1AR, only the *Phytophthora* elongation factor was amplified
281 at 1000 bp. Primers AV1F and AV1R only specifically amplified the PpHPAV 'region' of *P. palmivora* with
282 the PCR amplicon of 1000 bp.

283

284 *Sequence and phylogenetic analyses of multigenes*

285 Low intraspecific variation was observed in the ITS1 and ITS2 sequence data for all 24 isolates of *P. palmivora*.
286 Isolates of *P. palmivora* originating from oil palm in Colombia (PPC280574) showed a high similarity (97%
287 to 100% identity, based on BLAST report) with other isolates obtained from various hosts and regions,
288 including all six Malaysian isolates. Further assessments using phylogenetic analysis showed similar results.
289 The evolutionary history inferred using the Maximum Likelihood method based on the Tamura-Nei model
290 grouped all *P. palmivora* isolates into one clade (Clade 1) with a strong bootstrap value regardless of the host
291 and demographic origin of the isolates and other species in Clade 2 (Figure 1). The tree with the highest log

292 likelihood likelihood is shown. The tree was constructed using 37 nucleotide sequences and involved 801
293 nucleotides in the final dataset. Heuristic searches of the initial tree(s) were automatically calculated by
294 applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum
295 Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value.
296 Sub-branching of clade 1 was observed (bootstrap value 84%), which consisted of two isolates originating
297 from Malaysian durian (PPM4 and PPM5) and isolates from betel palm (Guam) (P11007), cocoa (Ghana)
298 (PPG8) and bamboo palm, USA (*P. arecea*=*P. palmivora*) (CBS148.88). There was no consistent pattern for
299 the origin/host of these isolates except that two isolates were from Malaysia. Interestingly, one isolate of *P.*
300 *palmivora* (PPM3) isolated from cocoa in Malaysia was separated from the large *P. palmivora* clade with a
301 high bootstrap value.

302 Initial tree(s) for the heuristic search using partial nucleotide sequences of *EF-1 α* , *β -tubulin*, *CoxI* and
303 *CoxII* were obtained by applying the Neighbour-Joining method to a matrix of pairwise distances estimated
304 using the Maximum Composite Likelihood (MCL) approach (Saitou and Nei 1987; Tamura et al. 2004). The
305 analyses involved 43 sequences with a total of 870 positions in the final dataset for *EF-1 α* (Figure 2) and 41
306 sequences with a total of 648 positions in the final dataset for *β -tubulin* (Figure 3). For both *CoxI* and *CoxII*,
307 the analysis involved a total of 38 sequences which incorporated 773 and 621 nucleotides, respectively (Figures
308 4 & 5). All the *P. palmivora* isolates (including *P. arecae*) were clustered in one clade (Clade 1) with bootstrap
309 values of more than 97% for all trees. Sub-branching of Clade 1 was observed for condensed trees with 50%
310 bootstrap value cut off for all datasets except *β -tubulin*. In the *EF-1 α* tree, isolates PPM4 and PPM5 were
311 grouped together in a sub-clade 2, branched out from Clade 1, similar to the ITS tree. The other Malaysian
312 isolates were distributed randomly in Clade 1. Some Colombian isolates were grouped in sub-clade 2 and sub-
313 clade 3 with other isolates from Ghana and Sri Lanka also randomly distributed in Clade 1. Sub-clades were
314 also observed with the *β -tubulin* tree but with low bootstrap values (<50%) (Figure 3). In the *CoxI* tree, two
315 isolates from Malaysia, PPM1 and PPM2, were grouped in a sub-clade (Figure 4). There were other sub-clades
316 but with lower than 50% bootstrap values. The *CoxII* tree also grouped all 27 isolates of *P. palmivora* in one
317 clade, but the clade was sub-branched into another sub-clade of 26 isolates with one isolate (P11007) separated
318 (Figure 5). All trees were drawn to scale, with branch lengths measured in the number of substitutions per site.

319 The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000
320 replicates) are shown next to the branches when ≥ 50 . The labeled sub-clade is with $\geq 50\%$ bootstrap value.

321 Interspecific variation among other species of *Phytophthora* was clearly observed using all nucleotide
322 datasets. *Phytophthora palmivora* is clearly distinguished from other species included in this study. Some
323 species with more than one isolate were grouped together into the same clade such as for *P. megakarya*. Isolate
324 CBS358.59, originally identified as *P. colocasiae* in this study, was always grouped with isolate CBS581.69
325 from Malaysia in all trees. Both original hosts of these isolates are rubber.

326

327 *Concatenated tree of ITS and other housekeeping genes*

328 The concatenated tree was constructed from sequences of five different markers (Figure 6). The reconstruction
329 of the tree was done using the same methods as previous trees. The tree involved 35 sequences from this study
330 and from GenBank[®] marked with an asterisk (*). There was a total of 3773 nucleotide positions in the final
331 concatenated dataset. As in other trees, all *P. palmivora* isolates were grouped in one clade, Clade 1. There are
332 three sub-clades branching out from Clade 1 with more than 50% bootstrap value. Observation of the members
333 of each sub-clade show no relationship in terms of host and demographic origin of the isolates involved.

334

335 *Phylogenetic analysis of the PpHPAV marker*

336 The initial tree(s) for the heuristic search from datasets of PpHPAV sequences were obtained by using
337 the Neighbour-Joining method to a matrix of pairwise distances estimated using the Maximum Composite
338 Likelihood (MCL) approach. The analysis involved 31 sequences with a total of 958 positions in the final
339 dataset (Figure 7). The final tree consists of several major clades. *Phytophthora palmivora* Colombian isolates
340 and Malaysian isolates were clearly separated in different clades. All the Colombian isolates were strongly
341 grouped (bootstrap value of 98%) in Clade 1 together with isolates from the USA, Ghana, Trinidad & Tobago,
342 Guam, Sri Lanka and India including *P. arecae* from the USA which later separated as an outgroup from the
343 rest of the Clade 1 members which were further grouped in sub-clade 1. Isolates from Malaysia and Indonesia
344 from different hosts of cocoa, durian, coconut and rubber clustered in several clades. Clade 2 consists of three
345 Malaysian isolates obtained from cocoa (PPM1, PPM2 and PPM3). Clade 3 consist of a mixture of Malaysian
346 and Indonesian isolates form coconut, cocoa and durian. Clade 4 also contains a mixture of Malaysian and

347 Indonesian isolates from various hosts. One isolate from South Korea (CBS111146) was also included in this
348 clade.

349

350 *Phylogenetic analyses of AFLP data*

351 Phylogenetic tree (s) constructed from individual AFLP datasets of marker *EcoRI-A/MseI-AG*, *EcoRI-*
352 *AC/MseI-AG* and *EcoRI-TA/MseI-AG* which each involved 75, 121 and 149 random markers of polymorphic
353 bands were able to separate Colombian and Malaysian isolates into two distinct clades (Figure 8) which was
354 further shown in the concatenated tree of the three datasets involved 345 random markers (Figure 9) where all
355 three Colombian isolates were grouped together in Clade 1, whilst the two Malaysian isolates were clustered
356 in Clade 2. Other species were clearly distinguished as outgroups.

357

358 *Pathogenicity Tests*

359 Detached leaf assay: No lesions were observed in the initial trials using green mature leaves and green
360 unopened spear leaves of 12 month old oil palms inoculated with zoospore suspensions (approximately 10^4
361 zoospores ml^{-1}) and mycelial plugs of oil palm pathogenic isolate PPC280574, both with and without
362 wounding, by the 5th day after inoculation. Brown lesions were observed at 4 days after inoculation using white
363 unopened spears on the lower part (whitish) nearer to the crown/growing point but not the with the upper
364 greenish part, but only with wounded leaves (Figure 10). The presence of *P. palmivora* in the diseased tissue
365 was confirmed by microscopic evaluation of the diseased tissue and re-isolation using selective media.
366 *Phytophthora palmivora* was not observed in control assays and there was no mycelial growth on the selective
367 media.

368

369 Glasshouse inoculation: No lesions were observed on any of the inoculated seedlings conducted in the winter.
370 Brown lesions was firstly observed on some of the inoculated seedlings at the end of May on the 6th-7th day
371 after inoculation using isolates P16385, CBS111346 and PPG1 (Figure 11). Subsequent inoculations with 12
372 isolates showed that all isolates can cause brown lesions at the inoculation sites but not all inoculated seedlings
373 formed lesions (Table 3). Similar findings were shown with the seedlings in another trial repeated with only
374 four isolates (Table 4). Lesions appeared to be localized on the wounded site and no further infection was

375 observed after two weeks of inoculation at the infection site. The size of the lesions was mostly small
376 (approximately 3 mm-15 mm) and did not expand or grow. Nevertheless, there were three seedlings (inoculated
377 with PPM4, PPM1 and CBS111346) that had larger infection areas, where half of the young spear leaf become
378 brown and infected. On all infected leaves, the diseased tissue become necrotic and dried out. After some time,
379 the necrotic tissue fell out leaving a hole in the leaf, but the rest of the leaf (the healthy tissue) kept on growing,
380 including the new healthy shoot. No recurrent infections were observed on any inoculated seedlings.

381

382 **DISCUSSION**

383 Studies on diversity, phylogenetics and polymorphisms among oomycetes, particularly *Phytophthora*, have
384 been carried out using various molecular tools including analysis of DNA sequences of target regions or genes.
385 The ITS, which is the non-coding spacer region between the 28S and 18S rDNA, has been shown to be
386 particularly useful such as in the work of Lee and Taylor (1992) and Cooke and Duncan (1997) where high
387 resolution of interspecific levels were achieved. However, intraspecific variations using this region are rather
388 limited and rarely encountered (Sorensen et al. 1998), although not impossible. For example, Cohen et al.
389 (2003) demonstrated some intraspecies variations and phylogenetic separation of *P. citrophthora*, whilst
390 Vinuesa et al. (2001) showed up to 16% variation for *Mycocalicium substantial* but only 1% for *M. albonigrum*.
391 In this current study, variation at the intraspecific level within 26 isolates of *P. palmivora* from various hosts
392 and demographic origins was not clearly observed in the DNA sequences of the ITS regions. The small
393 percentage of DNA nucleotide variations (0-3%) between some isolates might be due to errors during PCR and
394 sequencing even though effort was taken to minimise such errors. Analyses with 32 additional ITS sequences
395 of *P. palmivora* obtained from GenBank® also showed similar findings. Since the evolution of one gene may
396 not represent the entire genome (Villa et al. 2006) phylogenetic analyses using other genes; *CoxI*, *CoxII*, β -
397 *tubulin* and *EF-1 α* genes were included in this study.

398 *Cox* genes of subunit I and II code for enzymes that catalyse the terminal step in the electron transport
399 chain and are encoded in the mitochondria, which is considered generally to be more variable than nuclear
400 DNA and has proven to be good for studying the relationship at the sub-generic level for various taxa (Villa et
401 al. 2006). Phylogenetic relationships of the *Phytophthora* genus based on the *CoxI* and *CoxII* genes has been
402 established by Martin and Tooley (2003a), whilst Villa et al. (2006) used β -*tubulin* data along with *ITS* and

403 *CoxI*. Blair et al. (2008) used seven multi-locus markers (28S rDNA, 60S ribosomal protein L10, β -tubulin,
404 *EF- α 1*, *Enolase*, *heat shock protein 90* and *TigA gene fusion protein*) and found that β -tubulin provided the
405 highest level of phylogenetic variation across the *Phytophthora* genus. However, in this study, all the individual
406 phylogenetic trees reconstructed using sequence data for *CoxI*, *CoxII*, β -tubulin and *EF-1 α* , demonstrated
407 similar findings to the ITS, with low intraspecific variations in DNA sequences. The trees did not exhibit
408 consistent similarities in grouping based on demographic and host origin. A multiple loci sequences data
409 constructed from the combination of all five datasets to enhance the phylogenetic inference as suggested by
410 (Bininda-Emonds et al. 2001; Sanderson et al. 2003) and has been demonstrated in many studies such as
411 Baptiste et al. (2002), Kroon et al. (2004), Martin and Tooley (2003a) and Blair et al. (2008). However, the
412 multi-locus tree constructed from the five loci in this study showed no clear separation of *P. palmivora* isolates
413 from Malaysia, Colombia and other isolates from different demographic origin and hosts. These five molecular
414 markers shown to be suitable for inter-specific studies between species but not intra-specific evaluation within
415 species of *P. palmivora*.

416 This study was expanded by looking at genome level variation using AFLP which has the ability to
417 simultaneously screen many DNA regions distributed randomly throughout the genome rather than looking at
418 specific loci, although these are dominant markers, so they cannot differentiate homologous alleles, making it
419 less useful for studies that involve allelic states such as heterozygosity analyses (Mueller and Wolfenbarger
420 1999). AFLP analysis using some representative isolates of *P. palmivora* from Malaysia and Colombian using
421 three AFLP primer combinations was able to show some variations. The isolates from Colombian and Malaysia
422 were separated into different clades in the phylogenetic tree.

423 The results from the AFLP encouraged the exploration of other loci as molecular markers to study
424 variations among Colombian isolates and Malaysian isolates. One region of interest was the gene clusters or
425 regions encoding effector/avirulence proteins that are involved in the infection process and colonization of
426 plant tissue. The genome sequencing of *Phytophthora* species such as *P. infestans* has revealed a diverse and
427 large class of effectors (Bozkurt et al. 2012) such as *AVR3a* (Armstrong et al. 2005; Bos et al. 2009), *AVR1b*
428 (Shan et al. 2004) and *PiAVR4* (van Poppel 2009; van Poppel et al. 2008). The effector proteins are secreted
429 by the oomycetes to suppress the immune responses of the host plant (such as pathogen associated molecular
430 patterns (PAMPs) trigger immunity (PTI) triggered by their own elicitors. For example, *P. infestans* effector

431 AVR3a suppresses perception of the PAMP INFI through stabilization of the U-box protein CMPG1 (Fawke
432 et al. 2015). The AVR3a protein is encoded by avirulence gene *Avr3a* and belongs to a large, oomycete-specific
433 family of highly divergent effectors that share a conserved domain named RXLR-dEER (Tyler et al. 2006)
434 which triggers disease resistance and the hypersensitive response (HR) (Armstrong et al. 2005). The
435 corresponding resistance *R* gene of the host plant to *Avr3a* is the *R3a*, and *R* proteins generally activate
436 resistance responses effector-triggered immunity of the plant host (ETI).

437 The primers to amplify the unknown region of only *P. palmivora*, named as PpHPAV were designed
438 from the sequences of *P. infestans Avr4 (PiAvr4)* sequences which encodes a typical oomycete RXLR effector
439 molecule (van Poppel et al. 2008). The PpHPAV sequences did not match closely to DNA or protein sequences
440 in the GenBank® database, probably because whole genome sequencing and studies on effector proteins and
441 avirulence genes of *P. palmivora* have not yet been published and are still on going. Although the nature of the
442 PpHPAV sequences is vague, the locus was shown to have some intraspecific variation within *P. palmivora*
443 species, at least between Colombian and Malaysian isolates. Phylogenetic analyses using PpHPAV sequences
444 separated all the Colombian isolates into one clade along with other isolates except isolates from Malaysia,
445 Indonesia and South Korea, which were clustered in separated clades. However, these three clades did not
446 show any other characteristics based on host and origin, but the phylogenetic observation suggested that they
447 share common ancestry. It will be interesting to further explore isolates from South East Asia such as Thailand,
448 the Philippines and Myanmar to confirm the distinct nature of SE Asian isolates.

449 Pathogenicity studies using isolates from Malaysia, Colombia and others however, did not show any
450 significant differences in the level of aggressiveness against oil palm. In *in vitro* assays, infection was
451 successfully established on the very young spear leaves using the Colombian isolates PPC280574. The initial
452 symptoms of small brown lesions with water-soaking at the edge were observed at 3-4 days after inoculation,
453 which coincides with the symptoms described in several reviews such as Sarria (2013) and Torres et al. (2016).
454 Similar water-soaked symptoms were also described by Tri et al. (2015) on the jackfruit leaf inoculation using
455 the same species. Turner (1969a) reported that the water-soaked margin was only observed on inoculated
456 immature leaves of *Piper betle* and both upper and lower leaf surfaces can be inoculated. In our study, it was
457 observed that wounding of the spear was required for the infection to occur in contrast with the findings by
458 Sarria et al. (2016), where the infection readily occurred without wounding. However, Sarria et al. (2016) used

459 individual leaflets of the young spear instead of direct inoculation on the un-opened spear. Mohamed Azni et
460 al. (2016) also reported the need for wounding for infection to occur. The re-isolation of the diseased tissue
461 and microscopic evaluation confirmed the presence of *P. palmivora*.

462 Initially, we could not establish infection in the glasshouse through artificial inoculation of *P.*
463 *palmivora* on oil palm seedlings using the isolate originating from oil palm in Colombia believed to be
464 pathogenic to oil palm. Several trials were conducted including trials with modifications of inoculation methods
465 including increasing the inoculum (in term of volume, zoospore counts, combinations of mycelium,
466 sporangium, chlamydospores and zoospores), introduction of wounding at the stem base of the seedlings and
467 waterlogging the seedlings before and after inoculation. In order to avoid loss of virulence during sub-culturing,
468 the isolate was reactivated in the fruit (apple/pear) and re-isolated onto selective media prior to production of
469 zoospores. Positive infection on rubber leaves suggested the continued existence of the pathogenic nature of
470 the isolate. The artificial inoculation was then extended to other isolates originating from oil palm and cocoa
471 in Colombia, cocoa, durian, rubber in Malaysia, *Cymbidium* orchid (South Korea, betel palm (Guam), kentia
472 palm (California) and cocoa (Ghana), regardless of the failure to established infection using the isolate from
473 the oil palm as positive reference. It is believed that the infection of *P. palmivora* to the seedlings is affected
474 by the temperature because eventually infections were observed at the end of May, which was the beginning
475 of spring in the UK, and subsequent inoculations with the same isolates as tested before (with no infection),
476 showed positive infections when retested during the summer months.

477 The initial symptoms of brown lesions with water-soaked margins (observed on the seedlings
478 inoculated with Colombian and other isolates) coincided with the previous detached leaf assay and observations
479 from Sarria et al. (2016). However, the lesions appeared to be localized in our study as reported by Mohamed
480 Azni et al. (2016) with work using Malaysian isolates in Malaysia. The infection did not grow further in most
481 infected seedlings. In other words the Colombian, Malaysian and other isolates from different hosts all caused
482 mild symptoms, potentially equating to the non-lethal form of the disease that has been found in some parts of
483 the world including possibly Malaysia (Sharples (1928); Sharples (1928); Turner (1981), and the disease did
484 not progress to a severe form with the typical aggressive symptoms that had been found to occur in inoculation
485 tests in Colombia (Sarria 2013; Torres et al. 2010). Torres et al. (2010) reported 15% of the seedlings inoculated
486 with 40,000 zoospores developed into typical bud rot symptoms but none in our study even though up to

487 180,000 zoospores were used per seedling. The disease cycle of *Phytophthora* often involves primary and
488 secondary inoculum. Primary inoculum initiates the infection and upon successful infection, a second
489 generation of secondary inoculum is produced. The rate of propagation of secondary inoculum determines the
490 severity of the next infection (Drenth and Guest 2004). In the case of infection in the glasshouse in the UK,
491 there were some factors affecting the propagation of secondary inoculum including environmental conditions
492 such as temperature, humidity and maybe the presence or absence of other microbes as secondary invaders that
493 are different in the UK and Malaysia compared to Latin America. In terms of disease incidence, not all
494 seedlings inoculated with each isolate were infected. The incidence observed on the inoculated seedlings was
495 variable between and within isolates. Some difficulty was experienced in producing zoospores for each trial,
496 such that the inoculum strength in terms of zoospore could not be exactly standardized and was in the range of
497 $1-9 \times 10^4$ zoospore ml^{-1} for the first summer inoculation and $5-8 \times 10^3$ zoospore ml^{-1} for the second round
498 summer inoculation, and this may have affected the incidence scores between tests. However, the incidence
499 data is useful in providing information on the cross pathogenicity between isolates against oil palm seedlings
500 even though it may not be appropriate for showing the aggressiveness levels of each isolate.

501 Cross pathogenicity assays showed isolates from oil palm could cause infection in rubber and durian
502 leaves. Both inoculum sources of zoospores and mycelial plugs have the potential to infect the leaves and lesion
503 growth varied in each assay using the same isolates which might reflect the influence of many factors such as
504 humidity in the inoculation chamber, age/condition of the leaf and inoculum potential. Cross pathogenicity of
505 isolates from different hosts; coconut, cocoa, durian, rubber, bamboo palm, betel palm and orchid on both
506 durian and rubber leaves suggested that *P. palmivora* does not have specific strains adapted for each host as
507 observed for *Fusarium* sp., supporting the hypothesis of a broad host range for *P. palmivora* (Drenth and Guest
508 2004). Pongpisutta and Sangchote (2004) showed cross pathogenicity of *P. palmivora* isolates from durian
509 against black pepper and rubber leaves. However, not all *Phytophthora* species have broad host ranges.
510 Different species of *Phytophthora* may have different degrees of host specificity. Some species such as *P.*
511 *havea* have narrow ranges and *P. colocasiae* is very host specific to taro (*Colocasia esculenta*) (Drenth and
512 Guest 2004). Some species of *Phytophthora* seem to have both non-host and host-specific receptor-based
513 recognition systems for induction of encystment of zoospores by host surface components, therefore enabling
514 general and host specific pathogenicity, which enables them to invade compromised plants in the absence of

515 preferred hosts (Raftoyannis and Dick 2006a). However, there is also the possibility that the specificity of host
516 selection arises during the attempts at penetration and invasion of plant tissue and that the zoospore stage is
517 non-specific (Van West et al. 2002). Several reports with root diseases show that zoospores of *Phytophthora*
518 species are attracted to and encyst similarly on roots of susceptible and resistant seedlings of plants
519 (Raftoyannis and Dick 2006a). Raftoyannis and Dick (2006b) found that the relationship between encystment
520 of zoospores and disease development depends on the oomycete–plant combination.

521 Similar to the inoculation of oil palm young spear leaves, the inoculation on durian and rubber leaves
522 in the cross-pathogenicity assays using several isolates from various hosts including oil palm conducted in this
523 study also failed to established infection without wounding. Introduction of wounding in the artificial
524 inoculation is not new in pathogenicity studies of *Phytophthora* spp. using stems to facilitate infection and has
525 been shown by others especially when working with stem rots. Nevertheless, most studies with leaves usually
526 do not involve wounding and infections on the leaves become established without wounding such as in citrus
527 (Ann 1984), durian (Lim and Chan 1986) and jackfruit (Tri et al. 2015). Meanwhile, other researchers have
528 introduced wounding prior to inoculation such as Pongpisutta and Sangchote (2004). O’Gara et al. (2004a)
529 reported that *P. palmivora* was attracted to fresh wounds on the durian leaf and rapidly colonized the entire
530 leaf lamina when infection happened through the fresh wound and non-wounded durian leaves did not develop
531 disease symptoms reliably (O’Gara et al. 2004b). In contrast, Brooks (2008) found that there was no difference
532 in the infection of *P. colocasiae* on taro leaves.

533 It is hoped that more studies can be conducted to understand more on the pathogenicity and
534 aggressiveness of *P. palmivora* against oil palm. Artificial inoculation of oil palm seedlings using different *P.*
535 *palmivora* isolates should be repeated but with the same inoculum size for each isolate so that the
536 aggressiveness of the different isolates originating from different hosts and geographical regions can be
537 assessed without prejudice and probably can be correlated with the molecular characterization to see if the
538 isolates belonging to same clade have similar levels of aggressiveness against oil palm. It would also be good
539 if the assay can be conducted in a tropical environment; however, due to biosecurity constraints, it is difficult
540 to carry out such experiments in countries such as Malaysia and Colombia as it would involve introducing the
541 foreign isolates to the areas. However, the evaluation of the local isolates obtained from different hosts against
542 oil palm is possible.

543 REFERENCES

544

- 545 Abu-El Samen FM, Secor GA, Gudmestad NC (2003) Genetic variation among asexual progeny of
546 *Phytophthora infestans* detected with RAPD and AFLP markers. Plant Pathol 52:314-325.
547 <https://doi.org/10.1046/j.1365-3059.2003.00858.x>
- 548 Ahmed Y, D'Onghia AM, Ippolito A, Yaseen T (2013) First report of citrus root rot caused by *Phytophthora*
549 *palmivora* in Egypt. Plant Dis 98:155-155. <https://doi.org/10.1094/PDIS-02-13-0206-PDN>
- 550 Albertazzi-Leandro H, Bulgarelli-Mora JM, Chinchilla-López CM (2005) Onset of spear rot symptoms in oil
551 palm and contemporary events. ASD Oil Palm Papers:11-41.
- 552 Ann PJ (1984) Species, mating types and pathogenicity of *Phytophthora* distributed in citrus orchards in
553 Taiwan. Trans Br Mycol Soc 82:631-634. [https://doi.org/10.1016/S0007-1536\(84\)80103-5](https://doi.org/10.1016/S0007-1536(84)80103-5)
- 554 Aragaki M, Uchida JY (1980) Foliar stage of Phytophthora blight of macadamia. Plant Dis 64:483-484.
555 <https://doi.org/10.1094/PD-64-483>
- 556 Arcate JM, Karp MA, Nelson EB (2006) Diversity of Peronosporomycete (Oomycete) communities associated
557 with the rhizosphere of different plant species. Microb Ecol 51:36-50. <https://doi.org/10.1007/s00248-005-0187-y>
- 558
- 559 Armstrong MR et al. (2005) An ancestral oomycete locus contains late blight avirulence gene Avr3a, encoding
560 a protein that is recognized in the host cytoplasm. Proc Natl Acad Sci U S A 102:7766-7771.
561 <https://doi.org/10.1073/pnas.0500113102>
- 562 Awuah RT, Frimpong M (2002) Cocoa-based media for culturing *Phytophthora palmivora* (Butl.) causal agent
563 of black pod disease of cocoa. Mycopathologia 155:143-147.
564 <https://doi.org/10.1023/A:1020415109308>
- 565 Baptiste E et al. (2002) The analysis of 100 genes supports the grouping of three highly divergent amoebae:
566 Dictyostelium, Entamoeba, and Mastigamoeba. Proc Natl Acad Sci U S A 99:1414-1419.
567 <https://doi.org/10.1073/pnas.032662799>
- 568 Beuther E, Wiese U, Lukács N, Van-Slobbe WG, Riesner D (1992) Fatal yellowing of oil palms: Search for
569 viroids and double-stranded RNA. J Phytopathol 136:297-311. <https://doi.org/10.1111/j.1439-0434.1992.tb01312.x>
- 570
- 571 Bininda-Emonds ORP, Brady SG, Kim J, Sanderson MJ Scaling of accuracy in extremely large phylogenetic
572 trees. In: Pacific symposium on biocomputing, 2001. pp 547-558
- 573 Blaha G, Hall G, Warokka JS, Concibido E, Ortiz-Garcia C (1994) *Phytophthora* isolates from coconut
574 plantations in Indonesia and Ivory Coast: Characterization and identification by morphology and
575 isozyme analysis. Mycol Res 98:1379-1389. [https://doi.org/10.1016/S0953-7562\(09\)81067-8](https://doi.org/10.1016/S0953-7562(09)81067-8)
- 576 Blair JE, Coffey MD, Park SY, Geiser DM, Kang S (2008) A multi-locus phylogeny for *Phytophthora* utilizing
577 markers derived from complete genome sequences. Fungal Genet Biol 45:266-277.
578 <https://doi.org/10.1016/j.fgb.2007.10.010>
- 579 Boari AdJ (2008) Estudos realizados sobre o amarelecimento fatal do dendezeiro (*Elaeis guineensis* Jacq.) no
580 Brasil. Embrapa Amazônia Oriental Documentos. Embrapa Amazônia Oriental, Belém, Brazil
- 581 Boari AJ, Teixeira WG, Venturieri A, Martorano L, Tremacoldi CR, Carvalho KB (2012) Avanços nos estudos
582 sobre o amarelecimento fatal da palma de óleo (*Elaeis guineensis* Jacq.). In: Congresso Brasileiro de
583 Fitopatologia 45° Manaus, Brazil, 19 to 23 August 2012. Tropical Plant Pathology, vol 37. Brazilian
584 Phytopathological Society, Manaus, Brazil, p 4
- 585 Bos JIB, Chaparro-Garcia A, Quesada-Ocampo LM, McSpadden Gardener BB, Kamoun S (2009) Distinct
586 amino acids of the *Phytophthora infestans* effector AVR3a condition activation of R3a
587 hypersensitivity and suppression of cell death. Mol Plant Microbe Interact 22:269-281.
588 <https://doi.org/10.1094/MPMI-22-3-0269>
- 589 Bozkurt TO, Schornack S, Banfield MJ, Kamoun S (2012) Oomycetes, effectors, and all that jazz. Curr Opin
590 Plant Biol 15:483-492. <https://doi.org/10.1016/j.pbi.2012.03.008>
- 591 Brasier CM (1992) Evolutionary biology of Phytophthora: I: Genetic system, sexuality and the generation of
592 variation. Annu Rev Phytopathol 30:153-171. <https://doi.org/10.1146/annurev.py.30.090192.001101>
- 593 Brooks FE (2008) Detached-leaf bioassay for evaluating taro resistance to *Phytophthora colocasiae*. Plant Dis
594 92:126-131. <https://doi.org/10.1094/PDIS-92-1-0126>
- 595 Bruns TD et al. (1992) Evolutionary relationships within the fungi: Analyses of nuclear small subunit rRNA
596 sequences. Mol Phylogenet Evol 1:231-241. [https://doi.org/10.1016/1055-7903\(92\)90020-H](https://doi.org/10.1016/1055-7903(92)90020-H)

- 597 Bunting B, Georgi CDV, Milsum JN (1934) The oil palm in Malaya: Malayan Planting Manual No. 1.
598 Department of Agriculture, Kuala Lumpur
- 599 Chee KH (1975) Pathogenicity of *Phytophthora palmivora* from *Hevea brasiliensis*. Trans Br Mycol Soc
600 65:153-157. [https://doi.org/10.1016/S0007-1536\(75\)80196-3](https://doi.org/10.1016/S0007-1536(75)80196-3)
- 601 Chliyah M et al. (2013) *Phytophthora palmivora*: A new pathogen of olive trees in Morocco. Atlas J Biol
602 2:130-135.
- 603 Cohen S, Allasia V, Venard P, Notter S, Vernière C, Panabières F (2003) Intraspecific Variation in
604 *Phytophthora citrophthora* from Citrus Trees in Eastern Corsica. Eur J Plant Pathol 109:791-805.
605 <https://doi.org/10.1023/A:1026190318631>
- 606 Concibido-Manohar E (2004) *Phytophthora* diseases of coconut in the Philippines. In: Drenth A, Guest DI
607 (eds) Diversity and management of *Phytophthora* in Southeast Asia, vol 27. ACIAR Monograph vol
608 114. ACIAR, Canberra, Australia, p 115
- 609 Cooke DEL, Anderson B (2013) *Phytophthora infestans* and potato late blight in Europe. In: Lamour K (ed)
610 *Phytophthora: A Global Perspective*. CAB International, United Kingdom, pp 59-67
- 611 Cooke DEL, Drenth A, Duncan JM, Wagels G, Brasier CM (2000) A molecular phylogeny of *Phytophthora*
612 and related oomycetes. Fungal Genet Biol 30:17-32. <https://doi.org/10.1006/fgbi.2000.1202>
- 613 Cooke DEL, Duncan JM (1997) Phylogenetic analysis of *Phytophthora* species based on ITS1 and ITS2
614 sequences of the ribosomal RNA gene repeat. Mycol Res 101:667-677.
615 <https://doi.org/10.1017/S0953756296003218>
- 616 Crawford AR, Bassam BJ, Drenth A, MacLean DJ, Irwin JAG (1996) Evolutionary relationships among
617 *Phytophthora* species deduced from rDNA sequence analysis. Mycol Res 100:437-443.
618 [https://doi.org/10.1016/S0953-7562\(96\)80140-7](https://doi.org/10.1016/S0953-7562(96)80140-7)
- 619 Darus A (2000) Major diseases of oil palm. In: Basiron Y, Jalani BS, Chan KW (eds) *Advances in Oil Palm*
620 *Research*, vol 1. Malaysian Palm Oil Board, Bangi, Malaysia, pp 596-622
- 621 De Franqueville H (2003) Oil palm bud rot in Latin America. Exp Agr 39:225-240.
622 <https://doi.org/10.1017/S0014479703001315>
- 623 Dick MA, Williams NM, Bader MKF, Gardner JF, Bulman LS (2014) Pathogenicity of *Phytophthora pluvialis*
624 to *Pinus radiata* and its relation with red needle cast disease in New Zealand. N Z J For Sci 44:6.
625 <https://doi.org/10.1186/s40490-014-0006-7>
- 626 Drenth A, Guest DI (2004) *Phytophthora* in the tropics. In: Drenth A, Guest D (eds) *Diversity and management*
627 *of Phytophthora in Southeast Asia*. ACIAR Monograph vol 114. ACIAR, Canberra, Australia, pp 30-
628 41
- 629 Drenth A, Sendall B (2001) *Practical guide to detection and identification of Phytophthora*. CRC Tropical
630 Plant Protection, Australia
- 631 Elliott ML (2006) Bud rots of palm, IFAS Extension: Fact Sheet PP-220. Plant Pathology Department, Florida
632 Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida,
633 USA
- 634 Fawke S, Doumane M, Schornack S (2015) Oomycete interactions with plants: infection strategies and
635 resistance principles. Microbiol Mol Biol Rev 79:263-280. <https://doi.org/10.1128/MMBR.00010-15>
- 636 Felsenstein J (1985) Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783-
637 791. <https://doi.org/10.1111/j.1558-5646.1985.tb00420.x>
- 638 Förster H, Cummings MP, Coffey MD (2000) Phylogenetic relationships of *Phytophthora* species based on
639 ribosomal ITS 1 DNA sequence analysis with emphasis on Waterhouse groups V and VI. Mycol Res
640 104:1055-1061. <https://doi.org/10.1017/S0953756200003087>
- 641 Gallegly ME, Hong C (2008) *Phytophthora*: Identifying species by morphology and DNA fingerprints.
642 American Phytopathological Society (APS Press), St. Paul
- 643 Garofalo JF, McMillan RT (2000) *Phytophthora* bud-rot of palms in South Florida. In: Proceedings of Florida
644 State Horticultural Society, Florida USA, 1999. Florida State Horticultural Society, pp 110-111
- 645 Ginetti B, Moricca S, Squires JN, Cooke DEL, Ragazzi A, Jung T (2014) *Phytophthora acerina* sp nov., a new
646 species causing bleeding cankers and dieback of *Acer pseudoplatanus* trees in planted forests in
647 Northern Italy. Plant Pathol 63:858-876. <https://doi.org/10.1111/ppa.12153>
- 648 Hampl V, Pavlíček A, Flegr J (2001) Construction and bootstrap analysis of DNA fingerprinting-based
649 phylogenetic trees with the freeware program FreeTree: Application to trichomonad parasites. Int J
650 Syst Evol Microbiol 51:731-735. <https://doi.org/10.1099/00207713-51-3-731>
- 651 Hawksworth DL, Kirk PM, Sutton BC, Pegler DN (1995) *Ainsworth and Bisby's Dictionary of the Fungi*. 8th
652 edn. CAB International, Wallingford, UK

- 653 Henry J, Gutiérrez M, Torres J, Chinchilla C, Escobar R, Mata R, Arauz F (2015) Recovery of oil palms (*Elaeis*
654 *guineensis* Jacq.) affected by spear rots. ASD Oil Palm Papers:17-29.
- 655 Ho HH (2018) The taxonomy and biology of *Phytophthora* and *Pythium*. J Bacteriol Mycol 6:40–45.
- 656 Hu J et al. (2013) Genetically diverse long-lived clonal lineages of *Phytophthora capsici* from pepper in Gansu,
657 China. Phytopathology 103:920-926. <https://doi.org/10.1094/PHYTO-01-13-0016-R>
- 658 Ivors KL, Hayden KJ, Bonants PJM, Rizzo DM, Garbelotto M (2004) AFLP and phylogenetic analyses of
659 North American and European populations of *Phytophthora ramorum*. Mycol Res 108:378-392.
660 <https://doi.org/10.1017/s0953756204009827>
- 661 Jović J, Cvrković T, Mitrović M, Petrović A, Krstić O, Krnjajić S, Toševski I (2011) Multigene sequence data
662 and genetic diversity among ‘Candidatus *Phytoplasma ulmi*’ strains infecting *Ulmus* spp. in Serbia.
663 Plant Pathol 60:356-368. <https://doi.org/10.1111/j.1365-3059.2010.02383.x>
- 664 Kastelein P, van Slobbe WG, De Leeuw GTN (1990) Symptomatological and histopathological observations
665 on oil palms from Brazil and Ecuador affected by fatal yellowing. Neth J Plant Pathol 96:113-117.
666 <https://doi.org/10.1007/BF02005135>
- 667 Korabecna M (2007) The variability in the fungal ribosomal DNA (ITS1, ITS2, and 5.8 S rRNA gene): its
668 biological meaning and application in medical mycology. In: Méndez-Vilas A (ed) Communicating
669 Current Research and Educational Topics and Trends in Applied Microbiology, vol 2. Microbiology
670 Book Series. Formatex, Spain, pp 783-787
- 671 Kroon LPNM, Bakker FT, van den Bosch GBM, Bonants PJM, Flier WG (2004) Phylogenetic analysis of
672 *Phytophthora* species based on mitochondrial and nuclear DNA sequences. Fungal Genet Biol
673 41:766-782. <https://doi.org/10.1016/j.fgb.2004.03.007>
- 674 Lee SB, Taylor JW (1992) Phylogeny of five fungus-like protocistan *Phytophthora* species, inferred from the
675 internal transcribed spacers of ribosomal DNA. Mol Biol Evol 9:636-653.
676 <https://doi.org/10.1093/oxfordjournals.molbev.a040750>
- 677 Lim TK, Chan LG (1986) Fruit rot of durian caused by *Phytophthora palmivora*. Pertanika 9:269-276.
- 678 Maiti S, Sen C (1979) Fungal diseases of betel vine. PANS 25:150-157.
679 <https://doi.org/10.1080/09670877909411690>
- 680 Martin FN, Tooley PW (2003a) Phylogenetic relationships among *Phytophthora* species inferred from
681 sequence analysis of mitochondrially encoded cytochrome oxidase I and II genes. Mycologia 95:269-
682 284. <https://doi.org/10.1080/15572536.2004.11833112>
- 683 Martin FN, Tooley PW (2003b) Phylogenetic relationships of *Phytophthora ramorum*, *P. nemorosa*, and *P.*
684 *pseudosyringae*, three species recovered from areas in California with sudden oak death. Mycol Res
685 107:1379-1391. <https://doi.org/10.1017/S0953756203008785>
- 686 Martinez G (2009) Bud rot, sudden wilt, red ring and lethal wilt in oil palm in Central-South America. In:
687 unedited (ed) Proceeding of the International Workshop on Awareness, Detection and Control of Oil
688 Palm Devastating Diseases, Kuala Lumpur Convention Centre, Kuala Lumpur, Malaysia, 2009.
689 MPOB-IOPRI, pp 113-131
- 690 Martínez G, Sarria GA, Torres GA, Varon F, Romero HM, Sanz JI Advances in the research of *Phytophthora*
691 *palmivora*, the causal agent of bud rot of oil palm in Colombia. In: unedited (ed) Proceedings of
692 PIPOC 2009: Agriculture, Biotechnology and Sustainability Conference, Kuala Lumpur, Malaysia,
693 2009. Malaysian Palm Oil Board (MPOB), pp 875-890
- 694 Martínez G, Sarria GA, Varón F (2010) *Phytophthora palmivora* es el agente causal de la pudrición del cogollo
695 de la palma de aceite. Palmas 1:334-344.
- 696 Mohamed Azni INA, Ramachandran V, Idris ASS, Shamala (2016) *Phytophthora palmivora*: Pathogenicity
697 study of Malaysian isolates on oil palm, cocoa and durian. Paper presented at the 6th IOPRI-MPOB
698 International Seminar: Current Research and Management of Pests, Ganoderma, and Pollination in
699 Oil Palm for Higher Productivity, Medan, Indonesia, 27-29 September 2016
- 700 Mueller UG, Lipari SE, Milgroom MG (1996) Amplified fragment length polymorphism (AFLP)
701 fingerprinting of symbiotic fungi cultured by the fungus-growing ant *Cyphomyrmex minutus*. Mol
702 Ecol 5:119-122. <https://doi.org/10.1111/j.1365-294X.1996.tb00297.x>
- 703 Mueller UG, Wolfenbarger LL (1999) AFLP genotyping and fingerprinting. Trends Ecol Evol 14:389-394.
704 [https://doi.org/10.1016/S0169-5347\(99\)01659-6](https://doi.org/10.1016/S0169-5347(99)01659-6)
- 705 Nalim FA, Elmer WH, McGovern RJ, Geiser DM (2009) Multilocus phylogenetic diversity of *Fusarium*
706 *avenaceum* pathogenic on lisianthus. Phytopathology 99:462-468. <https://doi.org/10.1094/PHYTO-99-4-0462>
- 707

- 708 Navia EA, Ávila RA, Daza EE, Restrepo EF, Romero HM (2014) Assessment of tolerance to bud rot in oil
709 palm under field conditions. *Eur J Plant Pathol* 140:711-720. [https://doi.org/10.1007/s10658-014-](https://doi.org/10.1007/s10658-014-0491-9)
710 [0491-9](https://doi.org/10.1007/s10658-014-0491-9)
- 711 Nei M, Li W-H (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases.
712 *Proc Natl Acad Sci U S A* 76:5269-5273.
- 713 O'Gara E, Sangchote S, Fitzgerald L, Wood D, AngChing S, Guest DI, Drenth A (2004a) Infection biology of
714 *Phytophthora palmivora* Butl. in *Durio zibethinus* L. (Durian) and responses induced by phosphonate.
715 In: Drenth A, Guest DI (eds) Diversity and Management of Phytophthora in Southeast Asia. ACIAR
716 Monograph, vol 114. ACIAR, Canberra, Australia, pp 42-52
- 717 O'Gara E, Vawdrey L, Martin T, Sangchote S, van Thanh H, Guest DI, Drenth A (2004b) Screening for
718 resistance to Phytophthora. In: Drenth A, Guest DI (eds) Diversity and Management of Phytophthora
719 in Southeast Asia. ACIAR Monograph vol 114. ACIAR, Canberra, Australia, pp 194-199
- 720 Ordoñez ME, Jácome DA, Keil CB, Montúfar RJ, Evans TA (2016) First report of *Phytophthora palmivora*
721 causing bud rot on palmito (*Bactris gasipaes*) in Ecuador. *Plant Dis* 100:1248.
722 <http://dx.doi.org/10.1094/PDIS-11-15-1354-PDN>
- 723 Pavlicek A, Hrda S, Flegr J (1999) Free-Tree, freeware program for construction of phylogenetic trees on the
724 basis of distance data and bootstrap/jackknife analysis of the tree robustness: Application in the RAPD
725 analysis of genus *Frenkelia*. *Folia Biol* 45:97-99.
- 726 Pongpisutta R, Sangchote S (2004) Morphological and host range variability in *Phytophthora palmivora* from
727 durian in Thailand. In: Drenth A, Guest DI (eds) Diversity and Management of Phytophthora in
728 Southeast Asia. ACIAR Monograph vol 114. ACIAR, Canberra, Australia, p 53
- 729 Raftoyannis Y, Dick MW (2006a) Effect of oomycete and plant variation on zoospore cover and disease
730 severity. *J Plant Pathol*:95-101. <https://www.jstor.org/stable/41998286>
- 731 Raftoyannis Y, Dick MW (2006b) Zoospore encystment and pathogenicity of Phytophthora and Pythium
732 species on plant roots. *Microbiol Res* 161:1-8. <https://doi.org/10.1016/j.micres.2005.04.003>
- 733 Rahman MZ, Uematsu S, Suga H, Kageyama K (2015) Diversity of Phytophthora species newly reported from
734 Japanese horticultural production. *Mycoscience* 56:443-459.
735 <https://doi.org/10.1016/j.myc.2015.01.002>
- 736 Ritland CE, Ritland K, Straus NA (1993) Variation in the ribosomal internal transcribed spacers (ITS1 and
737 ITS2) among eight taxa of the *Mimulus guttatus* species complex. *Mol Biol Evol* 10:1273-1288.
738 <https://doi.org/10.1093/oxfordjournals.molbev.a040067>
- 739 Robideau GP et al. (2011) DNA barcoding of oomycetes with cytochrome c oxidase subunit I and internal
740 transcribed spacer. *Mol Ecol Resour* 11:1002-1011. [https://doi.org/10.1111/j.1755-](https://doi.org/10.1111/j.1755-0998.2011.03041.x)
741 [0998.2011.03041.x](https://doi.org/10.1111/j.1755-0998.2011.03041.x)
- 742 Saitou N, Nei M (1987) The neighbor-joining method - A new method for reconstructing phylogenetic trees.
743 *Mol Biol Evol* 4:406-425. <https://doi.org/10.1093/oxfordjournals.molbev.a040454>
- 744 Sanderson MJ, Driskell AC, Ree RH, Eulenstein O, Langley S (2003) Obtaining maximal concatenated
745 phylogenetic data sets from large sequence databases. *Mol Biol Evol* 20:1036-1042.
746 <https://doi.org/10.1093/molbev/msg115>
- 747 Sankar MS, Nath VS, Misra RS, Lajapathy Jeeva M (2013) Incidence and identification of cassava tuber rot
748 caused by *Phytophthora palmivora*. *Arch Phytopathology Plant Protect* 46:741-746.
749 <https://doi.org/10.1080/03235408.2012.751284>
- 750 Sarria GA, Martinez G, Varon F, Drenth A, Guest DI (2016) Histopathological studies of the process of
751 *Phytophthora palmivora* infection in oil palm. *Eur J Plant Pathol* 145:39-51.
752 <https://doi.org/10.1007/s10658-015-0810-9>
- 753 Sarria GV (2013) Nuevas evidencias del cumplimiento de los postulados de Koch en el estudio de las relaciones
754 entre *Phytophthora palmivora* y la pudrición del cogollo de la palma de aceite. *Revista Palmas* 34:73-
755 83.
- 756 Scibetta S, Schena L, Chimento A, Cacciola SO, Cooke DEL (2012) A molecular method to assess
757 Phytophthora diversity in environmental samples. *J Microbiol Methods* 88:356-368.
758 <https://doi.org/10.1016/j.mimet.2011.12.012>
- 759 Sdoodee R (2004) Phytophthora diseases of rubber. In: Drenth A, Guest DI (eds) Diversity and Management
760 of *Phytophthora* in Southeast Asia. ACIAR Monograph vol 114. ACIAR, Canberra, Australia, pp
761 136-142

762 Shan W, Cao M, Leung D, Tyler BM (2004) The Avr1b locus of *Phytophthora sojae* encodes an elicitor and a
763 regulator required for avirulence on soybean plants carrying resistance gene Rps1b. *Mol Plant*
764 *Microbe Interact* 17:394-403. <https://doi.org/10.1094/mpmi.2004.17.4.394>

765 Sharadraj KM, Mohanan RC (2014) A new detached coconut leaf let technique for bioassay of fungicides
766 against *Phytophthora palmivora* – The incitant of coconut bud rot. *Int J Plant Prot* 7:161-165.

767 Sharples A (1928) Palm diseases in Malaya. *Malayan Agricultural Journal* 16:313-360 pp.

768 Shimomoto Y et al. (2011) Pathogenic and genetic variation among isolates of *Corynespora cassiicola* in
769 Japan. *Plant Pathol* 60:253-260. <https://doi.org/10.1111/j.1365-3059.2010.02374.x>

770 Smith J, Flood J (2001) Epidemiology and population structure of *Phytophthora* species causing diseases of
771 coconut in Indonesia. CABI Bioscience UK Centre, Egham, Surrey, UK, United Kingdom

772 Sorensen RE, Curtis J, Minchella DJ (1998) Intraspecific variation in the rDNA ITS loci of 37-collar-spined
773 Echinostomes from North America: Implications for sequence-based diagnoses and phylogenetics. *J*
774 *Parasitol* 84:992-997. <https://doi.org/10.2307/3284633>

775 Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of
776 mitochondrial DNA in humans and chimpanzees. *Mol Biol Evol* 10:512-526.
777 <https://doi.org/10.1093/oxfordjournals.molbev.a040023>

778 Tamura K, Nei M, Kumar S (2004) Prospects for inferring very large phylogenies by using the neighbor-
779 joining method. *Proc Natl Acad Sci U S A* 101:11030-11035.
780 <https://doi.org/10.1073/pnas.0404206101>

781 Tamura K, Stecher G, Peterson D, Filipiński A, Kumar S (2013) MEGA 6: Molecular evolutionary genetics
782 analysis version 6.0. *Mol Biol Evol* <https://doi.org/10.1093/molbev/mst197>

783 Tashiro N, Uematsu S, Ide Y, Matsuzaki M (2012) First report of *Phytophthora palmivora* as a causal pathogen
784 of citrus brown rot in Japan. *J Gen Plant Pathol* 78:233-236. <https://doi.org/10.1007/s10327-012-0378-6>

785

786 Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: Improving the sensitivity of progressive multiple
787 sequence alignment through sequence weighting, position-specific gap penalties and weight matrix
788 choice. *Nucleic Acids Res* 22:4673-4680. <https://doi.org/10.1093/nar/22.22.4673>

789 Torres GA, Sarria GA, Martinez G, Varon F, Drenth A, Guest DI (2016) Bud rot caused by *Phytophthora*
790 *palmivora*: A destructive emerging disease of oil palm. *Phytopathology* 106:320-329.
791 <https://doi.org/10.1094/PHYTO-09-15-0243-RVW>

792 Torres GA, Sarria GA, Varon F, Coffey MD, Elliott ML, Martinez G (2010) First report of bud rot caused by
793 *Phytophthora palmivora* on African oil palm in Colombia. *Plant Dis* 94:1163-1163.
794 <https://doi.org/10.1094/PDIS-94-9-1163A>

795 Tri MV et al. (2015) Decline of jackfruit (*Artocarpus heterophyllus*) incited by *Phytophthora palmivora* in
796 Vietnam. *Phytopathol Mediterr* 54:275. http://dx.doi.org/10.14601/Phytopathol_Mediterr-15008

797 Tsao PH, Ocana G (1969) Selective isolation of species of *Phytophthora* from natural soils on an improved
798 antibiotic medium. *Nature* 223:636-638. <https://doi.org/10.1038/223636a0>

799 Türkölmez Ş, Çiftçi O, Serçe ÇU, Derviş S (2015) First report of *Phytophthora* crown and root rot of cherry
800 caused by *Phytophthora palmivora* in eastern Turkey. *Can J Plant Pathol* 37:390-396.
801 <https://doi.org/10.1080/07060661.2015.1055517>

802 Turner GJ (1969a) Leaf lesions associated with foot rot of *Piper nigrum* and *P. betle* caused by *Phytophthora*
803 *palmivora*. *Trans Br Mycol Soc* 53:407-IN401. [http://dx.doi.org/10.1016/S0007-1536\(69\)80098-7](http://dx.doi.org/10.1016/S0007-1536(69)80098-7)

804 Turner GJ (1969b) *Phytophthora palmivora* from piper betle in Sarawak. *Trans Br Mycol Soc* 52:411-418.
805 [http://dx.doi.org/10.1016/S0007-1536\(69\)80124-5](http://dx.doi.org/10.1016/S0007-1536(69)80124-5)

806 Turner PD (1960) Strains of *Phytophthora palmivora* (Butl.) from *theobroma cacao* L.: I. Isolates from West
807 Africa. *Trans Br Mycol Soc* 43:665-IN667. [http://dx.doi.org/10.1016/S0007-1536\(60\)80057-5](http://dx.doi.org/10.1016/S0007-1536(60)80057-5)

808 Turner PD (1981) *Oil Palm Diseases and Disorders*. Oxford University Press, Kuala Lumpur

809 Tyler BM et al. (2006) *Phytophthora* genome sequences uncover evolutionary origins and mechanisms of
810 pathogenesis. *Science* 313:1261-1266. <http://dx.doi.org/10.1126/science.1128796>

811 Uchida JY, Aragaki M (1991) *Phytophthora* diseases of orchids in Hawaii. University of Hawaii, Hawaii

812 Vaidya G, Lohman DJ, Meier R (2011) SequenceMatrix: Concatenation software for the fast assembly of
813 multi-gene datasets with character set and codon information. *Cladistics* 27:171-180.
814 <http://dx.doi.org/10.1111/j.1096-0031.2010.00329.x>

815 Van de Lande HL, Zadoks JC (1999) Spatial patterns of spear rot in oil palm plantations in Surinam. *Plant*
816 *Pathol* 48:189-201. <http://dx.doi.org/10.1046/j.1365-3059.1999.00331.x>

817 van Poppel PMJA (2009) The *Phytophthora infestans* avirulence gene PiAvr4 and its potato counterpart R4.
818 Wageningen University
819 van Poppel PMJA, Guo J, van de Vondervoort PJI, Jung MWM, Birch PRJ, Whisson SC, Govers F (2008) The
820 *Phytophthora infestans* avirulence gene Avr4 encodes an RXLR-dEER effector. Mol Plant Microbe
821 Interact 21:1460-1470. <https://doi.org/10.1094/MPMI-21-11-1460>
822 Van West P et al. (2002) Oomycete plant pathogens use electric fields to target roots. Mol Plant Microbe
823 Interact 15:790-798. <https://doi.org/10.1094/MPMI.2002.15.8.790>
824 Villa NO, Kageyama K, Asano T, Suga H (2006) Phylogenetic relationships of Pythium and Phytophthora
825 species based on ITS rDNA, cytochrome oxidase II and beta-tubulin gene sequences. Mycologia
826 98:410-422. <https://doi.org/10.1080/15572536.2006.11832676>
827 Vinuesa MdLA, Sanches-Puelles JM, Tibell L (2001) Intraspecific variation in *Mycocalicium subtile*
828 (*Mycocaliciaceae*) elucidated by morphology and the sequences of the ITS1-5.8 S-ITS2 region of
829 rDNA. Mycol Res 105:323-330. <https://doi.org/10.1017/S0953756201003604>
830 Waterhouse GM (1963) Key to the species of Phytophthora de Bary. Mycological Papers 92:1-22.
831 White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA
832 genes for phylogenetics. In: PCR Protocols: A Guide to Methods and Applications. Academic Press,
833 New York, pp 315-322
834 Zentmyer GA (1988) Origin and distribution of four species of Phytophthora. Trans Br Mycol Soc 91:367-
835 378. [http://dx.doi.org/10.1016/S0007-1536\(88\)80111-6](http://dx.doi.org/10.1016/S0007-1536(88)80111-6)

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Table 2 Details of isolates collected in this study

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No.	Isolate	Species based on the source database	IDENTIFY IN THIS STUDY	Origin	Ex-host	Source*	GeneBank Accession Number				
							ITS	<i>B-tubuli</i>	<i>CoxI</i>	<i>CoxII</i>	<i>EF-1a</i>
1	PPM1	<i>P. palmivora</i>	<i>P. palmivora</i>	Malaysia	<i>Theobroma cacao</i> (cocoa)	MPOB	KY19 7718	MH40 1213	MH76 0206	MH76 0244	MH76 0169
2	PPM2	unknown	<i>P. palmivora</i>	Malaysia	<i>Theobroma cacao</i> (cocoa)	MPOB	KY19 7719	MH40 1214	MH76 0207	MH76 0245	MH76 0170
3	PPM3	unknown	<i>P. palmivora</i>	Malaysia	<i>Theobroma cacao</i> (cocoa)	MPOB	KY19 7720	MH40 1215	MH76 0208	MH76 0246	MH76 0171
4	PPM4	unknown	<i>P. palmivora</i>	Malaysia	<i>Durio zibethinus</i> (durian)	MPOB	KY19 7721	MH40 1216	MH76 0209	MH76 0247	MH76 0172
5	PPM5	<i>P. palmivora</i>	<i>P. palmivora</i>	Malaysia	<i>Durio zibethinus</i> (durian)	MPOB	KY19 7722	MH40 1217	MH76 0210	MH76 0248	MH76 0173
6	PPM6	unknown	<i>P. palmivora</i>	Malaysia	<i>Theobroma cacao</i> (cocoa)	MPOB	KY19 7723	MH40 1218	na	na	na
7	P6948	<i>P. palmivora</i>	<i>P. palmivora</i>	Malaysia	<i>Hevea brasiliensis</i> (rubber)	WOGRC	KY47 5615	MH40 1219	MH76 0211	MH76 0249	MH76 0174
8	PPC280 574	<i>P. palmivora</i>	<i>P. palmivora</i>	Colombia, Tumaco	<i>Elaeis guineensis</i> (African oil palm)	CENIPA LMA	KY47 5616	MH40 1220	MH76 0212	MH76 0250	MH76 0175
9	P16828	<i>P. palmivora</i>	<i>P. palmivora</i>	Colombia, Central Zone	<i>Elaeis guineensis</i> (African oil palm)	WOGRC	KY47 5617	MH40 1221	MH76 0213	MH76 0251	MH76 0176
10	P16831	<i>P. palmivora</i>	<i>P. palmivora</i>	Colombia, Tumaco	<i>Elaeis guineensis</i> (African oil palm)	WOGRC	KY47 5618	MH40 1222	MH76 0214	MH76 0252	MH76 0177
11	P8513	<i>P. palmivora</i>	<i>P. palmivora</i>	Colombia	<i>Theobroma cacao</i> (cocoa)	WOGRC	KY47 5619	MH40 1223	MH76 0215	MH76 0253	MH76 0178
12	P0497	<i>P. palmivora</i>	<i>P. palmivora</i>	Colombia	<i>Theobroma cacao</i> (cocoa)	WOGRC	KY47 5620	MH40 1224	MH76 0216	MH76 0254	MH76 0179
13	IMI382 544	<i>P. palmivora</i>	<i>P. palmivora</i>	Indonesia	<i>Cocos nucifera</i> (coconut)	CABI Bioscience	KY47 5621	MH44 5343	MH76 0217	MH76 0255	MH76 0180

14	IMI382 528	<i>P. palmivora</i>	<i>P. palmivora</i>	Indonesia	<i>Cocos nucifera (coconut)</i>	CABI Biosciences	KY47 5622	MH44 5344	MH76 0218	MH76 0256	MH76 0181
15	P3767	<i>P. palmivora</i>	<i>P. palmivora</i>	Indonesia	<i>Cocos nucifera (coconut)</i>	WOGRC	KY47 5623	MH44 5345	MH76 0219	MH76 0257	MH76 0182
16	CBS236 .30	<i>P. palmivora</i>	<i>P. palmivora</i>	India	<i>Cocos nucifera (coconut)</i>	CBS- KNAW	KY47 5624	MH44 5346	MH76 0220	MH76 0258	MH76 0183
17	P16385	<i>P. palmivora</i>	<i>P. palmivora</i>	California, USA	<i>Howea forsteriana (kentia palm)</i>	WOGRC	KY47 5625	MH44 5347	MH76 0221	MH76 0259	MH76 0184
18	P11007	<i>P. palmivora</i>	<i>P. palmivora</i>	Guam	<i>Areca catechu (betel palm)</i>	WOGRC	KY47 5626	MH44 5348	MH76 0222	MH76 0260	MH76 0185
19	CBS179 .26	<i>P. palmivora</i>	<i>P. palmivora</i>	Sri Lanka	<i>Theobroma cacao (cocoa)</i>	CBS- KNAW	KY47 5627	MH44 5349	MH76 0223	MH76 0261	MH76 0186
20	CBS298 .29	<i>P. palmivora</i>	<i>P. palmivora</i>	Trinidad & Tobago	<i>Theobroma cacao (cocoa)</i>	CBS- KNAW	KY47 5628	MH44 5350	MH76 0224	MH76 0262	MH76 0187
21	PPG1	unknown	<i>P. palmivora</i>	Ghana	<i>Theobroma cacao (cocoa)</i>	OPRI	KY47 5629	MH44 5351	MH76 0225	MH76 0263	MH76 0188
22	PPG8	unknown	<i>P. palmivora</i>	Ghana	<i>Theobroma cacao (cocoa)</i>	OPRI	KY47 5630	MH44 5352	MH76 0226	MH76 0264	MH76 0189
23	PPG11	unknown	<i>P. palmivora</i>	Ghana	<i>Theobroma cacao (cocoa)</i>	OPRI	KY47 5631	MH44 5353	MH76 0227	MH76 0265	MH76 0190
24	PPG13	unknown	<i>P. palmivora</i>	Ghana	<i>Theobroma cacao (cocoa)</i>	OPRI	KY47 5632	MH44 5354	MH76 0228	MH76 0266	MH76 0191
25	CBS111 346	<i>P. palmivora</i>	<i>P. palmivora</i>	South Korea	<i>Cymbidium</i> spp. (orchid)	CBS- KNAW	KY47 5633	MH44 5355	MH76 0229	MH76 0267	MH76 0192
26	PPC261 4P	<i>P. palmivora</i>	<i>P. palmivora</i>	Colombia, Tumaco	<i>Elaeis guineensis (African oil palm)</i>	CENIPA LMA	MH40 1198	na	MH76 0230	MH76 0268	MH76 0193
27	PPC361 4L	<i>P. palmivora</i>	<i>P. palmivora</i>	Colombia, Tumaco	<i>Elaeis guineensis (African oil palm)</i>	CENIPA LMA	MH40 1199	na	MH76 0231	MH76 0269	MH76 0194
28	CBS148 .88	<i>P. arecae</i>	<i>P. arecae</i>	USA (Florida)	<i>Chamaedorea sefritzii (bamboo palm)</i>	CBS- KNAW	MH40 1200	na	MH76 0232	MH76 0270	MH76 0195
29	PPM7	unknown	<i>P. palmivora</i>	Malaysia	<i>Theobroma cacao (cocoa)</i>	MPOB	na	na	na	na	na
30	PPM8	unknown	<i>P. palmivora</i>	Malaysia	<i>Durio zibethinus (durian)</i>	MPOB	na	na	na	na	na
31	P19537	<i>P. palmivora</i>	<i>P. parasitica</i>	Colombia	<i>Elaeis guineensis (African oil palm)</i>	WOGRC	MH40 1208	MH76 0160	MH76 0233	MH76 0271	MH76 0196
32	P19538	<i>P. palmivora</i>	<i>P. parasitica</i>	Colombia	<i>Elaeis guineensis (African oil palm)</i>	WOGRC	MH40 1209	MH76 0161	MH76 0234	MH76 0272	MH76 0197

33	CBS358 .59	<i>P. palmivora</i>	<i>P. colocasiae</i>	Sri Lanka	<i>Hevea brasiliensis</i> (rubber)	CBS- KNAW	MH40 1210	MH76 0162	MH76 0235	MH76 0273	MH76 0198
34	PPG3	unknown	<i>P. megakarya</i>	Ghana	<i>Theobroma cacao</i> (cocoa)	OPRI	MH40 1202	MH76 0163	MH76 0236	MH76 0274	MH76 0199
35	PPG4	unknown	<i>P. megakarya</i>	Ghana	<i>Theobroma cacao</i> (cocoa)	OPRI	MH40 1203	MH76 0164	MH76 0237	MH76 0275	MH76 0200
36	PPG12	unknown	<i>P. megakarya</i>	Ghana	<i>Theobroma cacao</i> (cocoa)	OPRI	MH40 1204	MH76 0165	MH76 0238	MH76 0276	MH76 0201
37	PC01	<i>P. cryptogea</i>	<i>P. cryptogea</i>	unknown	unknown	UoN	MH40 1205	MH76 0166	MH76 0239	MH76 0277	MH76 0202
38	13-A2	<i>P. infestans</i>	<i>P. infestans</i>	United Kingdom	<i>Solanum tuberosum</i> (potato)	UoN	MH40 1206	na	MH76 0240	MH76 0278	MH76 0203
39	2009- 7654A	<i>P. infestans</i>	<i>P. infestans</i>	United Kingdom	<i>Solanum tuberosum</i> (potato)	UoN	MH40 1207	na	MH76 0241	MH76 0279	MH76 0204
40	CBS581 .69	<i>P. botryosa</i>	<i>P. botryosa</i>	Malaysia	<i>Hevea brasiliensis</i> (rubber)	CBS- KNAW	MH40 1211	MH76 0167	MH76 0242	MH76 0280	MH76 0205
41	PYT01	<i>Pythium aphanidermatum</i>	<i>Pythium aphanidermatum</i>	Unknown	unknown	UoN	MH40 1212	MH76 0168	MH76 0243	MH76 0281	na
42	CCO20 83	<i>P. palmivora</i>	<i>P. palmivora</i>	unknown	unknown	FERA	MH40 1201	na	na	na	na

854 *Abbreviations of culture centres and source agencies: CBS-KNAW, Westerdijk Fungal
855 Biodiversity Institute, Netherlands; CABI, The Centre for Agriculture and Bioscience International,
856 UK; WOGRC, The World Oomycetes Genetic Resource Collection, University of California,
857 Riverside, USA; MPOB, Malaysian Palm Oil Board, Malaysia; OPRI, The Oil Palm Research
858 Institute (OPRI) of Ghana, Ghana; CENIPALMA, Colombian Oil Palm Research Center, Colombia;
859 FERA, The Food and Environment Research Agency, UK; UoN, University of Nottingham, UK.

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Table 3 First summer inoculation using African oil palm seedlings (Tenera)

Isolates	Host and origin	No. of inoculated palms	No. of palms with lesions	% of seedlings with lesions	% of palms recovered after 6 months
Ctrl (dH2O)	-	5	0	0	100
PPC280574	Oil palm - Colombia	5	3	60	100
P16828	Oil palm - Colombia	5	2	40	100
P16831	Oil palm - Colombia	5	1	20	100
P8513	Cocoa - Colombia	5	1	20	100
PPM1	Cocoa - Malaysia	5	4	80	100
PPM4	Durian - Malaysia	5	3	60	100
P6948	Rubber - Malaysia	5	4	80	100
IMI382544	Coconut - Indonesia	5	2	40	100
CBS1113.46	Cymbidium - South Korea	5	4	80	100
P11007	Betel palm - Guam	5	3	60	100
P16385	Kentia palm - California	5	3	60	100
PPG1	Cocoa-Ghana	5	2	40	100

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878 Table 4 Second round summer inoculation using African oil palm seedlings (Tenera)

Isolates	Host and origin	No. of inoculated palms	No. of palms with lesions	% of seedlings with lesions	% of palms recovered after 6 months
Ctrl (dH2O)	-	10	0	0	100
PPM1	Cocoa - Malaysia	10	3	30	100
P6948	Rubber - Malaysia	10	4	40	100
PPC280574	Oil palm - Colombia	10	5	50	100
P8513	Cocoa - Colombia	10	5	50	100

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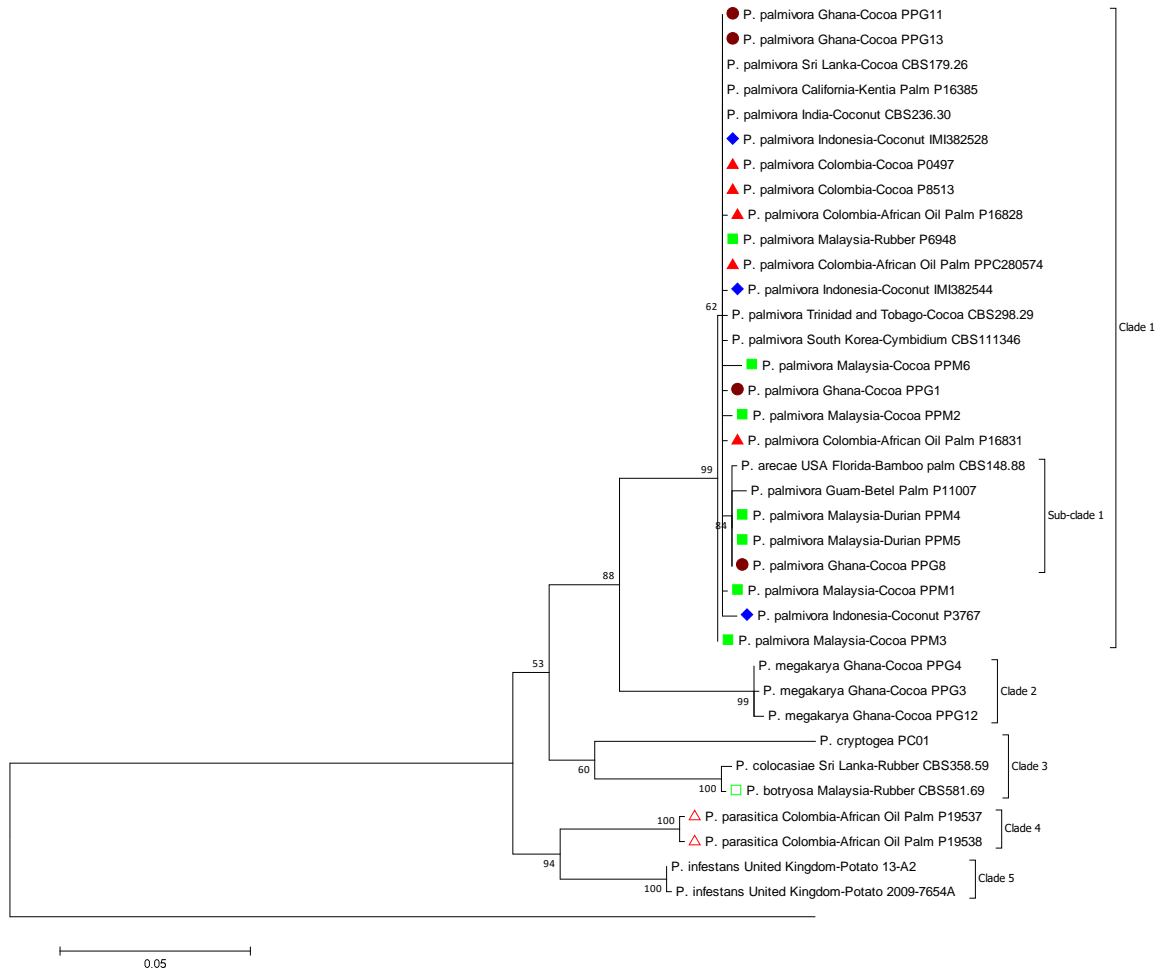


Figure 1 Molecular phylogenetic tree showing the relationship *P. palmivora* and other *Phytophthora* from different hosts and demographic origin constructed from ITS rDNA data using Maximum Likelihood method based on the Tamura-Nei model. The tree with the highest log likelihood (-3074.7150) is shown.

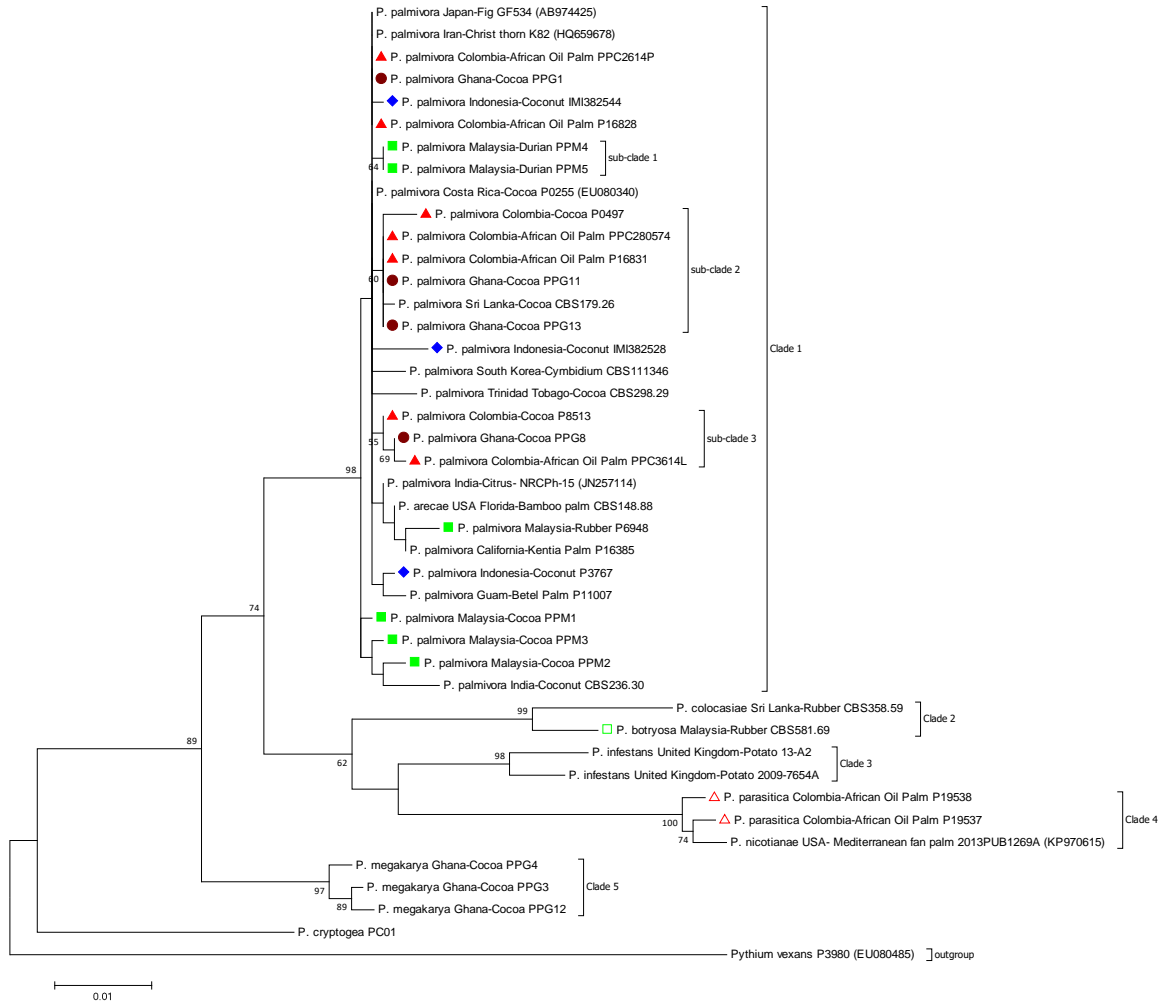


Figure 2 Molecular phylogenetic tree showing the relationship of *P. palmivora* and other *Phytophthora* from different hosts and demographic origin constructed from partial gene sequences of *translation elongation factor 1 alpha (EF-1 α)* using maximum likelihood method based on the Tamura-Nei model. Note: The tree with the highest log likelihood likelihood (-2832.2482) is shown.

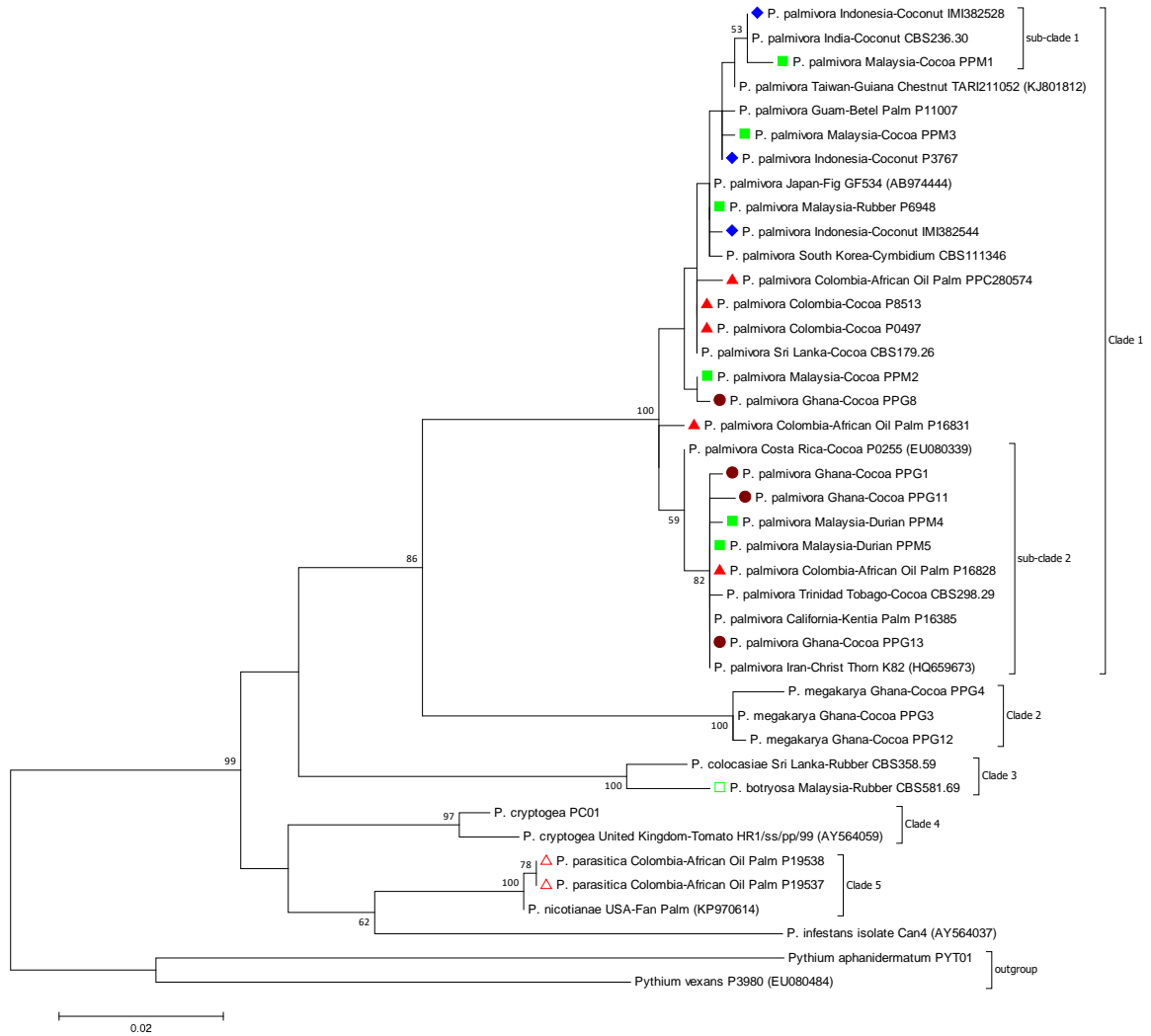


Figure 3 Molecular phylogenetic tree showing the relationship of *P. palmivora* and other *Phytophthora* from different hosts and demographic origin constructed from partial gene sequences of β -*tubulin* using maximum likelihood method based on the Tamura-Nei model. Note: The tree with the highest log likelihood likelihood (-2351.2154) shown.

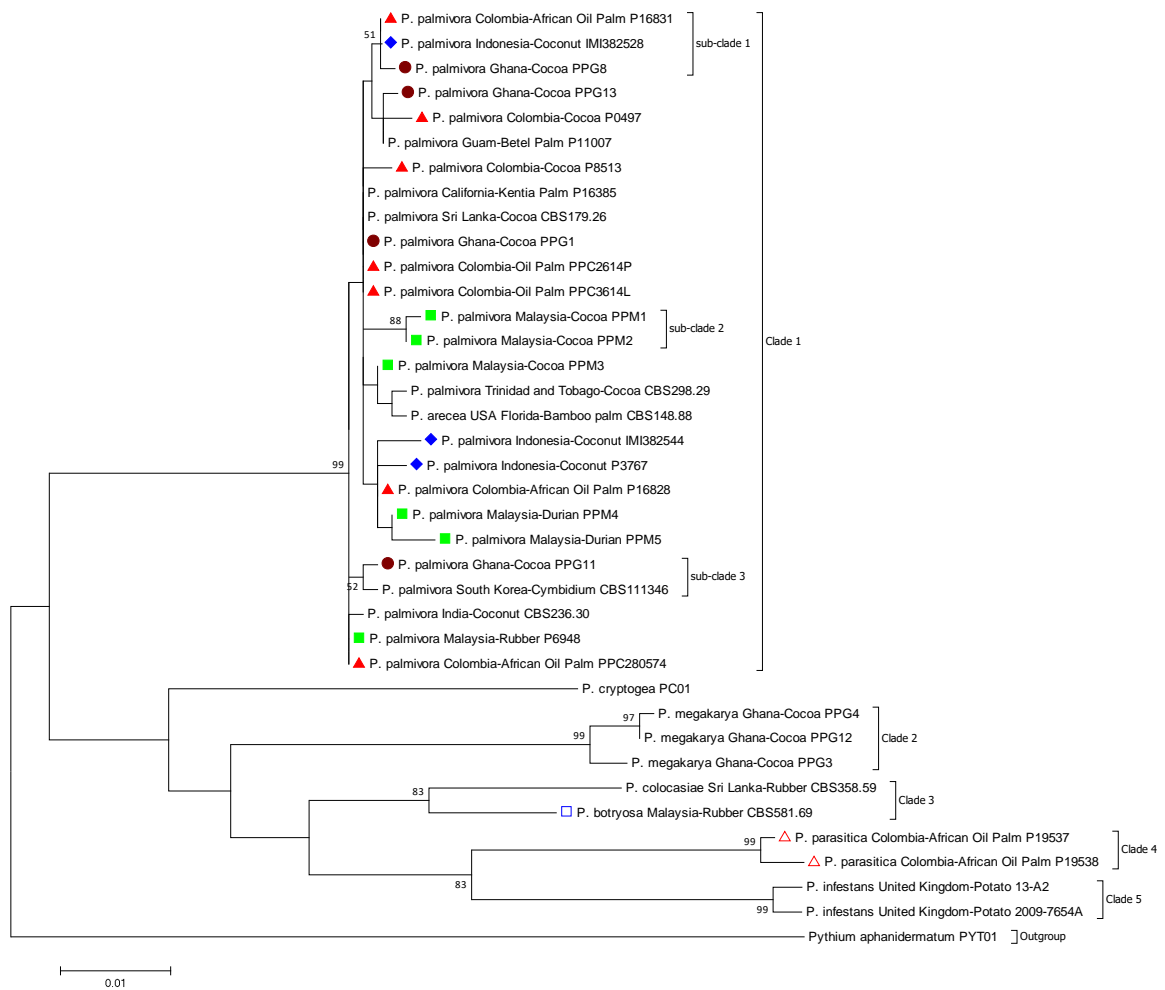


Figure 4 Molecular phylogenetic tree showing the relationship of *P. palmivora* and other *Phytophthora* from different hosts and demographic origin constructed from partial gene sequences of *cytochrome c oxidase subunit I (CoxI)* using maximum likelihood method based on the Tamura-Nei model. Note: The tree with the highest log likelihood likelihood (-2770.5889) is shown.

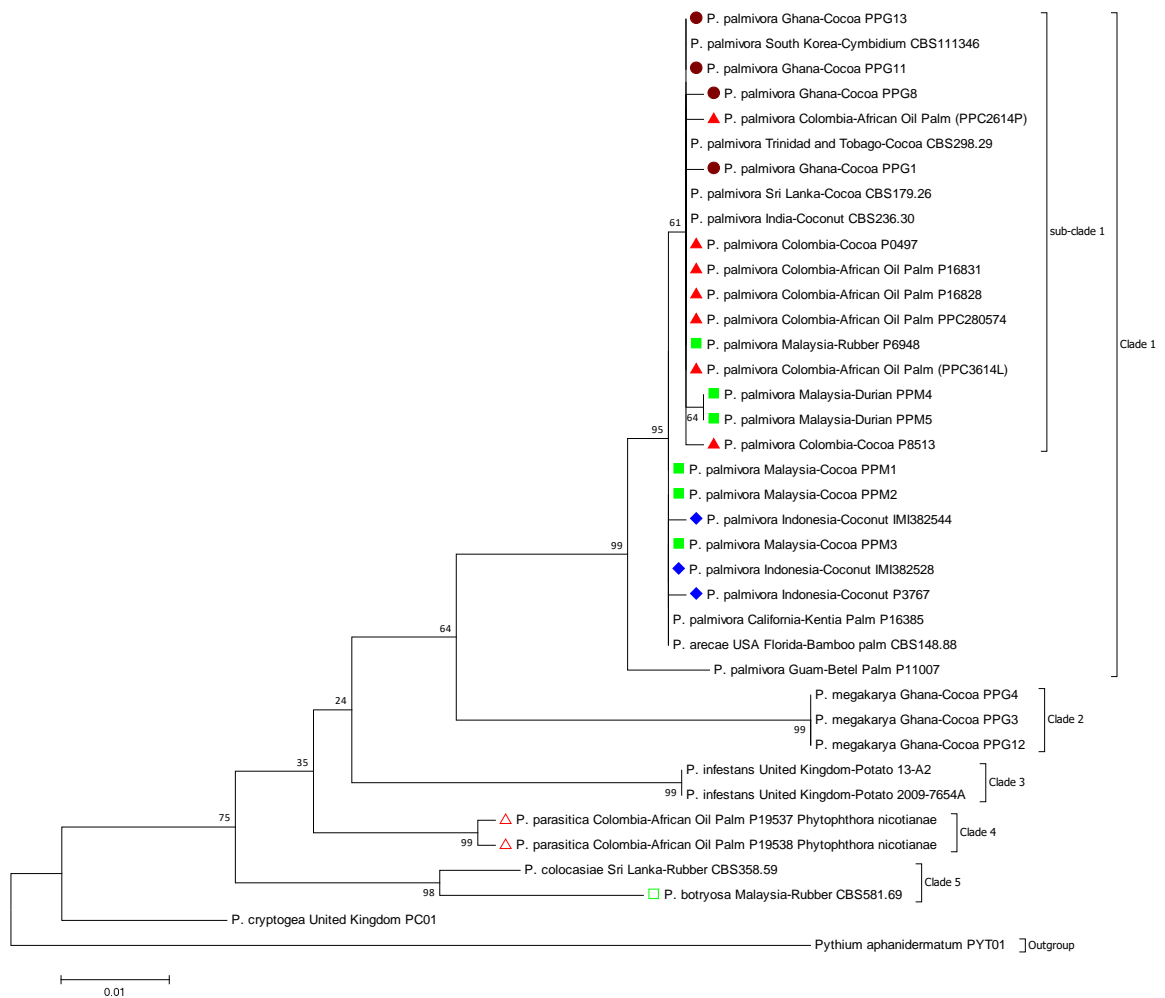


Figure 5 Molecular phylogenetic tree showing the relationship of *P. palmivora* and other *Phytophthora* from different hosts and demographic origin constructed from partial gene sequences of *cytochrome c oxidase subunit II (CoxII)* using maximum likelihood method based on the Tamura-Nei model.

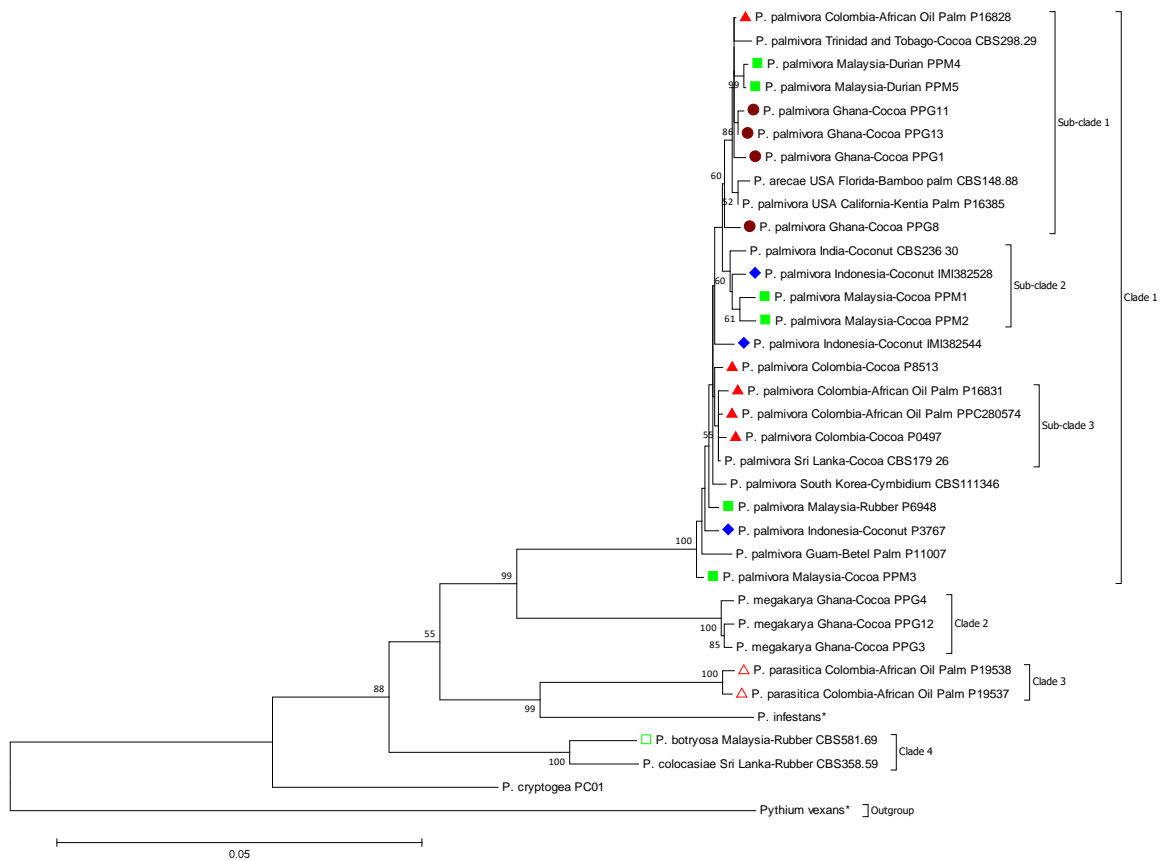


Figure 6 Molecular phylogenetic tree showing the relationship of *P. palmivora* and other *Phytophthora* from different hosts and demographic origin constructed from concatenated sequences of ITS, *EF-1 α* , *β -tubulin*, *CoxI* and *CoxII* using maximum likelihood method based on the Tamura-Nei model.

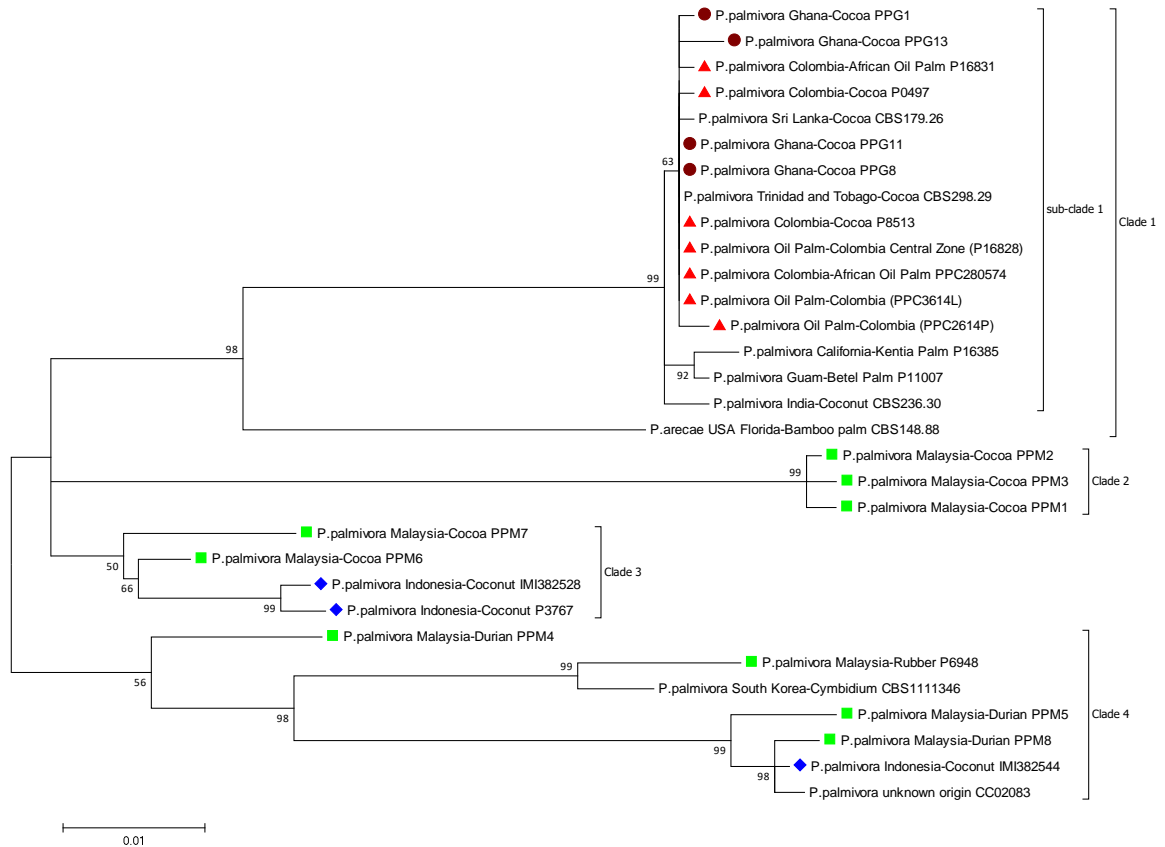
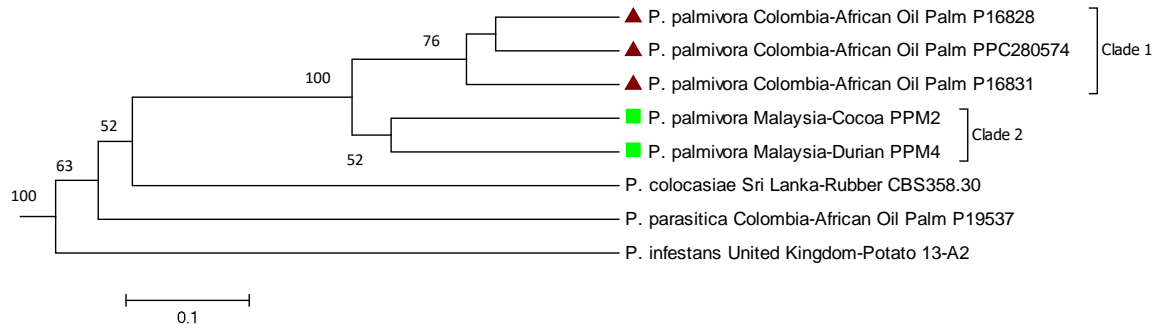
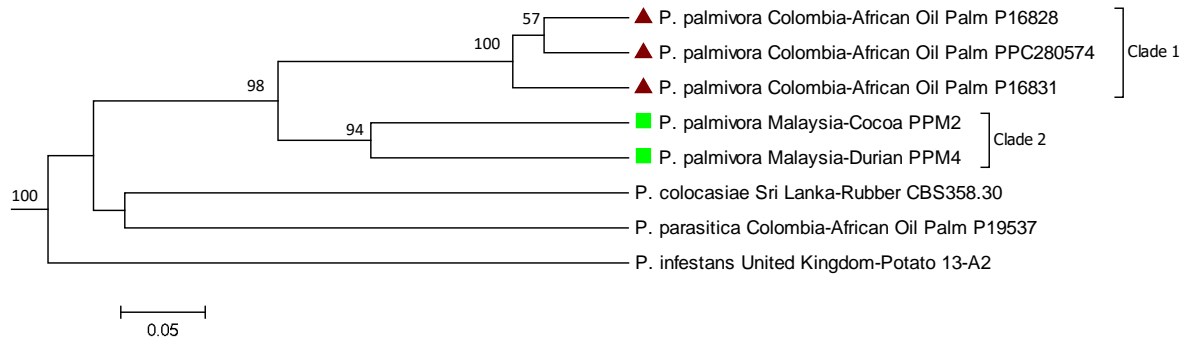


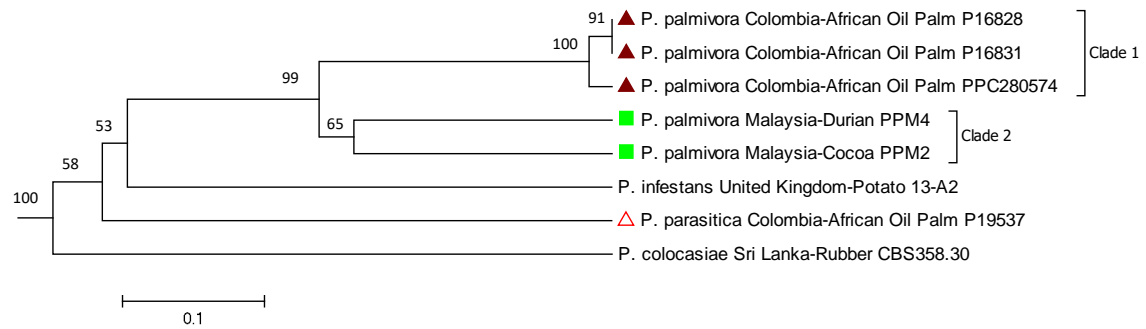
Figure 7 Molecular phylogenetic tree showing the relationship of *P. palmivora* isolated from oil palm originated from bud rot disease hotspot zone in Colombia with other isolates of *P. palmivora* from different hosts and demographic origin constructed from our new PpHPAV marker using maximum likelihood method based on the Tamura-Nei model. Note: The tree with the highest log likelihood likelihood is shown.



(a)



(b)



(c)

Figure 8 Phylogenetic tree constructed from AFLP data using primer (a) *EcoRI*-A/*MseI*-AG, b). *EcoRI*-AC/*MseI*-AG (c) *EcoRI*-TA/*MseI*-AG.

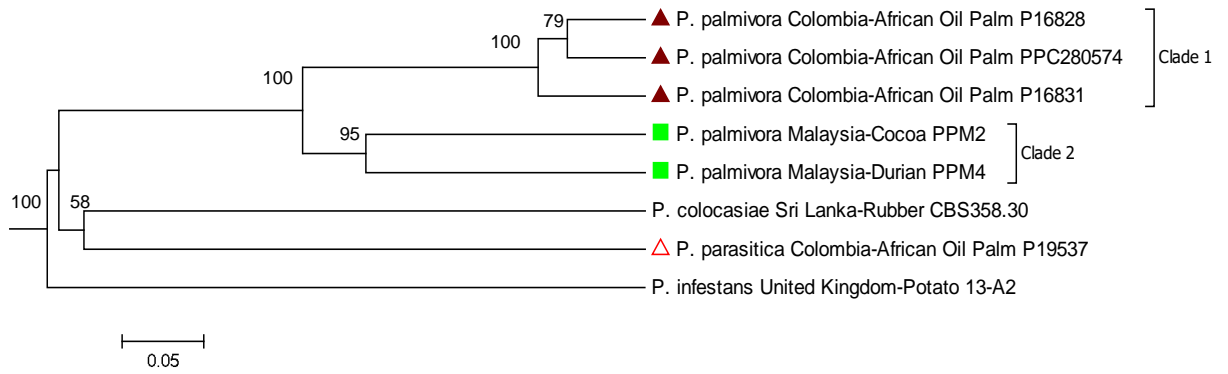


Figure 9 Phylogenetic tree constructed from concatenated AFLP data using three primer pairs of *EcoRI*-A/*MseI*-AG, *EcoRI*-AC/*MseI*-AG and *EcoRI*-TA/*MseI*-AG.

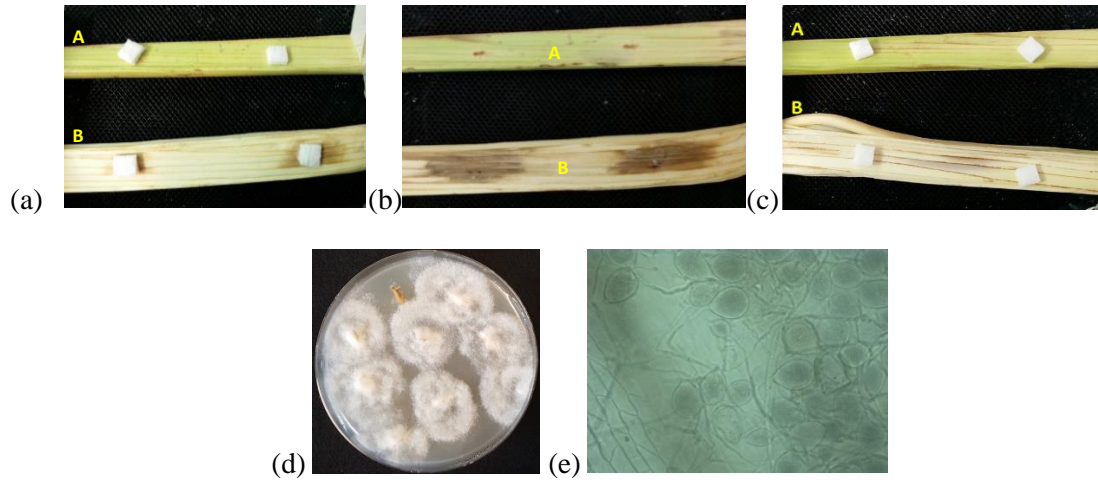


Figure 10 (a) Brown lesions on the inoculation site of young oil palm spear leaves observed at the 4th day of inoculation, (b) lesion at 5th day and (c) control assay with water and (d) Mycelial growth from the re-isolation of the diseased tissue (brown lesion) and (e) sporangia observed on diseased tissue at the inoculation site of *P. palmivora* (Microscopic magnification: 40x10).

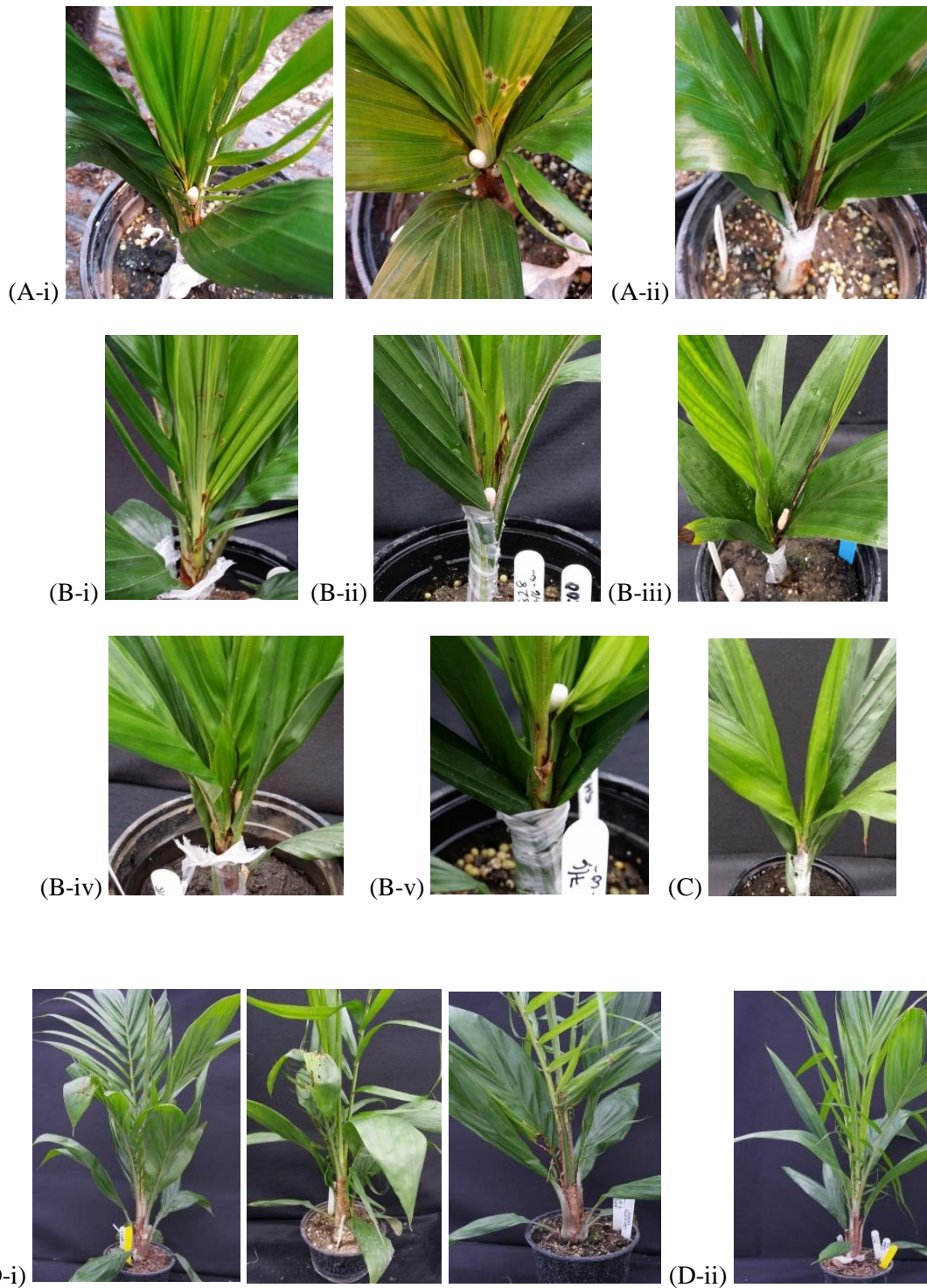


Figure 11 Lesions observed on infected oil palm seedlings inoculated with *P. palmivora* isolate P16835 (A-i) and CBS111346 (A-ii) carried out in the middle of May. Similar disease symptoms were also observed on the subsequent inoculation repeated with the same isolates as in the previous inoculation. Shown are some examples of the symptoms that appeared on the seedlings

inoculated with; B-i) PPC280574, B-ii) P16828, B-iii) PPM4, B-iv) PPM1, B-v) P6896 and C) Control (distilled water spiked with carrot juice). Infected seedlings at 6 months after inoculation with (D-i) *P. palmivora* zoospores (D-ii) distilled water + carrot juice (control).