

PHYTOPHTHORA PALMIVORA RELATIONSHIP ANALYSIS ON ITS rDNA PRIMERS USING MEGA11 SOFTWARE (NCBI DNA SEQUENCING LIBRARY STUDY)

ANALISIS KEKERABATAN PHYTOPHTHORA PALMIVORA PADA PRIMER rDNA ITS MENGGUNAKAN SOFTWARE MEGA11 (STUDI PUSTAKA DNA SEKUENSING NCBI)

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ABSTRACT

*Phylogenetic study of *Phytophthora palmivora* species utilizing nucleotide sequences of rDNA ITS and genes was conducted to evaluate the cladistics and evolution of kinship among *P. palmivora* species in order to support genetic diversity and linkages and also compare with sequences of mitochondrial COX1. The analysis included 61 nucleotide sequences of *P. palmivora* rDNA ITS and one outgroup as well as 56 nucleotides sequences of COX1 retrieved from the NCBI GeneBank, which were aligned and trimmed using MEGA 11 software. The Kimura-2 parameter model and Maximum-Likelihood approach were used to create a monophyletic tree with four major clades. The percentage of GC content in DNA sequences was less than 50%, influencing genetic diversity and adaptation throughout evolutionary time periods. Temperature, humidity, rainfall, soil moisture, and seasonal fluctuations all work together to generate optimal climatic and weather conditions for the establishment and development of *Phytophthora palmivora* in tropical climates.*

ABSTRAK

KATA KUNCI:

GC-content, keragaman
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filogenetik, *Phytophthora
palmivora*

Studi filogenetik *Phytophthora palmivora* dilakukan dengan menggunakan urutan nukleotida dari rDNA ITS dan gen untuk mengevaluasi kladistik dan evolusi hubungan kekerabatan antarspesies *P. palmivora*. Penelitian ini bertujuan untuk mendukung pemahaman tentang keragaman genetik dan hubungan kekerabatan, serta membandingkannya dengan urutan gen mitokondria COX1. Analisis melibatkan 61 urutan nukleotida rDNA ITS *P. palmivora* dan satu outgroup, serta 56 urutan nukleotida COX1 yang diperoleh dari NCBI GeneBank. Urutan-urutan tersebut disejajarkan dan dipotong menggunakan perangkat lunak MEGA11. Model Kimura-2 parameter dan pendekatan Maximum-Likelihood digunakan untuk membangun pohon monofiletik yang terdiri atas empat klad utama. Persentase kandungan GC pada urutan DNA yang kurang dari 50% memengaruhi keragaman genetik dan adaptasi selama periode evolusi. Faktor seperti suhu, kelembapan, curah hujan, kelembapan tanah, serta fluktuasi musiman bekerja secara sinergis menciptakan kondisi iklim yang optimal untuk pembentukan dan perkembangan *Phytophthora palmivora* di daerah beriklim tropis.

1. INTRODUCTION

Phytophthora palmivora is one of the significant pathogens worldwide, including in Indonesia. *P. palmivora* can attack various plants, including cocoa, oil palm, and horticultural crops (Muntana et al., 2022). This pathogen causes root rot, fruit rot, and leaf blight in cocoa plants (Rahmahwati, Khaeruni and Bande, 2021). The use of native rhizobacteria, such as GM 8/1 and GM 7/10, has been proven to inhibit the growth of *P. palmivora* (Muntana et al., 2022). Additionally, *Trichoderma sp.* has been shown to reduce *P. palmivora* inoculum on cocoa pod husks (Rahma et al., 2022). Certain cocoa clones, such as DR 1, ICS 13, and ICCRI 3, have also been identified to possess high resistance to *P. palmivora* (Rubiyo et al, 2020).

Several efforts have been made to reduce the incidence of diseases caused by *P. palmivora*, but controlling this disease remains challenging for farmers. The ability to identify the genetic diversity of pathogen isolates, the relationship between *P. palmivora* isolates on cocoa and alternative host plants, and the dynamics of pathogen populations in plantations all depend on establishing disease control strategies for *P. palmivora* in cocoa plantations (Rubiyo et al., 2020). Previous studies have discussed the development of molecular markers to identify *P. palmivora* (Darmono et al., 2016), identifying disease isolates on various plants (Barboza et al., 2020), and evaluating *P. palmivora* resistance to fungicides like metalaxyl (Kongtragoul et al., 2021). Identifying and studying *P. palmivora* using DNA sequencing, especially in the rDNA ITS region, has become essential (Robideau et al., 2011).

Internal transcribed spacers (ITS) of nuclear ribosomal DNA (rDNA) are widely known for their utility in many study fields. Most markers, including ITS1, ITS2, and the 5.8S region, have proven beneficial for population studies, species boundaries, and phylogenetic relationships (Marcilla et al., 2001; Gazis et al., 2011; Cheng et al., 2013). The commonly used method for intra- and inter-species relationship analysis is phylogenetic reconstruction, a standard approach in relationship analysis (Hidayat & Pancoro, 2016). The genetic relationships between groups of organisms can be observed through phylogenetic studies using molecular markers based on evolutionary lines (Hidayat & Pancoro, 2016). In constructing phylogenetics, the Kimura-2 model is used to find similarities between sequences. This model also has advantages in quickly and easily analyzing a group of sequences (Bhambri & Gupta, 2012). The aim of this research is to examine the relationships of *Phytophthora palmivora* from several countries based on rDNA ITS sequences using phylogenetic analysis with MEGA 11 software and data input from the NCBI (National Center for Biotechnology Information) GenBank <https://www.ncbi.nlm.nih.gov>.

2. MATERIALS AND METHOD

This research used secondary data in the form of *Phytophthora palmivora* rDNA ITS-1 nucleotide sequences obtained from GenBank via the NCBI (National Center for Biology Information) website. For comparison, several authentic species of *P. palmivora* were used, namely: **CPHST BL 105**, **CPHST BL 106**, and **CPHST BL 46**. The out-group species selected was *Phytophthora hedraindra* strain CBS 111725. The sequence data were aligned using ClustalW in MEGA 11. Phylogenetic tree reconstruction was performed using MEGA 11 software (Tamura et al., 2021) with the Maximum Likelihood method and a bootstrap analysis of 1000 replicates.

3. RESULT AND DISCUSSION

3.1 Phylogenetic Tree Analysis

Table 1 shows the number of 61 nucleotide sequences of the ITS-1 gene from *Phytophthora palmivora* species originating from various plants and countries, along with one outgroup species, *Phytophthora hedraindra*. Using the Kimura-2 model, an original phylogenetic tree was obtained (**Fig. 1.**). The tree broadly shows four main clades and one outgroup. Clade 1 from *Phytophthora palmivora* isolate 94P43 until *Phytophthora palmivora* isolate Dodonea 3; Clade 2 from *Phytophthora palmivora* isolate Ph13 until Ph15; Clade 3 *Phytophthora palmivora* isolate RYL1 S19 until *Phytophthora palmivora* strain RY 1-9; and the last was Clade 4 from *Phytophthora palmivora* isolate P80 until *Phytophthora palmivora* isolate 8B. Isolates which have the same code grouping within the same clade, such as the TARI isolate from Taiwan, the Ph isolate from Turkey, and the CL isolate from Thailand, indicating that these species share a common ancestor and form a monophyletic group. The cladistic analysis results show how these species are related and form a distinct group separate from other species in the phylogenetic tree. However, there is one exception: *Phytophthora palmivora* isolate Pth5, which originated in Turkey and attacks lavender is an outlier in clade 3, because all other individuals of the species come from Asian countries, specifically Malaysia and Thailand, and attack durian. Clade 1 groups with the authentic *P. palmivora* species, indicating that these species share a common ancestor and form a monophyletic group.

The nucleotide sequences used are rDNA ITS, where the rDNA ITS marker is excellent for hybridization experiments, distinguishing species, and resolving closely related taxa affiliations (Bargues & Mas-Coma, 2005). ITS-2 of rDNA has been widely used in research focusing on lymnaeid vectors, highlighting its importance as a molecular marker in that field (Bargues & Mas-Coma, 2005). The rDNA ITS marker shows high variability in sequence and length, making it a useful tool for comparing populations at inter- and intraspecific levels (Kazi et al., 2013). Additionally, rDNA ITS-1 has been found to be a suitable molecular marker for phylogenetic analysis between species and identifying different nematodes (Lin et al., 2011).

The rDNA gene, including the ITS region, has been used in various studies, ranging from phylogenetic analysis of cnidarians to genetic characterization of fish species, demonstrating its broad application and importance to molecular biology research (Merlo et al., 2012; Muhammad et al., 2021). This rDNA ITS marker is also very useful for identifying tick species, *anisakis* nematodes, and other organisms that can cause zoonoses, underscoring its significance in disease ecology and epidemiology (Zhu et al., 2007; Abouelhassan et al., 2019).

As a comparison, the mitochondrial COX1 marker was used alongside ITS rDNA. The COX1 tree contained only 56 species, which is 5 fewer species than were included in the ITS rDNA analysis. Interestingly, the COX1 tree showed a clear geographical clustering of the species, with groups forming based on their areas of origin (**Fig. 2.**). This was in contrast to the ITS rDNA tree, where the species were more intermixed without a strong geographical pattern. The phylogenetic analysis using maximum likelihood method on COX1 gene showed strong relationship based on the geographic origin. Isolates from Malaysia, Italy, Oceania, and Thailand were grouped in the first, second, and third clade respectively. This result is relevant with previous study that showed some marker is more suitable to determine the geographic origin (Masanto et al., 2019). The COX1 marker appeared to provide higher resolution for distinguishing between closely related *Phytophthora* species compared to the more variable ITS rDNA region.

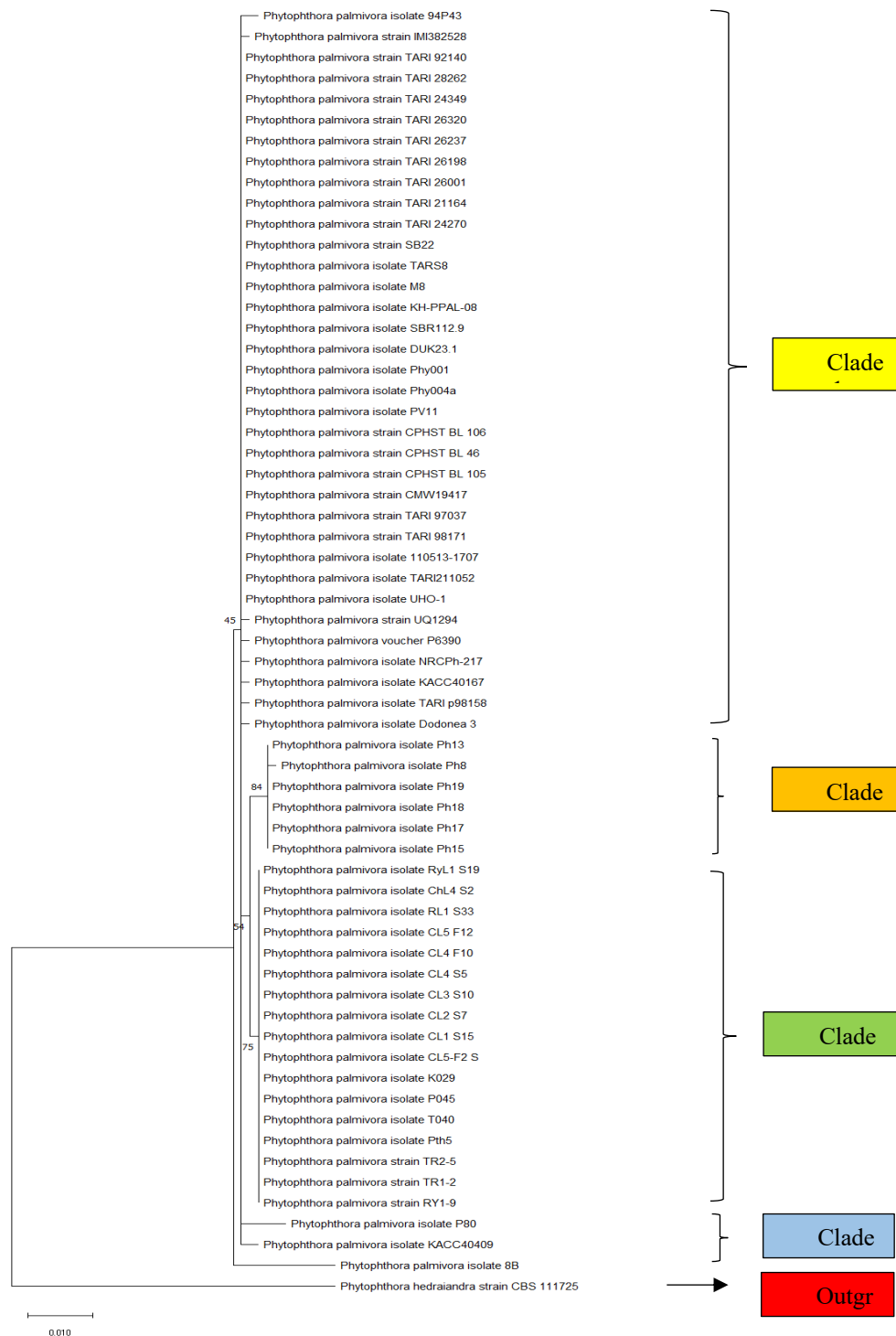


Fig. 1. Maximum likelihood tree based on rDNA ITS markers, the phylogenetic tree of *Phytophthora palmivora* shows 61 species in-group and 1 species out-group. MEGA 11 software was used to reconstruct the data. Bootstrap values from 1,000 replications are displayed on the branches.

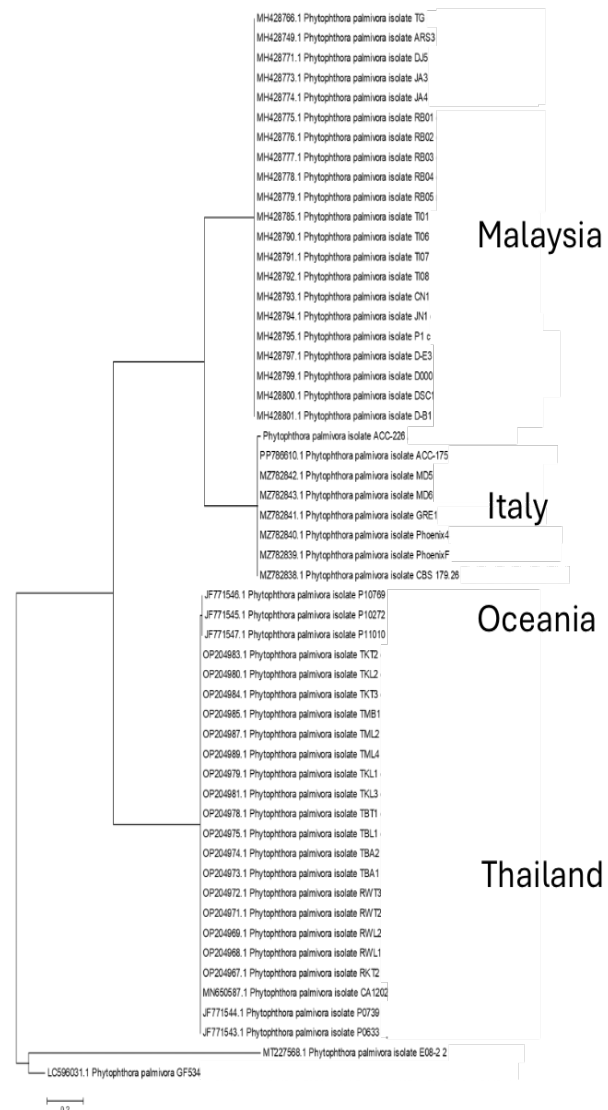


Fig 2. Phylogenetic tree constructed using maximum likelihood algorithm on COX1 gene of *Phytophthora palmivora* revealed a clustering based on the geographic origin.

3.2 %GC Content Analysis

Table 1. shows that %GC-content is fairly uniform in the range of 48.1% to 49.4%, or has a value of %GC below 50%. GC content in a DNA sequence is critical for many biological activities. It affects DNA stability, PCR amplification efficiency, genomic organization, and sequencing procedures. The GC content, which denotes the percentage of guanine (G) and cytosine (C) bases in a DNA sequence, is important in regulating the stability and melting point of the DNA molecule (Yunfei & Xunca, 2023). DNA sequences with extremely high or low GC content might hamper PCR amplification by forming secondary structures that inhibit DNA polymerase activity (Guido et al., 2016). Understanding a DNA sequence's GC content is critical for optimizing PCR settings and guaranteeing efficient target region amplification. A DNA sequence with GC content less than 50% can have serious consequences in molecular biology and genetics. DNA sequences with low GC content (usually less than 50%) are frequently connected with certain features and functions. One important consideration is the effect on DNA stability and secondary structure development. Sequences with lower GC content are less stable due to weaker hydrogen bonding between adenine-

Table 1. List of species acquired from the National Center for Biotechnology Information (NCBI) website

No	Isolate Name	Accession Number	Base Pairs	% GC content	Country	Host
1	Phytophthora palmivora isolate P80	L41384	827	49.1	Australia	-
2	Phytophthora palmivora isolate RyL1_S19	OR655102	819	48.4	Thailand	Durian
3	Phytophthora palmivora isolate ChL4_S2	OR655100	811	48.6	Thailand	Durian
4	Phytophthora palmivora isolate RL1_S33	OR655099	825	48.4	Thailand	Durian
5	Phytophthora palmivora isolate CL5_F12	OR655096	818	48.2	Thailand	Durian
6	Phytophthora palmivora isolate CL4_F10	OR655093	819	48.4	Thailand	Durian
7	Phytophthora palmivora isolate CL4_S5	OR655092	812	48.4	Thailand	Durian
8	Phytophthora palmivora isolate CL3_S10	OR655089	822	48.4	Thailand	Durian
9	Phytophthora palmivora isolate CL2_S7	OR655087	819	48.5	Thailand	Durian
10	Phytophthora palmivora isolate CL1_S15	OR655085	815	48.5	Thailand	Durian
11	Phytophthora palmivora isolate CL5-F2_S	OR655081	826	48.4	Thailand	Durian
12	Phytophthora palmivora isolate 8B	OR427291	839	48.7	India	Coconut
13	Phytophthora palmivora strain UQ1294	AF266780	787	48.7	Australia	-
14	Phytophthora palmivora strain CMW19417	HQ013213	786	48.9	Australia	Eucalyptus
15	Phytophthora palmivora strain CPHST BL 105	MG865559	825	48.6	India	Betel Nut palm
16	Phytophthora palmivora strain CPHST BL 46	MG865560	800	48.8	Costa Rica	Cacao
17	Phytophthora palmivora strain CPHST BL 106	MG865561	826	48.7	Indonesia	Coconut
18	Phytophthora palmivora isolate PV11	PP064982	821	48.8	Thailand	Orchid
19	Phytophthora palmivora isolate Ph15	KP985659	723	49.2	Turkey	Pear
20	Phytophthora palmivora isolate Ph17	KP985656	723	49.2	Turkey	Kiwi Fruit
21	Phytophthora palmivora isolate Ph18	KP985657	723	49.2	Turkey	Pistachio
22	Phytophthora palmivora isolate K029	OR051924	852	48.2	Malaysia	Durian
23	Phytophthora palmivora isolate P045	OR051920	823	48.6	Malaysia	Durian
24	Phytophthora palmivora isolate T040	OR051910	820	48.5	Malaysia	Durian
25	Phytophthora palmivora isolate Phy004a	KU170145	819	48.5	Ecuador	Peach Palm
26	Phytophthora palmivora isolate Phy001	KU170144	819	48.5	Ecuador	Peach Palm
27	Phytophthora palmivora isolate Ph19	KP985658	723	49.2	Turkey	Pomegranade
28	Phytophthora palmivora isolate Ph13	KF831195	818	48.8	Turkey	Cherry
29	Phytophthora palmivora isolate Ph8	KF723836	818	48.8	Turkey	Apricot
30	Phytophthora palmivora isolate DUK23.1	JX315271	825	48.7	France	Cacao
31	Phytophthora palmivora isolate SBR112.9	JX315268	825	48.7	France	Cacao
32	Phytophthora palmivora isolate KH-PPAL-08	JQ743200	777	49.2	India	Cassava
33	Phytophthora palmivora isolate Pth5	JF777117	787	48.7	Turkey	Lavender
34	Phytophthora palmivora isolate Dodonea 3	JF278081	822	48.7	Italy	Hopbush
35	Phytophthora palmivora isolate M8	GQ398157	879	48.7	Colombia	Oil Palm
36	Phytophthora palmivora isolate TARS8	DQ987922	796	48.7	Puerto Rico	Cacao
37	Phytophthora palmivora strain SB22	DQ182751	786	48.9	Thailand	Durian
38	Phytophthora palmivora strain TR2_5	DQ182748	787	48.7	Thailand	Durian
39	Phytophthora palmivora strain TR1_2	DQ182746	787	48.8	Thailand	Durian
40	Phytophthora palmivora strain RY1_9	DQ182744	787	48.7	Thailand	Durian
41	Phytophthora palmivora isolate KACC40409	AF228088	837	48.4	Korea	Orchid (Cymbidium sp)

42	Phytophthora palmivora isolate KACC40167	AF228087	837	48.3	Korea	Golden Cane Palm
43	Phytophthora palmivora isolate TARI p98158	MG255148	786	48.7	Taiwan	Betel Nut Palm
44	Phytophthora palmivora strain TARI 24270	GU111664	902	48.1	Taiwan	Orange
45	Phytophthora palmivora strain TARI 21164	GU111662	902	48.1	Taiwan	Papaya
46	Phytophthora palmivora strain TARI 26001	GU111659	902	48.1	Taiwan	Jujube
47	Phytophthora palmivora strain TARI 26198	GU111658	901	48.2	Taiwan	English Ivy
48	Phytophthora palmivora strain TARI 26237	GU111657	902	48.1	Taiwan	Umbrella Plant
49	Phytophthora palmivora strain TARI 26320	GU111655	902	48.1	Taiwan	Chinese Mahogany Orchid
50	Phytophthora palmivora strain TARI 24349	GU111654	902	48.1	Taiwan	(Cattleya hybrid)
51	Phytophthora palmivora strain TARI 28262	GU111653	896	48.4	Taiwan	Lilies Orchid
52	Phytophthora palmivora strain TARI 92140	GU111648	888	48.3	Taiwan	(Oncidium sp)
53	Phytophthora palmivora strain TARI 97037	GU111647	888	48.2	Taiwan	Mango
54	Phytophthora palmivora strain TARI 98171	GU111646	887	48.3	Taiwan	Avocado
55	Phytophthora palmivora isolate 110513-1707	KY447326	879	48.7	USA	Papaya
56	Phytophthora palmivora isolate NRCPh-217	KU877819	786	49	India	Orange
57	Phytophthora palmivora isolate TARI211052	KJ801810	786	48.9	Taiwan	Money Tree
58	Phytophthora palmivora isolate 94P43	AF467089	836	48.3	Indonesia	Cacao
59	Phytophthora palmivora strain IMI382528	KY475622	875	48.7	Indonesia	Oil Palm
60	Phytophthora palmivora voucher P6390	HQ261633	787	49	Indonesia	-
61	Phytophthora palmivora isolate UHO-1	MT644188	786	48.9	Indonesia	Cocoa
62	Phytophthora hedraiaandra strain CBS 111725	AY707987	792	49.4	Canada	-

thymine (A-T) base pairs than guanine-cytosine (G-C) base pairs (Benjamini & Speed, 2012). This decreased stability has the potential to disrupt a variety of molecular processes, including DNA replication, transcription, and translation.

Furthermore, DNA sequences with less than 50% GC content may have different interactions with proteins and other molecules. The GC concentration of DNA sequences has been demonstrated to alter the binding affinity of proteins, such as transcription factors, to certain genomic areas (Deng, 2000). In the context of gene regulation, regions with decreased GC content may have altered transcription factor binding sites, which could affect gene expression patterns. DNA sequences with less than 50% GC content can have an impact on PCR amplification and sequencing performance. DNA templates with low GC content may present difficulties during PCR amplification because secondary structures can hamper the progress of DNA polymerases (Qiao et al., 2022). Likewise, during sequencing library preparation, areas with low GC concentration may exhibit coverage biases, resulting in uneven sequencing depth and potential data interpretation issues (Araujo et al., 2015).

In terms of genome evolution and comparative genomics, DNA sequences with GC concentration less than 50% may reveal particular evolutionary tendencies. Variations in GC content across genomes can reveal information about species' evolutionary history and the selective pressures acting on their genomes (Nishida, 2012). The distribution of GC content across genomic areas can also have an impact on genome organization and chromatin structure, hence affecting gene expression regulation and genome function (Vinogradov, 2005). Lower GC content in DNA sequences can be attributed to selection against functionally harmful nonsynonymous mutations as well as

selection to reduce nutritional requirements in the DNA and protein pools (Hellweger et al., 2018). Furthermore, recombination via biased gene conversion (BGC) has been recognized as a major driver of genome evolution, alongside mutation, selection, and drift (Duret & Arndt, 2008). The evolution of GC content is strongly related to genome size and environmental factors (Veleba et al., 2016). The link between GC content and recombination rates is critical for understanding the development of GC content, particularly the preservation of isochore structures in vertebrates (Pracana et al., 2020). Recombination influences GC-content evolution via biased gene conversion, which favours G/C alleles over A/T alleles during homologous recombination (Kent et al., 2012). Recombination has been shown to influence the development of GC content in a variety of species, including bacteria and archaea (Ran et al., 2014).

More importantly, codon use optimization, nucleotide substitutions, and gene conversion all influence the evolution of GC content in DNA sequences (Meunier & Duret, 2004; Duret & Galtier, 2009; Pessia et al., 2012). The interplay of mutation bias, selection, and recombination causes GC content fluctuations among taxa with varied lifestyles (Zhang et al., 2020). Understanding the evolutionary repercussions of DNA methylation on GC content in vertebrate genomes sheds light on the mechanisms that drive GC content evolution (Mugal et al., 2015).

3.3 Analysis of the affected plants and their countries of origin

Analysis of the affected plants and their country of origin **Table 1.** shows that *P. palmivora* attacked not only plants in the Arecaceae family, or palm trees, but also forest trees, fruit plants, nuts, cocoa, and flowers. The countries that were targeted by *P. palmivora* expanded across the continent. Thailand, India, Indonesia, Malaysia, and Korea are examples of Asian countries where it can be found. It happens in Turkey, France, and Italy in Europe, and in Costa Rica, Ecuador, Colombia, Puerto Rico, the United States, and Canada in America. And that is happening on the Australian continent. Rotimi, Ikotun and Agbeniyi, in 2021 report that *P. palmivora* attacks occurred throughout the African continent, specifically in Nigeria, attacking cocoa plantations and causing black pod disease. However, no data has been identified that suggests *P. palmivora* attacked the Antarctic continent. It could be induced by climate circumstances that are not favourable to *P. palmivora* growth.

Phytophthora palmivora, a damaging plant pathogen that affects a variety of tropical crops, grows and develops in response to environmental and climate conditions. Several researches provide insights into the elements that produce favorable conditions for the proliferation of *P. palmivora*. *Phytophthora palmivora* thrives in environments with temperatures around 26°C and relative humidity levels exceeding 80%. These conditions promote additional rotting of plant tissues, which leads to disease development (Torres et al., 2010). Wet-season rainfall and wet soils are critical variables in the formation and development of *P. palmivora*. Adequate soil moisture and warm temperatures throughout the spring season can promote pathogen development (Aguayo et al., 2014). The climate during the wet season, particularly in December and January, has a considerable impact on tree development and can affect the growth of *P. palmivora* (Trouet et al., 2006). *Phytophthora* species, including *Phytophthora palmivora*, thrive on clayey and loamy soils with lower acidity and higher base saturation. These soil conditions can provide an ideal home for the pathogen (Jönsson et al., 2005). The interaction between *P. palmivora* and its host plants is critical to disease progression. Understanding the molecular mechanisms underlying these interactions will help us understand the pathogen's pathogenicity and host resistance (Overdijk et al., 2016). Plants vulnerability to *Phytophthora* species, particularly *P. palmivora*, fluctuates throughout the year. Temperature, rainfall, and humidity over different seasons can affect infection growth (Alvarez et al., 2009).

4. CONCLUSIONS

Overall, the phylogenetic tree in rDNA ITS is divided into four primary clades, with *Phytophthora palmivora* isolate Pth5 distinguishing itself in Clade 3 due to differences in country of origin and plants attacked. The internal transcribed spacers of nuclear ribosomal DNA are invaluable molecular markers across numerous fields due to their great variability, efficacy in species delimitation, and utility in phylogenetic analysis. Whereas the mitochondrial COX1 marker generated a phylogenetic tree that grouped *Phytophthora* species according to their geographic origins. GC content less than 50% in DNA sequences can influence evolutionary processes through a complex interplay of mutation bias, selection, recombination, and gene conversion mechanisms. These variables contribute to the dynamic growth of GC content in genomes, which influences genetic diversity and adaptation throughout evolutionary time scales. Finally, a combination of temperature, humidity, rainfall, soil moisture, and seasonal changes is necessary to create ideal climatic and weather conditions for the establishment and development of *Phytophthora palmivora* in tropical regions. Understanding these characteristics is crucial for disease control and mitigating the effects of *Phytophthora palmivora* on tropical crops.

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