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Research Article

Genetic Variation and Phylogenetic Analysis of Fungal Pathogens on Native *Dendrobium* in Lampung using DNA Barcoding

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Abstract
Dendrobium is one of the flagship collections of Liwa Botanical Garden, as well as an endemic flora of southern Sumatra that requires conservation. One of the major challenges in its conservation and potential development is the identification of diseases. Molecular approaches employing genetic variation and phylogenetic analyses with DNA barcoding markers can serve as an effective alternative. Genetic variation analysis is required to evaluate the level of intra and interspecific diversity of pathogens, while phylogenetic analysis is necessary to determine the evolutionary relationships among pathogenic fungal species. This study provides insights into the evolutionary patterns and adaptive potential of pathogens to their hosts at the molecular level. A total of 6 out 10 samples showing fungal infection symptoms, such as rust spots, necrosis, and others, were collected. Amplification of two selected samples, D23 and D24, revealed specific bands of approximately 300 bp. Sequence and phylogenetic analyses indicated similarity to isolates of the genera *Xylaria* and *Termitomyces*, with sequence lengths ranging from 513 to 671 bp, respectively. Genetic variation analysis through haplotype grouping showed that isolate D23 clustered with the *Xylariales* group, while isolate D24 clustered with the *Termitomyces* group. The findings of this study enrich the sequence data of pathogenic fungi, although with a limited success rate, and provide a molecular basis for the accurate conservation of native *Dendrobium* in the Liwa Botanical Garden through protection against disease infections.

Keywords: DNA barcoding, fungal pathogens, genetic variation, Lampung, native *Dendrobium*, phylogenetic analysis

1. INTRODUCTION

Dendrobium is one of the most widely distributed orchid genera in the world, consisting of approximately 1,600 species across Southeast Asia [1]. According to Gandawidjaya and Sastrapradja [2] about 275 species are found in Indonesia, including in Lampung. Liwa Botanical Garden as the only regional botanical garden carrying out *ex-situ* conservation in Lampung has developed the potential of native *Dendrobium* through exploration and collection activities [3], resulting in 48 accessions [4][5]. Conservation and development efforts for these orchids can be carried out through species identification [6]-[8] and protection against infectious diseases [9]-[13]. Based on our previous studies, native *Dendrobium* collections in the Liwa Botanical Garden have shown symptoms of infection by bacteria [9], fungi [10], and viruses [11]. Mahfut et al. [10] reported fungal infection symptoms in the form of necrotic spots on the upper leaf surface and blackening along the leaf margins. Analysis of plant resistance levels indicated that native *Dendrobium* species are the most susceptible to fungal infection, with a disease intensity of 74.11%. These findings suggest that fungal infections in the Liwa Botanical Garden collections are severe, thereby requiring rapid and accurate measures for the control of bacterial and fungal diseases.

Disease management of fungal infections in native *Dendrobium* at the Liwa Botanical Garden has thus far been carried out conventionally through the application of chemical pesticides based on visible infection symptoms [8]-[10]. However, this method is often ineffective due to frequent misdiagnosis. Alberts et al. [14] explained that a single disease may produce symptoms similar to those of other diseases, while the same disease may exhibit different symptoms in the same host plant. Therefore, a more accurate disease identification method is required, particularly through molecular approaches.

Molecular approaches commonly used in the identification of fungal diseases include genetic

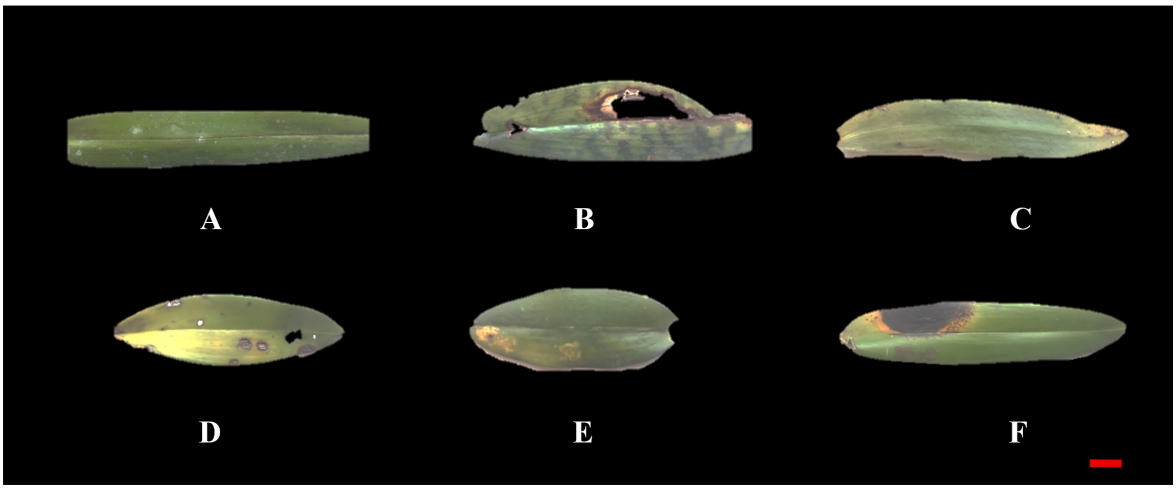

Figure 1. Variation of fungal infection symptoms on the leaves of native *Dendrobium* in Lampung. A. D11 showing RS symptoms, B. D23 showing N, C. D24 showing N+C, D. D30 showing N+C, E. D32 showing N+C, F. D33 showing N+C. RS = Rust spot, N = Necrotic, C = Chlorotic. Bar = 1 cm.

variation and phylogenetic analysis [15] with ITS markers of DNA barcoding [16]. Genetic variation analysis is required to evaluate the level of intra and interspecific diversity of fungal pathogens [17], thereby providing insights into their evolutionary patterns and potential adaptation to hosts, using DNA sequence data derived from ITS amplification [18]. Meanwhile, phylogenetic analysis is necessary to determine the evolutionary relationships among pathogenic fungal species [19][20], enabling accurate and reliable identification methods and supporting disease management strategies based on molecular biology [21].

Anisamaun et al. [15] reported the identification of fungal contaminants in *in vitro* cultures using the ITS marker, which included *Aspergillus*, *Penicillium*, *Fusarium*, *Trichoderma*, and *Cladosporium*. Phylogenetic analysis revealed an interspecific genetic distance of 0.205, dividing the fungi into three major groups, indicating homogeneous substitutions among the sequences. Similarly, Chaisiri et al. [22] conducted phylogenetic and haplotype network analyses of *Diaporthe eres* species in China based on multiple loci sequences, demonstrating that concatenated multi-locus DNA sequence analysis could resolve the taxonomic status of *D. eres*. Moreover, haplotype network analysis revealed a correlation between population diversity and both distribution and host range across China.

Previous studies have demonstrated that molecular approaches through genetic variation and phylogenetic analysis are effective for the identification of bacterial diseases [10]. Furthermore, Feng et al. [23] also reported that the rDNA-ITS marker exhibits high variability among species but remains conserved within the same species, thereby enabling more accurate identification. The present study aims to extend our previous findings [9][10] by developing a molecular approach for the identification of fungal diseases in native *Dendrobium* from Lampung. The results of this study are expected to enhance our understanding of epidemiology, which will subsequently provide a foundation for appropriate disease management and conservation strategies for native *Dendrobium* in the Liwa Botanical Garden.

2. MATERIALS AND METHODS

2.1. Materials

Survey and sample collection were conducted in the greenhouse of Liwa Botanical Garden following the methods described previously [9]-[11]. The collected samples consisted of leaves of native *Dendrobium* from Lampung exhibiting symptoms of fungal infection. The samples were labeled, preserved, and further analyzed in the laboratory.

2.2. Methods

2.2.1. DNA Genom Isolation, Amplification, and Sequencing of ITS

DNA was isolated using the

cetyltrimethylammonium bromide (CTAB) method following the protocol of Mahfut et al. [7]. Amplification was conducted with the universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') with a product size of 300 bp [24], adopting the procedure described previously [7]. PCR conditions consisted of 35 cycles with the following parameters: initial denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 90 s, and a final extension step at 72 °C for 5 min. The integrity of amplified DNA fragments was verified by 2% agarose gel electrophoresis at 60 V for 105 min with an electrode spacing of 19.4 cm. PCR products were visualized using a Gel Doc UV system, and sequencing was performed using the Sanger dideoxy method [7][24].

2.2.2. Data Analysis

The initial DNA sequence editing was manually corrected using GeneStudio Pro v2.2. The resulting consensus sequences were then compared with GenBank data using the Nucleotide BLAST program [7]. The ITS sequences with the highest similarity were subsequently aligned for intraspecific genetic analysis. Two additional samples were included as outgroups without altering the fragment length. Phylogenetic tree reconstruction was carried out using the maximum Likelihood (ML) method with Partition Finder 2.1.1, and 1,000 bootstrap replicates were run in IQtree2. Bayesian inference (BI) analysis was performed with MrBayes v3.2.6, applying the MCMC method for 4 million generations with sampling every 1,000 generations. The resulting consensus tree was visualized using FigTree v1.4.4, while genetic distances were calculated using the K-2P model in MEGA11. Intraspecific genetic variation was analyzed by identifying polymorphic sites, estimating haplotype diversity (*h*), and constructing a haplotype network with PopART v1.7. PCoA analysis was conducted using GenAIEx 6.503 [17][18].

3. RESULTS AND DISCUSSIONS

3.1. Sample Collection

A total of 10 leaf samples of native *Dendrobium* from Liwa Botanical Garden were collected, all showing symptoms of fungal infection. The variations in infection symptoms included rust spots, necrosis, and combined necrotic and chlorotic infections (Figure 1). Based on the analysis of infection symptom variation, the native *Dendrobium* collection at Liwa Botanical Garden was found to be severely infected, and plant resistance level analysis indicated susceptibility. Rust spot is a symptom characterized by small reddish-brown to orange spots on the leaf surface caused by rust fungal infection, which disrupts photosynthesis and weakens the plant [25]. Necrosis refers to tissue death manifested as brown to black spots on the leaves, usually resulting from fungal toxins or cell damage due to pathogen invasion [26]. Chlorosis is the symptom of leaf yellowing caused by chlorophyll degradation, indicating physiological disturbances in the plant as a result of fungal infection [27]. The variation in symptoms is caused by complex interactions among pathogen virulence, host plant resistance, and environmental conditions [10][28]. Plant resistance plays an important role in determining the intensity of disease infection [29], such that susceptible genotypes are more likely to exhibit severe symptoms compared to tolerant ones [30]. Environmental factors such as high humidity,

Table 1. Results of statistical analysis of phylogenetic relationships and genetic diversity of pathogenic fungi.

Parameters	D23	D24
Nucleotide diversity:	π = 0.33993	π = 1.53539
Number of segregating sites:	157	207
Number of parsimony-informative sites:	113	138
Tajima's D statistic:	D = 0.712343	D = 18.6725
	p (D >= 0.712343) = 0.253338	p (D >= 18.6725) = 0

Table 2. Genetic distance (p-distance) of the D23 isolate sequence.

	1	2	3	4	5	6	7	8	9	10	11
NR_147567.1											
NR_147516.1	0.036										
NR_153251.1	0.062	0.062									
NR_153201.1	0.030	0.041	0.057								
NR_153200.1	0.030	0.020	0.051	0.030							
NR_147520.1	0.080	0.063	0.097	0.096	0.080						
PX022201.1	0.263	0.265	0.242	0.250	0.264	0.235					
PV706673.1	0.254	0.220	0.227	0.271	0.241	0.230	0.352				
MF534953.1	1.273	1.292	1.308	1.343	1.294	1.269	1.306	1.422			
LS990581.1	1.273	1.292	1.308	1.343	1.294	1.269	1.306	1.422	0.000		
D23_5312090.1	1.753	1.685	1.701	1.779	1.714	1.756	1.861	1.791	1.796	1.796	

Table 3. Genetic distance (p-distance) of the D24 isolate sequence.

	1	2	3	4	5	6	7	8	9	10	11
PX022201.1											
PV706673.1	0.489										
ON557367.1	1.267	1.234									
KY809209.1	1.289	1.214	0.004								
KY809192.1	1.296	1.338	0.033	0.030							
AY232681.1	1.294	1.234	1.042	1.029	1.088						
KY809214.1	1.267	1.234	0.000	0.004	0.033	1.042					
KY809212.1	1.267	1.254	0.008	0.013	0.033	1.053	0.008				
KY809205.1	1.293	1.225	0.012	0.000	0.046	1.033	0.012	0.021			
KY809191.1	1.336	1.272	0.025	0.030	0.039	1.092	0.025	0.021	0.038		
D24_5312092.3	1.879	2.169	1.812	1.819	1.869	0.937	1.812	1.907	1.882	2.070	

rainfall, and temperature fluctuations [31] also contribute to disease development by creating favorable conditions for fungal spore growth and dissemination [9][11].

3.2. Amplification of rDNA-ITS

The DNA amplification using primers ITS1 and ITS4 showed a specific band of approximately 300 bp. This finding indicates that the two primers successfully amplified the DNA fragment corresponding to the expected target. Two representative samples, D23 and D24, were selected because they exhibited thicker and brighter bands compared with the other samples. Mahfut et al. [7] also reported the successful amplification of a 300 bp band using the same primers in orchid species. The similarity of these results demonstrates that ITS1 and ITS4 primers have high consistency and reliability in detecting the internal transcribed spacer (ITS) region in orchids, making them reliable molecular markers for identification and phylogenetic studies. Furthermore, the consistent fragment size provides additional evidence that this method is effective for characterizing both intraspecific and interspecific genetic variation in pathogenic fungi [15][16].

3.3. Result of Sequencing

The consensus sequence analysis of DNA from two fungal isolates, D23 and D24, revealed nucleotide sequence lengths of 513 and 671 bases, respectively. Sequence alignment of homologous sequences from the two pathogenic fungal isolates showed significant differences in genetic diversity and selection patterns. The data included species from the genera *Xylaria* and *Termitomyces*

originating from various regions. The differences in sequence length and genetic variation among isolates indicate a relatively high level of divergence, which may reflect evolutionary adaptations to different environments or hosts [32]. This finding is consistent with previous reports that pathogenic fungi from the genera *Xylaria* and *Termitomyces* exhibit broad ecological flexibility and complex adaptive mechanisms to ensure their survival. The detected selection patterns also suggest that environmental pressures and host interactions play an important role in shaping the genetic variation of these fungal populations [22] [33].

The statistical analysis of phylogenetic relationships and genetic diversity showed that isolate D23 had a nucleotide diversity (π) of 0.33993, while D24 had a value of 1.53539, indicating a higher level of genetic variation in the D24 group. In addition, the number of segregating sites and parsimony-informative sites in D24 was also greater than in D23, suggesting a more complex level of nucleotide variation. Tajima's D statistic for D23 was 0.712343 with a p-value of 0.253338, indicating no significant deviation from neutrality, whereas the very high Tajima's D value for D24 (18.6725, p = 0) suggested strong selective pressure or significant demographic events in that population. These findings imply that the D24 group has undergone more dynamic genetic variation compared to D23, and therefore may serve as a focus for further research in the context of adaptation and genetic diversification of this fungal species. The statistical analysis of phylogenetic relationships and fungal diversity is presented in Table 1.

These findings indicate that the D24 group has undergone more intensive evolutionary dynamics compared to D23, which is likely influenced by environmental factors, host interactions, or natural selective pressures from other pathogens in its habitat. The high genetic variation in D24 also demonstrates a greater potential for adaptation to changing environmental conditions and biotic pressures, making this group an important candidate for further investigation in the context of adaptive evolution and genetic diversification of pathogenic fungi [34]. Thus, these results not only confirm the differences in genetic diversity between the two isolates but also provide a scientific basis for a deeper understanding of the evolutionary mechanisms and ecological potential of each group [35]-[37]. The analysis of genetic distance among specimens in the matrix revealed a marked difference between groups D23 and D24, as well as with other fungal species analyzed. The genetic distances (p-distance) of the D23 and D24 fungal sequences are presented in Tables 2 and 3, respectively.

Based on the genetic distance values, D23 showed relatively high distances from other species, ranging from 1.68 to 1.86, which were consistently greater than the interspecific distances within the *Xylaria* group, generally ranging from 0.02 to 0.08. A similar pattern was observed for D24, which exhibited genetic distance values with other *Termitomyces* species ranging from 1.81 to 2.19, reflecting greater genetic differences compared to other core groups such as PV706673.1 or ON557367.1, where interspecific distances were

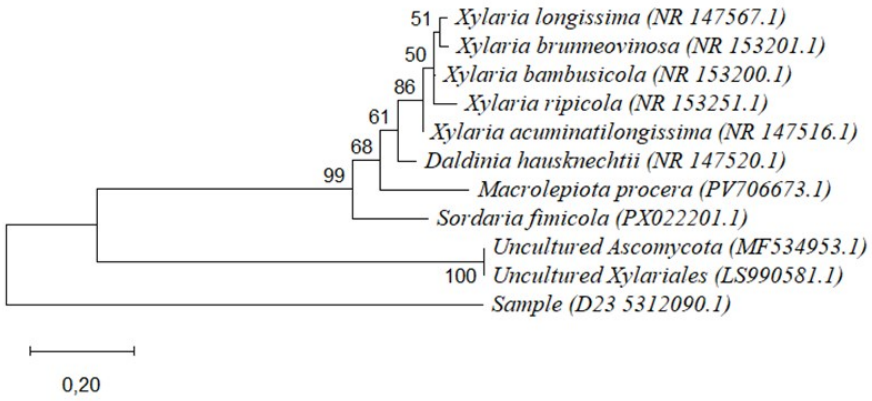

Figure 2. Phylogenetic tree reconstruction of the fungal group D23.

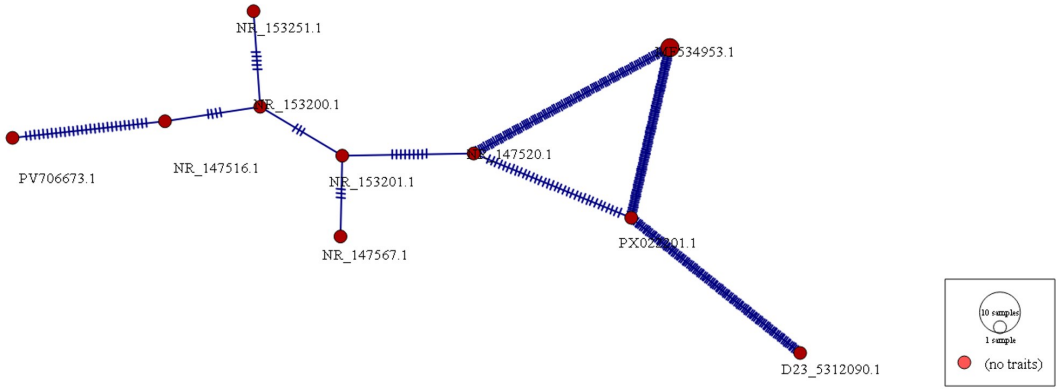

Figure 3. Haplotype network reconstruction of the D23 fungal group.

relatively low (approximately 0.004 to 0.489). The high genetic distance values between D23 and D24 and other species groups indicate significant genetic divergence within both the *Xylaria* and *Termitomyces* groups. This supports the hypothesis that D23 and D24 belong to distinct lineages and have undergone genetic isolation processes that promoted mutation accumulation and phylogenetic separation.

This high divergence can be interpreted as evidence that D23 and D24 may represent separate lineages or even candidate new species within the pathogenic fungi under study. Long-term genetic isolation may have led to the emergence of distinct morphological and physiological characteristics, which in turn could affect pathogen–host interactions and adaptation to specific environmental conditions [38]. These results are consistent with theories of sympatric and allopatric speciation, where environmental pressures and restricted gene flow are the main drivers of genetic diversification [18][19][39]. Thus, these findings not only highlight differences in the level of genetic diversity but also provide an important foundation for further taxonomic and phylogenetic studies to confirm the systematic status of D23 and D24.

Sample D23 (5312090.1) has a very close phylogenetic relationship with the groups uncultured *Xylariales* (LS990581.1) and uncultured *Ascomycota* (MF534953.1), as shown in the phylogenetic tree that places D23 in the same clade with these two sequences with 100% bootstrap support. This position indicates that sample D23 most likely belongs to the order *Xylariales*, but it cannot yet be identified at the genus or species level because it is closely related to sequences that have not been definitively characterized (uncultured). Taxonomically, this group is clearly separated from the clade occupied by the genera *Xylaria* (*longissima, brumeovinosa, bambusicola, ripicola, acuminatilongissima*), *Daldinia*, *Macroplepiota*, and *Sordaria*, indicating significant evolutionary divergence among these groups. The phylogenetic reconstruction of the fungal group D24 is presented in Figure 2.

These findings suggest that D23 may represent a new lineage or an undocumented taxon within the order *Xylariales*. This underscores the importance of exploring fungal diversity using molecular approaches, particularly to uncover cryptic species or groups of *uncultured fungi* that are often overlooked in morphology-based identification [40]. Furthermore, the clear distinction between D23 and the main *Xylaria* clade indicates possible ecological adaptations or specific evolutionary pressures that shaped a unique divergence pathway in this isolate [23][41][42].

The visualization of the haplotype network for sample D23 also formed a distinct node directly connected to two ‘uncultured’ sequences, the same as in the phylogram (MF534953.1 and LS990581.1). The connecting pathway between D23 and the two haplotypes consisted of only a few mutations, indicating a very close evolutionary relationship and the possibility of sharing a common ancestor within the *Xylariales* group that has not yet been comprehensively described. The reconstruction of the haplotype network for the D24 fungal group is presented in Figure 3.

The nodes forming a distinct cluster with only a few mutations separating them in the haplotype network support the phylogenetic finding that D23

is not part of the main *Xylaria* clade nor of other groups outside the 'uncultured' *Xylariales*. The presence of thick branches and few connecting nodes in the haplotype network also indicates low genetic differentiation among haplotypes within the clade, suggesting a low level of genetic diversity or possibly reflecting isolates originating from similar habitats or sources. The combined phylogenetic and haplotype network analyses consistently demonstrate that sample D23 is closely related to unidentified *Xylariales* sequences and is genetically distinct from the *Xylaria* group and other analyzed genera.

These findings are significant as they highlight the potential presence of a novel taxon or cryptic species within the order *Xylariales* that has remained undetected through classical morphological approaches. The low genetic diversity observed in the D23 group may reflect evolutionary stability or specific adaptations to particular environmental conditions, such as restricted host substrates or specialized microhabitats [43]. Conversely, the reduced variation could also indicate a population bottleneck or homogeneous selective pressures within its environment. Therefore, further investigations using multilocus genetic markers and molecular ecological approaches are necessary to confirm the taxonomic position of D23 and to elucidate its potential ecological and evolutionary roles within fungal communities in its native habitat [24][44].

The phylogenetic tree shows that sample D24_5312092.3 is clearly grouped within the *Termitomyces* clade, clustered together with other *Termitomyces* species such as *T. heimii*, *T. clypeatus*, and *T. intermedius* with high bootstrap support, indicating a strong phylogenetic relationship among them. The position of sample D24, which is slightly more distant from the main clade, suggests a considerable genetic divergence while still remaining within the *Termitomyces* group. This indicates that sample D24 represents a unique variant or haplotype within the genus, yet remains closely related to its nearest species. The phylogenetic reconstruction of the D24 fungal group is presented in Figure 4.

Bhunjun et al. [21] also reported that the genus *Termitomyces* exhibits a high level of genetic diversity, largely driven by ecological interactions with termites as symbiotic hosts. The genetic variation observed in D24 may reflect local adaptation to specific environmental conditions or selective pressures arising from the symbiotic relationship with termites. The presence of this unique haplotype further supports the hypothesis that diversification within *Termitomyces* is driven by coevolutionary mechanisms and limited gene flow among populations [18][45].

The haplotype network supports the results of the phylogenetic tree by showing D24_5312092.3 as a separate yet connected node, indicating the presence of mutations or genetic variations that distinguish it from other haplotypes within the *Termitomyces* group. This network illustrates the genetic interconnections among haplotypes, with line thickness reflecting the degree of relatedness. The position of D24 as a unique haplotype suggests potential genetic isolation or specific evolutionary adaptations that warrant further investigation. The reconstructed haplotype network of the D24 fungal

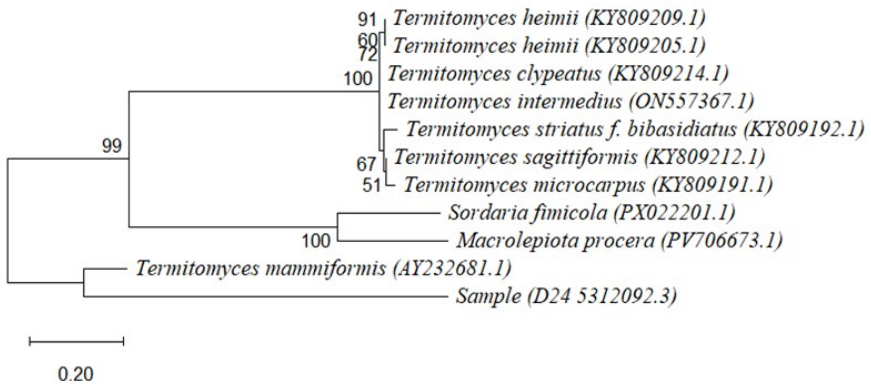

Figure 4. Phylogenetic tree reconstruction of the fungal group D24.

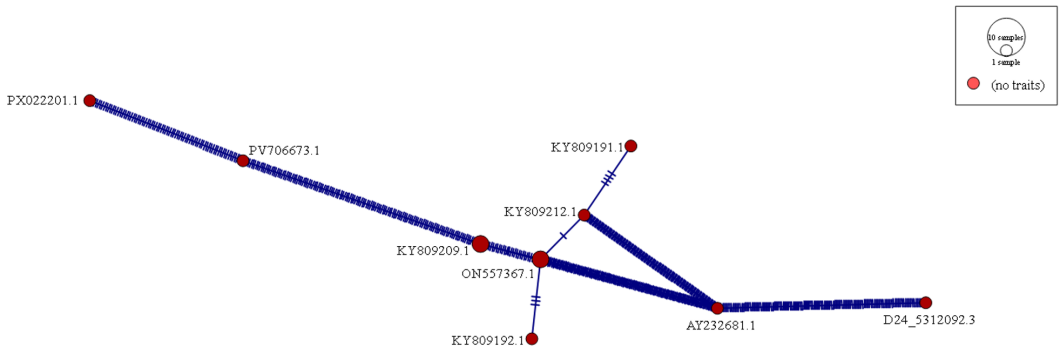

Figure 5. Haplotype network reconstruction of the D24 fungal group.

group is presented in Figure 5.

The presence of a separated node in the haplotype network can be interpreted as the result of environmental selection pressures, differences in microhabitat ecology, or restricted gene flow among populations. In fungal evolution, such patterns often reflect ongoing diversification processes, with unique haplotypes serving as indicators of the emergence of new lineages [9][46]. Moreover, the genetic differences observed in D24 may also indicate adaptations to specific hosts or changes in ecosystem conditions that influence reproductive dynamics and spore dispersal. Thus, haplotype network analysis not only corroborates the phylogenetic results but also provides additional perspectives on the evolutionary mechanisms shaping genetic diversity within the genus *Termitomyces*. This opens opportunities for further research, such as examining ecological associations with termite hosts, conducting population-level studies on a broader geographic scale, and performing functional analyses of genes potentially involved in environmental adaptation.

4. CONCLUSIONS

A total of 10 *Dendrobium* samples from the Liwa Botanical Garden showing fungal infection symptoms such as rust spots and necrosis were analyzed. DNA amplification of two selected isolates, D23 and D24, revealed specific bands of approximately 300 bp, with sequence lengths of 513 bp and 671 bp, respectively. Phylogenetic analysis showed that isolate D23 clustered with the *Xylariales* group, while isolate D24 clustered with the *Termitomyces* group, supported by sequence similarity to reference isolates. Genetic variation analysis through haplotype grouping confirmed distinct evolutionary lineages, with D24 exhibiting higher nucleotide diversity and evidence of stronger selective pressure compared to D23. These findings enrich the molecular sequence data of pathogenic fungi associated with native *Dendrobium* and provide a genetic basis to support accurate conservation strategies and disease management efforts for orchid collections in the Liwa Botanical Garden.

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M. M., A. S. P. and S. W.

Conflicts of Interest

The authors declare no conflict of interest.

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DECLARATION OF GENERATIVE AI

Not applicable.

REFERENCES

[1] A. Burzacka-Hinz, M. Dudek, N. Olędrzyńska, A. M. Naczk, and D. L. Szlachetko. (2025). "Evolution of Morphological Traits of Dendrobium Sensu Lato (Orchidaceae): An Attempt to Resolve Phylogenetic Relationships in Nominal and Morphologically Convergent Sections". *BMC Plant Biology.* **25** (1): 239. 10.1186/s12870-025-06263-w.

[2] D. Gandawidjaya and S. Sastrapradja. (1980). "Plasma Nutfah Dendrobium Asal Indonesia". *Buletin Kebun Raya Indonesia.* **4** (4): 113-125.

[3] A. S. Putra, A. Syarif, Mahfut, S. R. Sulistiyanti, and M. Hasibuan. (2024). "Performance Evaluation of Fuzzy Logic System for Dendrobium Identification Based on Leaf Morphology". *Journal of Applied Data Sciences.* **5** (4): 1520-1529. 10.47738/jads.v5i4.224.

[4] Mahfut, M. Riniarti, and E. Kurniawaty. (2025). "Morphology and Phytochemical Characterization of Native Dendrobium Used as a Herbal Medicine in Lampung, Indonesia". *SABRAO Journal of Breeding and Genetics.* **57** (2): 587-598. 10.54910/sabrao2025.57.2.17.

[5] S. Wahyuningsih, Mahfut, and D. S. Putri. (2025). "Genetic Stability and Phytochemical Properties of Dendrobium nobile Lindl. as an Antiviral Using RAPD". *SABRAO Journal of Breeding and Genetics.* **57** (3): 924-934. 10.54910/sabrao2025.57.3.5.

[6] Mahfut. (2021). "Identification and Efforts to Control Infection of Odontoglossum Ringspot Virus (ORSV) on Orchid". *International Journal of Engineering, Science and Technology.* **1** (1): 25-29. 10.52088/ijesty.v1i1.38.

[7] Mahfut, A. Syarif, and S. Wahyuningsih. (2025). "Molecular Phylogeny of rDNA-ITS on Native Dendrobium in Lampung". *Journal of Multidisciplinary Applied Natural Science.* **5** (2): 553-563. 10.47352/jmans.2774-3047.263.

[8] Mahfut, T. T. Handayani, S. Wahyuningsih, and Sukimin. (2021). "Identification of Dendrobium (Orchidaceae) in Liwa Botanical Garden Based on Leaf Morphological Characters". *Journal of Tropical Biodiversity and Biotechnology.* **6** (1): 1-6. 10.22146/jtbb.59423.

[9] Mahfut, A. Anggeiny, S. Wahyuningsih, T. T. Handayani, and Sukimin. (2020). "Identification of Disease and Efforts to Protect Native Orchid Plants Against Bacterial Infection in Liwa Botanical Garden". *Journal of Physics: Conference Series.* **1641** : 012098. 10.1088/1742-6596/1641/1/012098.

[10] Mahfut, M. V. T. Panjaitan, S. Wahyuningsih, T. T. Handayani, and Sukimin. (2021). "Identification of Disease and Efforts to Protect Natural Orchid Plants Against Fungal Infection in the Liwa Botanical Garden". *International Journal of Engineering, Science and Technology.* **1** (1): 30-35. 10.52088/ijesty.v1i1.39.

[11] Mahfut. (2020). "Identification and Detection of Odontoglossum Ringspot Virus on Native Orchid Collections of Nurseries in Java, Indonesia". *Journal of Physics: Conference Series.* **1641** : 012075. 10.1088/1742-6596/1641/1/012075.

[12] L. Anbiya, Mahfut, S. Wahyuningsih, and Suratman. (2024). "Optimum Temperature for Detection of TrAP and Rep Genes Using SPG1/SPG2 Primer Design on Red Chili (Capsicum annuum L.)". *AIP Conference Proceedings.* **2970** : 050034. 10.1063/5.0208192.

[13] A. Z. Septiana, Mahfut, T. T. Handayani, and Suratman. (2024). "Survey of Tomato Yellow Leaf Curl Virus (TYLCV) Infection on Solanum lycopersicum L. in Lampung, Indonesia". *AIP Conference Proceedings.* **2970** : 050027. 10.1063/5.0208190.

[14] B. Alberts, A. Johnson, and J. Lewis. (2002). "Molecular Biology of the Cell". Garland Science, New York.

[15] D. A. Animasaun, C. D. Nnamdi, O. I. Ipinmoroti, S. Oyedeji, E. A. Olonya, R. Krishnamurthy, and J. A. Morakinyo. (2022). "Molecular Identification and Phylogenetic Analysis of Fungi Contaminants Associated with In Vitro Cultured Banana Based on ITS Region Sequence". *HAYATI Journal of Biosciences.* **29** (3): 288-300. 10.4308/hjb.29.3.288-300.

[16] J. Xu. (2016). "Fungal DNA Barcoding". *Genome.* **59** (11): 913-932. 10.1139/gen-2016-0046.

[17] M. N. Maphosa, E. T. Steenkamp, A. M. Kanzi, S. V. Wyk, L. D. Vos, Q. C. Santana, T. A. Duong, and B. D. Wingfield. (2022). "Intra-Species Genomic Variation in the Pine Pathogen Fusarium circinatum". *Journal of Fungi.* **8** (7): 657. 10.3390/jof8070657.

[18] D. Parker, N. V. Meyling, and H. H. D. F. Licht. (2023). "Phenotypic Variation and Genomic Variation in Insect Virulence Traits Reveal Patterns of Intraspecific Diversity in a Locust-Specific Fungal Pathogen". *Journal of Evolutionary Biology.* **36** : 1438-1454. 10.1111/jeb.14214.

[19] D. Wang, L. Jun, N. Jorinde, I. F. S. Gomes, H. Jacquemyn, and V. S. F. T. Merckx. (2022). Root-associated Fungi in Orchidaceae: Diversity, Phylogeny, Ecology, and Outstanding Questions. *bioRxiv*. **16** : 519622. 10.1101/2022.12.16.519622.

[20] Mahfut, A. Tolangara, H. Ahmad, R. Rasyid, and Z. Ahmad. (2023). "Variation of Resistance and Physiological Response of Orchid from Induction Trichoderma to Infection Odontoglossum Ringspot Virus (ORSV)". *Asian Journal of Plant Sciences.* **22** (3): 496-505. 10.3923/ajps.2023.496.505.

[21] C. S. Bhunjun, A. J. L. Phillips, R. S. Jayawardena, I. Promputtha, and K. D. Hyde. (2021). "Importance of Molecular Data to Identify Fungal Plant Pathogens and Guidelines for Pathogenicity Testing Based on Koch's Postulates". *Pathogens.* **10** (9): 1096. 10.3390/pathogens10091096.

[22] C. Chaisiri, X. Liu, Y. Lin, Y. Fu, F. Zhu, and C. Luo. (2021). "Phylogenetic and Haplotype Network Analyses of Diaporthe eres Species in China Based on Sequences of Multiple Loci". *Biology.* **10** (3): 179. 10.3390/biology10030179.

[23] S. Feng, Y. Jiang, S. Wang, M. Jiang, Z. Chen, Q. Ying, and H. Wang. (2015). "Molecular Identification of Dendrobium Species (Orchidaceae) Based on the DNA Barcode ITS2 Region and Its Application for Phylogenetic Study". *International Journal of Molecular Sciences.* **16** (9): 21975-21988. 10.3390/ijms160921975.

[24] M. Korabečná, V. Liška, and K. Fajfrlík. (2003). "Primers ITS1, ITS2 and ITS4 Detect the Intraspecies Variability in the Internal Transcribed Spacers and 5.8S rRNA Gene Region in Clinical Isolates of Fungi". *Folia Microbiologica.* **48** : 233-238. 10.1007/BF02930961.

[25] T. K. Bag, P. Dutta, M. Hubballi, R. Kaur, M. Mahanta, A. Chakraborty, G. Das, M. Kataky, and R. Waghunde. (2024). "Destructive Phytophthora on Orchids: Current Knowledge and Future Perspectives". *Frontiers in Microbiology.* **14** : 1139811. 10.3389/fmicb.2023.1139811.

[26] W. C. Tsao, Y. H. Li, Y. H. Tu, Y. S. Nai, T. C. Lin, and C. L. Wang. (2025). "Identification and Molecular Detection of the Pathogen of Phalaenopsis Leaf Yellowing Through Genome Analysis". *Frontiers in Microbiology.* **15** : 1431813. 10.3389/fmicb.2024.1431813.

[27] A. Jain, S. Sarsaiya, J. Chen, Q. Wu, Y. Lu, and J. Shi. (2021). "Changes in Global

Orchidaceae Disease Geographical Research Trends: Recent Incidences, Distributions, Treatment, and Challenges". *Bioengineered.* **12** (1): 13-29. 10.1080/21655979.2020.1853447.

[28] D. Asadudin, Mahfut, S. Wahyuningsih, N. Nurcahyani, F. R. Lumbanraja, and B. Irawan. (2025). "Morphological Characteristics of the Pitcher Plant (Nepenthes spp.) in Lampung, Indonesia". *SABRAO Journal of Breeding and Genetics.* **57** (5): 2057-2068. 10.54910/sabrao2025.57.5.26.

[29] M. Arsitalia, Mahfut, F. R. Lumbanraja, B. Irawan, and N. Nurcahyani. (2026). "Stomatal Morpho-Anatomical Characterization of the Native Coelogyne in Liwa Botanical Garden, Lampung, Indonesia". *SABRAO Journal of Breeding and Genetics.* **58** (1): 338-347. 10.54910/sabrao2026.58.1.31.

[30] L. Anbiya, Mahfut, F. R. Lumbanraja, S. Wahyuningsih, and N. Nurcahyani. (2026). "Morphological Characterization and Kinship Analysis of the Dendrobium from Liwa Botanical Garden". *SABRAO Journal of Breeding and Genetics.* **58** (1): 205-214. 10.54910/sabrao2026.58.1.19.

[31] Mahfut, H. E. Sari, and P. Kendari. (2024). "Habitat Identification and Phenetic Analysis of Bryophyta in Lampung, Indonesia". *AIP Conference Proceedings.* **2970** : 050014. 10.1063/5.0208187.

[32] Mahfut, M. Sari, H. M. Akin, S. Wahyuningsih, and B. Irawan. (2025). "Molecular Characterization and Effectiveness Cross-Protection of Weak Strains Against Super-Infection Malignant Strains Cucumber Mosaic Virus (CMV) on Cayenne Pepper (Capsicum annuum L.) in Lampung, Indonesia". *Jurnal Hama dan Penyakit Tumbuhan Tropika.* **25** (1): 63-73. 10.23960/jhptt.12563-75.

[33] S. Wahyuningsih, Mahfut, and L. Anbiya. (2026). "Stomata Characterization of Native Dendrobium in Liwa Botanical Garden". *Journal of Multidisciplinary Applied Natural Science.* **6** (2): 592-603. 10.47352/jmans.2774-3047.347.

[34] Mahfut, P. Kendari, R. Bangsawan, and E. Susiyanti. (2024). "Agronomic Characteristics of Sugarcane Cultivar GMP3 Mutants Induced Through Colchicine". *SABRAO Journal of Breeding and Genetics.* **56** (3): 1083-1094. 10.54910/sabrao2024.56.3.16.

[35] Mahfut, P. Kendari, E. Susiyanti, A. N. Ahyar, and R. Bangsawan. (2024). "Molecular Characterization of the Sugarcane Cultivar GMP3 Mutants Induced Through Colchicine Using PCR-RAPD Markers". *SABRAO Journal of Breeding and Genetics.* **56** (3): 940-950. 10.54910/sabrao2024.56.3.4.

[36] Mahfut, P. Kendari, A. Syarif, S. Wahyuningsih, and E. Susiyanti. (2023). "Stomata Characters of Sugarcane (Saccharum officinarum L.) Mutants of GMP3 Variety at PT Gunung Madu Plantations, Lampung, Indonesia". *Journal of Tropical Biodiversity and Biotechnology.* **8** (3): 1-12. 10.22146/jtbb.79860.

[37] M. Y. Chung, M. Y. Chung, J. Merilä, J. Li, K. Mao, J. López-Pujol, Y. Tsumura, M. G. Chung, and M. G. Chung. (2023). Neutral and adaptive genetic diversity in plants: An overview. *Frontiers in Ecology and Evolution*. **11**. 10.3389/fevo.2023.1116814

[38] S. Nuraini, Mahfut, and R. Bangsawan. (2024). "Germination of Bud Chips 5 Varieties of Commercial Sugar (Saccharum officinarum L.) at PT Gunung Madu Plantation". *AIP Conference Proceedings.* **2970** : 050037. 10.1063/5.0208193.

[39] Y. Simamora, Mahfut, and R. Bangsawan. (2024). "Germination Test of Commercial Variety of Sugarcane (Saccharum officinarum L.) in the End Planting Month Using Bud Chips at PT Gunung Madu Plantations". *AIP Conference Proceedings.* **2970** : 050029. 10.1063/5.0208191.

[40] L. L. Aritonang, Mahfut, and A. N. Ahyar. (2024). "Acclimatization of Sugarcane (Saccharum officinarum L.) the Results of Tissue Culture at PT Gunung Madu Plantations". *AIP Conference Proceedings.* **2970** : 050004. 10.1063/5.0208181.

[41] A. Y. Putera, I. Mawardi, M. Ananda, D. I.

Sephanti, O. P. U. Rajagukguk, D. P. Pubianty, Mahfut, A. N. Ahyar, R. Bangsawan, and E. Susiyanti. (2024). "The Effect of Colchicine on Anatomic Stomata Characteristics of 24 Mutants of RGM 01-1869 Varieties in PT Gunung Madu Plantations". *AIP Conference Proceedings.* **2970** : 050038. 10.1063/5.0208535.

[42] D. Asadudin, I. P. Mulya, M. A. Nugraha, S. N. A. Hasanudin, Mahfut, E. Susiyanti, and A. N. Ahyar. (2024). "Anatomic Characterization of 24 Mutant Varieties of GMP6 in PT Gunung Madu Plantations". *AIP Conference Proceedings.* **2970** : 050039. 10.1063/5.0208463.

[43] Mahfut, M. Ananda, E. Susiyanti, and R. Agustina. (2025). "Screening of Sugarcane Commercial GMP Varieties Tolerant to Drought Stress Based on Molecular Detection of P5CS Gene". *Journal of Multidisciplinary Applied Natural Science.* **5** (1): 119-129. 10.47352/jmans.2774-3047.233.

[44] Mahfut, V. D. A. Sari, A. Widyawan, S. Wahyuningsih, and B. Irawan. (2025). "Phenotypic Stability of Mutant Sugarcane (Saccharum officinarum L.) Tolerant to Drought Stress Based on Variations in Morphological Traits at the Greenhouse Scale". *International Journal of Agricultural Technology.* **21** (3): 959-972. 10.63369/ijat.2025.21.3.959-972.

[45] H. E. Sari, Mahfut, A. Widyawan, E. Susiyanti, S. Wahyuningsih, and B. Irawan. (2025). "Sugarcane Commercial Cultivars with Drought Stress Tolerance on In Vitro and Greenhouse Scales". *SABRAO Journal of Breeding and Genetics.* **57** (1): 137-148. 10.54910/sabrao2025.57.1.14.

[46] Mahfut, A. Setiawan, M. Sari, V. E. Sijabat, V. A. P. Siregar, and Z. Ahmad. (2023). "Molecular Characterization of Mycorrhiza and Its Potential as Biocontrol". *SABRAO Journal of Breeding and Genetics.* **55** (6): 2092-2104. 10.54910/sabrao2023.55.6.21.