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Research Article

Micromorphological Characterization and DNA Barcoding of *Durio macrantha* Kosterm. from the Bogor Botanical Garden Collection

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ABSTRACT

Durio macrantha is an endemic species first discovered in 1981 in Gunung Leuser National Park, North Sumatra, and has been conserved at the Bogor Botanical Garden (BBG) since 1994. This study aims to characterize the micromorphology and DNA profile of *D. macrantha* using DNA barcoding with *rbc*L and ITS gene markers. Sample of *D. macrantha* were collected from the BBG for analysis. Micromorphological analysis revealed that the leaves exhibit three vein types (primary, secondary, and tertiary) with a pentagonal venation pattern and a tri-veinlet pattern. Additionally, eight types of trichomes were observed on the abaxial surface. Molecular characterization showed that the *rbc*L sequence was 563 bp long, with nucleotide composition T (27.9%), C (21.7%), A (27.9%), and G (22.6%). The ITS sequence was 960 bp long, with nucleotide composition T (15.4%), C (33.4%), A (19.0%), and G (32.2%). BLAST analysis of both *rbc*L and ITS genes revealed a high level of similarity of around 100% between *D. macrantha* and *Durio zibethinus*. This study provides fundamental data supporting the conservation and further research of *D. macrantha*, particularly in morphological and molecular aspects.

Keywords: BLAST analysis, conservation biology, micromorphology, molecular characterization, nucleotide composition.

Introduction

Durio macrantha Kosterm. is the only species of *Durio* (Malvaceae) endemic to Sumatra, occupying a unique taxonomic position among congeners that are primarily distributed in Kalimantan and other parts of Southeast Asia (POWO, 2025). First described by Kostermans in 1992, this species is known only from Gunung Leuser National Park, a key biodiversity hotspot in North Sumatra (GBIF Secretariat, 2025; Kostermans, 1992). Its highly restricted natural range highlights its ecological vulnerability and the urgent need for targeted conservation efforts and scientific investigation.

The Bogor Botanical Gardens (BBG) serve as a vital center for *ex situ* conservation of tropical plant diversity, including threatened durian species such as *D. macrantha*, locally referred to as “durian dok” (Aprilianti, 2019). Initially discovered in 1981 and introduced to BBG in 1994, the species is currently maintained as two

living specimens within the collection. Compared to the widely cultivated *Durio zibethinus*, *D. macrantha* is characterized by a taller stature (20–30 m), larger flowers, higher fruit productivity, a milder aroma, and the absence of nectar (Kostermans, 1992). Despite these unique traits, comprehensive data on its morphological and phylogenetic characteristics remain limited, underscoring the necessity for further research.

To enhance conservation efforts for *D. macrantha*, an integrated approach combining morphological and molecular methods is essential. Micromorphology analysis aims to confirm the identity of plant type or species which involves detailed examination of features such as trichomes, pollen, seeds, and vegetative organ (Barthlott & Neinhuis, 1997; Shehata et al., 2024). According to Salma (1999), the micromorphology characteristics of the genus *Durio*, particularly the variation in trichome types, provide an effective basis for identifying the majority of species and for distinguishing taxa at the infraspecific level. The leaf surface features are predominantly controlled by genetic mechanisms and exhibit minimal influence from environmental factors.

However, plant morphological characters can be influenced by ecological and environmental conditions (rainfall, temperature, and soil composition), which may result in less distinctive traits (Alcántara-Ayala et al., 2020; Ekanayaka et al., 2025). Therefore, molecular methods including DNA barcoding, phylogenetic analysis, and genome sequencing are recognized as rapid, reliable and efficient approaches for species identification and for addressing taxonomic challenges posed by cryptic species (Ekanayaka et al., 2025). DNA barcoding using *rbc*L and ITS sequences is a reliable tool for accurately identifying and assessing the genetic diversity of plant species (Purty & Chatterjee, 2016). Currently, no molecular data for *D. macrantha* is available in GenBank, and its conservation status is categorized as Data Deficient by the IUCN (Rahman, 2021). Therefore, this study aims to conduct micromorphological and molecular characterization of *D. macrantha* to support conservation initiatives and expand the existing database on durian diversity in Indonesia.

Materials and Methods

Sampling of D. macrantha

Fresh mature leaves of *D. macrantha* were collected from the BBG under the collection number XIX.F.86a. The samples used in this study were healthy, free from pests, and showed no signs of fungal infestation. After collection, the leaves were stored in a sealed container with ice gel to maintain freshness during transport from the field.

Micromorphology Analysis

Observations were conducted qualitatively and quantitatively using the references from Corpuz et al. (2007). Specific adaxial leaf structures, such as veins, venation patterns, and veinlet patterns, were described following Ellis et al. (2009) and Hickey (1973), while abaxial leaf characteristics focused on trichomes were analyzed according to Salma (1999).

Isolation of Genomic DNA

Genomic DNA was extracted using the GeneJET Plant Genomic DNA Kit (K0791) following the manufacturer's protocol. Fresh leaf tissue (50 mg) was manually ground using a mortar and pestle and mixed with 500 µl Lysis Buffer A, 50 µl Lysis Buffer B, and 20 µl RNase A in a 1.5 ml microcentrifuge tube. The mixture was vortexed for 30 sec and incubated at 65°C for 10 min with periodic inversion. Next, 130 µl Precipitation Solution was added, mixed, and incubated at -20°C for 5 min before centrifugation at 13,000 × *g* for 5 min to separate the supernatant. A total of 450 µl of the supernatant was transferred to a new tube, combined with 400 µl Plant gDNA Binding Solution and 400 µl of 96% ethanol, mixed thoroughly, and transferred to a spin column. The mixture was

centrifuged at 6,000 × *g* for 1 min, and the filtrate was discarded. The process was repeated until the entire sample was filtered. The spin column was then sequentially washed with 500 µl Wash Buffer I (8,000 × *g*, 1 min) and 500 µl Wash Buffer II (13,000 × *g*, 3 min), followed by an additional 1-min centrifugation at 13,000 × *g* to dry the membrane. DNA was eluted with 50 µl Elution Buffer, incubated at room temperature for 5 min, and centrifuged for 1 min at 8,000 × *g*. The elution step was repeated twice before discarding the spin column. The extracted DNA was stored at -20°C for further analysis.

PCR Amplification and DNA Detection

PCR amplification was performed in a total reaction volume of 50 µl, comprising 25 µl PCR Mix MayTaq, 5 µl primers (forward and reverse), 6 µl DNA template, and 14 µl ddH_2O. The reaction mixture was placed in a 1.5 ml microtube and centrifuged for 30 sec to ensure thorough mixing. The primer sequences used for *rbc*L gene amplification were *rbc*L aF (5'-ATGTCACCACAAACAGAGACT AAAGC-3') and *rbc*L aR (5'-GTAAAA TCAAGTCCACCRCG-3') (Bafeel et al., 2012), while for the ITS gene, AB101F (5'-ACGAATTCATGGTCCGGTGAAGTGT TCG-3') and AB102R (5'-TAGAATTCC CCGGTTCGCTCCCCGTTAC-3') were used (Cheng et al., 2016). PCR amplification was conducted using a Thermal Cycler with the following conditions: an initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 15 sec, annealing at 52°C for the *rbc*L marker or 58°C for the ITS marker for 15 sec, and extension at 72°C for 10 sec, with a final extension at 72°C for 5 min.

Following PCR amplification, 5 µl of the PCR product was separated on a 1% agarose gel and visualized using 1 µl of GelRed. Electrophoresis was conducted at 100 V for 45 min, and DNA bands were observed under UV light.

DNA Sequencing and Data Analysis

DNA sequencing was performed by 1st Base Malaysia using the Sanger sequencing method. The obtained *rbc*L and ITS sequences were assembled into contigs using the ClustalW method (Thompson et al., 1994) and the assembled contigs were subsequently submitted to the NCBI GenBank. Sequence homology was determined through BLAST analysis (https://blast.ncbi.nlm.nih.gov/) of the *rbc*L and ITS sequences (Aprilianingsih et al., 2022). A total of eighteen *rbc*L sequences and twenty ITS sequences from *Durio* species obtained from the BLAST results were downloaded for phylogenetic tree construction with *Theobroma cacao* was used as an outgroup. Multiple sequence alignment was performed using the MUSCLE algorithm (Edgar, 2004). The phylogenetic tree was constructed using the maximum likelihood (ML) method with 1,000 bootstrap replications to enhance the reliability of the phylogenetic analysis. All analysis were conducted using MEGA11 software (Tamura et al., 2021).

Results and Discussion

Micromorphological Characters of D. macrantha

The morphological observations of *D. macrantha* during the vegetative phase indicate that this species belongs to the dicotyledonous plant group. This classification is supported by its pinnate venation pattern, where the primary vein extends toward the leaf apex, while secondary veins branch from the primary vein and reconnect with it. Additionally, tertiary veins exhibit a reticulate pattern, forming connections with the secondary veins (Figure 1). These morphological characteristics align with the findings of Fujita & Mochizuki (2006), who reported that dicotyledonous plants typically possess a pinnate venation pattern composed of primary, secondary, and tertiary veins.

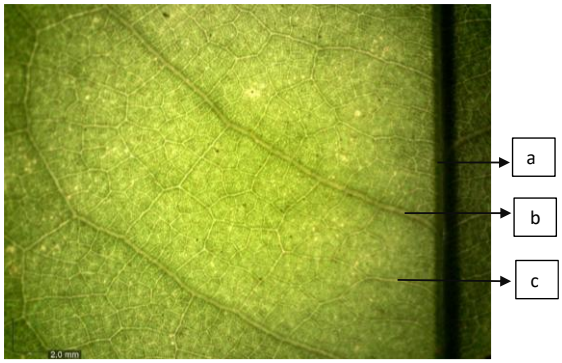

Fig. 1. Vein patterns on leaves of *Durio macrantha*. (a) primary vein, (b) secondary vein, (c) tertiary vein.

The leaf venation pattern represents a form of morphological diversity influenced by various factors, including leaf shape, cell division patterns, auxin activity, and leaf surface area (Fujita & Mochizuki, 2006). Detailed observations revealed that the tertiary veins of *D. macrantha* exhibit a regular reticulate venation pattern, characterized by a relatively uniform areola formation. According to Hickey's classification (1973), the areola shape in this species falls into the pentagonal type, with veinlets exhibiting more than one branching. Furthermore, the observed veinlet pattern in *D. macrantha* corresponds to a tri-veinlet tertiary vein structure (Figure 2).

Durio species generally possess trichomes on the leaf surface, particularly on the abaxial side (Priyanti et al., 2015). Trichomes are modifications of epidermal cells that exhibit variations in shape, structure, and function. Based on the number of constituent cells, trichomes are classified as unicellular or multicellular, while in terms of secretion function, they are categorized as glandular or non-glandular. Glandular trichomes contain secretory tissues that facilitate the secretion of specific substances, whereas non-glandular trichomes lack secretory tissues and primarily serve as a physical barrier against biotic and abiotic stressors (Tozin et al., 2016).

The present analysis identified trichomes in *D. macrantha* as belonging to the non-glandular category, with eight distinct types observed: complex peltate scale, simple peltate scale, flat stellate hair, three-armed stellate, four-armed stellate, five-armed stellate, seven-armed stellate, and eleven-armed stellate (Figure 3). These trichomes play a crucial role in plant protection by mitigating environmental stressors such as high temperatures, solar radiation, and water loss from the leaf surface (Werker, 2000). The distinct morphological characteristics of trichomes provide valuable taxonomic markers for the identification and classification of species within the genus *Durio*.

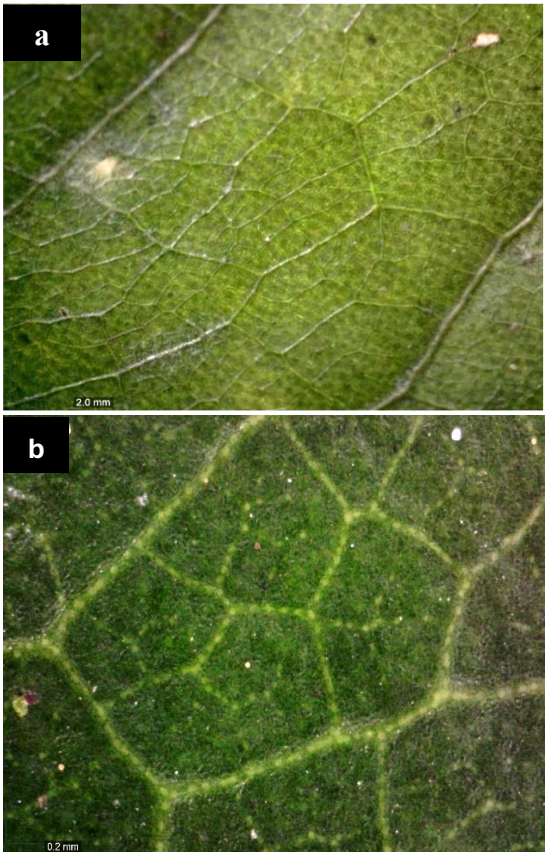

Fig. 2. Visualization of the characteristic patterns in *Durio macrantha*. (a) areola patterns, (b) veinlet patterns.

Molecular Characterization of D. macrantha Using rbcL and ITS Markers

The *D. macrantha* sample was successfully extracted and amplified using the *rbc*L and ITS primers (Figure 4). Molecular characterization revealed that

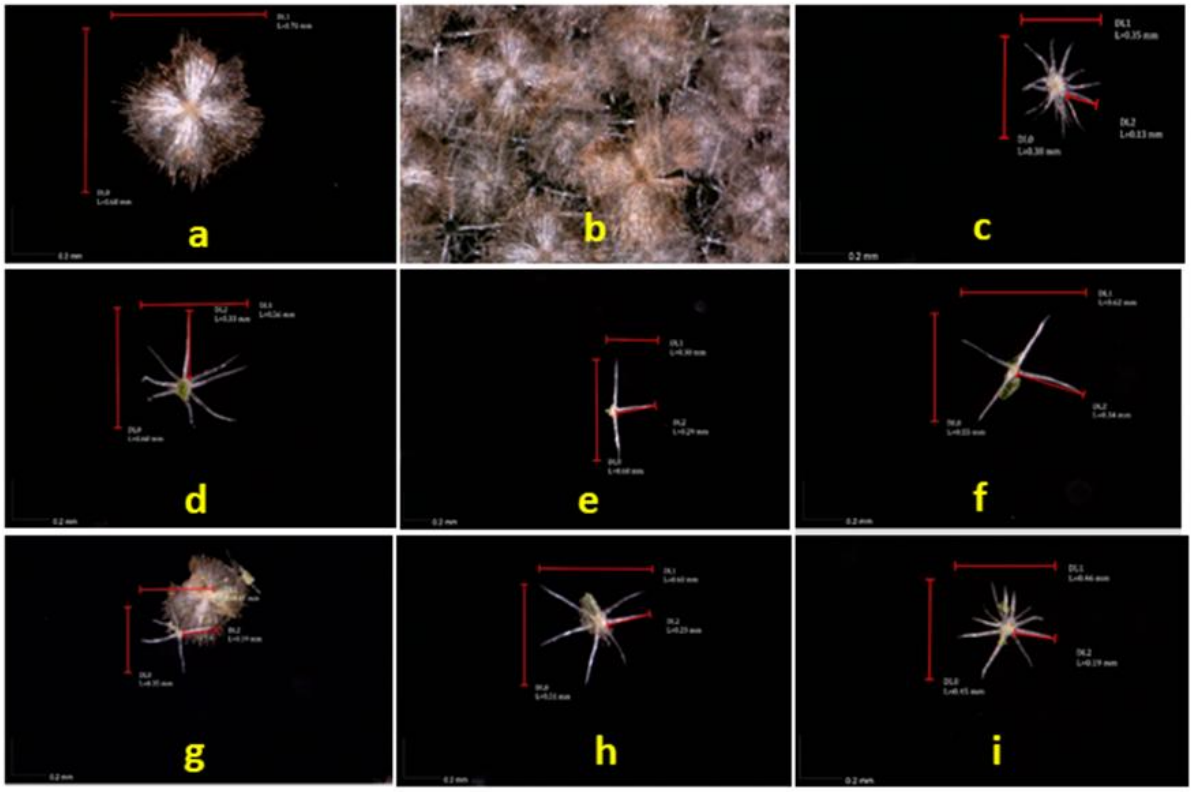

Fig. 3. Types of trichomes observed on *Durio macrantha* leaves. (a) complex peltate scale, (b) complex peltate collection, (c) simple peltate scale, (d) flat stellate hair, (e) three-armed stellate, (f) four-armed stellate, (g) five-armed stellate, (h) seven-armed stellate, (i) eleven-armed stellate, (j) trichomes on swollen structures, (k) trichomes on leaf stalks.

the *rbc*L sequence length was 563 bp, with a nucleotide composition of thymine (T) 27.9%, cytosine (C) 21.7%, adenine (A) 27.9%, and guanine (G) 22.6%. In comparison, the ITS sequence length was 960 bp, with a nucleotide composition of T (15.4%), C (33.4%), A (19.0%), and G (32.2%) (Figure 5). The *rbcL* sequence had a GC content of 44.2%, whereas the ITS sequence showed a higher GC content of 65.6%. Both sequences were successfully submitted to the NCBI GenBank under accession numbers PV524687 (*rbc*L) and PV495751 (ITS).

BLAST Analysis and phylogenetic relationship

BLAST analysis of *D. macrantha* samples against the NCBI database showed identical percentage identity results for both the *rbc*L sequence and the ITS region (Figure 6), with 100% sequence similarity to *D. zibethinus*. The query cover value reached 100% for *rbc*L sequence and was also relatively high for the ITS region at 87%. These high identity percentages, along with the substantial query cover values, indicate a very close related between *D. macrantha* and *D. zibethinus*.

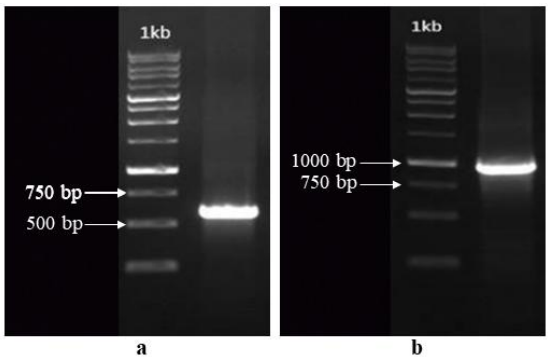

Fig. 4. Electrophoresis profile of *Durio macrantha*. PCR products amplified using: (a) *rbc*L marker, (b) ITS marker.

This study successfully reconstructed the phylogenetic tree of *D.*

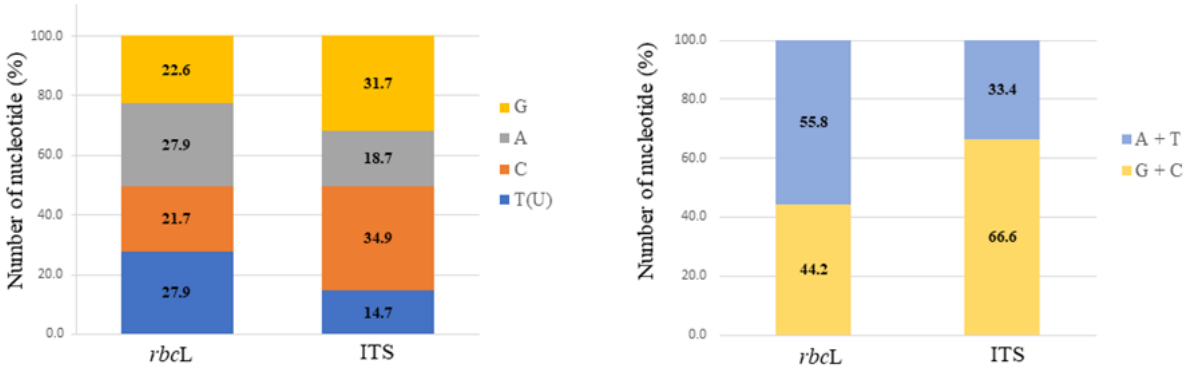

Fig. 5. Nucleotide composition of *Durio macrantha* in the *rbc*L sequence and ITS region.

macrantha and its close relatives within the genus *Durio* using the Maximum Likelihood algorithm (Figure 7). The tree was generated based on the top five BLAST hits of *D. macrantha* sequences, selected according to percentage identity, as well as additional *Durio* species sequences available in the NCBI GenBank database. Phylogenetic analyses of both the *rbc*L gene and the ITS region consistently showed that *D. macrantha* is positioned very closely to *D. zibethinus* in the resulting trees.

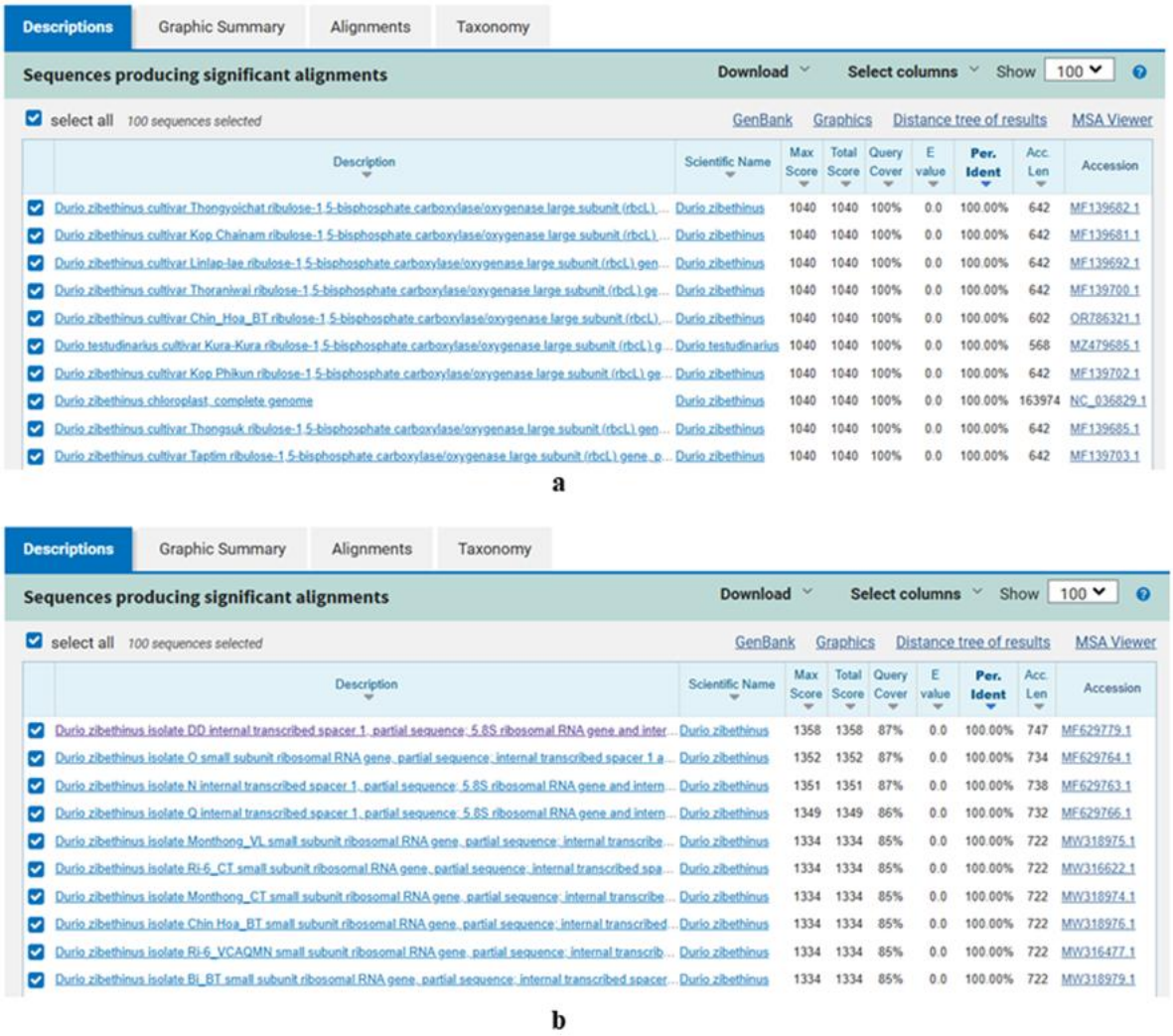

Fig. 6. BLAST analysis of *Durio macrantha* samples against the NCBI database. (a) *rbc*L gene sequence, (b) ITS region sequence.

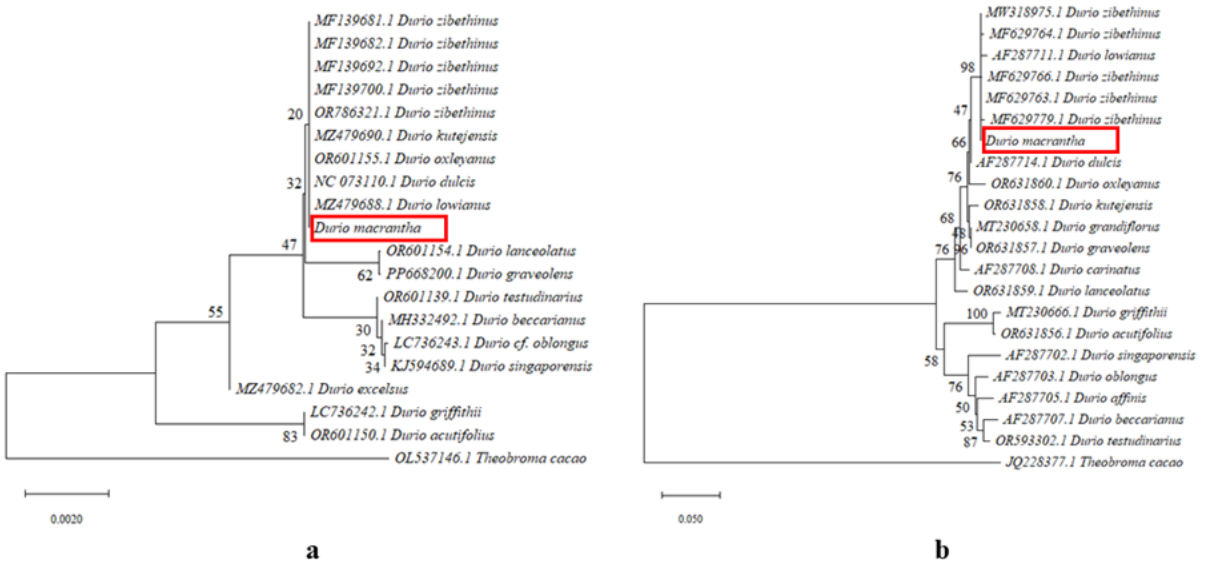

Fig. 7. Phylogenetic tree of *Durio macrantha* and its close relatives within genus *Durio*. (a) *rbc*L sequence, (b) ITS region. The phylogenetic tree was constructed using the Maximum Likelihood algorithm with 1,000 bootstrap replications. Numbers above the branches indicate bootstrap support values, while species codes represent accession numbers from the NCBI Gene Bank. The red box highlights the position of *D. macrantha* in the tree. The Scale bars represent genetic distance.

Upon closer examination, the phylogenetic tree based on the *rbc*L sequence was unable to clearly resolve species boundaries. This limitation is evident from the extremely low genetic distances (in some cases, zero) and the clustering of multiple distinct species within the same clade. Furthermore, the *rbc*L-based tree exhibited low bootstrap support values, indicating weak confidence in the branching patterns and suggesting potential bias in species delineation. As noted by Felsenstein (1985), higher bootstrap values indicate greater confidence in the placement of branches within a phylogenetic tree, whereas lower values correspond to greater uncertainty regarding the accuracy of those positions. In this tree, *D. macrantha* clustered with all five *D. zibethinus* sequences from the top BLAST hits, as well as with *D. kutejensis* (MZ479690.1), *D. oxleyanus* (OR601155.1), *D. dulcis* (NC073110.1), and *D. lowianus* (MZ479688.1), supported by a low bootstrap value of 20. The low bootstrap support values on this clade (<50) indicate an uncertain arrangement of taxa within the branch (Berry and Gascuel, 1996).

In contrast, the phylogenetic tree based on the ITS region showed a more robust and well-supported structure. This was reflected in higher bootstrap values across several branches and clearer species groupings. In this tree, *D. macrantha* grouped with the same five *D. zibethinus* sequences (top BLAST hits) and *D. lowianus* (AF287711.1), supported by a high bootstrap value of 98 and a very low genetic distance (<0.005). These findings strongly support a close phylogenetic relationship between *D. macrantha* and *D. zibethinus*.

The use of the *rbc*L marker for species identification and phylogenetic analysis within the *Durio* genus in this study proved to be relatively weak. This finding supports previous research on *Durio* relatives by Magandhi et al. (2024), which also reported weak and potentially biased phylogenetic tree construction when using *rbc*L for taxonomic resolution. Similar results were observed in the case of bamboo (Awaliah & Polosoro, 2024), which demonstrated very low variability

among species and even genera, rendering *rbc*L ineffective for species-level identification. This limitation may be attributed to the functional role of *rbc*L as a gene encoding the RuBisCo enzyme, which is essential for carbon fixation during photosynthesis (Nurhasanah et al., 2019). Due to this conserved function, *rbc*L tends to have a low mutation rate and thus produces limited genetic variation among closely related species (Basith, 2015; Hollingsworth et al., 2011). Consequently, while *rbc*L is useful for identifying species at the genus level, its resolution power at the intraspecific or closely related species level is often suboptimal (Gielly & Taberlet, 1994).

On the other hand, the ITS region is more variable, allowing for higher-resolution species identification and making it a more effective marker for phylogenetic analysis at the intraspecific level. This increased variability is due to the high mutation rate of the ITS region, which is a non-coding sequence and thus more prone to genetic changes caused by recombination events during cell division (Cheng et al., 2016). As a non-coding region, the ITS evolves more rapidly, enhancing its effectiveness in distinguishing species with close phylogenetic relationships (Kress & Erickson, 2007).

Overall, this study found that *D. macrantha*, an endemic species of Sumatra restricted to Gunung Leuser National Park, is closely related to *D. zibethinus* in terms of genetic affinity. It is hoped that these findings will contribute valuable information to the conservation efforts and future development of durian varieties.

Conclusion

The leaf micromorphology of *D. macrantha* is characterized by three types of veins—primary, secondary, and tertiary—with a distinct pentagonal venation and tri-veinlet pattern. Eight types of trichomes were identified on the abaxial leaf surface. Molecular analysis using *rbc*L and ITS gene markers revealed sequence lengths of 563 bp and 960 bp, respectively, with specific nucleotide compositions. BLAST results indicated a high level of genetic similarity between *D. macrantha* and *D. zibethinus*. These findings contribute valuable morphological and molecular data that support the conservation and further study of *D. macrantha*.

Conflict of Interest

We confirm that we have no conflict of interest regarding any financial, personal, or other affiliations with individuals or organizations related to the subject matter discussed in the manuscript.

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