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PLASTOME REPORT

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The complete chloroplast genome of *Durio zibethinus* L. cultivar Ri6 (Helicteroideae, Malvaceae)

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ABSTRACT

Durian, a member of the Malvaceae family, is famous for its delicious fruits, which have strong scents and are rich in nutrients. In this study, we sequenced and characterized the complete chloroplast genome of *Durio zibethinus* L. 1774 cultivar Ri6, a popular durian cultivar in Vietnam, using the Illumina Hiseq platform. The results showed a circular chloroplast genome composed of a large single copy of 96,115 bp, a small single copy of 20,819 bp, and two inverted repeat regions of 24,185 bp. This genome consisted of 79 protein-coding genes, 30 transfer RNA genes, and four ribosomal RNA genes. The overall GC content of this genome was 35.7%. Phylogenetic analysis inferred from 78 protein-coding regions revealed monophyly of *Durio* species and a close relationship between *D. zibethinus* cultivar Ri6 and cultivar Mongthong. This study provides essential information for further studies examining genetic population, breedings, and species identification among *Durio* taxa and cultivars.

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Durian; king fruit; Malvaceae; phylogeny; plastome

Introduction

Durian (*Durio zibethinus* L. 1774) is a Malvaceae tropical tree, distributed mainly in the Southeast Asia region (POWO, 2023). The durian fruits are rich in nutrients (i.e. fat, sugar, and vitamins) and contain various flavonoids, ascorbic acid, and carotenoids (Aziz and Jalil 2019). These contents revealed antioxidant features and health benefits (Arancibia-Avila et al. 2008; Ho and Bhat, 2015; Ali et al. 2020; Arsa et al. 2021). There are several varieties and cultivars of durians of which genetic variations and phylogeny have been explored (Nyffeler and Baum, 2000; Nyffeler and Baum, 2001; Santoso et al. 2013; Santoso et al. 2017; Khang et al. 2021; Mursyidin 2022). Additionally, the draft genome and transcriptome data of durian were reported and provided essential data for evolutionary studies of *Durio* and related species (Teh et al. 2017)

The chloroplast is an essential organelle of land plants and contains a circular genome that encodes different genes for photosynthesis (Dobrogojski et al. 2020). Additionally, the chloroplast genome exhibited a useful role in elucidating the phylogeny of angiosperms and genetic engineering (Daniell et al. 2016; Gitzendanner et al. 2018). In Malvaceae, complete chloroplast genomes of various species have been published (Cvetković et al. 2021). The complete chloroplast genomes of *D. zibethinus* and *D. oxleyanus* were recently reported (Cheon et al. 2017; Wong et al. 2022). Therefore, in this study, we sequenced and characterized the complete chloroplast genome of *D. zibethinus* cultivar Ri6 using the next-generation sequencing method. Additionally, the phylogenetic analysis inferred from 78 protein-coding regions of *D. zibethinus* and 43 related species in Malvaceae was reconstructed using the Maximum likelihood and Bayesian inference methods (Ronquist et al. 2012; Huelsenbeck, 2019; Minh et al. 2020).

Materials and methods

Fresh young leaves of *D. zibethinus* L. 1774 cultivar Ri6 were collected at Cho Lach, Ben Tra Province, Vietnam (N10°15'23.1", E106°08'52.3") and dried in silica gel beads (Figure 1). No specific permission was required to collect *D. zibethinus* cultivar Ri6 because this species is considered a common plant in Vietnam. The voucher specimen was stored at the Institute of Food and Biotechnology, Can Tho University (contact person: Dr. Nguyen Pham Anh Thi; email: npathi@ctu.edu.vn; under voucher number BRDI-THI 20230701-001). The total genomic DNA was extracted from dried leaves using a Dneasy Plant Mini Kit (Qiagen, USA). Agarose gel electrophoresis and DNA spectrophotometers were used to check the quality of the DNA sample. The high-quality DNA (showing a clear band on the agarose gel and having concentration over 100 ng/ul; A260/280 and A260/230 ratios in a range of 1.8–2.0 and 2.0–2.2, respectively) was

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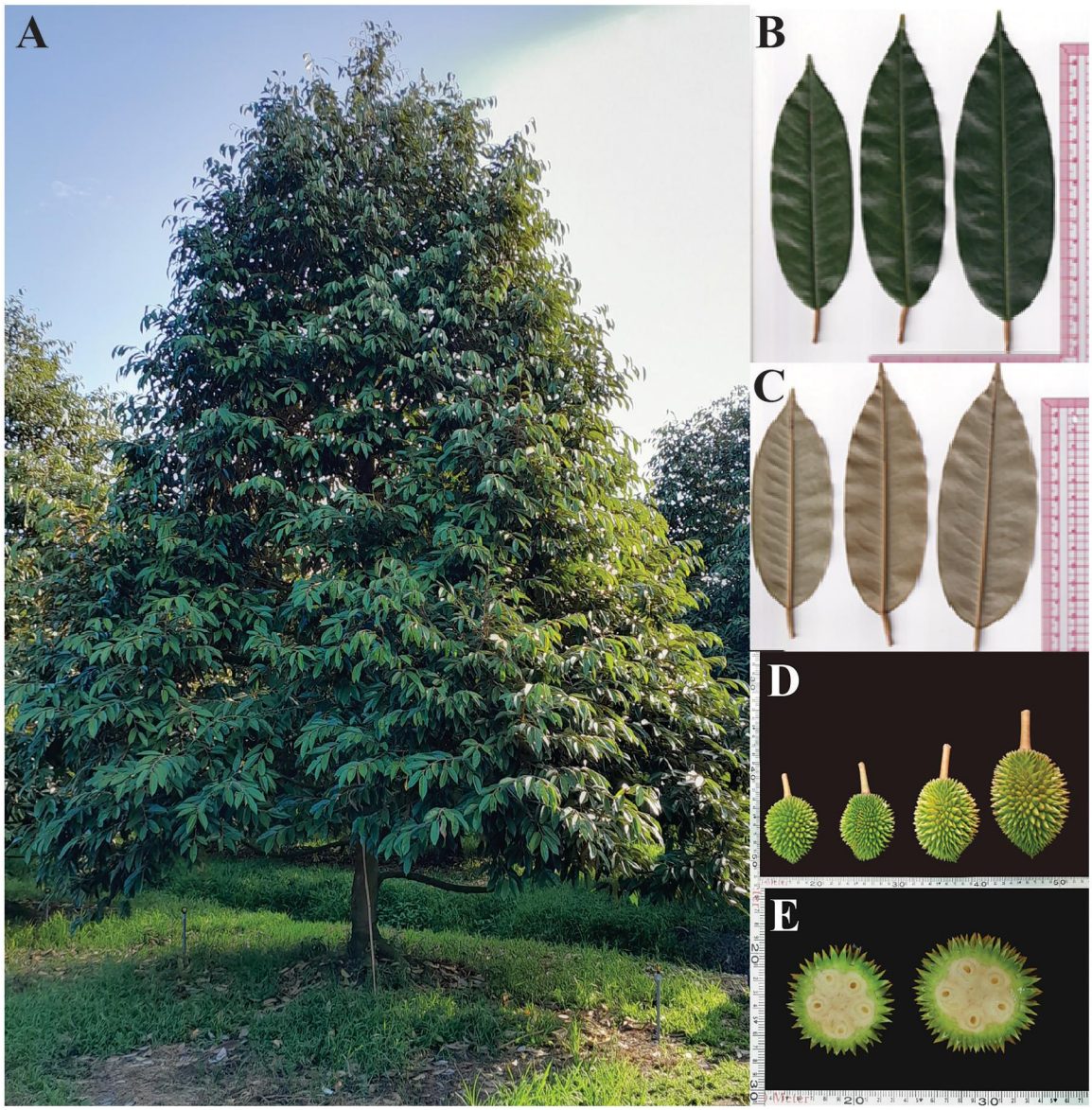

Figure 1. The photo of *Durio zibethinus* cultivar Ri6. A. The whole plant. B. The adaxial surface of leaves. C. The abaxial surface of leaves. D. The young fruits. E. The fruits cut in half. Note: the tree branch type is intermediate. The leaf is oblong; the upper surface is dark green, and the lower surface is beige or brown. The fruit is green and oval with five carpels.The photo was self-taken by the first authors (Nguyen Pham Anh Thi) at Cho Lach, Ben Tre province, Vietnam.

used for preparing a sequencing library with a TruSeq Nano DNA Sample Preparation Kit (Illumina, USA). The library was used for the Illumina HiSeq platform to generate paired-end reads of 150 bp in length. The complete chloroplast genome of *D. zibethinus* cultivar Ri6 was completed using NOVOPlasty (Dierckxsens, Mardulyn and Smits, 2016). Gene composition and gene map of *D. zibethinus* cultivar Ri6 chloroplast genome were annotated using Geseq program and OGDRAW v1.3.1, respectively (Tillich et al. 2017; Greiner et al. 2019). The complete chloroplast genome of *D. zibethinus* cultivar Ri6 (average coverage depth = 642x, Supplementary Figure S1) was deposited to GenBank with accession number OR731187. For phylogenetic analysis, 78 protein-coding regions were extracted from 44 chloroplast genomes of Malvaceae species with sequences of *Dipterocarpus littoralis* Blume 1825 and *Muntingia calabura* L. 1753 as outgroups. The extracted sequences were then aligned using MUSCLE embedded in Geneious Prime 2022.2 (www.geneious.com). The best model for the dataset was identified as TVM + I + G (Akaike information criteria) using jModeltest 2.1.10 (Darriba et al. 2012). The maximum likelihood phylogenetic tree was reconstructed using IQTREE with 1000 ultrafast bootstraps (Minh et al. 2020). Meanwhile, MrBayes 3.2.7a was used to construct a Bayesian inference phylogenetic tree with 1,000,000 generations (Ronquist et al. 2012). The phylogenetic tree was illustrated using Figtree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

Results

The complete chloroplast genome of *D. zibethinus* cultivar Ri6 was 165,304 bp in length, and the GC content was 35.7% (Figure 2). This quadripartite genome included a large-single copy (LSC, 96,115 bp), a small-single copy (SSC, 20,819 bp), and two inverted repeat regions (IR, 24,185 bp). This genome contained 79 protein-coding genes, 30 tRNA genes, and four

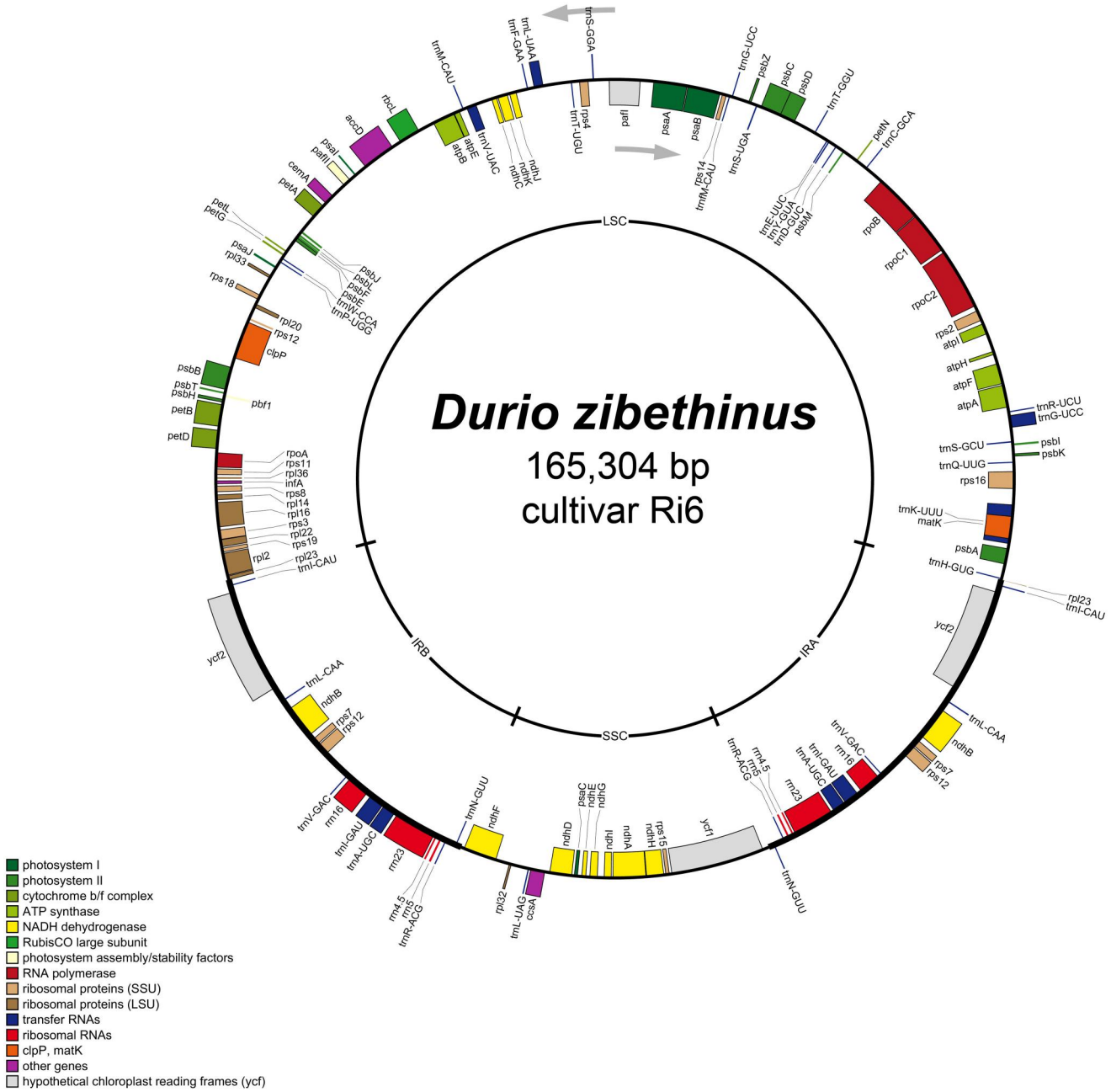

Figure 2. Map of the *D. zibethinus* cultivar Ri6 chloroplast genome. The arrows showed the direction of transcription including genes inside the circle that are transcribed clockwise, and those outside the circle are counterclockwise transcribed otherwise. The inner circle correspond to locations of LSC (large single copy), SSC (small single copy), and IRA/IRB (inverted repeat) regions. Different functional groups are signed according to the colored legend.

rRNA genes, of which 15 regions (including *trn*N_GUU, *trn*R_ACG, *rrn*5S, *rrn*4.5S, *rrn*23S, *rrn*16S, *trn*A-UGC, *trn*I_GAU, *trn*V_GAC, *rps*12, *rps*7, *ndh*B, *trn*L_CAA, *ycf*2, and *trn*I_CAU) were fully duplicated. There were nine protein-coding genes containing one intron and two protein-coding genes having two introns (Supplementary Figure S2). The *rps*12 gene was a trans-splicing gene (Supplementary Figure S3). The junction between SSC and IR regions is located within *trn*N_GUU - *ndh*F intergenic space, whereas the boundary of LSC and IR regions is distributed within the *rpl*23 gene (Figure 2). The results of Maximum likelihood and Bayesian inference methods revealed the same topology trees. Additionally, phylogenetic analysis revealed a monophyly of Helicteroideae taxa and a close relationship between *Reevesia* and *Durio* members (Figure 3). Within the *Durio* genus, *D. zibethinus* Ri6 exhibited a sister relationship to *D. zibethinus* Mongthong with moderate support values (bootstrap value = 85, posterior probability = 0.98). The pairwise identity between *D. zibethinus* Ri6 and *D. zibethinus* is 98.8% similarity. Specifically, there were 89 indel and substitution sites, which were located mostly in non-coding regions. The *ycf*1, *ycf*2, *acc*D, and *rpo*B exhibited variable sites between two durians.

Discussion and conclusion

In this study, the complete chloroplast genome of *D. zibethinus* cultivar Ri6 was characterized and did not possess special

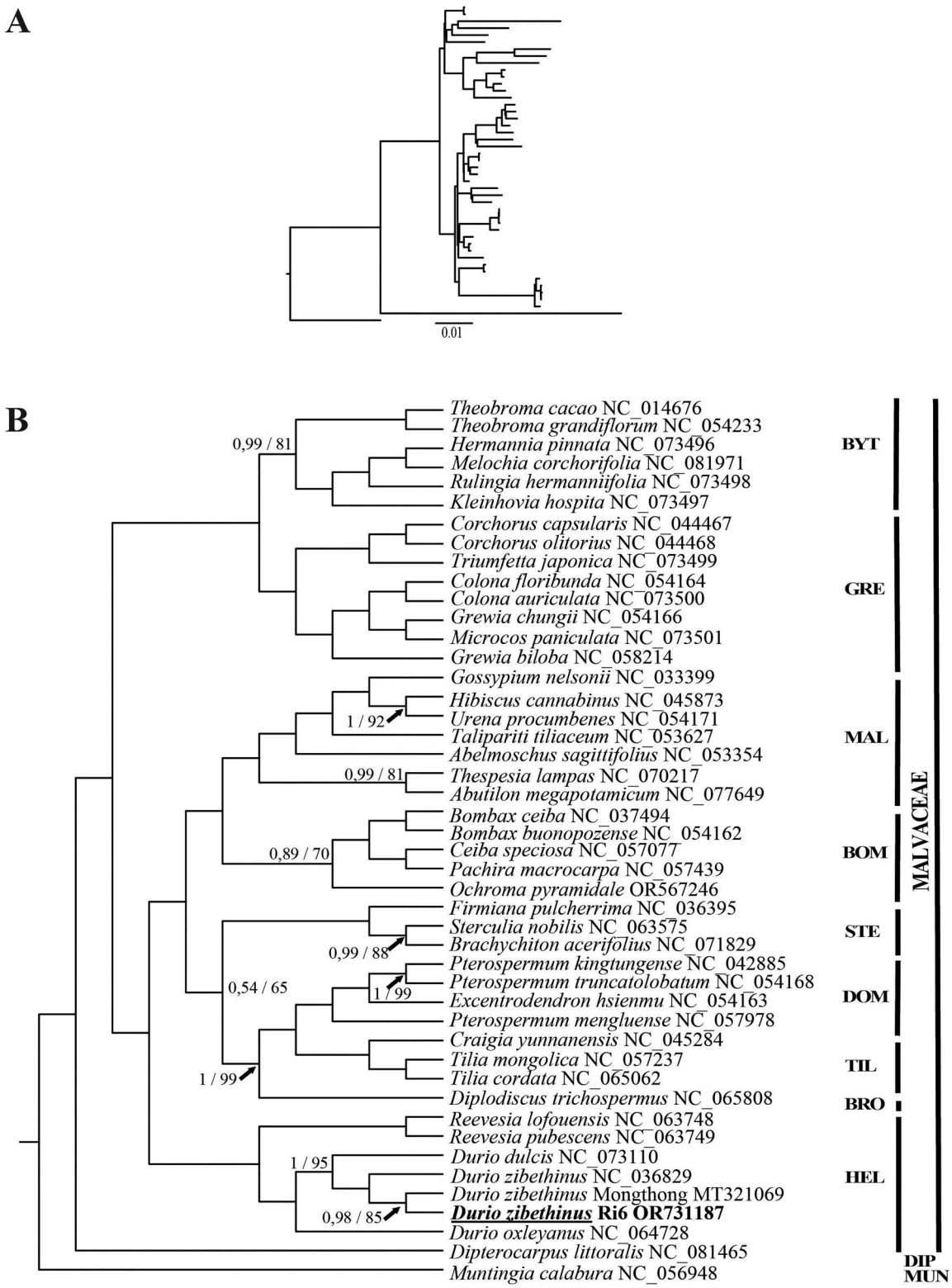

Figure 3. Phylogenetic tree of *D. zibethinus* cultivar Ri6 and related species inferred from 78 protein-coding regions of chloroplast genomes using maximum likelihood and Bayesian inference methods. A. The Bayesian infererence method based phylogenetic tree. B. The cladogram of phylogenetic tree with the bootstrap values < 100 and posterior probabilitiy values < 1. Note: the best substitution model was TVM + I + G (Akaike information criteria). The numbers indicate the bootstrap values and posterior probabilities. The scale bar means the expected number of nucleotide substitutions per site. The chloroplast genome of *dipterocarpus littoralis* and *Muntingia calabura* was used as an outgroup. BOM: Bombacoideae; BRO: Brownlowioideae; BYT: Byttnerioideae; DOM: Dombeyoideae; GRE: Grewioideae; HEL: Helicteroideae; MAL: Malvoideae; STE: Sterculioideae; TIL: Tilioideae; DIP: Dipterocarpaceae; MUN: Muntingiaceae. The following sequences were used: *Colona floribunda* NC_054164 (Wang et al. 2021); *Gossypium nelsonii* NC_033399 (Chen et al. 2017); *Durio zibethinus* NC_036829 (Cheon et al. 2017); *Durio zibethinus* Ri6 OR731187 (this study); *Abelmoschus sagittifolius* NC_053354 (Li et al. 2020); *Pachira macrocarpa* NC_057439 (Xu et al. 2020); *Theobroma cacao* NC_014676 (Abdullah et al. 2020); *Grewia biloba* NC_058214 (Xu et al. 2021); *Melochia corchorifolia* NC_081971 (No reference); *Theobroma grandiflorum* NC_054233 (Niu et al. 2019); *Abutilon megapotamicum* NC_077649 (No reference); *Reevesia lofouensis* NC_063748 (No reference); *Brachychiton acerifolius* NC_071829 (No reference); *Urena procumbenes* NC_054171 (Wang et al. 2021); *Craigia yunnanensis* NC_045284 (Wariss et al. 2019); *Bombax ceiba* NC_037494 (Gao et al. 2018); *Grewia chungii* NC_054166 (Wang et al. 2021); *Ochroma pyramidale* OR567246 (No reference); *Bombax buonopozense* NC_054162 (Wang et al. 2021); *Corchorus capsularis* NC_044467

Figure 3. (*Continued*)
(Fang et al. 2021); *Pterospermum truncatolobatum* NC_054168 (Wang et al. 2021); *Microcos paniculata* NC_073501 (No reference); *Colona auriculata* NC_073500 (No reference); *Pterospermum kingtungense* NC_042885 (Wang et al. 2018b); *Triumfetta japonica* NC_073499 (No reference); *Hibiscus cannabinus* NC_045873 (Chen et al. 2020); *Hermannia pinnata* NC_073496 (No reference); *Durio oxleyanus* NC_064728 (Wong et al. 2022); *Taliparitiliaceum* NC_053627 (Qiu et al. 2021); *Durio zibethinus* Mongthong MT321069 (Shearman et al. 2020); *Durio dulcis* NC_073110 (No reference); *Tilia mongolica* NC_057237 (Zheng et al. 2021); *Pterospermum mengluense* NC_057978 (Guan-Song et al. 2021); *Excentrodendron hsienmu* NC_054163 (Wang et al. 2021); *Firmiana pulcherrima* NC_036395 (Wang et al. 2018a); *Sterculia nobilis* NC_063575 (No reference); *Ceiba speciosa* NC_057077 (Huang et al. 2019); *Reevesia pubescens* NC_063749 (No reference); *Tilia cordata* NC_065062 (Yan et al. 2022); *Corchorus olitorius* NC_044468 (Fang et al. 2021); *Thespesia lampas* NC_070217 (No reference); *Rulingia hermanniifolia* NC_073498 (No reference); *Dipterocarpus littoralis* NC_081465 (No reference); *Diplodiscus trichospermus* NC_065808 (Wu et al. 2023); *Kleinhovia hospita* NC_073497 (No reference); *Muntingia calabura* NC_056948 (No reference).

features (i.e. gene deletion, gene duplication, and inversion) in comparison to other Helicteroideae species (Cheon et al. 2017; Quan et al. 2019; Wong et al. 2022). However, the border between the large single copy and inverted repeat regions of *D. zibethinus* cultivar Ri6 located within *rpl*23 compared to *rpl*23-*trn*I_CAU intergenic spacer in *D. zibethinus* and *D. oxleyanus*, suggesting a unique character of *D. zibethinus* cultivar Ri6. Previously, a study found a lack of one IR region in the chloroplast genome of *D. zibethinus* cultivar Mongthong based on long-read Pacbio data (Shearman et al. 2020). Therefore, more complete chloroplast genomes of durian cultivars should be characterized to verify this loss of IR region. The phylogenetic analysis inferred from 78 protein-coding genes revealed the monophyly of nine subfamilies of Malvaceae. These relationships were also found in a previous study inferred from whole chloroplast genomes (Cvetković et al. 2021). Within *Durio* genus, *D. zibethinus* cultivar Ri6 had a close relationship to the cultivar Mongthong, suggesting genetic variations among durian cultivars. Previous genetic studies of *D. zibethinus* cultivars focused on local samples (Nyffeler and Baum, 2000; Nyffeler and Baum, 2001; Santoso et al. 2013; Santoso et al. 2017; Khang et al. 2021; Mursyidin 2022). Therefore, further studies examining more durian cultivars are needed to explore genetic variations and develop molecular markers for each cultivar. This study provides initial data for exploring chloroplast genomes of *D. zibethinus* cultivars, which are helpful in tracing the genetic variations of durians.

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Author's contribution

NPAT and DTK conceived the conception and design; NPAT, TGH, and DTK collected and determined the samples; TGH, NPAT and HDKD conducted the experiments, analyzed the data; TGH and HDKD wrote the draft manuscript; NPAT and DTK revised the draft manuscript. All authors agreed to the final form of this manuscript.

Ethical approval

The collection of *Durio zibethinus* cultivar Ri6 does not require specific permissions or licenses from the government and local governors. The authors alone are responsible for the content and writing of this article.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability statement

The genome sequence data that support the findings of this study are openly available in GenBank of NCBI at https://www.ncbi.nlm.nih.gov] (https://www.ncbi.nlm.nih.gov/) under accession no. OR731187. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA1060435, SRR27403059, and SAMN39229674, respectively.

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