Characterization of the plastid genome of *Cratoxylum* species (Hypericaceae) and new insights into phylogenetic relationships

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To expand the genomic information of Hypericaceae, particularly on *Cratoxylum*, we characterized seven novel complete plastid genomes (plastomes) of five *Cratoxylum* and two of its allied taxa, including *C. arborescens*, *C. formosum* subsp. *formosum*, *C. formosum* subsp. *pruniflorum*, *C. maingayi*, *C. sumatranum*, *Hypericum hookerianum*, and *Triadenum breviflorum*. For *Cratoxylum*, the plastomes ranged from 156,962 to 157,792 bp in length. Genomic structure and gene contents were observed in the five plastomes, and were comprised of 128–129 genes, which includes 83–84 protein-coding (CDS), 37 tRNA, and eight rRNA genes. The plastomes of *H. hookerianum* and *T. breviflorum* were 138,260 bp and 167,693 bp, respectively. A total of 110 and 127 genes included 72 and 82 CDS, 34 and 37 tRNA, as well as four and eight rRNA genes. The reconstruction of the phylogenetic trees using maximum likelihood (ML) and Bayesian inference (BI) trees based on the concatenated CDS and internal transcribed spacer (ITS) sequences that were analyzed separately have revealed the same topology structure at genus level; *Cratoxylum* is monophyletic. However, *C. formosum* subsp. *pruniflorum* was not clustered together with its origin, raising doubt that it should be treated as a distinct species, *C. pruniflorum* based on molecular evidence that was supported by morphological descriptions.

The family Hypericaceae Jussieu comprises nine genera and over 500 species worldwide. In general, members of Hypericaceae are further categorized into three different tribes viz. Cratoxyleae Bentham & J.D. Hooker, Hypericeae Choisy, and Vismieae Choicy. As the smallest tribe in the family, two genera are recognized in Cratoxyleae, viz. *Cratoxylum* Blume and the monotypic genus, *Eliea* Cambess. At present, there are seven accepted species of *Cratoxylum* Blume (Hypericaceae, Malpighiales), and three of them are recognized with at least two intraspecific identities. Members of *Cratoxylum* are native to the tropical Asia region, widespread from India through South China to Malesia and are commonly soughed for their wood as a source of timber and charcoal production. The great adaptability in harsh environments and fast-growing performance has warrant some of these species as potential replanting species that are useful for peatland rehabilitation strategies.

Despite the potential as useful rehabilitation agents in peat swamp forests, genetic studies on *Cratoxylum* are limited. Genetic data of *Cratoxylum* are only restricted to short gene sequences derived from the plastid, mitochondrial and nuclear regions of either *C. arborescens* (Vahl) Blume or *C. cochinchinense* (Lour.) Blume as representative species of its genus in the reconstruction of the phylogenetic tree of Malpighiales. The lack of the phylogenetic studies among species of *Cratoxylum* has hindered our understanding of this genus at its genetic level.

The plastid genome (plastome) is a valuable resource for molecular taxonomy research. Angiosperm plastomes are circular haploid genomes with a large single copy (LSC) region, two inverted repeats (IR), and a small

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single copy (SSC) region that are typically small in between 110–240 kbp in length\textsuperscript{10}. Recently, researchers have shown great interest in obtaining the complete plastome sequences through next-generation sequencing technique. This is because when compared to short gene sequences, genome-scale datasets that are used in phylogenetics contain larger number of single nucleotide polymorphisms, which could contribute to the reconstruction of a well-supported phylogenetic tree\textsuperscript{11}. Owing to the advancement in sequencing technique and the availability of useful bioinformatic programs to aid in the assembly and annotation of the plastomes, to date, many complete plastome sequences have been made available publicly to decipher ambiguous phylogenetic relationships in complicated genera\textsuperscript{2,13}. On the other hand, the nuclear DNA internal transcribed spacer (ITS) region has served to be useful in revealing the biparental inheritance of plants at a nuclear genome level\textsuperscript{15}. Among all nuclear genes, amplification of the ITS sequence is known to be easier, and the genetic information provided is also useful to delimit individuals at an intraspecific level\textsuperscript{15}.

Although records on published complete plastomes are increasing substantially over the years, genome data for Hypericaceae is still lacking. To date, published records on the complete plastome sequences of Cratoxylum are only limited to \textit{C. cochinchinense}, in which at least three genome sequences of different accessions are available in the NCBI GenBank database (as of September 2021). In view of the need to expand the genomic information of Hypericaceae, we further sequenced and characterized the plastomes of five taxa of \textit{Cratoxylum}, including \textit{C. arborescens}, \textit{C. formosum} subsp. \textit{formosum} (Jack) Benth. & Hook.ex Dyer, \textit{C. formosum} subsp. \textit{pruniflorum} (Kurz) Gogelein, \textit{C. maingayi} Dyer, and \textit{C. sumatrana} (Jack) Blume, as well as two closely-related species, \textit{Hupericum hookerianum} Wight & Arn. and \textit{Triadenium breviflorum} Wall. ex Dyer. In order to reveal the phylogenetic relationship among species of \textit{Cratoxylum}, we further performed phylogenetic analysis using the plastid protein-coding sequence (CDS) dataset and the nuclear DNA internal transcribed spacer (ITS) sequence region. The findings of this work will serve as important reference for the phylogenetic and evolutionary studies of Hypericaceae and Malpighiales.

Results and discussion
Plastome features. All seven plastomes obtained from this study exhibited a typical quadripartite structure, which comprised of a large single-copy (LSC) and a small single-copy (SSC) region that are separated by a pair of inverted repeats (IR) (Fig. 1). Plastome sizes were between 156,962 bp (\textit{C. formosum} subsp. \textit{pruniflorum} and 157,792 bp (\textit{C. arborescens}) among the five taxa of \textit{Cratoxylum}, while \textit{H. hookerianum} and \textit{T. breviflorum} were 138,260 bp and 167,693 bp in length, respectively (Table 1). A total of 128–129 genes were predicted in the plastome of the five taxa of \textit{Cratoxylum}, which comprised of 83–84 CDS, 37 tRNA, and eight rRNA genes. \textit{Cratoxylum formosum} subsp. \textit{formosum} and \textit{C. formosum} subsp. \textit{pruniflorum} were short of one CDS compared to the other three species of \textit{Cratoxylum}, which was the \textit{rpl32} gene that should be located at the SSC region (Table 2). There were 15 genes, including nine CDS and six tRNA genes, that contained one intron, while two genes, \textit{clpP} and \textit{ycf3}, contained two introns.

Although the gene content in plastome of \textit{Cratoxylum} was consistent across the five taxa examined in this study, there was an inversed gene block arrangement detected in the LSC region, between \textit{rbcL} and \textit{trnK-UUU} genes (Fig. 1). The inversed gene block was approximately 55,000 bp in length, containing 28 CDS and 19 tRNA genes. By comparing to other plastomes of closely related families, we identified that the gene arrangement for the gene block in \textit{C. cochinchinense}, \textit{C. formosum} subsp. \textit{formosum}, and \textit{C. formosum} subsp. \textit{pruniflorum} was similar to those of Bonnetiaceae, Calophyllaceae, Chrysobalanaceae, and Clusiaceae, i.e. \textit{Bonnetia paniculata} (GenBank accession no. MK995182), \textit{Curasia heterocarpa} (GenBank accession no. MW853787), \textit{Garinia mangostana} (GenBank accession no. KX822787), and \textit{Liciana micrantha} (GenBank accession no. KX180080); while the gene arrangement for the gene block in \textit{C. arborescens}, \textit{C. maingayi}, and \textit{C. sumatrana} was identical to those of Podostemaceae, i.e. \textit{Marathrum capillaceum} (GenBank accession no. MN165813) and \textit{Tristicha trifaria} (GenBank accession no. MK995179). This finding was congruent with a previous work, in which gene block inversion between \textit{rbcL} and \textit{accD} was observed in two clusioid families, including Hypericaceae and Podostemaceae, as well as Papilionoideae\textsuperscript{46}. For \textit{H. hookerianum} and \textit{T. breviflorum}, a total of 110 and 127 genes were predicted, including 72 and 82 CDS, 34 and 37 tRNA, as well as four and eight rRNA genes, respectively. The GC content of the plastome for the five taxa of \textit{Cratoxylum} ranged between 36.1 and 36.3%, while GC content for the plastomes of \textit{H. hookerianum} and \textit{T. breviflorum} was 38.1% and 37.4%, respectively.

Short and large sequence repeats. Simple sequence repeats (SSRs) or microsatellites were short tandem repeats of 1–6 nucleotides and motifs at a specific locus are present in all genomes, particularly eukaryotes. Besides being developed as genome markers for the use in marker assisted selection, kinship, breeding, etc., SSRs contribute to the performance of important regulatory functions with the variation in their lengths at the coding regions\textsuperscript{17,18}. In this study, the total SSRs detected in the plastomes of \textit{C. arborescens}, \textit{C. cochinchinense}, \textit{C. formosum} subsp. \textit{formosum}, \textit{C. formosum} subsp. \textit{pruniflorum}, \textit{C. maingayi}, and \textit{C. sumatrana} were 170, 103, 95, 95, 104, and 96, respectively (Fig. 2). The mononucleotide repeats were most abundant among all repeat types, ranging between 69 (\textit{C. formosum} subsp. \textit{formosum}) and 80 (\textit{C. arborescens}); the frequency of mononucleotide repeat type A/T was greater than the repeat type C/G. It was worth noting that pentanucleotides were only found present in \textit{C. arborescens}, including two AAATTT/AATTT and one AAAAT/ATTT repeat type. Large repeats were only recorded in forms of forward as well as palindromic repeats in the six plastomes assessed. All plastomes were identified with 25 each for both repeats, except for \textit{C. sumatrana} that has 24 forward repeats and 26 palindromic repeats.

Expansion and contraction of the IR regions. The genes adjacent to the IR junctions in the plastome of the six taxa of \textit{Cratoxylum} examined displayed identical gene content (Fig. 3). The genes adjacent to the junc-

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tion LSC/IRb (JLB) were rpl22 and rps19, with rps19 located across JLB. However, for the junction LSC/IRa (JLA), rps19 was intact in the IRA region, while trnH of the LSC region was recorded crossing over JLA. The ycf1 gene was placed across the junction SSC/IRA (JSA) for all six taxa of *Cratoxylum* examined, but for the junction SSC/IRb (JSB), only the ycf1 gene of four taxa of *Cratoxylum*, including *C. cochin chinense*, *C. formosum* subsp. *formosum*, *C. maingayi*, and *C. sumatranum*, were detected placing across JSB. The ycf1 of *C. arborescens* was still intact in the IRb region, while ycf1 gene of *C. formosum* subsp. *pruniflorum* was identified to be short in length and presumed to be a pseudogene, was located in the SSC region. When analyzed together with the six taxa of *Cratoxylum*, the gene content adjacent to the 1R junctions in *H. hookerianum* and *T. breviflorum* exhibited some variations when compared to *Cratoxylum*. At JLA and JLB, the gene contents of *T. breviflorum* was similar to those of *Cratoxylum*, in which rps19 and trnH were placed across JLB and JLA, respectively. However, for *H.*
### Table 1. General characteristics of complete plastid genomes of the seven taxa of Hypericaceae obtained in this study.

| Collector and collection number | Source of origin | Plastome features | GenBank accession number |
|---------------------------------|-----------------|-------------------|-------------------------|
| Cratoxylum arborescens          | Syazwan; SAS678 Selangor, Malaysia | 84 37 8 157,792 36.1 MZ703418 MZ674200 |
| Cratoxylum formosum subsp. formosum | A. Chaveerach; 1089 Udonthani, Thailand | 83 37 8 156,978 36.3 MZ703419 MZ674201 |
| Cratoxylum formosum subsp. pruniflorum | A. Chaveerach; 1099 Udonthani, Thailand | 84 37 8 157,089 36.3 MZ703415 MZ674202 |
| Cratoxylum maingayi             | Syazwan; SAS679 Selangor, Malaysia | 84 37 8 157,358 36.2 MZ703417 MZ674203 |
| Cratoxylum sumatranum          | A. Chaveerach; 1091 Udonthani, Thailand | 84 37 8 157,089 36.3 MZ703415 MZ674204 |
| Hypericum hookerianum         | A. Chaveerach; 1092 Chiang Mai, Thailand | 72 34 4 138,260 38.1 MZ714015 MZ703053 |
| Triadenum breviflorum         | Zhang et al.; TanCM704 Jiangxi, China | 82 37 8 167,693 37.4 MZ714016 OM980718 |

### Table 2. Genes predicted in complete plastid genome of the five taxa of Cratoxylum used in this study. Genes that contain duplicates are indicated in parenthesis. *Indicates gene containing single intron; **Indicates gene containing two introns; *Indicates gene not found in Cratoxylum formosum subsp. formosum and C. formosum subsp. pruniflorum.

| Category                      | Group of function                  | Genes                                                                 |
|-------------------------------|------------------------------------|----------------------------------------------------------------------|
| Self-replication related genes| Large subunit of ribosome proteins | rpl2(×2)*, rpl14, rpl16*, rpl20, rpl22, rpl23(×2), rpl32*, rpl33, rpl36 |
|                               | Small subunit of ribosomal proteins| rps2, rps3, rps4, rps7(×2)*, rps8, rps11, rps12, rps14, rps15, rps18, rps19(2) |
|                               | DNA-dependent RNA polymerase       | rpoA, rpoB, rpoC1*, rpoC2                                              |
|                               | rRNA genes                         | trnA-U-GC(×2)*, trnC-GCA, trnD-GUC, trnE-UUC, trnF-GAA, trnM-CAU, trnG-GCC, trnG-UCC*, trnH-GUG, trnL-CAU(×2), trnQ-GAU(×2), trnK-UUU*, trnL-CAA(*)2, trnL-CAA*, trnL-UAG, trnM-CAU, trnN-GUU(×2), trnR-UGG, trnQ-UUG, trnR-ACG(×2), trnR-UCU, trnS-GCU, trnS-GGA, trnS-UGA, trnT-GGU, trnT-UGU, trnV-GAC(2), trnV-UAC*, trnW-CCA, trnY-GUA |
|                               | rRNA gene                          | trnK-U-G(2), trnS(5(2), trnS16(5(2), trnS23(×2) |
| Photosystem I                 |                                   | psaA, psaB, psaC, psaK, psaL, psaI                                      |
| Photosystem II                |                                   | psbA, psbB, psbC, psbD, psbE, psbF, psbH, psbL, psbM, psbN, psbT, psbZ  |
| NADH oxidoreductase           |                                   | ndhA*, ndhB(2)(×2), ndhC, ndhD, ndhE, ndhF(2), ndhG, ndhH, ndhI, ndhJ, ndhK |
| Cytochrome b6/i complex       |                                   | petA, petB*, petD*, petE, petG, petL, petN                             |
| Cytochrome c synthesis        |                                   | ccsA                                                                 |
| ATP synthase                  |                                   | atpA, atpB, atpE, atpF*, atpH, atpI                                    |
| Rubisco                       |                                   | rbcL                                                                 |
| Other genes                   | Maturase                           | matK                                                                 |
|                               | Protease                           | clpP**                                                                |
|                               | Envelope membrane protein          | cemA                                                                  |
|                               | Subunit acetyl-CoA-carboxylase     | accD                                                                  |
| Unknown function genes        | Conserved hypothetical chloroplast reading frames | ycf1, ycf2(×2), ycf3**, ycf4                                         |

hookerianum, the trnN gene was placed in the LSC region, next to JLB, while rbcL of the LSC region was the closest gene next to JLA. Both H. hookerianum and T. breviflorum had the ndhF genes located in the IR regions, adjacent to JSA and JSB, while at the SSC region, ndhA and rps15 were placed next to JSA in H. hookerianum and T. breviflorum, respectively.

**Comparative genomic analysis.** Genome comparison analysis of the complete plastome sequences revealed high conservatism across all taxa of Cratoxylum, with C. cochinchinense as the reference genome (Fig. 4). A small gap that resembles a variation in form of deletion, was observed at the intergenic spacer region psbH-petA of C. formosum subsp. formosum, C. formosum subsp. pruniflorum, and C. sumatranum. When compared to H. hookerianum and T. breviflorum, at least six large gaps could be observed across the plastome, indicating great variations in nucleotide sequence at genus level. These gaps were located at the trnQ-UUG-trnK-UUU, clpP, trnR-ACG-ndhF, trnB-ACG-rps15, and two ycf2 regions. The multiple sequence alignment of the six taxa of Cratoxylum was 169,031 bp in length, containing 1447 singletons and 22,090 parsimony informative sites. There were at least 1678 indel events identified, including a total of 23,364 indel sites.

**Phylogenetic inference.** The total length of the multiple sequence alignment of the concatenated CDS dataset was 89,82, while it was 807 bp before trimming and 723 bp after trimming for the ITS dataset. As both
the ML and BI tree exhibited identical topology structure in both datasets, only the ML tree was presented in this study, with the BI posterior probability included at each of the branch nodes. In the CDS-tree, all branch nodes were well-supported (BS: ≥ 75; PP: ≥ 0.95); the phylogenetic relationship among all taxa included in the study was well-resolved (Fig. 5A). For *Cratoxylum*, all six taxa revealed a monophyletic relationship. *Cratoxylum arborescens* was recorded to diverge first from the other taxa, followed by *C. cochinchinense*, *C. formosum* subsp. *pruniflorum*, and *C. maingayi*. *Cratoxylum formosum* subsp. *formosum* was placed at the tip of the branch with *C. sumatranum*. For the ITS dataset, a similar tree topology was observed when compared to the tree reconstructed using the CDS-dataset (Fig. 5B); a monophyletic relationship was also observed in *Cratoxylum*, in which the molecular placement of all six taxa of *Cratoxylum* in the ITS-tree was identical to those presented in the CDS-tree. The phylogenetic relationship among all taxa of *Cratoxylum* used in this study was also well-resolved when using the ITS dataset (BS: ≥ 75; PP: ≥ 0.95).

**Conflicts on taxonomic identity of *C. formosum* subsp. *Pruniflorum***. It was noteworthy that *C. formosum* subsp. *pruniflorum* was not clustered together with its original, *C. formosum* subsp. *formosum* in the phylogenetic trees reconstructed using both the nuclear and plastid regions. Despite the ITS sequences, which
are biparental inherited, could indicate possible hybridization in *C. formosum* subsp. *pruniflorum*; however, the well-resolved phylogenetic tree based on the maternal inherited plastid genes indicated that *C. formosum* subsp. *formosum* and *C. formosum* subsp. *pruniflorum* should be treated as two natural groups. Based on the literatures, *C. formosum* subsp. *pruniflorum* was first regarded as a distinct species, *Hypericum prunifolium* Wall19. However, the species has undergone several taxonomic revisions, before it was recognized as a subspecies to *C. formosum* in 19674. Based on the description, the author emphasized that the key to differentiate between *C. formosum* subsp. *pruniflorum* and its original is based on the occurrence of an indumentum; both taxa exhibited high morphological similarities. Although it should not be a key morphological characteristic to differentiate the two taxa, *C. formosum* subsp. *pruniflorum* comes with pubescent sepals, while *C. formosum* subsp. *formosum* is glabrous at all parts, and they were geographically defined and hardly overlapping. Other morphological characteristics that were proposed to delimit *C. formosum* subsp. *pruniflorum* from *C. formosum* subsp. *formosum* were—the former has rusty, tomentose young twigs, pedicels and calyx, while the latter is glabrous; the former has short and truncated hypogynous scale, which is 0.7–0.8 mm long, while the latter has a linguiform hypogynous scale that is 2 mm long; the former has capsule that is ovoid-shaped and comes with 54–58 seeds, while the latter has ellipsoid-shaped capsule that is 36–46 seeded20.

To identify the genetic distance between *C. formosum* subsp. *pruniflorum* and its original based on the complete plastome and the ITS sequences, we conducted pairwise distance analysis on the complete plastome and ITS sequences and analyzed them separately. We found that the intraspecific pairwise distance was 0.00351, which was greater than the interspecific pairwise distance between *C. formosum* subsp. *pruniflorum* and *C. maingayi* (0.00159) at plastome level, while intraspecific pairwise distance was 0.0502, which was longer than the interspecific pairwise distance between *C. formosum* subsp. *pruniflorum* and *C. sumatranum* (0.0358) as well as *C. maingayi* (0.0316) at the ITS level. There was no report on natural hybridization in *Cratoxylum*; despite that the pairwise distance was not a suitable parameter to tell closely related species apart, it was generally accepted that intraspecific pairwise distance of a species should be less than that of the interspecific pairwise distance under a regular basis21,22. On the other hand, the chromosome count in *C. cochinchinense* is n = 1123, while *C. formosum* subsp. *formosum* is known to be n = 724. In general, the chromosome count in diploid plant species was often conserved intraspecifically under natural circumstances25. *Cratoxylum cochinchinense* was proposed to be conspecific to *C. formosum* at one time due to their identical morphological features, but the proposal was later denied; morphological variations between the two species were distinct in terms of their tree size, color of the bark, leaf structure, leaf shape, and staminal bundle of the flower4. Thus, we commented that cytology studies on *C. formosum* subsp. *pruniflorum* could provide useful insights to the genetic identity of the subspecies when compared to its original. Nevertheless, the finding between conventional taxonomic classification and molecular phylogenetic analysis in *Cratoxylum* was partly incongruent; the taxonomic identity of *C. formosum* subsp. *pruniflorum* to be accepted as a reduced taxon under *C. formosum* warrants further taxonomic revision.
on this genus. Based on the molecular evidence in this study, we believed that *C. formosum* subsp. *pruniflorum* should be considered as a natural group, and the species name *Cratoxylum pruniflorum* Kurz should be reinstated.

**Conclusion**

This study has contributed to the expansion of genome data in Hypericaceae, with the characterization of novel plastomes of five taxa of *Cratoxylum*, as well as one each from *Hypericum* and *Triadenum*. The findings obtained from the well-resolved phylogenetic trees reconstructed using both the CDS and ITS datasets have provided insight to the molecular placement and evolution of *Cratoxylum*, in which the taxonomic identity of *C. formosum* subsp. *pruniflorum* to be recognized as a subspecies under *C. formosum* was questionable. Nonetheless, the molecular data obtained in this study will be a valuable resource for gaining a better understanding of Hypericaceae taxonomy and phylogeny.

**Materials and methods**

**Plant materials.** Fresh leaves of five taxa of *Cratoxylum* species, including *C. arborescens*, *C. formosum* subsp. *formosum*, *C. formosum* subsp. *pruniflorum*, *C. maingayi*, and *C. sumatranum*, as well as *Hypericum hookerianum* and *Triadenum breviflorum* (Supplementary Fig. 1) were collected from natural populations and ex-situ sites (Table 1). All the experiments were performed in accordance with relevant guidelines and regulations. The identities of each specimen were confirmed by the corresponding authors prior to specimen collection. Leaf specimens were kept in ziplock bags filled with silica gels and transported to respective local laboratories for total genomic DNA extraction.
DNA extraction, genome sequencing and assembly. Total genomic DNA was conducted using DNeasy Plant Mini Kit (QIAGEN, Germany), based on the manufacturer's protocol. The purity and quantity of the DNA extract were estimated using Qubit™ 4 Fluorometer (Thermo Fisher Scientific, USA). Next-generation sequencing was conducted on an Illumina NovaSeq platform (Illumina, USA) to obtain 350-bp paired-end reads. The NGS QC Toolkit v2.3 was used to trim off the adapter sequences and the plastome was assembled using NOVOPlasty v2.7.2 with the rbcL gene of *C. cochinchinense* (GenBank accession no. MN399961) as the seed sequence. The assembled plastome was annotated and the inverted region junctions were identified using

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**Figure 5.** Phylogenetic trees of *Cratoxylum* and its allied taxa based on the concatenated protein-coding sequences derived from the plastid genome (A), as well as the nuclear DNA internal transcribed spacer (ITS) sequences (B). The phylogenetic tree was constructed using both maximum likelihood (ML) and Bayesian inference (BI). Bootstrap support (BS) and posterior probabilities (PP) that are considered reliable (BS: ≥ 75; PP: ≥ 0.95) are indicated with an asterisk (*).
Repeat analysis. In order to provide a better understanding between the plastomes of all species of *Cratoxylum* available online, the complete plastome sequence of *C. cochinchinense* (GenBank accession no. MN399961) was downloaded from the NCBI GenBank database. Subsequent genome comparative analyses were conducted with the inclusion of the genome data of this species. Using MISA-web, the SSRs of each plastome were identified. The minimum number of repeat parameters were set for 10, 4, 4, 3, 3, and 3 for mono-, di-, tri-, tetra-, penta-, and hexanucleotide motifs, respectively. The large repeats, which includes the forward, palindrome, reverse, and complement repeats, were identified using REPutter, in which the minimum repeat size was set at 30 bp and a Hamming distance of 3.

Genome comparative and sequence divergence analyses. To detect the expansion and contraction of the IR region in the plastomes, the boundaries and junctions of the IR regions were visualized using IRscope. To reconstruct the plastome sequences, the plastome boundaries and junctions were visualized using FigTree v.1.4.4. The plastome sequences obtained from this study were deposited into the NCBI GenBank database, under the accession numbers MZ703415—MZ703419, and MZ714015—MZ714016.

Polymerase chain reaction and Sanger sequencing. To obtain the ITS (ITS1-5.8S-ITS2) sequences of the five taxa of *Cratoxylum*, as well as *H. hookeriannum* and *T. breviflorum* used in this study, polymerase chain reaction (PCR) was carried out using a pair of universal primers, ITS5 and ITS4, and ITS4′-GGA AGT AAA AGT CGT AAC. PCR amplification was carried out using an ABI 3730 DNA Analyzer (Applied Biosystems, USA). The resulting amplicons were verified via gel electrophoresis and viewed under the UV machine prior to being sent for direct Sanger sequencing at both ends using an ABI 3730 DNA Analyzer (Applied Biosystems, USA). The resulting sequences were aligned and manually edited using MEGA7 to obtain the clean sequences that will be subjected to phylogenetic analysis. The ITS sequences obtained from this study were deposited into the NCBI GenBank database under the accession numbers MZ674200—MZ674204, MZ703053, MZ703415–MZ703419, MZ714015–MZ714016, and OM980718.

Phylogenetic reconstruction. The reconstruction of the CDS-based phylogenetic tree was conducted based on the concatenated CDS sequences of 14 taxa, in which eight are from Hypericaceae, while seven closely-related species, *Benettia paniculata* (Clusiaceae; GenBank accession no. MK995182), *Carapa heterocarpa* (Calophyllaceae; GenBank accession no. MW853787), *Garinia mangostana* (Clusiaceae; GenBank accession no. KX822787), *Licania micrantha* (Chrysobalanaceae; GenBank accession no. KX180080), *Marthrum capillaceum* (Podostemaceae; GenBank accession no. MN165813), *Messua ferra* (Calophyllaceae; GenBank accession no. NC_049111), as well as *Tristicha trifaria* (Podostemaceae; GenBank accession no. MK995179) that belong to Malpighiales were analyzed together. *Avverrhoa carambola* (Oxalidaceae; GenBank accession no. KX364202) of Oxalidales was included as outgroup. Plastome sequences were aligned using MAFFFT v7 and phylogenetic analysis was conducted using both maximum likelihood (ML) and Bayesian inference (BI) method. For ML analysis, a generalized-time reversible (GTR) model with gamma (+ G) (= GTR + G) was set and an ML tree was reconstructed using RAxML v8.2.11 under 1000 bootstrap replicates. BI analysis was conducted using the MrBayes v3.2.7a pipeline available in the CIPRES Science Gateway. A mixed substitution type and a 4 by 4 Protein Model (ML) function embedded in MEGA7 calculated that the Tamura 3-parameter (T92) model was the optimal DNA substitution model. All sites were included in the analysis and calculation was conducted with 1000 bootstrap replicates. For BI analysis, calculations were performed using MrBayes v3.2.7a following the same parameters and settings as mentioned above.

Data availability The data that support the findings of this study are openly available in GenBank of NCBI at https://www.ncbi.nlm.nih.gov, accession number (MZ674200-MZ674204, MZ703053, MZ703415–MZ703419, MZ714015–MZ714016, and OM980718). The raw NGS data that support the findings of this study are available from the corresponding author, A.C., upon reasonable request.
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Conceptualization, S.Y.L., A.C. and R.S.; methodology, R.S., U.A.; formal analysis, R.S., S.K., S.Y.L.; resources, P.S. and S.A.S.; data curation, P.S., S.Y.L.; writing—original draft preparation, R.S., S.K.; writing, review and editing, S.A.S., S.Y.L., A.C.; supervision, T.T., A.C.; funding acquisition, R.S., A.C. All authors have read and agreed to the published version of the manuscript.

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