Research paper

Plastome phylogenomics of the East Asian endemic genus Dobinea

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A B S T R A C T

Dobinea is a dioecious genus endemic to East Asia that consists of two extant species: Dobinea delavayi and Dobinea vulgaris. Although the genus is morphologically distinct, its phylogenetic position remains controversial. In this study, we investigated the phylogenetic relationships between Dobinea and related taxa by sequencing the whole plastome DNA sequences for both extant species of Dobinea and comparing them to published plastomes within Sapindales. The complete plastomes of D. vulgaris and D. delavayi were 160,683 and 160,154 base pairs (bp) in length, including a pair of inverted repeat regions (IRs, 26,889 and 26,759 bp) divided by the large single-copy region (LSC, 87,962 and 87,555 bp) and small single-copy region (SSC, 18,943 and 19,081 bp), and identically encoded 113 unique genes (79 protein-coding genes, 30 rRNAs, and 4 rRNA genes). Plastid phylogenomic analyses showed that Dobinea was a well-supported monophyletic unit and sister to the clade including tribes Anacardieae and Rhoideae, which suggests that Dobinea is a member of Anacardieae. In addition, molecular dating inferred D. delavayi and D. vulgaris diverged approximately 10.76 Ma, suggesting the divergence between these two species may have been driven by the intensification of the Asian summer monsoon and the establishment of distinct monsoon regimes in East Asia.

1. Introduction

East Asia, harboribout 600 endemic genera (Manchester et al., 2009), exhibits a high level of floristic endemism in seed plants. The significant endemism in East Asia florflora can be attributed to the persistence of a large number of relict taxa (paleo-endemism), as well as the emergence of substantial novel lineages (neo-endemism) (Manchester et al., 2009; Lu et al., 2018). Representatatives of paleo-endemism include Ginkgo L., Metasequoia Hu & W. C. Cheng, Cyclocarya Iljinsky, Davidia Baill., Tetragonolobus Oliv.; an example of a neo-endemic genus is Rodgersia A. Gray, which originated in Northeast Asia during the Pliocene and extensively diversified in southwest China during the Pleistocene (Ma et al., 2017). The occurrence of many neo-endemic lineages in East Asia is most likely contributed to the establishment of Asian monsoon in the Neocene (Lu et al., 2018; Wen et al., 2014), as the climatic changes created significant habitat heterogeneties (Jacques et al., 2011; Santosh, 2011; Wan et al., 2007; Yao et al., 2011; Zhang et al., 2012), which may have triggered extensive lineage diversification (Axelrod et al., 1998; Wu et al., 2005). Therefore, the evolutionary histories of these endemic lineages can provide useful insights into the origin and evolution of floristic endemism and broaden our understanding of the geological history and climatic oscillations in East Asia. In recent years, several evolutionary studies on such endemic genera have been presented (e.g., Appelhans et al., 2016; Deng et al., 2016; Hu et al., 2017; Ma et al., 2017; Xie et al., 2018). However, these case studies only represent a small fraction of the real diversity of East Asian endemic genera, and the evolutionary history of most East Asian endemic genera remains poorly understood.

The genus Dobinea Buchan-Hamilton ex D. Don is endemic to East Asia. The genus only includes two living species, namely, Dobinea delavayi Baillon and Dobinea vulgaris Buchan-Hamilton ex D. Don (Fig. 1), which occur in southwest China and the eastern Himalayas (Fig. 2) (Min and Barford, 2008; Singh et al., 2011). D. delavayi is a perennial herb occurring in thickets or
grasslands, whereas *D. vulgaris* is a shrub mainly growing in evergreen broad-leaved forests (Min and Barfod, 2008). Although their growth forms and habitats are different, they share distinctive morphological characters; specifically, the female flowers of both species lack petals and their single carpel is adnate to foliose bract. Because of this morphological distinctiveness, *Dobinea* is easy to recognize but difficult to classify taxonomically. Since the establishment of the genus, its taxonomic affinities have been controversial (Don, 1825; Engler, 1892; Morot, 1889; Pan et al., 2008; Radlkofer, 1888, 1890). Based on the growth form and leaf morphology, *Dobinea* was firstly referred to the family Sapindaceae by Don (1825). Subsequently, Radlkofer (1888) transferred it to the family Anacardiaceae based on anatomical evidence. Due to their distinctive morphological characters (e.g., apetalous female flowers with a single carpel adnate to foliose bract), Morot (1889) established the family Podoonaceae to accommodate *Dobinea*. However, Radlkofer (1890) argued there were no solid evidence to maintain Podoonaceae as a separate family. Engler (1892) followed this suggestion and established the tribe Dobineae within Anacardiaceae to accommodate this genus.

A high resolution and well-supported phylogeny could provide a valuable framework to resolve the disputes in the systematic placement of *Dobinea*. Based on plastid *rbcL* and nuclear Internal Transcribed Spacer (ITS) DNA sequences, the phylogenetic analyses by Pan et al. (2008) recovered *Dobinea* as a monophyletic unit within Anacardiaceae. Nevertheless, because both DNA regions lack sufficient sequence variation, the support and resolution of the phylogenetic trees are relatively low and the relationships between *Dobinea* and related taxa remain unresolved. Therefore, additional molecular data are needed to reconstruct a robust phylogeny.

Plastid genome (plastome) DNA sequences possess the highly variable characters required to reconstruct robust phylogenies in plants (Attigala et al., 2016; Kane et al., 2012; Tonti-Filippini et al., 2017). With the advent of the next-generation sequencing technologies, plastome DNA sequences have been widely used to resolve the historically difficult problems in plant phylogenetics (e.g., Huang et al., 2016; Jansen et al., 2007; Ji et al., 2019; Moore et al., 2007, 2010; Nock et al., 2011; Parks et al., 2009; Ruhsam et al., 2015; Yang et al., 2013; Yang et al., 2019). In this study, we firstly characterized the complete plastome DNA sequences for both extant species of *Dobinea*. Next, we investigated the phylogenetic relationships between *Dobinea* and related taxa based on the complete plastomes dataset. Finally, we dated the divergence of *Dobinea* to investigate the evolutionary history of the genus.
2. Material and methods

2.1. Plant material, DNA extraction, and plastome sequencing

Samples of Dobinea delavayi and D. vulgaris were collected from the wild. The voucher information is shown in Supplementary Table S1. Genomic DNA was isolated from ~20 mg silica-gel-dried leaf tissues using the CTAB method (Doyle and Doyle, 1987). Genomic DNA was fragmented into 500 bp to construct a pair-end library. Illumina libraries were prepared according to manufacturer’s protocol (Illumina, San Diego, CA, USA), and then sequenced on the Illumina HiSeq 2000 system.

2.2. Plastome assembly, annotation, and comparison

Both reference-based and de novo assembly strategies were employed for plastome assembly. First, the Illumina raw data were used to assemble the complete plastome with GetOrganelle pipeline (Jin et al., 2018), using the plastome sequence of Mangifera indica L. (GenBank accession: NC_035239) as reference (Pan et al., 2008). Next, the plastomes were assembled with the de novo assembler NOVOPlasty v3.8.3 (Dierckxsens et al., 2017) with a k-mer size of 31 and rcl sequence. In the case of Dobeliai (EU123469) as the seed. To validate the accuracy of plastome assembly, the plastomes of a given species recovered by different assembly strategies were compared using Geneious v.10.2 (Kearse et al., 2012).

Initial annotations of plastomes were performed with the online software DOGMA (Wyman et al., 2004). The start and stop codons and intron positions were manually corrected according to the reference plastome sequence of M. indica in Geneious v10.2 (Kearse et al., 2012). The tRNA genes were further verified by trRNascan-SE 1.21 with the default parameters (Schattner et al., 2005). The annotated plastomes were illustrated using the online program OrganellarGenomeDRAW (Lohse et al., 2007). Sequence divergence between the plastomes of D. delavayi and D. vulgaris was compared using the mVISTA tool (Frazer et al., 2004), with the default parameters.

2.3. Phylogenetic analyses

To examine the phylogenetic position of Dobinea, we reconstructed a phylogenetic tree of the order Sapindales, using 55 complete plastome sequences publicly available, including representatives of two genera of Burseraceae, five genera of Melliaceae, two genera of Simaroubaceae, five genera of Anacardiaceae, ten genera of Sapindaceae, eight genera of Rutaceae, and the two newly sequenced Dobinea species (Table S2). Gossypium stoksii Mast. (Malvales) was used as the outgroup to root the phylogenetic tree, according to the results of Gadek et al. (1996). The alignment was performed with MAFFT software (Katoh et al., 2012), and adjusted manually when necessary. The ambiguous positions and insertions presented in one or few nucleotides in matrix were excluded from phylogenetic analyses. Unambiguous alignment was subjected to Maximum-Likelihood analyses (ML) and Bayesian Inference (BI). The ML phylogeny was reconstructed in the program RAxML-HPC (Stamatakis, 2006) with the GTR+G model. The ML phylogeny was reconstructed in the program RAxML-HPC (Drummond and 2007) using a maximum likelihood substitution model. The ML phylogeny was reconstructed in the program RAxML-HPC (Drummond and 2007) using a maximum likelihood substitution model. The ML phylogeny was reconstructed in the program RAxML-HPC (Drummond and 2007) using a maximum likelihood substitution model.

3. Results

3.1. Plastome features of Dobinea

Illumina sequencing obtained a total of 31,505,300 and 31,529,868 paired-end clean reads for Dobinea vulgaris and D. delavayi. Of these, 1,475,181 and 2,028,403 reads were mapped to the final assembly (Table S3). The overall sizes of assembled complete plastomes of D. vulgaris and D. delavayi were 160,683 and 160, 154 bp. Both plastomes showed typically quadripartite structure, including a pair of inverted repeat regions (IRs, 26,889 and 26,759 bp) divided by the large single-copy region (LSC, 87,962 and 87,555 bp) and small single-copy region (SSC, 18,943 and 19,081 bp) (Fig. 3). The plastomes of Dobinea encoded 113 unique genes, including 79 protein-coding genes, 30 tRNAs, and 4 rRNA genes, of which twenty genes were duplicated in the IR regions. Among these unique genes, 12 protein-coding genes (apf, ndhA, ndhB, petB, petD, rpl16, rpl2, rpoC1, rps12, rps16, clpP, and ycf3), and six tRNAs (trnG-UCC, trnG-UCC, trnL-UAA, trnM-UAC, and trnV-UAC) contained introns (Table S4). The whole plastome of D. vulgaris and D. delavayi exhibited 99.9% sequence identity. Plastome-wide comparative analysis using mVISTA identified only 568 variation sites among the 160,961 alignment positions (Fig. 4).

3.2. Phylogenetic analyses and divergence time estimation

The tree topologies resulted from ML and BI analysis were identical. As indicated in Fig. 5, the fifty-five Sapindales taxa were fully resolved as three well-supported major clades. Clade I contains Burseraceae and Anacardiaceae, which was placed at basal position of the phylogenetic trees (PP = 1.00, BS = 100). Clade II is represented only by Sapindaceae (PP = 1.00, BS = 100). Clade III included Melliaceae, Simaroubaceae, and Rutaceae (PP = 1.00, BS = 100), in which the sister group Simaroubaceae and Rutaceae diverged from Meliaceae with strong support (PP = 0.99, BS = 100). In addition, all the Sapindales families (Sapindaceae, Meliaceae, Simaroubaceae, Rutaceae, Burseraceae, and Anacardiaceae) were recovered as fully supported (PP = 1.00, BS = 100) monophyletic lineages by our phylogenomic analyses.
Within Anacardiaceae, four well-supported lineages (PP = 1.00, BS = 100), corresponding to the four tribes (Spondieae, Anacardieae, Rhoideae, and Dobineeae) recognized by Engler (1892), were recovered. Among them, the tribe Spondieae was the first clade that split from the rest of Anacardiaceae (PP = 1.00, BS = 100), and Dobinea (tribe Dobineeae) was sister to the clade including tribes Anacardieae and Rhoideae (PP = 1.00, BS = 100). Tree topology clearly indicates that the genus Dobinea was phylogenetically distinct from the members of Sapindaceae. Furthermore, molecular dating analysis revealed that D. delavayi and D. vulgaris diverged approximately 10.76 Ma (95% HPD: 5.10–17.76 Ma) (Fig. 6).

4. Discussion

4.1. Relationships among Sapindales families

The order Sapindales, containing nine well-recognized families, i.e., Anacardiaceae, Biebersteiniaceae, Burseraceae, Kirkia-ceae, Meliaceae, Nitrariaceae, Rutaceae, Sapindaceae and Simaroubaceae, is a member of core eudicots (Angiosperm phylogeny group, 2016). Although several studies have proposed inter-familial relationships within Sapindales (e.g., Clayton et al., 2007; Gadek et al., 1996; Lin et al., 2018; Mueller et al., 2003, 2007; Mueller-Riehl et al., 2016), the relationships between the families Meliaceae, Rutaceae, Sapindaceae, and Simaroubaceae remain unresolved. The phylogenetic analyses of Mueller-Riehl et al. (2016) based on rbcl, atpB and trnL-trnF DNA sequences showed that Rutaceae was sister to the clade including Meliaceae and Simaroubaceae, with these together being sister to Sapindaceae. However, the phylogenetic analyses by Gadek et al. (1996) based on trnL-F sequence, as well as by Lin et al. (2018) based on 79 plastid genes revealed that Meliaceae was sister to the clade formed by Rutaceae and Simaroubaceae; Sapindaceae was not closely related to the clade consisting of Rutaceae, Meliaceae and Simaroubaceae. Our plastid phylogenomic analyses indicate that Meliaceae and the clade consisting of Simaroubaceae and Rutaceae robustly clustered into a group (PP = 1.00, BS = 100), which is, in turn, sister to Sapindaceae (PP = 0.94, BS = 98). The relationships recovered in this study are different from those of previous studies (Gadek et al., 1996; Lin et al., 2018; Mueller et al., 2007; Mueller-Riehl et al., 2016). With more variable characters and/or much larger taxon sample, our analyses provide new insights into the inter-familial relationships within Sapindales.

4.2. Phylogenetic position of Dobinea

Similar to the previous study of Pan et al. (2008), the monophyly of Dobinea was strongly supported by the plastome-based phylogeny. In our analyses the genus was not phylogenetically related to members of the family Sapindaceae, but fully nested within Anacardiaceae with high support values (PP = 1.00, BS = 100). This robustly supports the taxonomic treatment of
Dobinea as a member of Anacardiaceae (Radlkofer, 1888) rather than placing it into Sapindaceae (Don, 1825) or the separate family Podoonaceae (Forman, 1973; Hutchinson, 1973; Morot, 1889). The close relationships between Dobinea and other Anacardiaceae genera can be justified by the anatomical evidence: Dobinea harbor resin canals in the phloem, which is likely one of the morphological synapomorphies to recognize the family Anacardiaceae (Radlkofer, 1888). Nevertheless, Dobinea is morphologically distinctive in that female flowers lack petals and its single carpel is adnate to foliose bract, which is distinctly different from any other taxa within Anacardiaceae. This justifies the establishment of the tribe Dobineeae to accommodate the genus by Engler (1892).

The plastome-based phylogeny well resolved the inter-tribal relationships within Anacardiaceae. The tribes Spondieae, Anacardieae, and Rhoideae recognized by Engler (1892) were recovered as well-supported monophyletic units. The tribe Spondieae was recovered as the earliest diverged clade. This finding is congruent with previous studies that used rbcL and ITS sequences (Pan et al., 2008), two nuclear and seven plastid DNA regions (Xie et al., 2014), and two nuclear sequences and two plastid DNA regions (Yang et al., 2016). The basally branching position of Spondieae inferred Fig. 4. Identity plot comparing the plastomes of two Dobinea species. Gray arrows indicate the position and direction of each gene. Black lines define the percentage of identity, ranging from 50 to 100%. Genome regions are color-coded as protein coding (exon), rRNA (UTR), tRNA (UTR), and conserved non-coding regions (CNS).
Fig. 5. Tree topology results from maximum likelihood (ML) and Bayesian inference (BI) analyses based on whole plastomes. Numbers represent Bayesian posterior probabilities (PP) and maximum likelihood bootstrap (BS).

Fig. 6. Divergence time estimation based on plastome DNA sequences. The red number above the tree branch indicates mean divergent age. Horizontal blue bars on each node indicate the 95% confidence interval of divergence time. Numbers on the Time Axis indicate million years ago (Ma). A: calibrated age = 70 ± 5.0 Ma; B: calibrated age = 49.1 ± 2.1 Ma.
in this study can be further supported by fossil evidence: the earliest fossil record for Anacardiaceae belongs to Spondieae, which was known from the Lower Eocene London Clay flora of southern England (Manchester et al., 2009). In addition, Dobinea was sister to the clade including Anacardiaceae and Rhoideae. The affinity between them were also supported by the anatomical characters: Dobinea and Anacardiaceae share similar carpel morphology and structure (Wannan and Quinn, 1991), while Dobinea and Rhoideae have the same endocarp structure (Wannan and Quinn, 1990).

4.3. Species divergence within Dobinea

The intensification of the Asian summer monsoon since the late Miocene established a humid climate in subtropical East Asia (Jacques et al., 2011; Wan et al., 2007; Zhang et al., 2012) and caused the expansion of forests in East Asia (Santosh, 2011; Yao et al., 2011); these processes played an essential role in driving and promoting a remarkable evolutionary radiation of plants (Chen et al., 2018; Lu et al., 2018; Sun and Wang, 2005; Wen et al., 2014). For instance, the intensification of Asian summer monsoon may have created largely unoccupied and favorable habitats for the genus Saussurea DC. (Asteraceae), which facilitated intensive lineage diversification in this genus (Wang et al., 2009). Previous studies also suggested that species diversification in Lepisorus (J. Sm.) Ching (Polypodiaceae) (Wang et al., 2012), Rheum L. (Polygonaceae) (Sun et al., 2012), Paris L. (Melanthiaceae) (Ji et al., 2019), Stewartria L. (Theaceae) (Lin et al., 2019) and Prinsepia Royle (Rosaceae) (Ma et al., 2019) was also correlated with the intensification of the monsoonal climate in East Asia.

Our molecular dating inferred that Dobinea species diverged 10.76 Ma (95% HPD: 5.10–17.76 Ma), around the late Miocene, when the Asian summer monsoon greatly intensified (Sun et al., 2001; Sun and Wang, 2005). This implies that the divergence between the two extant Dobinea species was most likely driven by the intensification of the Asian summer monsoon. The intensification of the Asian summer monsoon established distinct monsoon regimes in East Asia (Li et al., 2008; Yao et al., 2011). Since then, the Himalayan region (distribution area of D. vulgaris, Fig. 2) have been mainly governed by the Indian monsoon, whereas the Yunnan-Guihu plateau (distribution area of D. delavayi, Fig. 2) have been affected by both the Indian and Pacific monsoons (Li et al., 2008; Yao et al., 2011). The divergence of monsoonal regimes may have triggered the vicariance between D. delavayi and D. vulgaris.

Author contributions

YJ conceived and designed the research. CL collected and analyzed the data. CL and YJ wrote the manuscript. YJ, LJ, SW and ZY read and approved the manuscript. All of the authors read and approved the manuscript.

Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pld.2020.05.002.

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