The Complete Plastomes of Five Hemiparasitic Plants (Osyris wightiana, Pyrularia edulis, Santalum album, Viscum liquidambaricolum, and V. ovalifolium): Comparative and Evolutionary Analyses Within Santalales

Xiaorong Guo1, Changkun Liu2, Guangfei Zhang1, Wenhua Su*1, Jacob B. Landis3, Xu Zhang4, Hengchang Wang*4 and Yunheng Ji2,5

1 Institute of Ecology and Geobotany, Yunnan University, Kunming, China, 2 CAS Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China, 3 Department of Botany and Plant Sciences, University of California, Riverside, Riverside, CA, United States, 4 CAS Key Laboratory of Plant Germplasm Enhancement and Specialty Agriculture, Wuhan Botanical Garden, Chinese Academy of Sciences, Wuhan, China, 5 Yunnan Key Laboratory for Integrative Conservation of Plant Species with Extremely Small Population, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China

Most species of Santalales (the sandalwood order) are hemiparasites, including both facultative and obligate hemiparasites. Despite its rich diversity, only a small fraction of the species in the sandalwood order have sequenced plastomes. The evolution of parasitism–associated plastome reduction in Santalales remains understudied. Here, we report the complete plastomes of three facultative hemiparasites (Pyrularia edulis, Cervantesiaceae; Osyris wightiana, and Santalum album, Santalaceae), and two obligate hemiparasites (Viscum liquidambaricolum and Viscum ovalifolium, Viscaceae). Coupled with publicly available data, we investigated the dynamics of plastome degradation in Santalales hemiparasites. Our results indicate that these hemiparasites can be characterized by various degrees of plastome downsizing, structural rearrangement, and gene loss. The loss or pseudogenization of ndh genes was commonly observed in Santalales hemiparasites, which may be correlated to the lifestyle shift from photoautotroph to hemiparasitism. However, the obligate hemiparasites did not exhibit a consistently higher level of gene loss or pseudogenization compared to facultative hemiparasites, which suggests that the degree of plastome reduction is not correlated with the trophic level facultative or obligate hemiparasitism. Instead, closely related taxa tend to possess highly similar plastome size, structure, and gene content. This implies the parasitism-associated plastome degradation in Santalales may evolve in a lineage-specific manner.

Keywords: parasitism, plastome degradation, gene loss, pseudogenization, Cervantesiaceae, Santalaceae, Viscaceae
INTRODUCTION

Chloroplast, which possess an independent genome (the plastome), is the key organelle in plant cells for photosynthesis and carbon assimilation, as well as the biosynthetic pathways of starch, fatty acids, pigments, and amino acids (Palmer, 1985; Neuhau and Emes, 2000; Daniell et al., 2016). Due to the essential roles of chloroplast in green plants, their plastomes are characterized by high conservatism in terms of genome size, structure, gene content, and organization (Daniell et al., 2016). A typical angiosperm plastome encodes 113 unique genes arranged in a quadripartite structure, consisting of a large single copy (LSC, 80–90 kbp) region and a small single copy (SSC, 16–27 kbp) region separated by a pair of inverted repeats (IRs, 20–30 kbp) regions (Wicke et al., 2011). Nevertheless, the lifestyle transition from autotrophy to parasitism leads to varying degrees of plastome modification, including decreasing size, physical or functional loss of genes, and structural rearrangement (reviewed by Neuhau and Emes, 2000; Wicke and Naumann, 2018). As a result, the plastomes of parasitic plants are significantly divergent from their autotrophic relatives (Krause, 2008; Wicke et al., 2013, 2016; Petersen et al., 2015; Frailey et al., 2018; Schneider et al., 2018; Shin and Lee, 2018; Wicke and Naumann, 2018).

There are approximately 4,500 parasitic species documented in 20 families of flowering plants (Nickrent, 1997, 2002; Heide–Jørgensen, 2008), and parasitism likely has evolved at least 11 or 12 times in angiosperms (Barkman et al., 2007; Westwood et al., 2010). Hemiparasitic plants, different from holoparasitic plants (non-photosynthetic parasites) that obtain all nutrition and energy from the host plants, comprise more than 90% of plant parasites and are capable of photosynthesis (Nickrent, 1997; Heide–Jørgensen, 2008; Tešitel, 2016). They hence display a lower degree of plastome degradation than holoparasitic plants, and their plastomes are often within the size range of regular angiosperm plastomes (Wicke and Naumann, 2018).

Hemiparasitic plants can be further divided into facultative and obligate hemiparasites: an obligate cannot survive without exploiting a host, while a facultative does not rely on its host throughout its entire life cycle (Nickrent, 1997; Heide–Jørgensen, 2008). A previous study revealed that facultative hemiparasites may have relatively slight plastome degradation compared with obligate hemiparasites (Petersen et al., 2015). However, the currently available sequenced plastomes of hemiparasitic plants only represent a small fraction of the species diversity. Thus, it remains uncharacterized to what extent their plastomes are divergent, and whether facultative/obligate parasitism is correlated with different levels of plastome degradation.

Comparative and phylogenetic analysis of parasitic plastomes can provide insight into the evolutionary reduction concomitant with the lifestyle shift from autotrophy to parasitism (Wicke et al., 2016). Under a phylogenetic framework, reconstructing an evolutionary pathway of plastome degradation in relation to parasitism becomes feasible (Wicke and Naumann, 2018). The sandalwood order (Santalales) consists of 18 families, approximately 160 genera and 2,200 species (Nickrent et al., 2010; Su et al., 2015), which cover all lifestyles from autotrophy to hemiparasitism and holoparasitism (Nickrent, 1997, 2002; Der and Nickrent, 2008; Su et al., 2015). Most species in Santalales are hemiparasites, including both facultative and obligate (Nickrent, 1997, 2002; Heide–Jørgensen, 2008). Therefore, it provides an ideal system to investigate the reductive plastome evolution in hemiparasitic plants.

In this study, we sequenced and assembled the complete plastomes of three facultative hemiparasites: Pyrularia edulis (Cervantesiaceae), Osyris wightiana, and Santalum album (Santalaceae), and two obligate hemiparasites: Viscum liquidambaricolum, and Viscum ovalifolium (Viscaceae) in Santalales using a genome skimming approach (Straub et al., 2012). We aim to (1) characterize the newly sequenced plastomes, (2) determine structural shifts, gene loss and/or pseudogenization events in hemiparasites by comparing with autotrophic lineages, and (3) investigate the extent and progression of the plastome reduction in both facultative and obligate hemiparasites.

MATERIALS AND METHODS

Plant Sampling, DNA Extraction, and Illumina Sequencing

Specimen and leaf tissues of P. edulis, O. wightiana, S. album, V. liquidambaricolum, and V. ovalifolium were sampled with vouchers deposited in the herbarium of Kunning Institute of Botany, CAS (information see Supplementary Table S1). Genomic DNA was extracted from ca. 50 mg of silica gel dried leaves using a modified CTAB method (Doyle and Doyle, 1987). Purified DNA was sheared to fragments with an average length of 350 bp, followed by ligation of adaptors for library amplification according to the manufacturer’s guideline (Illumina, San Diego, CA, United States). The shotgun library for each species was sequenced using a 2 × 150 Illumina HiSeq 2500 system at BGI (Wuhan, Hubei, China).

Plastome Assembly, Annotation and Comparison

Original shotgun reads were filtered using the FASTX–Toolkit1 to remove adaptors and reads with ambiguous bases. Both reference-based and de novo strategies were employed to assemble the plastomes. Firstly, the clean reads were assembled using CLC genome assembler v. 4.0β (CLC Inc., Aarhus, Denmark) with default parameter setting. The plastome of Osyris alba was used as reference to P. edulis, O. wightiana and S. album, and the plastome of Viscum album was selected as reference to both V. ovalifolium and V. liquidambaricolum. Genbank accessions of the reference plastomes are provided in Supplementary Table S2. All contigs were aligned to the reference by BLAST searches. The plastid contigs were contigued according to the reference and connected with overlapping terminal sequences to yield the entire plastome. Secondly, the program NOVOPlasty v2.7.0 (Dierckxsens et al., 2017) was used

1http://hannonlab.cshl.edu/fastx_toolkit
for de novo assembly of plastomes with the k-mer size set at 31. The plastid rbcL gene of *Taxillus chinensis* (KY996492) was used as the seed to recover the plastomes. The plastomes of a given species recovered from different assembly strategies were compared using Geneious V10.2 (Kearse et al., 2012) to validate the accuracy of assembly.

Annotation of plastid genes were performed with Dual Organellar Genome Annotator database (Wyman et al., 2004). All protein-coding genes were checked with a BLAST search against the NCBI protein database. The protein-coding genes with one or more frame shift mutations or premature stop codons were annotated as pseudogenes. Start and stop codons and intron/exon boundaries for protein-coding genes were checked manually. Genes putatively annotated as transfer RNA (tRNA) were further verified by tRNAscan-SE 1.21 (Schattner et al., 2005) with default parameters. All fully annotated plastomes were deposited in the NCBI GenBank database under the accession number MK675807–MK675811 (Supplementary Table S1).

The published plastomes of Santalales (Petersen et al., 2015; Su and Hu, 2016; Li et al., 2017; Yang et al., 2017; Liu et al., 2018; Shin and Lee, 2018; Zhu et al., 2018; Jiang et al., 2019; Chen et al., 2020) were downloaded from NCBI GenBank (Supplementary Table S2). Together with the newly generated plastomes, the gene content, boundaries of IRs/LSC and IRs/SSC of Santalales hemiparasites were compared using Geneious V10.2 (Kearse et al., 2012). Any putative gene losses detected in the newly generated plastomes were further verified by extracting intact sequences of the corresponding genes from the autotrophic *Erythropalum scandens* plastome and conducting local BLAST searches against the sequencing reads of each species. To investigate structural rearrangement in Santalales plastomes, the plastomes were progressively aligned by the multiple genome alignment software Mauve 2.3.1 (Darling et al., 2010), with the NCBI GenBank database as the seed to recover the plastomes. The plastomes of *E. scandens* were compared using local BLAST searches against the illumina paired-end sequencing generated over 30 million clean reads for each species. The mean coverage of the plastome sequencing was 6,518.67X for *O. wightiana*, 573.28X for *P. edulis*, 4314.96X for *S. album*, 134.61X for *V. liquidambaricum*, and 592.48X for *V. ovalifolium*. The high levels of sequencing coverage guaranteed the accuracy of plastome assembly (Supplementary Table S3). For each species, the reference-based and de novo strategies generated almost identical plastomes, with only slight differences in plastome sizes (<5 bp).

Similar to other Santalales hemiparasites, the five newly sequenced plastomes had a typical quadripartite structure, consisting of a pair of IRs, a LSC, and a SSC (Figure 1). The size of these plastomes ranged from 128,601 bp (*V. liquidambaricum*) to 147,544 bp (*O. wightiana*), with the overall GC content varying from 36.1% (*V. liquidambaricum* and *V. ovalifolium*) to 38.3% (*P. edulis*) (Table 1). Among the five plastomes, the total gene numbers as well as protein-coding, tRNA, and rRNA genes that constituted each are all slightly different (Table 2).

The plastomes of *O. wightiana* and *S. album* retained 101 potentially functional genes (Table 2). The pseudogenization of *infA*, *ndhA-E*, *ndhG-H*, and *ndhK*, as well as the deletion of *ndhF*, *ndhI*, and *ndhJ*, were observed in *O. wightiana*. In *S. album*, *infA*, *ndhB-E*, *ndhG*, and *ndhK* were identified as pseudogenes, and *ndhA* and *ndhH* had been deleted. Although the loss of all *ndh* genes except for *ndhB* (pseudogenized) was detected in *P. edulis*, *V. liquidambaricum*, and *V. ovalifolium*, the additional loss of *infA*, *trnG-UCC*, and *trnV-UAC*, as well as the pseudogenization of *cssA*, were only identified in *V. liquidambaricum* and *V. ovalifolium* (Supplementary Table S4). Also, the loss of *trnR-ACG* and pseudogenization of *matK* was observed in *P. edulis* and *V. ovalifolium*. These gene losses were confirmed using corresponding sequences of *E. scandens* for local BLAST searches against the illumina reads of each species. As the result of gene loss and pseudogenization, a total of 100, 97 and 95 putatively functional genes were observed in *P. edulis*, *V. liquidambaricum*, and *V. ovalifolium*, respectively.

Comparison of Plastome Size and GC Content of Santalales Hemiparasites
Based on the plastomes sequenced in this study, as well as those reported previously, we compared the plastome size and GC content of Santalales hemiparasites with those of *E. scandens* (Table 1). All hemiparasite plastomes, except for that of *Ximenia americana* (156,834 bp), are smaller than the 156,154 bp of the autotrophic outgroup. The LSC length of the Santalales hemiparasites ranged from 65,171 bp (Arceuthobium) to 87,816 bp (*X. americana*), whereas the length of IR and SSC regions varied from 12,381 bp (*S. album*) to 32,691 bp (*X. americana*), and 15 bp (*Malania oleifera*) to 14,081 bp (*O. wightiana*), respectively. These regions of the hemiparasites (except for *X. americana*), were reduced in size to varying degrees compared to those of *E. scandens* (84,799 bp).
in LSC, 26,394 bp in each IR, and 18,576 bp in SSC). Also, the hemiparasite plastomes had varying GC content from 34.9% (A. sichuanense) to 38.3% (P. edulis). In each plastome, LSC, SSC, and IR regions had an uneven distribution of GC content. Specifically, the IR regions had the highest GC (40.4–47.9%), followed by LSC (30.1–36.1%). The SSC region exhibited the lowest GC content (23.7–40.0%).

**Structural Shifts in Santalales Hemiparasitic Plastomes**

The junctions of IR/LSC and IR/SSC in the plastomes of Santalales hemiparasites are variable (Figure 1). The SSC region has been extremely contracted to 15 bp in M. oleifera (Ximeniaceae), and 265 bp in A. sichuanense (Viscaceae). A significant IR contraction to the ycf2 at the IR/LSC junctions was observed in the plastomes of Schoepfiaceae. The IR/LSC boundaries of all Loranthaceae species are in rpl2, and the IR/SSC junctions fall into trnL-UAG and ycf1. Within Santalaceae, the IR falls into rps19 in LSC, and in ccsA and the trnR-trnN intergenic spacer in SSC. The plastomes of Cervantesiaceae and C. manillana (Opiliaceae) share the same IR/LSC boundaries with species of Santalaceae, while their IRb/SSC junction shifted to the intergenic spacer between trnN-GUU and rrn5S, and trnL-UAG and trnN-GUU. The expansion of IR regions in Viscaceae reached rpl22-trnH-rps3 cluster in the IR/LSC boundaries, and their IR/SSC fall into ycf1, as well as the intergenic spacer between trnN-GUU and rpl32. The result of the multiple plastome alignment is illustrated in Figure 2. Compared with the autotrophic E. scandens plastome, no structural rearrangement was found in the LSC and IR regions of Santalales hemiparasites. However, multiple rearrangements were found in the IR/SSC boundaries, and SSC regions in species of Amorphogynaceae, Cervantesiaceae, Santalaceae, and Viscaceae.
Comparison of Plastome Gene Content and Organization

The examined Santalales hemiparasites had divergent gene content in their plastomes (Table 2 and Supplementary Table S4 and Figure 3). The plastomes of Dendrotrophe varians, O. alba, O. wightiana, S. album, S. fragrans, and Schoepfia jasminodora encoded the highest number of potentially functional genes with a total of 101, including 67 protein-coding, 30 tRNA, and 4 rRNA genes. Comparatively, the lowest number of putatively functional genes was observed in A. sichuanense, which has 54 protein-coding, 23 tRNA, and 4 rRNA genes. The functional or physical losses of the plastid-encoded NAD(P)H–dehydrogenase (NDH) complex is commonly shared by all Santalales hemiparasites, while the loss of plastid infA was observed in Cervantesiaceae, Loranthaceae, Opiliaceae, Santalaceae, and Viscaceae. In addition, the deletion of trnV–UAC genes was shared by species of Loranthaceae, Schoepfiaceae, Viscaceae, and Ximeniaceae. The further loss of trnG–UCC was observed in Loranthaceae, Viscaceae, and Ximeniaceae. The family specific losses of rpl32, rps15, rps16, and trnK–UUU were observed in Loranthaceae and Ximeniaceae, and the further deletion of essential photosynthetic genes (psaC, psbA, I, K, L and M, petB, and ccsA) was identified in Ximeniaceae.

Phylogenetic Analysis

Maximum-likelihood analysis (Figure 3) resolved the autotrophic taxa E. scandens (Erythrophalacaceae) as the early diverging clade of the sandalwood order, and recovered the hemiparasites in three major clades (BS = 100%). Notably, the obligate hemiparasites did not form a monophyletic group, with obligate hemiparasitism evolving at least twice from facultative...
GLAD SOMEONE IS SPEAKING TABLE 2 | Comparison of the plastome gene content of Santalales hemiparasites with Erythropalum scandens.

| Species                  | Lifeform* | Total plastid genes | Potentially functional genes | Functional protein–encoding genes | tRNA | rRNA | Deleted genes | Pseudogenes |
|--------------------------|-----------|---------------------|-----------------------------|----------------------------------|------|------|---------------|-------------|
| Erythropalum scandens    | Autotroph | 113                 | 113                         | 79                               | 30   | 4    | 0             | 0           |
| Osyris alba              | FH        | 110                 | 101                         | 67                               | 30   | 4    | 3             | 9           |
| Osyris wightiana         | FH        | 110                 | 101                         | 67                               | 30   | 4    | 3             | 9           |
| Santalum album           | FH        | 108                 | 101                         | 67                               | 30   | 4    | 5             | 7           |
| Champereia manillana     | FH        | 105                 | 100                         | 66                               | 30   | 4    | 8             | 5           |
| Dendrotrophe varians     | OH        | 105                 | 100                         | 67                               | 30   | 4    | 8             | 4           |
| Ximenia americana        | FH        | 103                 | 102                         | 68                               | 30   | 4    | 10            | 1           |
| Pyrularia edulis         | FH        | 102                 | 100                         | 67                               | 29   | 4    | 11            | 2           |
| Schoepfia fragrans       | FH        | 101                 | 101                         | 68                               | 29   | 4    | 12            | 0           |
| Schoepfia jasmindora     | FH        | 101                 | 101                         | 68                               | 29   | 4    | 12            | 0           |
| Viscum yunnanense        | OH        | 101                 | 97                          | 65                               | 28   | 4    | 13            | 3           |
| Viscum minimum           | OH        | 100                 | 98                          | 66                               | 28   | 4    | 13            | 3           |
| Viscum album             | OH        | 100                 | 97                          | 65                               | 28   | 4    | 13            | 3           |
| Viscum coloratum         | OH        | 100                 | 96                          | 64                               | 28   | 4    | 13            | 4           |
| Viscum liquidambaricumol | OH        | 100                 | 97                          | 65                               | 28   | 4    | 13            | 3           |
| Viscum ovalifolium       | OH        | 100                 | 97                          | 65                               | 28   | 4    | 13            | 3           |
| Viscum crassulae         | OH        | 99                  | 98                          | 66                               | 28   | 4    | 13            | 3           |
| Pyrularia sinensis       | FH        | 97                  | 94                          | 62                               | 28   | 4    | 16            | 3           |
| Taxillus subchuenensis   | OH        | 96                  | 91                          | 63                               | 27   | 4    | 17            | 2           |
| Scurrula rotchoides      | OH        | 96                  | 91                          | 60                               | 27   | 4    | 17            | 5           |
| Macrosolen sp.           | OH        | 96                  | 94                          | 63                               | 27   | 4    | 17            | 2           |
| Macrosolen tricolor      | OH        | 96                  | 95                          | 64                               | 27   | 4    | 17            | 1           |
| Taxillus chinensis       | OH        | 95                  | 94                          | 63                               | 27   | 4    | 18            | 1           |
| Scurrula parasitica      | OH        | 95                  | 94                          | 63                               | 27   | 4    | 18            | 1           |
| Tolypanthus maclurei     | OH        | 94                  | 93                          | 64                               | 25   | 4    | 19            | 1           |
| Dendrophthoe pentandra   | OH        | 94                  | 92                          | 63                               | 25   | 4    | 19            | 2           |
| Loranthus tanakae        | OH        | 94                  | 90                          | 61                               | 25   | 4    | 19            | 4           |
| Taxillus nigrans         | OH        | 94                  | 92                          | 62                               | 26   | 4    | 19            | 2           |
| Helianthera parasitica   | OH        | 93                  | 92                          | 63                               | 25   | 4    | 20            | 1           |
| Malania oleifera         | FH        | 85                  | 84                          | 58                               | 24   | 4    | 28            | 1           |
| Arecurthobium sichuanense| OH        | 84                  | 81                          | 54                               | 23   | 4    | 29            | 3           |

*Life form: FH, facultative hemiparasite; OH, obligate hemiparasite.

parasitism. Of those hemiparasites, Ximeniaceae (M. oleifera and X. Americana) was resolved as the first diverging branch. Within the second clade, Loranthaceae were recovered as monophyletic (BS = 100%), which was sister to Schoepfiaceae (BS = 100%). Within the third clade, C. manillana (Opiliaceae) is sister to clade comprised of Santalaceae + Cervantesiaceae, D. varians (Amphorogynaceae), and Viscaceae. The predicted gene deletions and gene inactivation shared by specific branches is shown in the tree topology. We also compared the plastome size among close species. The results indicate that closely related taxa possess similar-sized plastomes as well as relatively analogous gene content.

**DISCUSSION**

**Plastome Downsizing and Structural Shifts**

The lifestyle transition from autotrophy to parasitism often triggers reductive plastome evolution (Wicke and Naumann, 2018). Compared with the autotrophic E. scandens, the plastomes of Santalales hemiparasites have reduced in size by approximately 6–31%. Among them, X. americana possessed the largest plastome (156,834 bp). Contrarily, in the most reduced plastome (107,526 bp) of A. sichuanense, a total of 29 genes have been lost. The observation of more genes missing and a smaller sized plastome (Table 2), suggests that gene loss can be partly responsible for the plastome downsizing in Santalales hemiparasites.

We also found that the decrease of plastome size in Santalales is accompanied with a length reduction of non-coding regions, including intergenic regions between functional genes and introns (Table 1). A similar phenomenon has been shown in the hemiparasitic Cuscuta species (McNeal et al., 2007). A previous study proposed that the downsizing of non-coding regions have evolved to prompt the efficiency of energy and nutrition utilization, particularly in disadvantageous environments (Wu et al., 2009). Because of their nutritional reliance on host plants, reduction of non-coding regions may reinforce their fitness to the partial heterotrophic lifestyle.
Inverted repeat expansion and contraction frequently occur in angiosperm plastomes. These events often cause the lineage-specific gain or loss of a small number of genes in the IR/LSC, and IR/SSC junctions (Plunkett and Downie, 2000; Chumley et al., 2006). Although large-scale IR expansions and contractions are only observed in a few autotrophic species (Chumley et al., 2006), dramatic IR shifts commonly occur in the plastomes of both holoparasites (Bungard, 2004; Maier et al., 2007; McNeal et al., 2007; Krause, 2008; Naumann et al., 2016; Samigullin et al., 2016; Sidonie et al., 2016; Schneider et al., 2018) and hemiparasites (Li et al., 2013, 2017; Su and Hu, 2016; Cho et al., 2018; Frailey et al., 2018; Liu et al., 2018; Park et al., 2018; Shin and Lee, 2018; Jiang et al., 2019; Chen et al., 2020). Similarly, significant IR and SSC contractions were found in Schoepfiaceae, M. oleifera and A. sichuanense (Figure 1), respectively. Moreover, multiple rearrangements occurred in the SSC regions in hemiparasitic plastomes of Amphorogynaceae, Cervantesiaceae, Santalaceae, and Viscaceae (Figure 2). This suggests that the evolution of parasitism has caused prominent structural changes in the plastomes of some Santalales hemiparasites.

Missing and Pseudogenized Genes

The lifestyle transition from autotrophy to parasitism always leads to prevalent gene losses from the plastomes (Neuhaus and Emes, 2000; Wicke et al., 2013, 2016; Wicke and Naumann, 2018). Compared to autotrophic species, Santalales hemiparasites have experienced significant gene deletion and pseudogenization (Table 2). The series of reductive mutations involves ndh genes, infA, matK, rpl32, rpl33, rps15, rps16, ycf1, as well as several photosynthesis-associated genes (psaC, psbA, I, K, L and M, petB, and ccsA) and tRNAs (Figure 3 and Supplementary Table S4).

The ndh genes encode subunits of the NADH dehydrogenase complex, which mediates photosystem I electron recycle and facilitates chlororespiration in plant cells (Yamori and Shikanai, 2016). In general, the ndh genes are the earliest functional losses in the plastomes of hemiparasites (Maier et al., 2007; Wicke et al., 2013, 2016; Wicke and Naumann, 2018). In Santalales hemiparasites, all eleven ndh (A–K) genes have either been deleted or pseudogenized, suggesting that the plastid NDH pathway is not essential in these species. It is noteworthy that the physical or functional losses of these plastid–encoded ndh genes does not occur exclusively in parasitic or heterotrophic plants. The degeneration of ndh loci have been observed in a wide spectrum of autotrophic lineages, such as Gnetales and Pinaceae of gymnosperm (Wakasugi et al., 1994; McCoy et al., 2008; Braukmann et al., 2009; Wu et al., 2009), the early diverging eudicots (Sun et al., 2016, 2017), the eudicot families Cactaceae (Sanderson et al., 2015) and Geraniaceae (Blazier et al., 2011), the aquatic monocot Najas (Peredo et al., 2013), and epiphytic orchids (Chang et al., 2006; Lin et al., 2015, 2017). Given the independent loss of this pathway in many plant groups, Frailey et al. (2018) proposed that the plastid ndh genes may have been selected against in these photoautotrophic lineages. Also, a previous study revealed the NDH complex–related genes have been concomitantly deleted from plastid and nuclear genomes in some orchids, and proposed that the mutation in photoautotrophic plants may increase the possibility to evolve a heterotrophic life history (Lin et al., 2017). Given that all ndh genes are retained in their autotrophic relative (E. scandens), we hypothesize that the degradation of these genes from plastomes of Santalales hemiparasites may be associated with the lifestyle shift from photoautotroph to hemiparasitism. The eco-physiological consequences of the functional and physical losses of the plastid NDH pathway in Santalales hemiparasites, especially under stress conditions, merit to be furtherly examined (Chen et al., 2020).
Another commonly degraded plastid gene in Santalales is \textit{infA}; this mutation was detected in all Santalales hemiparasites except for Ximeniaceae and Schoepfiaceae. The loss or pseudogenization of \textit{infA} occurs in the plastomes of many holoparasitic plants (Wicke et al., 2011, 2013, 2016; Wicke and Naumann, 2018). With respect to hemiparasitic plants, reduction of this gene has been exclusively observed in Santalales (Petersen et al., 2015; Li et al., 2017; Yang et al., 2017; Liu et al., 2018; Shin and Lee, 2018; Zhu et al., 2018; Jiang et al., 2019; Chen et al., 2020). Given that pseudogenization or deletion of \textit{infA} gene has also been identified in a wide diversity of photoautotrophic plant lineages (Shinozaki et al., 1986; Millen et al., 2001; Ahmed et al., 2012), previous studies revealed that \textit{infA} is likely to be one of the most mobile plastid genes in flowering plants, which are often transferred to and maintained in the nucleus (Millen et al., 2001; Ahmed et al., 2012). Therefore, it is possible that \textit{infA} has been likewise transferred to the nucleus, and the degradation of this gene in Santalales hemiparasites may not be correlated to the evolution of parasitism.

Besides the functional or physical losses of \textit{ndh} loci and \textit{infA}, further plastome degradations were found in some Santalales hemiparasites. For example, the loss of \textit{rps15}, \textit{rps16}, and \textit{rpl32} was commonly shared by all Loranthaceae and Ximeniaceae species (Figure 3). Similarly, losses of these plastid ribosomal protein-coding genes have been observed in diverse lineage of autotrophic angiosperms (e.g., Saski et al., 2005; Jansen et al., 2007; Wicke et al., 2011, 2013, 2016; Wicke and Naumann, 2018).
tRNAs tend to be deleted from hemiparasitic plastomes. The degradation of may be dispensable even at an early parasitic stage. Following the import of tRNAs from the cytoplasm may be relatively easy (Wicke and Naumann, 2018). Therefore, plastid tRNA genes may be dispensable even at an early parasitic stage. Following the degradation of ndh genes, those non-essential genes such as tRNAs tend to be deleted from hemiparasitic plastomes.

Plastid genes that encode photosystem (psa/psb), cytochrome complex (pet), and heme attachment (ccsA) are key genes involved in photosynthesis (Wicke et al., 2011; Wicke and Naumann, 2018). Loss or pseudogenization of those core photosynthesis genes had been commonly found in holoparasitic angiosperms (e.g., Funk et al., 2007; McNeal et al., 2007; Wicke et al., 2016; Wicke and Naumann, 2018). According to the model of reductive plastome evolution associated with parasitism (Wicke et al., 2016; Wicke and Naumann, 2018), functional losses of the photosynthesis–associated genes (e.g., psa/psb genes, pet genes, ccsA, cemA) may occur around the boundary to holoparasitism. However, the observation of ccsA pseudogenization in Viscaceae species, as well as the loss of psaC, psbA, I, K, L and M, petB, and ccsA in M. oleifera, imply that the degradation of these core photosynthesis genes might initiate in hemiparasites that still heavily rely on photosynthesis. Because of their great importance for photosynthesis, further studies are needed to clarify whether these genes have been functionally transferred to the nuclear genome (Chen et al., 2020).

**Lineage–Specific Plastome Degradation**

A previous study suggested that the varying nutritional dependences on a host may influence the reductive evolution of hemiparasitic plastomes (Petersen et al., 2015). Distinct from facultative hemiparasite, an obligate hemiparasite cannot complete the life cycle without coming into contact with a host (Heide–Jørgensen, 2008). As a result, their plastomes may be expected to possess a higher level of degradation. Similar to a previous study (Chen et al., 2020), we found that the range of plastome size of the Santalales facultative hemiparasites (118,743–156,834 bp) largely overlaps the range for obligate ones (107,526–140,666 bp). We also observed that some facultative and obligate plants exhibited the same degree of gene deletion and pseudogenization. For instance, *Pyrularia* species (facultative) and *D. varians* (obligate hemiparasites) maintained almost identical gene content. In addition, a total of 28 genes has been deleted from the facultative hemiparasite, *M. oleifera*. The level of plastome degradation is higher than the majority of obligate hemiparasites (except for *A. sichuanense*) in Santalales. This suggests that obligate hemiparasitism may not lead to a higher level of physical or functional loss of plastid genes than facultative one. Therefore, the degree of plastome degradation may not be correlated with the lifeform of facultative or obligate.

The relationships within the sandalwood order recovered in this study received high support values (Figure 3), which are highly congruent with previous studies (Der and Nickrent, 2008; Nickrent et al., 2010; Su et al., 2015; Chen et al., 2020). Based on the phylogenetic relationships recovered in this study, we found that closely related taxa, for instance, within the same genera or family tend to possess high level of similarity in plastome size, structure, and gene content. We hence hypothesize that plastome degradation in Santalales hemiparasites may evolve in a lineage–specific manner.

**DATA AVAILABILITY STATEMENT**

The newly sequenced plastomes in this study were deposited in the NCBI GenBank database under the accession numbers MK675807–MK675811 (Supplementary Table S1).

**AUTHOR CONTRIBUTIONS**

YJ, XG, HW, and WS designed the research. XG, CL, and GZ collected and analyzed the data. XG and YJ wrote the manuscript. HW, XZ, and JL discussed the results and revised the manuscript.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2020.00597/full#supplementary-material

**TABLE S1** | Voucher information and GenBank accession.
**TABLE S2** | Lifeform and GenBank accession of published Santalales plastomes.
**TABLE S3** | Summary of Illumina sequencing of the five Santalales hemiparasites.
**TABLE S4** | Comparison of plastome gene content among Santalales hemiparasites.
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Conflict of 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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