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Elucidating the evolutionary dynamics of parasitism in *Cuscuta*: in-depth phylogenetic reconstruction and extensive plastomes reduction

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Abstract

Background The genus *Cuscuta* L. (Convolvulaceae), commonly known as dodder, is a holoparasite plant that relies on host plants for nutrition, leading to significant genomic changes, particularly in plastomes. This dependency has led to significant reductions and modifications in their plastomes compared to autotrophic plants. In contrast to the well-conserved plastomes of photosynthetic plants, *Cuscuta* exhibits substantial genomic reductions reflecting the loss of photosynthetic functions and associated genes.

Result This study examines eight plastomes within *Cuscuta* and reconstructs the phylogenetic relationships among 40 *Cuscuta* taxa using five other genera as an outgroup. The size of plastid genome varies significantly, with the smallest being 60 kb and the largest 121 kb, highlighting extensive genomic reduction. In special cases, the subgenera *Cuscuta* exhibit the loss of inverted repeats, distinguishing from them other subge within the *Cuscuta* genus. This reduction is most pronounced in genes related to photosynthesis, such as *atp*, *pet*, *psa*, *psb*, and *ycf* genes, particularly in the subg. *Grammica* (Lour.) Peter. The study also notes the frequent and independent loss of the plastid genes *infA*, *rpl23*, *rpl32*, *rps15*, and *rps16* across various angiosperm lineages, often involving transfer to the nuclear genome. In parasitic plants like *Cuscuta*, the *ndh* genes, crucial for photosynthesis, are often lost. The study also highlights that in the subg. *Grammica*, the *matK* and *rpo* genes, along with *trnR*-ACG genes, are lost in parallel, indicating that these parasitic plants do not need *matK* and *rpo* genes after the loss of *ndh* genes for survival. Analysis of selective relaxation pressure on plastid genes shows a reductive trend, with genes such as *atp*, *pet*, *psa*, *psb*, *rpo*, and *ycf* progressively becoming pseudogenes over time, with housekeeping genes like *rpl* and *rps* expected to follow. However, the pseudogenization process is specific to the subg. *Grammica*, *Pachystigma* (Engelm.) Baker & C.H.Wright, and *Cuscuta*, rather than in the subg. *Monogynella* (Des Moul.) Peter, Engl. & Prantl (ancient clade species).

Conclusion The study of *Cuscuta* plastomes reveals the profound impact of parasitism on genome evolution, highlighting the complex interplay of gene retention and loss through phylogenomic approaches. This research enriches our understanding of plant genome evolution and the intricate host-parasite relationships. It also sheds light on the evolutionary history and genomic adaptations of *Cuscuta*, illustrating the diverse strategies enabling subg. *Grammica*, *Pachystigma*, *Cuscuta*, and *Monogynella* thrive as parasitic species. These findings provide valuable insights into the molecular mechanisms underlying parasitism and its impact on plastid genome organization.

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Keywords Dodder, Parasite plants, *Cuscuta*, Plastomes, Gene loss and gene reduction, IR loss

Introduction

The genus *Cuscuta* L. (Convolvulaceae), commonly known as dodder, is a stem holoparasite and one of the most well-known and widely spread parasitic plants within the angiosperm lineage. This genus comprises about 200 species characterized by their unique parasitic lifestyle, which has led to significant morphological and genetic adaptations [1–6]. Unlike most plants, *Cuscuta* lacks the ability to photosynthesize effectively, relying instead on host plants for nutrients and energy. This adaptation is reflected in the structure and content of their plastomes, which have undergone considerable reductions and modifications compared to those of autotrophic plants [7–9].

The plastomes, commonly referred to as the chloroplast genome in photosynthetic plants, is a small, circular DNA molecule typically 120–160 kb in size and containing about 100–120 genes [10]. The plastomes of photosynthetic land plants are often well-preserved in terms of size, gene content, and sequence order due to intense purifying selection [11]. These genes are primarily involved in photosynthesis, gene expression, and various biosynthetic pathways. The reduction in the plastomes of parasitic plants is correlated with the loss of photosynthetic function and the corresponding decrease in genes related to these processes [12–16]. In parasitic plants like *Cuscuta*, the plastomes exhibits significant reductions and alterations, a phenomenon observed in other parasitic and mycoheterotrophic plants as well [17]. Given the significance of plastid as the site of photosynthesis in the cell, the genomic alterations seen due to a shift to a parasitic lifestyle are especially noticeable in the plastomes of heterotrophic plants. In some parasite plants, like *Rafflesia* R.Br. ex Gray (Rafflesiaceae), the plastomes appear to have been completely lost [18].

Plastid genomes provide valuable insights into the evolutionary history and relationships of plant species. In case of *Cuscuta*, phylogenetic studies have revealed intriguing patterns of genome evolution, such as gene loss, pseudogenization, and genome rearrangements [6]. Often, the loss of plastid genes is compensated by their transfer to the nucleus, altering the function from the plastid to the nuclear genome [19]. These studies have also highlighted the close evolutionary relationships between *Cuscuta* species and their divergence from other members of the Convolvulaceae family [20–22]. The use of molecular markers from the plastomes, along with nuclear and mitochondrial genomes, has enhanced our understanding of the phylogenetic placement and evolutionary adaptations of angiosperms [7, 23].

Advances in next-generation sequencing (NGS) technologies have facilitated the comprehensive analysis of plastomes in parasitic plants, enabling researchers to uncover the extent of genomic reduction and modifications in genus *Cuscuta*. Comparative genomics approaches have been particularly useful in identifying conserved and divergent regions across different species within the genus of holoparasite Orobanchaceae [24]. These approaches not only shed light on the evolutionary dynamics of plastomes in parasitic plants but also provide potential targets for genetic studies aimed at understanding host-parasite interactions [25].

Furthermore, this study of *Cuscuta* plastomes has broader implications for understanding plastomes evolution in parasitic plants. By examining the patterns of gene loss and retention, researchers can infer the selective pressures and functional constraints that shape the evolution of plastomes in parasitic lineages [26]. This knowledge contributes to our broader understanding of plant genome evolution and the complex interplay between host and parasite in parasitic plant systems [27].

In conclusion, the plastomes of *Cuscuta* offer a fascinating example of how parasitism can drive genome reduction and modification. The dynamic nature of plastomes and the evolutionary adaptations involving gene loss and transfer between cellular compartments highlight the evolutionary trajectory of plastomes, reflecting a complex interplay of gene retention, loss, and functional transfer to the nuclear genome. Phylogenetic analyses based on plastomes have provided significant insights into the evolutionary history and relationships of *Cuscuta* species, highlighting the impact of parasitic lifestyle on genome evolution. Ongoing research in this area promises to further elucidate the genetic and evolutionary mechanisms underlying holoparasite and mycoheterotrophic plants, with implications for plant biology, ecology, and evolution [24, 28, 29].

Results

Evolutionary phylogenetic analysis of *Cuscuta*

The resulting tree of IQ-TREE topology revealed that the subg. *Monogynella* is the most ancient sister group within genus *Cuscuta*, with subg. *Cuscuta* forming a sister clade to the subg. *Grammica* + *Pachystigma*. Both tree of IQ-TREE and ASTREL-III were supported by 100% bootstrap values, indicating strong support for the phylogenetic relationships within the different subgenera of *Cuscuta* (Figure S1). Phylogenetic analysis of genus *Cuscuta*, encompassing a total of 45 species, revealed relationships among its four

subg. *Grammica*, *Pachystigma*, *Cuscuta*, and *Monogynella*. The subg. *Grammica* is the largest and most diverse within *Cuscuta*. Phylogenetic analyses consistently indicate that subg. *Grammica* forms a monophyletic group, suggesting a single common ancestor and a coherent evolutionary lineage. Phylogenetically, the subg. *Pachystigma* is distinct from the subg. *Monogynella* and *Grammica*, supporting its classification as a separate lineage within *Cuscuta*. The subg. *Cuscuta* shows a closer relationship to the most ancient clade subg. *Monogynella* than to the more recently diverged subg. *Grammica* and *Pachystigma*. The subg. *Monogynella* is considered the most basal clade within *Cuscuta*. Phylogenetic studies support the early divergence of subg. *Monogynella*, consistent with its distinct morphological and genetic characteristics. The basal position of subg. *Monogynella* suggests it retains more ancestral traits, both morphologically and genetically, compared to the other subg. Based on the tree of IQ-TREE, further analyses were conducted to examine genome size and gene content.

Dynamic structural variation in plastid genome

The assembled plastome genomes of eight *Cuscuta* species exhibit a typical circular structure, comprising two inverted repeat (IR) regions, a large single-copy (LSC) region, and a small single-copy (SSC) region. Among the species, *C. japonica* possesses the largest plastid genome, while *C. gronovii* has the smallest (Table 1). The greatest extent of gene loss was observed in *C. cephalanthi*, a member of subg. *Grammica*, compared to other species within the genus *Cuscuta*. The average GC content across the plastomes of these species is approximately 37% (Table 1). The comparison of 40 species plastome size varies from among *Cuscuta* genus in between Convolvaceae family. That ranges from largest size was *C. exaltata* (*Monogynella*) with 125,373 bp to *C. erosa* (*Grammica*) with 60,959 bp (Sheet S2). Phylogenetic framework provides

valuable insights into evolutionary relationships and genomic characteristics of subg-*Cuscuta* that are lost in IR region. Other than that, the smallest IR observed in *C. erosa* and other members of its branch in subg. *Grammica*, and the largest single IR was observed in *C. nitida* E.Mey. ex Choisy at 18,135 bp (Fig. 1 and Table 1). Genus *Cuscuta* retained an average GC% of 37.43%. However, when the SSR region is small, they maintain the lowest GC% in *C. epithymum* (L.) L. (35.1%) and *C. europaea* L. (35.2%) compared to other species in *Cuscuta* (Sheet S2). The whole genome alignment produced six local colinear blocks (LCB) compared to the four subg. of genus *Cuscuta* and *I. batatas* L. There are breakages between *trnH*-GUG-*rps2* (around 19 kb), *trnC*-GCA-*trnT*-GGU (around 5 kb), *psbD*-*rps4* (around 2 kb), *trnT*-UGU-*trnF*-GAA (around 15 kb), *trnM*-CAU-*psbE*, and *petL*-*trnN*-GUU (around 49 kb) (Figure S2). The whole genome alignment also showed a decrease in the SSC region in *Cuscuta*, specifically in the subg. *Grammica*, compared to *I. batatas*.

Extensive plastid gene reduction

To study the evolutionary fates of the lost plastid genes in *Cuscuta*, a comparison was conducted with other species in the Convolvulaceae. The outgroup shows the independent pseudogene formation or loss of the *infA* gene in other Convolvulaceae members, whereas it is completely loss in *Cuscuta* (Fig. 2 and Sheet S3). In the subg. *Monogynella*, there is an independent loss of the *cemA* gene in *C. epithymum*, *ccsA* gene in *C. europaea*, and *clpP1* gene in *C. pedicellata*. In the subg. *Pachystigma*, *C. africana* Willd. has only lost the *rpoC2* gene, whereas the entire subg. *Grammica* has lost all RNA polymerase (*rpo*) genes, including *rpoA*, *rpoB*, *rpoC1*, and *rpoC2*. The maturase K (*matK*) gene and the *trnR*-ACG gene are lost only in the subg. *Grammica*. Additionally, in *C. indecora* Choisy, there is a loss of the *rpl36* gene, along with other sister group such as *C. bonafortunae* Costea

Table 1 Plastomes organization of eight *Cuscuta* species using DNA-sequencing

Species	Length	GC %	LSC	IR	SSC	CDS	Gene	tRNA	rRNA
<i>Cuscuta campestris</i> Yunck	86,749	37.7	50,978	14,354	7,063	60	68	28	8
<i>Cuscuta cephalanthi</i> Engelm	78,062	37.8	51,287	9,967	6,841	58	66	28	8
<i>Cuscuta chinensis</i> Lam	86,920	37.6	50,565	14,617	7,121	61	71	28	8
<i>Cuscuta glomerata</i> Choisy	78,959	37.7	52,185	9,961	6,852	60	68	28	8
<i>Cuscuta gronovii</i> Willd. ex Schult	78,903	37.8	52,058	10,009	6,827	61	73	28	8
<i>Cuscuta japonica</i> Choisy	120,994	38.3	79,498	16,554	8,388	68	80	32	8
<i>Cuscuta pentagona</i> Engelm	86,380	37.9	50,958	14,200	7,022	61	70	28	8
<i>Cuscuta polygonorum</i> Engelm	78,940	37.7	52,155	9,950	6,885	60	68	28	8

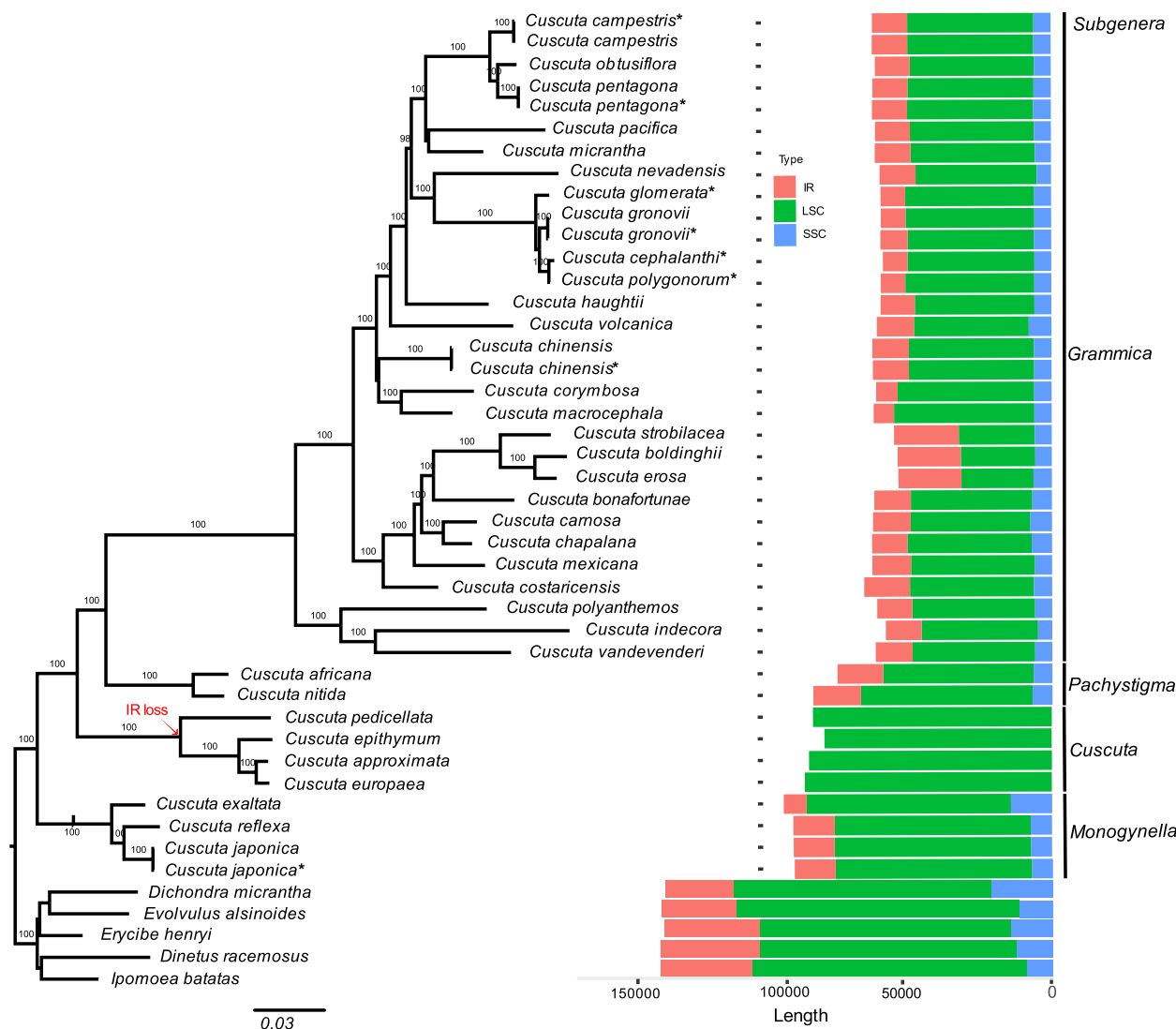


Fig. 1 Genome size variation among 40 *Cuscuta* species. The phylogenetic tree was used for relating the size variation from the subg. *Monogynella* to *Grammica* of *Cuscuta*. The species marked with an asterisk were analyzed in this study

& I.García, *C. erosa*, *C. boldinghii* Urb., and *C. strobilacea* Liebm. In special cases, *C. mexicana* Yunck. loses its functional properties in the *rbcL* and *cemA* genes, while *C. carnosa* Costea & I.García have lost the *psbF* and *psbM* genes. Furthermore, *C. bonafortunae* has lost the *psbF*, *psbI*, and *psbL* genes. Most gene loss occurs in *C. erosa*, *C. boldinghii*, and *C. strobilacea*, which are smaller in size due to the loss of most genes involved in the photosynthesis system and other molecular functions, including *ccsA*, *cemA*, *psa*, *psb*, *rbcL*, *rpl32*, and *ycf* genes. This represents more gene loss compared to other species in *Cuscuta*. Frequent loss of *psaI* and *rpl32* is noted in the clade leading from *C. macrocephala* Schaffner ex Yuncker to *C. campestris* Yunck. in the subg. *Grammica*.

Genes involved in ATP synthesis show that the entire subg. *Grammica* has lost the *atpE* gene (except *C. chinensis*) and some species, including *C. campestris*, *C. boldinghii*, *C. bonafortunae*, *C. chapalana*, and *C. mexicana*, have lost the *atpF* gene. The *ycf1* gene is lost in the clade leading to *C. polygonorum* and *C. campestris* in the subg. *Grammica*. Several tRNA genes, such as *trnA*-UGC, *trnG*-GAU, *trnI*-GAU, *trnK*-UUU, *trnL*-UAA, and *trnV*-UAC, are lost in all *Cuscuta* species, with *trnR*-ACG loss being specific to the subg. *Grammica* (Fig. 2). In the subg. *Grammica*, the *rpo*, *matK*, and *trnR*-ACG genes are lost in parallel in the latest diverged clade. The phylogenetic tree represents the frequent loss of the *cemA* and *ccsA* genes among the four subgenera.

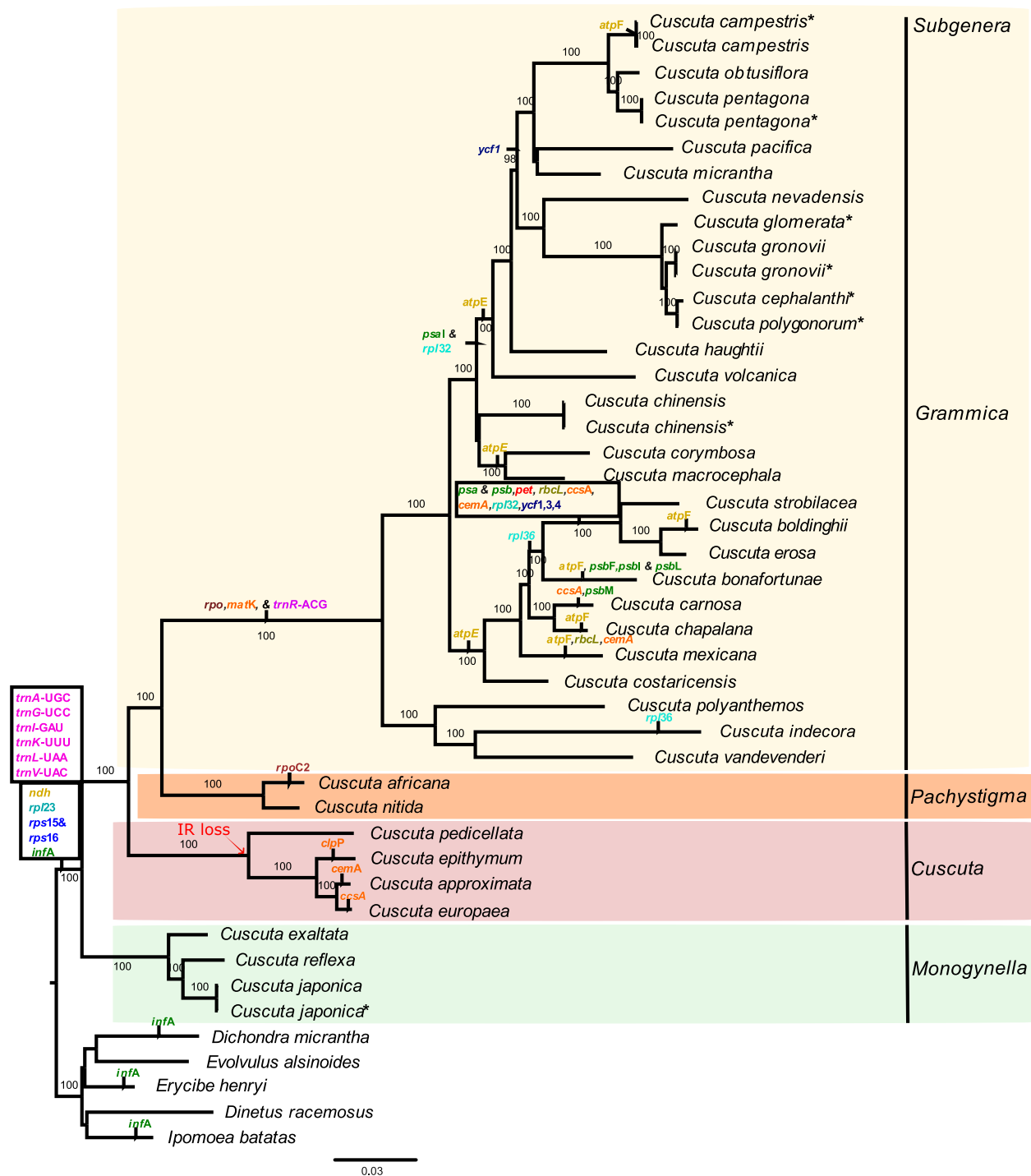


Fig. 2 Plastid protein-coding gene and tRNA gene loss in *Cuscuta* species

Evolutionary genomics adaptation by selective pressure
The k was estimated using a likelihood ratio test (LRT), indicating whether the selection was relaxed or negative (purifying) selection ($k < 1$) and intensified or positive selection ($k > 1$) in *Cuscuta* compared to autotrophic

plants. This approach provided robust insights into the evolutionary dynamics and selection pressures within this group. The rate of evolution in the holoparasite *Cuscuta*, compared with autotrophic plants, was analyzed using synonymous and non-synonymous ratios with the

RELAX tool. The RELAX model predicts an elevated or higher substitute rate in 59 plastid protein-coding genes. Among them, the *accD*, *ycf4*, *psaB*, *psaC*, *petD*, *petG*, *rbcl*, *rps7*, *rps18*, *ycf1*, and *ycf2* genes show a $k < 1$ ratio, indicating significant relaxation force between the test and reference groups. In contrast, the *atpA*, *atpB*, *rpl2*, *rpl16*, *rpl20*, *rps3*, *rps11*, *clpP*, *psaI*, *psbF*, *psbH*, *psbJ*, and *psbK* genes exhibit a $k > 1$ ratio, representing higher intensification force in genus *Cuscuta* (Fig. 3). The *atpH*, *atpI*, *rpl33*, *rps12*, *rps15*, *rps19*, *cemA*, *cssA*, *psb*, *pet*, and *ycf* genes do not strongly support relaxation selection, however, they indicate negative selection rather than intensified selective pressure. Based on selective pressure

analysis, the *pet*, *psb*, and *ycf* genes will follow strong relaxed selection in *Cuscuta* (Sheet S4). Most intensified genes are in *psb* (*psbF*, *psbH*, *psbJ*), and *psbK*) compared to *psa*, which shows strong relaxation in the *psaB* and *psaC* genes. ATP synthesis genes like *atpA* and *atpB* are under very strong intensified selection, unlike *atpH*, *atpF*, and *atpI*, which are under relaxed selection.

Discussion

The genus *Cuscuta*, also known as dodder, is a holoparasite plant that attaches to host plants to obtain nutrition [30]. Parasitic plants often undergo significant genomic changes due to their dependence on host plants

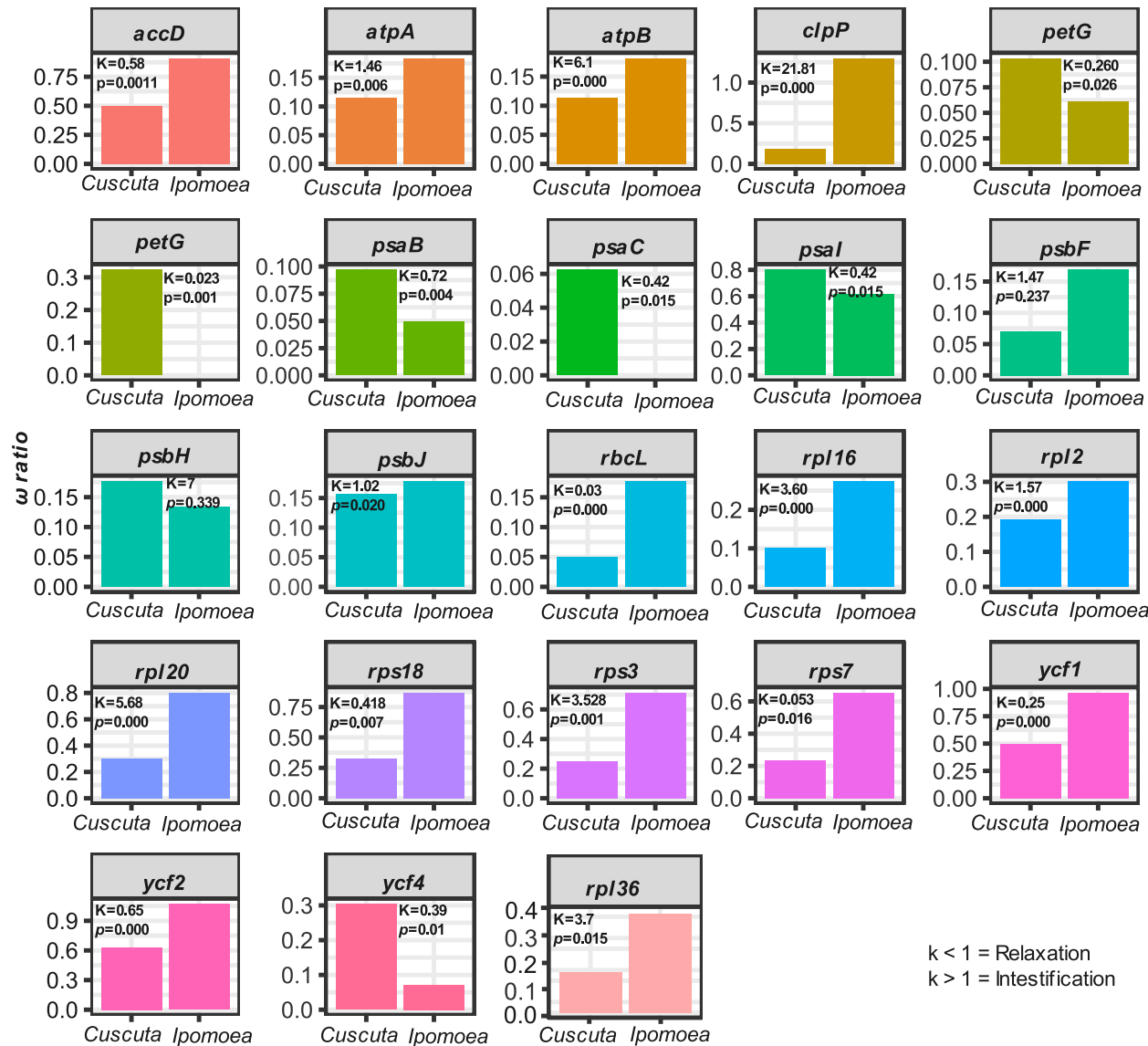


Fig. 3 The comparison of genus *Cuscuta* and *Ipomoea batatas* (Convolvulaceae) species shows a significant intensified or relaxed gene force representing using k (co-efficient value) using RELAX analysis

for nutrients, leading to the loss or reduction of photosynthetic functions. As a result, parasitic plants, which derive nutrients and water from their hosts, often exhibit reduced or altered plastomes. Genomic changes play a significant role in influencing the physiology, parasitic behavior, and interactions of parasitic plants with their host plants. The specialized parasitic behavior of parasitic plants which enables them to control host plants and seize resources is supported by genetic alterations in these plants. These modifications influence the physiology and evolutionary dynamics of the plants, enabling intricate interactions with their hosts [31–34]. The biological implications and evolutionary significance of genome reduction and gene loss in *Cuscuta*, the subgenera *Cuscuta* and *Monogynella* are largely attributed to the absence or low level of thylakoids and chlorophyll contents. This reduction in genomic content highlights the evolutionary adaptations that enable these plants to conserve energy and resources by eliminating non-essential genes, while optimizing their parasitic mechanisms for survival [35, 36]. In this study, we completed the plastomes of eight species to reconstruct the phylogenetic tree. The analysis utilized datasets from NCBI, which included 32 *Cuscuta* species and representatives from other five other genera within in the Convolvulaceae family [8, 20, 21, 23, 37]. This study encompasses plastome data from the 37 *Cuscuta* species, including the newly sequenced plastomes of *C. cephalanthi*, *C. glomerata*, and *C. polygonorum* for the first time.

Estimation of extensive divergence of genus *Cuscuta*

The phylogenetic relationships among the subg. of *Cuscuta*, namely *Grammica*, *Pachystigma*, *Cuscuta*, and *Monogynella*, supports the Banerjee and Stefanović and reveal interesting evolutionary insights within this genus [8, 9, 21]. Recent studies have utilized plastomes sequences and nuclear ribosomal DNA markers to reconstruct these relationships. subg. *Grammica* is the largest and most diverse within *Cuscuta* [38]. Phylogenetic analyses indicate that it forms a monophyletic group that place *Monogynella* as a basal ancestor clade to the subg. *Cuscuta*, *Pachystigma*, and *Grammica* [9, 23]. The loss of photosynthetic genes is particularly significant in the subg. *Grammica*, reflecting its adaptation to a parasitic lifestyle [9]. Although less diverse than subg. *Grammica*, subg. *Pachystigma* also shows notable genomic adaptations to parasitism. It is phylogenetically distinct from both subg. *Monogynella* and *Grammica*, supporting its classification as a separate lineage within *Cuscuta*. The subg. *Monogynella* is considered the most basal clade within *Cuscuta*, exhibiting fewer speciation events compared to the subg. *Grammica* and *Pachystigma*. This also suggests that the species are within subg. *Monogynella*

may have gone extinct [37, 39]. Phylogenetic studies support its early divergence, consistent with its distinct morphological and genetic characteristics [40]. Overall, phylogenetic analysis of four subg. demonstrate the dynamic nature of *Cuscuta* evolution, driven by ecological pressures and parasitic adaptation, resulting in significant genomic changes such as gene loss and pseudogenization [21–23, 41, 42]. The comparison of 40 *Cuscuta* plastomes sizes reveals a variation ranging from a minimum of 43 kb to a maximum of 121 kb among *Cuscuta* species, which is typical of non-green plants [11, 23]. In many cases of extensive genomic reduction, nearly all taxa show some degree of reduction in overall plastomes size and gene content, particularly in photosynthesis-related gene systems [43, 44]. In *C. strobilaceae*, *C. boldinghii*, and *C. erosa*, the plastomes show the most significant gene loss, particularly in the *pet*, *psa*, and *psb* gene groups, along with the *yfc* genes, resulting in genome reduction compared to other *Cuscuta* genomes [8, 45, 46]. This might be due to the more extensive divergence of holoparasite lineages within the subg. *Grammica* compared to the subg. *Pachystigma*, *Cuscuta*, and *Monogynella* of *Cuscuta*.

Plastomes shrinkage: a parallel gene loss

In parasitic angiosperms, the plastomes often undergoes significant reduction due to the loss of photosynthetic function. This reduction involves both physical shrinkage and loss of genes that are no longer essential for the parasite's survival. Gene loss in these parasitic plants is often accompanied by accelerated evolutionary rates and structural rearrangements. Massive gene loss or reduction in size has been observed in parasitic plants such as *Orobancha* L. (Orobanchaceae) and *Balanophora* J.R.Forst. & G.Forst. (Balanophoraceae), and there has been a complete loss of the plastomes in *Rafflesia* R.Br. ex Gray. (Rafflesiaceae) [18, 47–50]. The *infA* gene, which codes for the translation initiation factor 1 (IF-1), is notable for its unusual pattern of loss in angiosperms. Comprehensive analyses of plastomes sequences from various angiosperms further support the frequent and independent loss of the *infA* gene. The function of the lost *infA* gene in the plastomes is often compensated by a nuclear-encoded gene [19, 51, 52]. The loss of the *rps15* gene in angiosperms reflects a trend of plastomes evolution involving gene loss, pseudogenization, and transfer to the nuclear genome. In *Hypericum* Tourn. ex L. (Hypericaceae), the absence of the *rps15* gene is part of a broader pattern of plastid gene loss or transfer. Similarly, *Najas* L. (Hydrocharitaceae) and *Corydalis* DC. (Papaveraceae) show significant plastomes changes, including the loss of the *rps15* gene, driven by ecological and evolutionary pressures

[53–55]. The loss of the *rps16* genes from the plastomes has been observed in various plant lineages, indicating a significant evolutionary trend. The *rps16* gene in plastomes represents an extensive genomic reduction observed in both parasitic and some non-parasitic plants. It encodes the ribosomal protein S16, a component of the small subunit of the ribosome. Its loss has been documented in several plant lineages and is often compensated for by nuclear-encoded genes. It is absent in some members of the Apiaceae and Campanulaceae, indicating multiple independent losses within angiosperms [43, 53, 56–60]. The loss of the *rpl23* gene in plastomes is common among various plants. In *Hypericum*, it has become a pseudogene due to frameshift mutations, indicating that its function has transferred to the nuclear genome. Similarly, in *Vicia* L. (Fabaceae), it is also a pseudogene, aligning with the pattern of gene loss and nuclear transfer. This highlights the dynamic nature of plastomes and the complex evolutionary adaptations involving gene retention, loss, and functional transfer between cellular compartments [53, 61–64]. The *rpl32* gene encodes a ribosomal protein that is part of the plastid ribosome's small subunit. However, in several lineages of angiosperms, it has been lost from the plastomes and transferred to the nuclear genome, or in some cases, entirely lost. This phenomenon has been observed in *Euphorbia* L. (Euphorbiaceae), *Hepatica* Mill., *Thalictrum* Tourn. ex L. (Ranunculaceae), *Hypericum*, and *Populus* L. (Salicaceae) [53, 56, 60, 65, 66]. The *rpl32* has been found to undergo either transfer to the nuclear genome or complete loss, with significant evolutionary implications in angiosperms. This highlights the occurrence and implications of the *rpl32* gene loss and transfer, reflecting broader trends of plastomes evolution in this diverse group of plants. The *ndh* genes are thylakoid membrane-associated genes that comprise 11 complex genes in the plastomes [7, 67–69]. The loss of plastid *ndh* genes in parasite plants is a well-documented phenomenon, reflecting their specialized lifestyle and reduced dependence on photosynthesis. The set of 11 *ndh* genes has been found to be lost in many species within the angiosperm phylogeny, especially in non-green and some photosynthetic members such as *Corydalis* (Papaveraceae), *Drakaea* Lindl. (Orchidaceae), *Euphorbia*, *Erodium* L'Hér. (Geraniaceae), *Gentiana* Tourn. ex L. (Gentianaceae), *Simmondsia* Nutt. (Simmondsiaceae), and *Vanilla* Plum. ex Mill. (Orchidaceae) [55, 70–78]. The loss of the plastid *ndh* genes in parasitic flowering plants is a notable example of genome reduction associated with a parasitic lifestyle. The *ndh* genes have completely loss in cell of organism and there is no evidence of presents or replacement of nuclear *ndh* genes [7, 44, 79]. The *rpl36* gene encodes a

ribosomal protein that is part of the plastid ribosome. Its loss is often associated with the transfer of its function to the nuclear genome [74]. While this gene loss has been observed only five species of the subg. *Grammica* and is nonessential under normal growth conditions, it becomes crucial for plant survival and affects leaf morphology when the *rpl36* gene is lost [80]. The observed *rpl36* gene discordance and evolutionary rate heterogeneity of plastid genes suggest that different evolutionary pressures and ecological adaptations have shaped the plastomes within the subg. *Grammica*. In the subg. *Grammica*, the parallel loss of the *rpo*, *matK*, and *trnR-ACG* genes indicate that parasitic plants no longer require these genes after losing the *ndh* genes. The loss of the four *rpo* genes, which encode the core subunits of plastid-encoded RNA polymerase (PEP), is an important aspect of plastomes evolution. These genes are crucial for the transcription of plastid genes, particularly those involved in photosynthesis [81]. Genome rearrangements can lead to the loss of the *rpo* genes, which are crucial for encoding the α subunits of RNA polymerase, as observed in *Annona* L. (Annonaceae; *rpoA*), with all the *rpo* gene losses observed only in the subg. *Grammica* [82, 83]. The loss of introns and their associated maturase (such as *matK* gene) in the subg. *Grammica* plastomes reflect a trend towards genomic reduction. This phenomenon is common among parasitic plants like *Cuscuta*, which tend to lose non-essential genes over evolutionary time. The parallel loss of *trnK-UUU* introns and their maturase K in *Cuscuta* reflects a specialized adaptation to a parasitic lifestyle and provides insights into the dynamics of plastomes evolution in this unique genus [84]. In contrast, two ATP-synthesis complex genes undergoing pseudogenization, which frequent pseudogenization or loss of the *atpE* gene and random loss of the *atpF* gene in the subg. *Grammica*. This parallel pseudogenization or loss of the *atp* genes is also observed in *Orobancha* [44, 48, 85]. In conclusion, based on selective pressure and frequent gene loss in the phylogenetic tree, the subsequent loss of the *infA*, *rps15*, *rps16*, *rpl23*, and *rpl32* genes is common in the angiosperm lineage. The next reductive steps include the loss of the 11 *ndh* genes, followed by the loss of *rpo*, *matK*, and *trnR-ACG* genes in *Cuscuta*. In the latest sister clade group species, only subg. *Grammica* shows *atp*, *pet*, *psa*, and *psb* gene groups that play roles in encoding subunits of the photosynthesis, ATP-synthesis complex, cytochrome *b₆f* complex, unlike in the subg. *Cuscuta*, *Pachystigma*, and *Monogynella*. The *rpl36* gene loss is specific to *Cuscuta* in the latest diverged species of the subg. *Grammica*. The final reductive stage might involve the loss of hypothetical genes like *ycf* and house-keeping genes like *rpl* and *rps*, as well as metabolic

function genes like *accD*, *ccsA*, *cemA*, and *clpP1*. This pattern supports previous studies that have observed similar gene loss in the plastomes [16, 45, 86, 87].

Diverged evolutionary strategies in parasite

The phylogenetic analysis reveals a pattern of gene loss across different subg. of *Cuscuta*, with the most significant reductions observed in the subg. *Grammica*. Key findings include the frequent and independent loss of genes like *infA*, *rpl23*, *rpl32*, *rps15*, and *rps16* across various angiosperm lineages, and often compensated by nuclear-encoded genes. The study also documents the specific loss of the *rpl36* gene in *Cuscuta* and the significant reduction of the *atp*, *matK*, *ndh*, *pet*, *psa*, *psb*, and *rpo* genes. These genomic changes highlight the complex interplay of gene retention, loss, and transfer in the evolution of plastomes. The findings provide valuable insights into the evolutionary trajectory of plastomes in parasitic plants and underscore the role of selective pressures in shaping these genomes. The phylogenetic relationships and genomic characteristics of the subg. *Grammica*, *Pachystigma*, *Cuscuta*, and *Monogynella* illustrate the diverse evolutionary strategies employed by these lineages to adapt to parasitic lifestyles. This study underscores the intricate interplay between gene loss and selective pressures that have shaped the plastomes of *Cuscuta*, providing valuable insights into the evolutionary mechanisms underpinning parasitism in this genus.

Conclusion

The plastome sequence of *Cuscuta* provides valuable insights into plastome evolution across its four subgenera. This analysis reveals several unusual evolutionary phenomena, including significant genome reduction and the loss of the inverted repeat (IR) region within certain *Cuscuta* subgenera. Specifically, the recently diverged *Grammica* subgenus exhibits extensive gene and intron loss, particularly affecting genes typically involved in photosynthesis, transcription, and RNA processing in the chloroplast genome. In addition, the evolutionary forces acting on the *Cuscuta* plastome indicate a strong purifying selection compared to non-parasitic plastomes. This purifying selection correlates with substitution rates and reflects the co-evolution of structural changes within the plastome. These changes impact most functional genes, aligning with the shift toward an obligatory parasitic lifestyle. Importantly, the loss of genes and genome reduction in parasitic *Cuscuta* appears to occur in a step-wise, progressive manner, rather than through rapid or random gene loss. This research highlights how the gradual reduction of the plastome is intricately linked to the adaptation of *Cuscuta* to its parasitic mode of life, with

evolutionary rates and selection forces playing key roles in this process.

Materials and methods

DNA sequencing and plastome construction

Eight species of *Cuscuta* were collected and prepared as specimens for a study. Four species, the *C. cephalanthi*, *C. glomerata*, *C. gronovii*, and *C. polygonorum* were collected in the USA and processed as specimens at Gachon University, South Korea. An additional four species *C. japonica*, *C. campestris*, *C. chinensis*, and *C. pentagona* were collected in South Korea (Sheet S1). Total genomic DNA was extracted from the stems (haustoria) of these eight *Cuscuta* species using a modified cetyltrimethylammonium bromide (CTAB) method [88]. NGS was performed using Illumina Miseq platform (Illumina, Seoul, South Korea). The raw NGS data, consisting of 2×300 bp paired end reads, were cleaned by trimming adapter sequences, removing duplicated and chimeric reads, and trimming ends with a probability of error greater than 0.03 per base was performed by Geneious Prime 2022.10.2 (Sheet S1) [89]. The cleaned reads were then used for de novo genome assembly with Geneious employing the Velvet assembler [89, 90]. To construct the plastomes of *Cuscuta* species, further analysis of the raw reads was conducted, using *C. japonica* Choisy (GenBank accession No. MH780080) as a reference to extract plastid genome-related reads. The Geneious tool, with the Bowtie tool option, was used to map the reads that matched the plastid DNA sequence [89, 91]. Finally, de novo assembly was performed to complete the circular plastomes. The constructed genome was annotated using GeSeq, with tRNA annotation by tRNAscan-SE, and circular genome visualization was generated using OGDRAW [91–94]. The nucleotide sequence data have been deposited in the GenBank database under the accession number (PQ165168–PQ165175).

Constructing phylogenetic tree

We conducted a comprehensive phylogenetic analysis involving 45 species, including eight newly sequenced species from this study and 37 species obtained from NCBI data. This analysis encompassed four species from the subg. *Monogynella*, four from the subg. *Cuscuta*, two from the subg. *Pachystigma*, and 30 species from the subg. *Grammica* of the genus *Cuscuta*. Additionally, five outgroup species from the Convolvulaceae—*Ipomoea*, *Dinetus*, *Erycibe*, *Evolvulus*, and *Dichondra*—were included to enhance the resolution at the genus level of *Cuscuta*. We constructed a maximum likelihood (ML) phylogenetic tree using both IQ-TREE and ASTRAL-III, utilizing 18 protein-coding genes from the plastomes (Figure S1). Multiple sequence alignments (MSA) were

constructed using Geneious and Transalign, employing MAFFT with the L-INS-i algorithm [95] and the BLOSUM62 scoring matrix [96]. Maximum likelihood (ML) methods were employed to construct phylogenetic trees using IQ-TREE [97]. The GTR+I+G model was selected as the best-fit model for the analysis. Individual gene trees were combined using the cat tool, and the combined tree was further analyzed using ASTRAL-III to construct the tree [98]. The phylogenetic trees were visualized using FigTree (<http://tree.bio.ed.ac.uk/software/figtree/>), allowing for detailed annotation and interpretation of the results. Bootstrap values and posterior probabilities were used to assess the robustness of the tree nodes, ensuring the reliability of the phylogenetic inferences.

Selection analysis

We conducted a detailed analysis to identify plastid protein-coding genes exhibiting significant evidence of purifying or positive selection using adaptive branch-site tests (absREL) implemented in HYPHY v2.5.61 [99, 100]. This analysis utilized a combination of CDS-Transalign alignments, of both tree of ASTRAL-III, and IQ-TREE for individual genes selection analysis. To further investigate the intensity of selection, we employed random effects likelihood approximations (RELAX), also implemented in HYPHY v2.5.61 [101]. RELAX is a powerful tool in phylogenetics used to detect and quantify changes in selection intensity, particularly in *Cuscuta* and related outgroups within our datasets. This method tests for relaxation (negative selection) or intensification (positive selection) of selection along designated test branches compared to a reference set by fitting three dN/dS rate categories under the alternative model to both test and reference branch sets. The analysis then infers a "selection intensity parameter" (k), where $k < 1$ indicates a relaxation of selection and $k > 1$ indicates an intensification of selection relative to the reference set. This method is particularly useful for identifying shifts in selective pressures on specific lineages. Our reference set included lineages from *Dinetus* Buch.-Ham. ex D.Don, *Evolvulus* L., *Erycibe* Roxb., *Dichondra* J.R.Forst. & G.Forst., and *Ipomoea* L. We used a MSA in FASTA format for the RELAX selection analysis [99, 102]. The tree of IQ-TREE for this analysis was utilized, and the phylotree.js tool (<https://phylo tree.hyphy.org/>) was employed to select the test and reference datasets [103]. These selected datasets were then analyzed to determine significant selection pressures between *Cuscuta* (a holoparasite) and autotrophic plants within the Convolvulaceae family.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12864-025-11324-3>.

Supplementary Material 1: Figure S1. Comparison of phylogenetic relationship between ASTRAL-III and IQ-TREE using 18 protein coding sequences.

Supplementary Material 2: Figure S2. MAUVE-Genome alignment of four *Cuscuta* subgenera-*Monogynella*, *Cuscuta*, *Pachystigma* and *Grammica* for LCB in rearrangements.

Supplementary Material 3: Sheet S1. Genome -DNA sequencing, information of Total reads and coverage for this study. Sheet S2. Characterization of genome, gene and tRNA listed information of 40 genus *Cuscuta* species. Sheet S3. The plot of gene loss among 40 species of *Cuscuta* to the *I. batatas*. Sheet S4. 59 genes for Significant of RELAX or Intensification selection under bold in *Cuscuta*. K: Relax coefficient, LRT: Likelihood ratio Test, p : probability, ω : dN/dS ratio value, AIC-c: Akaike information criterion -corrected.

Acknowledgements

We extend our gratitude to the Animal and Plant Quarantine Agency, Ministry of Agriculture, Food, and Rural Affairs, for their valuable support and contributions to this project. Also, we would like to thank Kenneth M. Cameron at University of Wisconsin-Madison (United States of America) for collecting and providing the plant material for this study.

Authors' contributions

S-JC performed the experiments, analyzed the data, prepared figures and tables, and wrote the initial draft. KK, JJ, and HOK collected the plant materials, designed the species sampling, performed the experiments, and co-wrote the manuscript. JHK designed the experiments and revised the manuscript. All authors agree with the content of the manuscript. All authors have contributed to the manuscript and approved the submitted version.

Funding

This work was supported by a grant (Project No. PQ2022B007) from the Animal and Plant Quarantine Agency, Ministry of Agriculture, Food and Rural Affairs, Republic of Korea.

Data availability

Data is provided within the manuscript files. And it is available in NCBI database.

Declarations

Ethics approval and consent to participate

The study including plant samples complies with relevant institutional, national, and international guidelines and legislation. No specific permits were required for plant collection. The study did not require ethical approval or consent, as no endangered or protected plant species were involved.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Received: 23 July 2024 Accepted: 4 February 2025

Published online: 12 February 2025

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