BMC Plant Biology

RESEARCH Open Access

Plastid phylogenomics of *Robinsonia* (Senecioneae; Asteraceae), endemic to the Juan Fernández Islands: insights into structural organization and molecular evolution

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Abstract

Background The genus *Robinsonia* DC. (tribe Senecioneae, Asteraceae) endemic to the Juan Fernández Islands in Chile is one of the most conspicuous insular plant groups in the world. Unlike typical herbaceous Asteraceae plants, these plants demonstrate spectacular and unusual rosette tree growth forms as shown by the alpine giant senecios (genus *Dendrosenecio*, tribe Senecioneae) endemic to the East African mountains. However, monophyly of the genus and phylogenetic relationships among species of *Robinsonia* as well as their plastome evolution remain elusive. This study aims to explore their phylogeny, species diversification, and molecular evolution based on the complete plastome sequences in the context of adaptive radiation on oceanic islands.

Results The insular *Robinsonia* plastomes are highly conserved in their structures and organization of contents. Five divergence hotspots as potential chloroplast markers and five positively selected coding genes (*acc*D, *ndh*F, *rpo*A, *ycf*1, and *ycf*2) are identified. *Robinsonia* plastomes has an overall nucleotide diversity higher than that of the sky island *Dendrosenecio*, but much lower than herbaceous *Senecio*. Phylogenetic analysis demonstrates the monophyly of *Robinsonia* and identifies two major infrageneric lineages. Both *Robinsonia* and *Dendrosenecio* are deeply nested within large genus *Senecio*.

Conclusions While plastid genomes of *Robinsonia* are highly conserved, their sequences strongly demonstrated the monophyly of the genus and inferred robust interspecific relationships, including herbaceous *Senecio* and woody *Dendrosenecio*. Different sets of positively selected chloroplast genes, five for *Robinsonia* and two for *Dendrosenecio*, may play an important role in the adaptation strategies of these fascinating woody species in insular and continental sky island habitats. Overall phylogenetic positions and sister lineages of *Robinsonia* and *Dendrosenecio* require additional study based on broader sampling of *Senecio*.

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Keywords Adaptive radiation, Asteraceae, Critically endangered, Insular woodiness, Juan Fernández Islands, Plastome evolution

Introduction

Oceanic islands offer the opportunity to understand the evolutionary processes underlying rapid species diversification subsequent to the island colonization of immigrant populations by means of long-distance dispersal. The most commonly described evolutionary process in island biogeography is speciation associated with adaptive radiation called cladogenesis [1, 2]. Adaptive radiation connotes the entry of a single ancestor into a variety of habitats followed by species diversification and ecological shift. An initial immigrant population splits into morphologically and ecologically distinct evolutionary lineages through adaptation to divergent habitats available on an island (or an archipelago) free from competition and predation followed by inherent genetic variability release [1]. There have been numerous documented examples of island adaptive radiation, including the *Lobelia* complex (Campanulaceae) [3] and silversword alliance [4] in Hawaii; *Scalesia* (Asteraceae) in the Galápagos Islands [5–7]; *Echium* (Boraginaceae) [8, 9], *Aeonium* (Crassulaceae) [10, 11], and the woody *Sonchus* alliance (Asteraceae) [12–15] in the Canary Islands.

The Juan Fernández Islands, located 667 km off the west coast of Chile, are of volcanic origin and comprise two major islands and a much smaller island: Robinson Crusoe Island (also known as Isla Masatierra, 48 km^2); Alejandro Selkirk Island (Isla Masafuera, 50 km^2), and Santa Clara Island (2 km^2, close to Robinson Crusoe Island). Robinson Crusoe Island is estimated to be approximately four million years old and Alejandro Selkirk Island is estimated one million years old [16]. The older island, Robinson Crusoe, is closer to Chile and the younger Alejandro Selkirk is 181 km west of Robinson Crusoe. The archipelago harbors a small, but unique flora with high levels of endemic flowering plants, including one family (Lactoridaceae), 10 genera and 105 species (approximately 14% and 67% at the generic and specific levels, respectively) [17, 18]. The Juan Fernández Islands are well suited for addressing evolutionary questions due to having only two major islands, their small sizes, the small number of endemic species, and their proximity to the major source areas in continental South America [17]. The most spectacular radiations in the Juan Fernández Islands have been in the family Asteraceae, having evolved tree-like insular habits called rosette trees in three groups of genera from three different tribes: *Centaurodendron* Johow and *Yunquea* Skottsb. of Cardueae, *Dendroseris* D. Don of Lactuceae, and *Robinsonia* DC. of Senecioneae. Whereas the first two genera contained only three species, the other two genera comprised 11 species for *Dendroseris* and eight for *Robinsonia*. Genus *Robinsonia* includes eight unique rosette dioecious shrub (or tree) species with extensive morphological and ecological diversity [19, 20].

Robinsonia species are found mostly on the older and nearer Robinson Crusoe Island, with only *R. masafuerae* Skottsb. found on Alejandro Selkirk Island. Morphological [19] and genetic [21, 22] data indicate that *R. masafuerae* originated following dispersal from Robinson Crusoe of an *R. evenia*-like ancestor. *Robinsonia* exhibits a range of morphological variation accompanied by adaptation to diverse habitats (Fig. 1). Despite a suite of biological features shared by all species, such as rosette tree habit, dioecious breeding system and same chromosome numbers ($n=20$) [23–25], species vary considerably in habit (from a subshrub 1 ~ 2 m tall to a true tree, 5 ~ 6 m or more), and their habitats vary from epiphytism on tree ferns in very moist forests (*R. evenia*) to growth on montane scrubland and open cliff faces (*R. gayana*) [1, 19, 26].

As shown on *Robinsonia* species, one of the most prominent convergent aspects of island floras is the relatively high proportion of woody species in otherwise herbaceous lineages, a phenomenon known as insular woodiness [1, 27–29]. Insular woodiness, the evolutionary transition from herbaceous toward the woody condition on islands, are well exemplified by the Hawaiian silverswords (*Dubautia* and *Argyroxiphium*) and woody violets (*Viola*), as well as the Macaronesian tree lettuces (*Sonchus*) and viper buglosses (*Echium*) [4, 8, 9, 12–15, 30]. The spectra of growth forms have been considered adaptive modes, and woody insular representatives of predominantly herbaceous plants have likely evolved woodiness to develop tree-or shrub-like habits on islands. However, this tendency can also be observed in continental areas, most conspicuously in the equatorial highlands of Africa, Malaysia, and South America [1]. Insular woodiness and its evolutionary drivers remain poorly understood because the evidence is diverse for different island lineages, resulting in several hypotheses, such as aseasonal climate, competition for sunlight, drought resistance, and/or lack of large native herbivores [1, 31, 32]. The tribe Senecioneae (Asteraceae) encompasses several genera including the largest genus *Senecio* (ca. 1,250 species) [33, 34], and exhibits enormous variation in life-history strategies and morphology [35]. The tendency of Asteraceae to be represented on oceanic islands by woody species is well demonstrated by Senecioneae species with prominent examples in *Robinsonia* on the Juan Fernández Islands and *Dendrosenecio* on East African sky islands. The woody habit (accumulation of

Fig. 1 Representative species of *Robinsonia*. A, *R. berteroi*; B, C, *R. evenia*; D, *R. gracilis*; E, *R. masafuerae*; F, *R. thurifera*; G, *R. gayana*. In C-E, heads from female (left) and male (right) plants for comparisons. Photo credit: Tod F. Stuessy, The Ohio State University, USA

considerable secondary xylem) might be achieved in herbaceous Senecioneae when uniform conditions, typical of some oceanic islands, release plants from their seasonal cycles of growth [36].

Chloroplasts in plant cells play a crucial role in sustaining life on earth through the process of photosynthesis and oxygen release. They encode house-keeping and photosynthesis-associated proteins, serving as the active metabolic centers in cellular reactions to their environment [37]. Metabolites that are synthesized in chloroplasts are important for plant adaptation to environmental stress, such as drought [38], salinity [39], extreme temperature [40], high light [41] and heavy metal stress [42]. Plastid genomes are widely used to infer plant phylogeny and evolutionary history, as the reduced cost of next-generation sequencing and improved genomic analyses have facilitated the inclusion of massive amounts of data. These data revealed considerable genome-wide variation, which increased the phylogenetic resolution, especially at lower taxonomic levels, recent divergence, and rapid radiation. In contrast, conservative genome evolution yields limited sequence variation, which hinders phylogenetic resolution; therefore, plastome sequencing is now an efficient option for increasing phylogenetic resolution at lower taxonomic levels in plant phylogenetic and population genetic analyses [43].

In this study, we sequenced and assembled the whole plastid genomes of the genus *Robinsonia* to better understand their organization and evolution, as well as to reevaluate phylogenetic relationships inferred from previous studies. Specifically, we inferred the phylogenetic relationships within *Robinsonia* to hypothesize subgeneric, sectional, and species relationships, with inclusion of currently available complete plastome sequences of several *Senecio* and *Dendrosenecio* species. We also performed comparative plastome analyses to determine the structure, gene content, and rearrangements of plastid genomes of *Robinsonia*. Their genomic characteristics were subsequently compared with those of tropical

alpine herbaceous *Senecio* and woody *Dendrosenecio* species in East Africa to understand the genomic similarities and differences among herbaceous and woody Senecioneae plastomes evolved on oceanic islands and continental mountainous highlands (sky islands). Lastly, this study identified positively selected genes of plastid genomes that can give us some insights into adaptation strategies in insular and continental sky island habitats. In addition, several highly variable chloroplast regions, as useful markers for population genetic or phylogeographic studies of *Robinsonia* and *Senecio*, are identified.

Materials and methods

Plant materials and DNA extraction

Plant materials of *Robinsonia* species used in this study were previously collected in the field during four expeditions of the Universidad de Concepción, Chile and Ohio State University, USA to the Juan Fernández Islands. All but one species of *Robinsonia* were included, representing the two subgenera, *Rhetinodendron* and *Robinsonia*. The materials of *R. macrocephala* of subg. *Robinsonia* section *Symphyochaeta* were not available, as it is now thought to be extinct. As acknowledged in previous studies [44–47], the *Robinsonia* samples are representatives of the collections from the Robinson Crusoe National Park under the permission issued by CONAF (Corporación National Forestal). The fresh leaves were either dried (placed in sealable plastic bags with silica gel) or placed on ice and retained at 4 °C until extracted in the laboratory at The Ohio State University, Columbus, Ohio, USA. Total genomic DNAs were extracted using the DNeasy Plant Mini kit (Qiagen, Valencia, CA, United States) at Sungkyunkwan University laboratory following the manufacturer's instruction. The vouchers were deposited in the OS (Herbarium of Ohio State University) and WU (Herbarium of Universität Wien) (Table 1).

Plastome sequencing, assembly, and annotation

Illumina paired-end (PE) genomic libraries with a fragment size of 550 base pairs (bp) were prepared and sequenced using the Illumina HiSeq platform (Illumina, Inc., San Diego, Ca, USA) at Macrogen Corporation (Seoul, Korea). The sequence contigs were assembled using the de novo genomic assembler, Velvet 1.2.10 [48], and annotation was performed using GeSeq [49], ARAGORN v1.2.36 [50], and RNAmmer 1.2 Server [51]. Each draft annotation was then manually investigated and corrected whenever necessary using Geneious v8.1.6 (Biomatters Ltd., Auckland, New Zealand), performing a BLAST search by comparing with homologous genes in *Nicotiana tabacum* (NC001879) and *Senecio vulgaris* (NC046693) from the GenBank database at the National Center for Biotechnology Information (NCBI) as references. The complete plastome sequences of seven *Robinsonia* species were registered in GenBank under the accession numbers NC085195 (*R. berteroi*, Collection # 11238); NC085197 (*R. gayana*, Collection # 19251 J); NC085201 (*R. thurifera*, Collection # 11161); NC085198 (*R. gracilis*, Collection # 19138); NC085196 (*R. evenia*, Collection # 19283 F); NC085199 (*R. masafuerae*, Collection # 19637); and NC085200 (*R. saxatilis*, Collection # 11186). OGDRAW was used to draw circular plastid genome maps [52].

Comparative plastome analyses and identification of highly divergent regions

We performed several comparative plastome analyses of *Robinsonia* species on the Juan Fernández Islands in the Pacific Ocean, comparing with herbaceous *Senecio*

Table 1 Genomic features of the complete plastid genomes of seven *Robinsonia* species sequenced and analyzed in this study

Taxa	Collection no.	GenBank accession no.	Total plastid size (bp) / GC content (%)	LSC size (bp) / GC content (%)	IR size (bp) / GC content (%)	SSC size (bp) / GC content (%)	No. of genes	No. of protein-coding genes	No. of tRNA genes	No. of rRNA genes
Robinsonia* Subgenus *Rhetinodendron										
R. berteroi	11,238	NC085195	151,239/37.2	83,334 /35.3	24,824/42.9	18,257/30.4	130	87	37	6
Robinsonia* Subgenu s*Robinsonia										
Section *Symphyochaeta*										
R. macrocephala		not available due to extinction								
Section *Robinsonia*										
R. gayana	19,251 J	NC085197	151,330/37.2	83,429/35.3	24,817/42.9	18,267/30.5	130	87	37	6
R. saxatilis	11,186	NC085200	151,247/37.6	83,352/35.3	24,815/42.9	18,265/30.5	130	87	37	6
R. thurifera	11,161	NC085201	151,330/37.2	83,429/35.3	24,817/42.9	18,267/30.5	130	87	37	6
Section *Eleutherolepis*										
R. evenia	19,283 F	NC085196	151,246/37.2	83,352/35.3	24,816/42.9	18,262/30.4	130	87	37	6
R. gracilis	19,138	NC085198	151,278/37.2	83,377/35.3	24,816/42.9	18,269/30.4	130	87	37	6
R. masafuerae	19,637	NC085199	151,328/37.2	83,428/35.3	24,816/42.9	18,268/30.4	130	87	37	6

LSC: large single copy region; SSC: small single copy region; IR: inverted repeat

and woody *Dendrosenecio* plastomes from highland East Africa. This comparison aimed to investigate the similarities and differences among herbaceous and woody plastomes that convergently evolved as woody growth forms on both of oceanic islands and continental sky islands. The sequences of six *Dendrosenecio* and five *Senecio* plastomes reported by Gichira et al. [53] were obtained from GenBank for genomic comparison; i.e., *D. battiscombei*, *D. brassiciformis*, *D. elgonensis*, *D. johnstonii*, *D. keniodendron*, and *D. meruensis* for woody *Dendrosenecio* and *S. moorei*, *S. keniophytum*, *S. purtschelleri*, *S. schweinfurthii*, and *S. roseiflorus* for herbaceous *Senecio* plastomes (see Table 1 in Gichira et al. for their genomic features) [53]. The level of codon usage bias was determined by the relative synonymous codon usage (RSCU; the relative frequency of occurrence of the synonymous codon for a specific amino acid) value calculated from the codon usage frequency using MEGA7 [54]. To evaluate the pressure of natural selection on the protein-coding genes of the seven plastomes, site-specific models implemented in EasyCodeML [55] were used in the preset running mode based on the CodeML algorithms. Selective pressure has been inferred by the ratio of nonsynonymous and synonymous substitution rates (denoted as $\omega = dN/dS$), with $\omega = 1$ indicating neutral mutations; $\omega < 1$, purifying selection; and $\omega > 1$, diversifying positive selection. Positively selected sites were presented based on the fit of seven codon substitution models (M0, M1a, M2a, M3, M7, M8, and M8a) with heterogeneous ω values across sites implemented in EasyCodeML using likelihood ratio tests (LRT) [56, 57]. Overall sequence divergence was estimated using the mVISTA online program [58] (https://genome.lbl.gov/vista/mvista/submit.shtml) employing the LAGAN alignment mode [59]. Nucleotide diversity (Pi) was calculated using sliding window analysis (window length = 1000 bp and step size = 200 bp excluding sites with alignment gaps) to detect the most divergent regions (i.e., mutation hotspots) in DnaSP [60].

Phylogenetic analysis

Phylogenetic relationships of the newly sequenced accessions of *Robinsonia* were investigated in the context of their relationships with the complete plastid sequences of other closely related species obtained from GenBank, including *Senecio* (nine species), *Dendrosenecio* (12 species), and two other related genera in subtribe Senecioninae (*Jacobaea* and *Pericallis*). Two species of *Ligularia* from subtribe Tussilagininae were used as outgroups. In total, 32 plastid genomes were used to generate maximum likelihood (ML) trees based on both the complete plastid genome sequences and concatenated sequences of protein coding genes with 1000 replicate bootstrap (BS) analyses using IQ-TREE [61] after alignment using MAFFT v.7 [62]. The complete plastid genome sequences were partitioned for genic (protein, tRNAs, and rRNAs coding genes) and non-coding intergenic regions. The best fit evolutionary models were chosen as TVM+F+I for the concatenated sequences of 80 protein coding genes, HKY+F+I for tRNA, HKY+F for rRNAs, and K3Pu+F+I+G4 for noncoding intergenic regions, which were scored according to Bayesian information criterion scores and weights by testing 88 DNA models using ModelFinder [63] implemented in IQ-TREE.

Results

Gene content, order, and organization of the plastomes of *Robinsonia*

The seven plastomes of *Robinsonia* species (*R. berteroi*, *R. evenia*, *R. gayana*, *R. gracilis*, *R. masafuerae*, *R. saxatilis* and *R. thurifera*) were highly conserved in gene content and arrangement, displaying 99.7% pairwise sequence similarity despite the morphological and ecological differences among them. The total lengths of the seven *Robinsonia* plastomes ranged from 151,239 (*R. berteroi*, subg. *Rhetinodendron*) to 151,330 bp (*R. gayana* and *R. thurifera*, subg. *Robinsonia*), which were slightly longer than *Dendrosenecio* (average 150 bp shorter) species [53]. Each of the seven *Robinsonia* plastomes consisted of four typical plastid regions: LSC (83,334−83,429 bp), SSC (18,257−18,269 bp), and IR regions (24815−24,824 bp), sharing the same genes and similar gene contents at all adjacent junctions among the four regions (Figs. 2 and 3). The overall guanine-cytosine (GC) content of each plastid genome was 37.2%, with LSC, SSC, and IRs regions having 35.3%, 30.4−30.5%, and 42.9% GC contents, respectively. Each of the seven cp. genomes contained 130 genes, including 87 protein-coding genes (excluding pseudogenes), six rRNA genes, and 37 tRNA genes (Tables 1 and 2). Eighteen genes, including seven tRNA genes contained introns. Three genes, *clp*P, *rps*12, and *ycf*3, had two introns. The *trn*K-UUU tRNA gene harbored the largest intron, containing the *mat*K gene. In total, 17 genes were duplicated in the IR regions, including seven tRNAs, three rRNAs, and seven protein genes. The trans-splicing gene *rps*12, consisting of three exons, was located in the LSC region of exon 1, whereas exons 2 and 3 were imbedded in the IR regions. Incompletely duplicated parts of *ycf*1 and *rps*19 in the IR regions were considered pseudogenes in all the cp genomes sequenced in this study.

Comparative plastome analyses: codon usage , positive selection, sequence divergence and mutation hot spot

The total size of all protein coding genes (single copies, excluding repeated genes) in the seven *Robinsonia* plastomes was 68,214−68,232 bp, encoding 22,738−22,744 codons including stop codons. Their

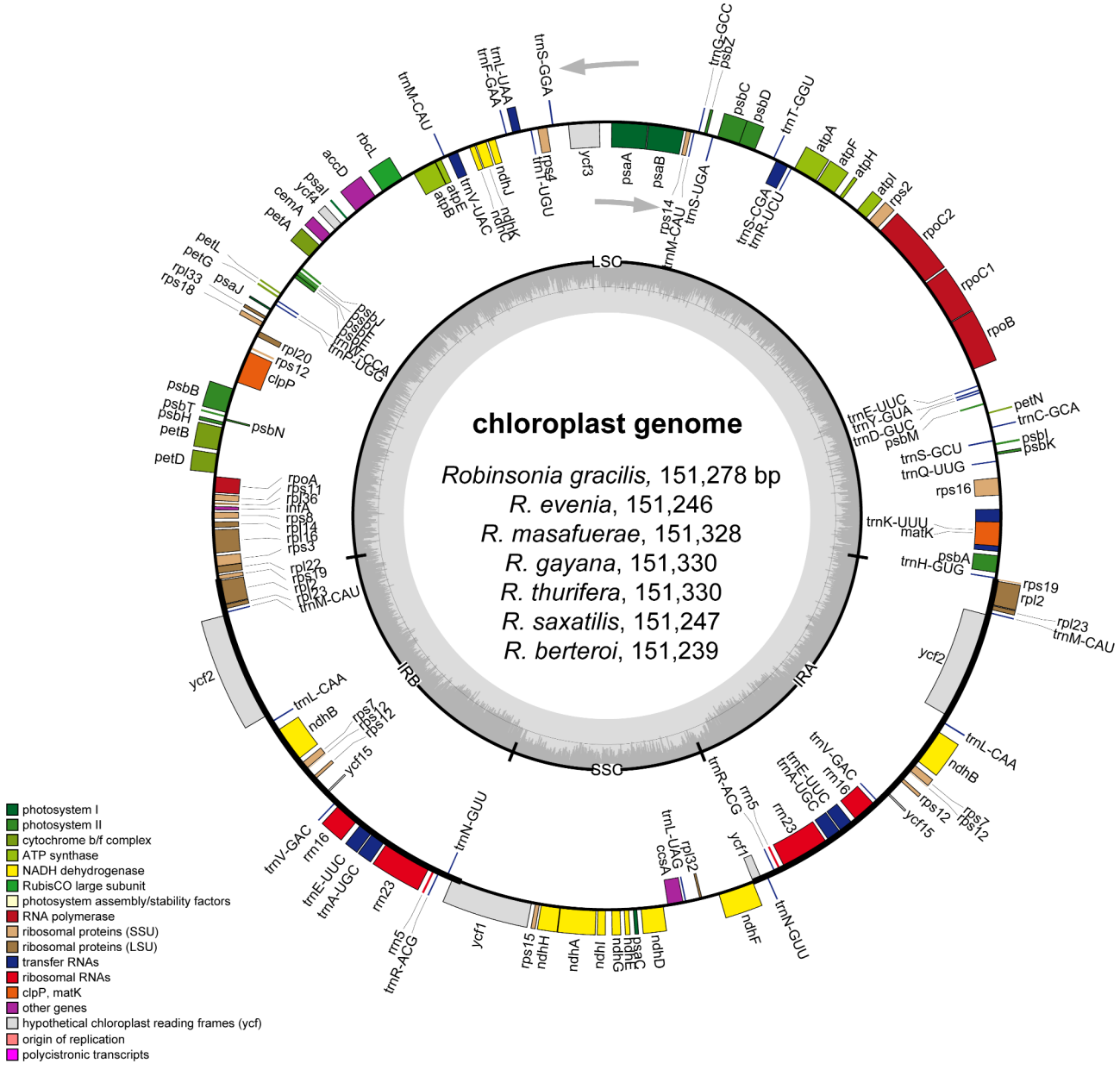

Fig. 2 Gene maps of the plastid genomes of seven *Robinsonia* species sequenced and analyzed in this study. The genes inside and outside of the circle are transcribed in the clockwise and counterclockwise directions, respectively. Genes belonging to different functional groups are shown in different colors. The thick lines indicate the extent of the inverted repeats (IRs) that separate the genomes into small single copy (SSC) and large single copy (LSC) regions

average number of codon usage was smaller than that of *Dendrosenecio* (22,815−22840 for single copies of coding genes; 26,293−26,382 for all coding genes), but slightly larger than *Senecio* (22,640−22,689; 26,067−26,177) in Gichira et al.'s [53]. The patterns of frequently used codons and their RSCU values were consistent among the seven *Robinsonia* plastomes (Fig. 4). The highest RSCU value was indicated in the usage of UUA codon for leucine (1.94) followed by AGA for arginine (1.85−1.86), while the lowest ones, CUC for leucine (0.34−0.35) and AGC (0.34−0.35) for Serine followed by CUG for leucine (0.36). The codons AUG (M) and UGG (W), which encode methionine and tryptophan, respectively, showed no bias (RSCU=1) (Supplementary table S1).

From seven *Robinsonia* plastomes, we identified five coding genes potentially evolved under positive selection, *acc*D, *ndh*F, *rpo*A, *ycf*1, and *ycf*2 using the pairwise comparison of codon substitution models, M7 (beta) vs. M8 (beta and ω>1). They were presented with a significant posterior probability (p) more than 0.95 indicated with an asterisk, * ($P \geq 0.95$) or ** ($P \geq 0.99$), calculated using the Bayes empirical Bayes (BEB) test [57]. On the other

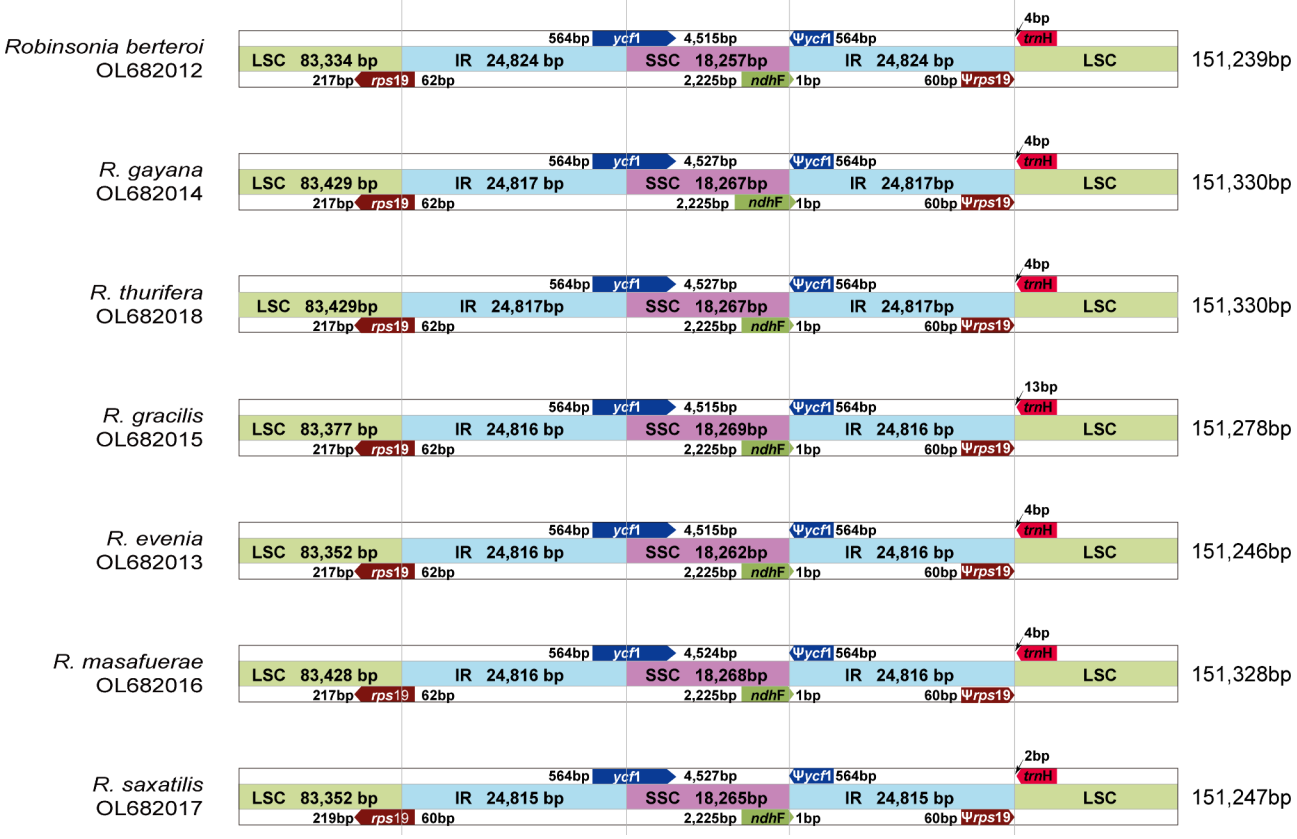

Fig. 3 Comparison of the border positions of the large single copy (LSC), small single copy (SSC), and inverted repeat (IR) regions among seven *Robinsonia* plastid genomes. Gene names are indicated in boxes, and their lengths in the corresponding regions are displayed above the boxes. Ψ indicates a pseudogene

hand, two coding genes, *atp*A and *rpo*C2 were found in the six *Dendrosenecio* plastomes undergoing positive selection on continental sky islands, whereas none were found in the five sympatric *Senecio* plastomes occurring in Mt. Kenya (Table 3).

The divergence of the seven *Robinsonia* plastomes was assessed using the mVISTA platform [58], with the annotated sequences of *Robinsonia berteroi* as a reference. The mVISTA graph exhibited a high degree of synteny and gene order conservation among the plastomes of *Robinsonia*, sharing almost identical coding regions (except *ycf*1) and more variable noncoding and intron regions (Fig. 5). However, the results of mVISTA analysis including six *Dendrosenecio* and five *Senecio* together with *Robinsonia* plastomes indicated different patterns of polymorphic sites among the three groups, mostly from noncoding and intron regions, even though all of them revealed similar synteny in the coding regions (except *pet*B and *ycf*1) (Supplementary fig. S1). The overall nucleotide diversity value (Pi) of the seven *Robinsonia* plastomes (average 0.00092, ranging from 0 to 0.00505) calculated using DnaSP [60] was higher than that of the six *Dendrosenecio* plastomes (average 0.00058, ranging from 0 to 0.0112). However, their diversity was much lower than five *Senecio* plastomes (average 0.00357, ranging from 0 to 0.0142). Among the seven *Robinsonia* plastomes, the SSC region containing *ycf*1, which is known to have high diversity in plants [47, 64–66], showed the highest nucleotide diversity (0.001668), whereas the lowest value was observed in the IR boundary regions (0.00032). Five divergence hotspots among the *Robinsonia* plastomes have been suggested as potential plastid markers for the phylogenetic studies of *Robinsonia* species and closely related *Senecio* groups. Four intergenic hotspot regions (*rps*16-*trn*Q, *trn*C-*pet*N, *atp*A-*trn*R, and *rps*18-*rpl*20), and one protein coding region (*ycf*1) were identified in LSC and SSC regions (Fig. 6).

Phylogenetic analysis

ML analyses were conducted based on sequences of complete plastomes (132,037 aligned nucleotide bp) and protein coding genes (68,573 bp) of the 32 representative Asteraceae plastomes, including *Senecio*, *Dendrosenecio*, and *Robinsonia* species. Both ML phylogenetic trees provided good resolution of inter-generic and inter-specific relationships, sharing congruent topology with slight variances in BS values (Fig. 7and Supplementary fig. S2). *Robinsonia* and *Dendrosenecio* were monophyletic with

Table 2 Genes present in the complete plastid genomes of seven *Robinsonia* species sequenced in this study

Category	Group	Genes
Photosynthesis	photosystem_I	*psa*A, *psa*B, *psa*C, *psa*I, and *psa*J
	photosystem_II	*psb*A, *psb*B, *psb*C, *psb*D, *psb*E, *psb*F, *psb*H, *psb*I, *psb*J, *psb*K, *psb*L, *psb*M, *psb*N, *psb*T, and *psb*Z
	NADH_dehydrogenase	*ndh*A*, *ndh*B(× 2)*, *ndh*C, *ndh*D, *ndh*E, *ndh*F, *ndh*G, *ndh*H, *ndh*I, *ndh*J, and *ndh*K
	cytochrome_b/f_complex	*pet*A, *pet*B*, *pet*D*, *pet*G, *pet*L, and *pet*N
	ATP_synthase	*atp*A, *atp*B, *atp*E, *atp*F*, *atp*H, and *atp*I
	Large_subunit_of_Rubisco	*rbc*L
Self-replication	Large_subunits_of_ribosome	*rpl*2(× 2)*, *rpl*14, *rpl*16*, *rpl*20, *rpl*22, *rpl*23(× 2), *rpl*32, *rpl*33, and *rpl*36
	Small_subunits_of_ribosome	*rps*2, *rps*3, *rps*4, *rps*7(× 2), *rps*8, *rps*11, *rps*12 (× 2)**, *rps*14, *rps*15, *rps*16*, *rps*18, and *rps*19
	DNA-dependent_RNA_polymerase	*rpo*A, *rpo*B, *rpo*C1*, and *rpo*C2
	translation initiation factor	*inf*A
	Ribosomal_RNAs	*rrn*5(× 2), *rrn*16(× 2), and *rrn*23(× 2)
	Transfer_RNAs	*trn*A-UGC(× 2)*, *trn*C-GCA, *trn*D-GUC, *trn*E-UUC(× 3)*, *trn*F-GAA, *trn*G-GCC, *trn*H-GUG, *trn*K-UUU*, *trn*L-CAA(× 2)*, *trn*L-UAA, *trn*L-UAG, *trn*M-CAU(× 4), *trn*N-GUU(× 2), *trn*P-UGG, *trn*Q-UUG, *trn*R-ACG(× 2), *trn*R-UCU, *trn*S-CGA, *trn*S-GCU*, *trn*S-GGA, *trn*S-UGA, *trn*S-GGU, *trn*T-UGU, *trn*V-GAC(× 2), *trn*V-GCA, *trn*W-CCA, and *trn*Y-GUA
Other genes	Maturase	*mat*K
	Protease	*clp*P**
	Envelope_membrane_protein	*cem*A
	Acetyl-CoA_carboxylase	*acc*D
	C-type_cytochrome_synthesis_gene	*ccs*A
Genes of unknown function	Proteins_of_unknown_function	*ycf*1, *ycf*2(× 2), *ycf*3**, *ycf*4, *ycf*15(× 2)

(× N) indicates the genes that have N copies. * and ** indicate genes containing one and two introns, respectively.

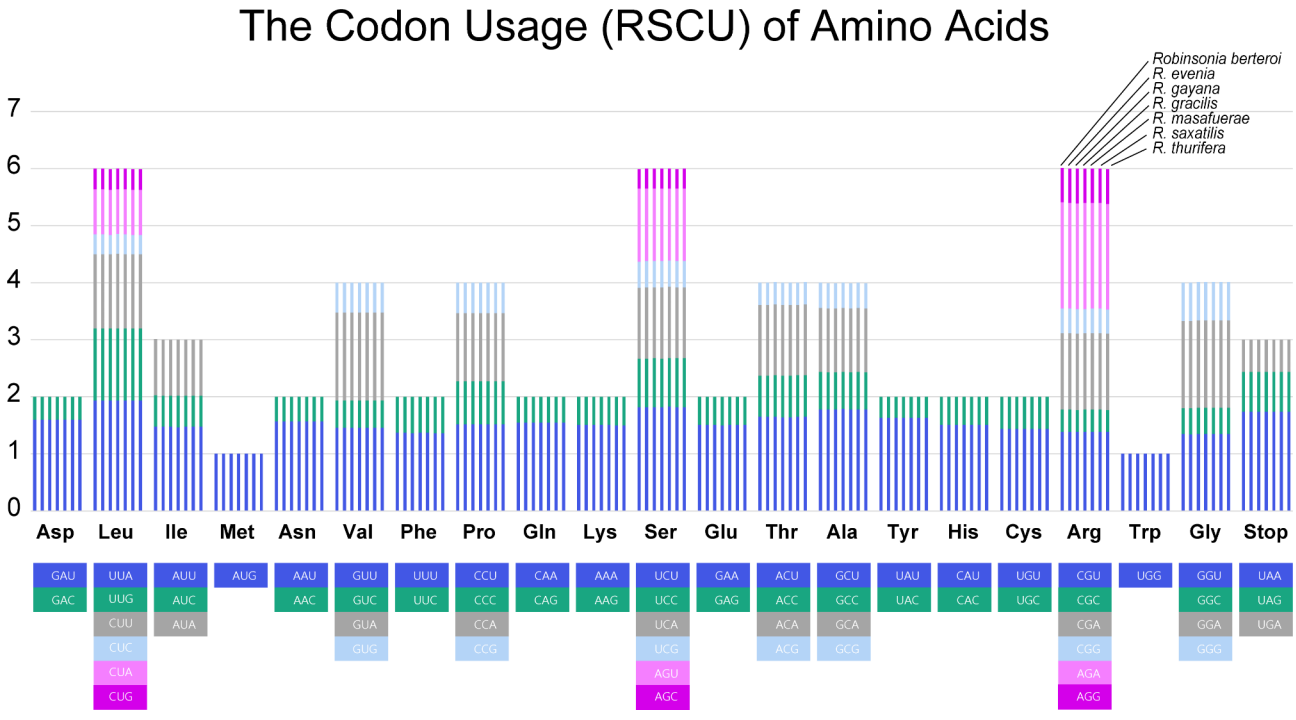

Fig. 4 The relative synonymous codon usage (RSCU) of the protein-coding genes in chloroplast genomes of seven *Robinsonia* species. The codon usages of amino acids are plotted along the x-axis, while the stacked RSCU values in each bar column are plotted along the y-axis respectively. Each amino acid contains seven clustered bar columns representing seven species; 1st column through 7th column for *R. berteroi*, *R. evenia*, *R. gayana*, *R. gracilis*, *R. masafuerae*, *R. saxatilis*, and *R. thurifera*

Table 3 Positively selected sites having *dN/dS* values > 1 detected in seven *Robinsonia* and six *Dendrosenecio* plastomes

Gene name	Models	np	ln L	Model compared	Likelihood ratio test *p*-value	Positively selected sites
***Robinsonia* plastomes (*R. berteroi*, *R. evenia*, *R. gayana*, *R. gracilis*, *R. masafuerae*, *R. saxatilis*, and *R. thurifera*)**						
accD	M8	16	-1964.763594	M7 vs. M8	0.667566011	39 S 0.962*, 116 I 0.962*
	M7	14	-1965.167711			
ndhF	M8	16	-2893.891812	M7 vs. M8	0.001549017	491 F 0.970*
	M7	14	-2900.361947			
rpoA	M8	16	-1327.780011	M7 vs. M8	0.628296557	95 V 0.962*, 280 R 0.962*
	M7	14	-1328.244754			
ycf1	M8	16	-6754.608911	M7 vs. M8	0.010754360	432 N 0.991**
	M7	14	-6759.141355			
ycf2	M8	16	-8899.330842	M7 vs. M8	0.517702255	794 D 0.976*, 831 G 0.976*, 1234 E 0.976*
	M7	14	-8899.989197			
***Dendrosenecio* plastomes (*D. battiscombei*, *D. brassiciformis*, *D. elgonensis*, *D. johnstonii*, *D. keniodendron*, and *D. meruensis*)**						
atpA	M8	14	-2016.260099	M7 vs. M8	0.557743556	123 I 0.977*, 477 S 0.977*, 508 G 0.977*
	M7	12	-2016.843955			
rpoC2	M8	14	-5520.953083	M7 vs. M8	0.447862506	278 R 0.984*, 717 I 0.984*, 1039 L 0.984*, 1040 N 0.984*
	M7	12	-5521.756352			

Significant posterior probability value for positively selected sites are indicated with * for >0.95 and ** >0.99

np is number of parameters, lnL, the log-likelihood values, and LRT p-value, Likelihood Ratio Test p-value

robust support (100% BS, respectively), whereas *Senecio* was not monophyletic. Within the ingroup species of the subtribe Senecioninae against the outgroup species of the subtribe Tussilagininae (*Ligularia intermedia* and *L. fischeri*), two major clades were revealed, with *Robinsonia* and *Dendrosenecio* embedded in different clades, suggesting that *Robinsonia* is not closely related to *Dendrosenecio*. Interestingly, *Robinsonia* clustered with *Senecio* species collected from East Africa, and the monophyletic clade of *Dendrosenecio* was more closely related to other *Senecio* species occurring primarily in Asia than to the sympatric African *Senecio* species. These relationships, however, hinge on a broader sampling of Senecio species in a future study. Within the monophyletic clade of all extant *Robinsonia* species, the plastome sequences did not support the current subgeneric classification of *Robinsonia* into two subgenera: *Rhetinodendron* and *Robinsonia*. *Robinsonia berteroi* of monotypic subg. *Rhetinodendron* was in a sister relationship with section *Robinsonia* of subg. *Robinsonia* (98% of BS) rather than a sister to whole subg. *Robinsonia*; two subgenera are not reciprocally monophyletic. Within subg. *Robinsonia*, two sections of *Robinsonia* (*R. gayana*, *R. thurifera*, and *R. saxatilis*) and *Eleutherolepis* (*R. gracilis*, *R. masafuerae* and *R. evenia)* were resolved into distinct clades. *R. saxatilis* initially described as being the closest to *R. evenia* and treated under the section *Eleutherolepis* [20] was nested in the section *Robinsonia* instead of *Eleutherolepis* (100% BS) on both the full sequences and coding genes of plastomes (Fig. 7and Supplementary fig. S2).

Discussion

Phylogenetic relationships of *Robinsonia*

Robinsonia species have been variously recognized at the genus level: *R. berteroi* (DC.) R.W. Sanders, Stuessy & Martic was initially recognized under distinct genera, that is, initially described as *Balbisia berteroi* DC [67–69]. and then as *Rhetinodendron berteroi* (Dcne.) Hemsl [70–72]. Skottsberg (1921) also recognized the monotypic genus *Rhetinodendron* Meisn. with *Rhetinodendron berteroi* and the genus *Robinsonia* DC. with six species [26]. He divided *Robinsonia* into subgenera and sections as follows: subg. *Symphyochaeta* (DC.) Skottsb. (*R. macrocephala* Dcne.) and subg. *Eleutherochaeta* (DC.) Skottsb. with two sections, i.e., section *Symphyolepis* Skottsb. (*Robinsonia gayana* Dcne. and *R. thurifera* Dcne.) and section *Eleutherolepis* DC. (*R. evenia* Phil., *R. masafuerae* Skottsb. and *R. gracilis* Dcne.). Later, Sanders et al. (1987) provided an explicit phylogenetic hypothesis using primarily morphological characteristics and suggested that all species of *Robinsonia* species should be recognized as a monophyletic genus resulting from a single introduction [19]. The genus *Rhetinodendron* was merged with *Robinsonia* as the monotypic subg.

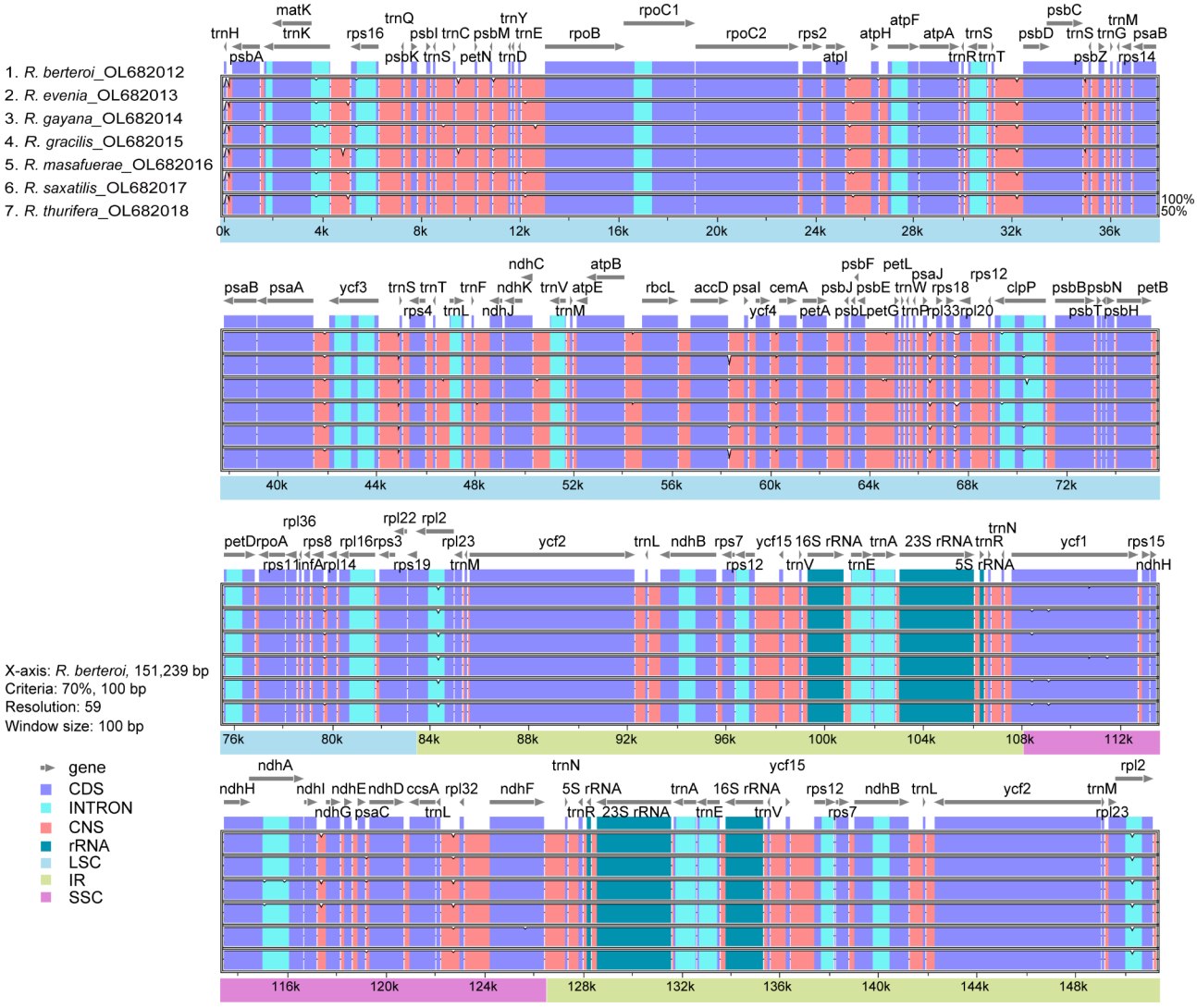

Fig. 5 Comparison of the plastid genomes of seven *Robinsonia* species, against *R. berteroi* by mVISTA. Grey arrows indicate genes with their orientation and position. Genome regions are color-coded as blue blocks for the conserved coding genes (exon), aqua blue blocks for introns, and orange blocks for the conserved non-coding sequences in intergenic regions (CNS). Thick lines below the alignment indicate the quadripartite regions of genomes; LSC region is in light blue, IR regions, in beige, and SSC region, in pink

Rhetinodendron (along with *R. berteroi*) based on its strong similarity to *Robinsonia* species in terms of flavonoid chemistry [73] and morphology [19]. The remaining six species were placed under subg. *Robinsonia*, which is divided into sections *Symphyochaeta* (*R. macrocephala*), *Robinsonia* (*R. gayana* and *R. thurifera*) and *Eleutherolepis* (*R. evenia*, *R. gracilis*, and *R. masafuerae*). Danton (2006) described *Robinsonia saxatilis* Danton from Robinson Crusoe Island and referred it to section *Eleutherolepis* DC. of subg. *Robinsonia* [20]. Unfortunately, several species of *Robinsonia* are highly threatened by extinction because of their restricted ranges and small population sizes. *Robinsonia macrocephala* was once known from several locations in Masatierra [26], but has not been found elsewhere despite repeated searches on several expeditions and is now thought to be extinct [74]. *Robinsonia berteroi* was once reported from eight localities [26], but is now known from only one mature individual found in 2015 and other individuals (between one and 15) in inaccessible places where prospecting efforts should be made. Thus, *R. berteroi* is categorized as Critically Endangered (CR, DS 06/2017 MMA) instead of Extinct (EX) on the IUCN Red List of Threatened Species [75].

Molecular phylogenetic analyses of *Robinsonia* generally supported the relationships based on the morphological characters suggested by Sanders et al. (1987) [19]. Although the phylogenetic study using restriction site mutations in cpDNA and IGS of nuclear rDNA failed to resolve the relationships among *Robinsonia* species

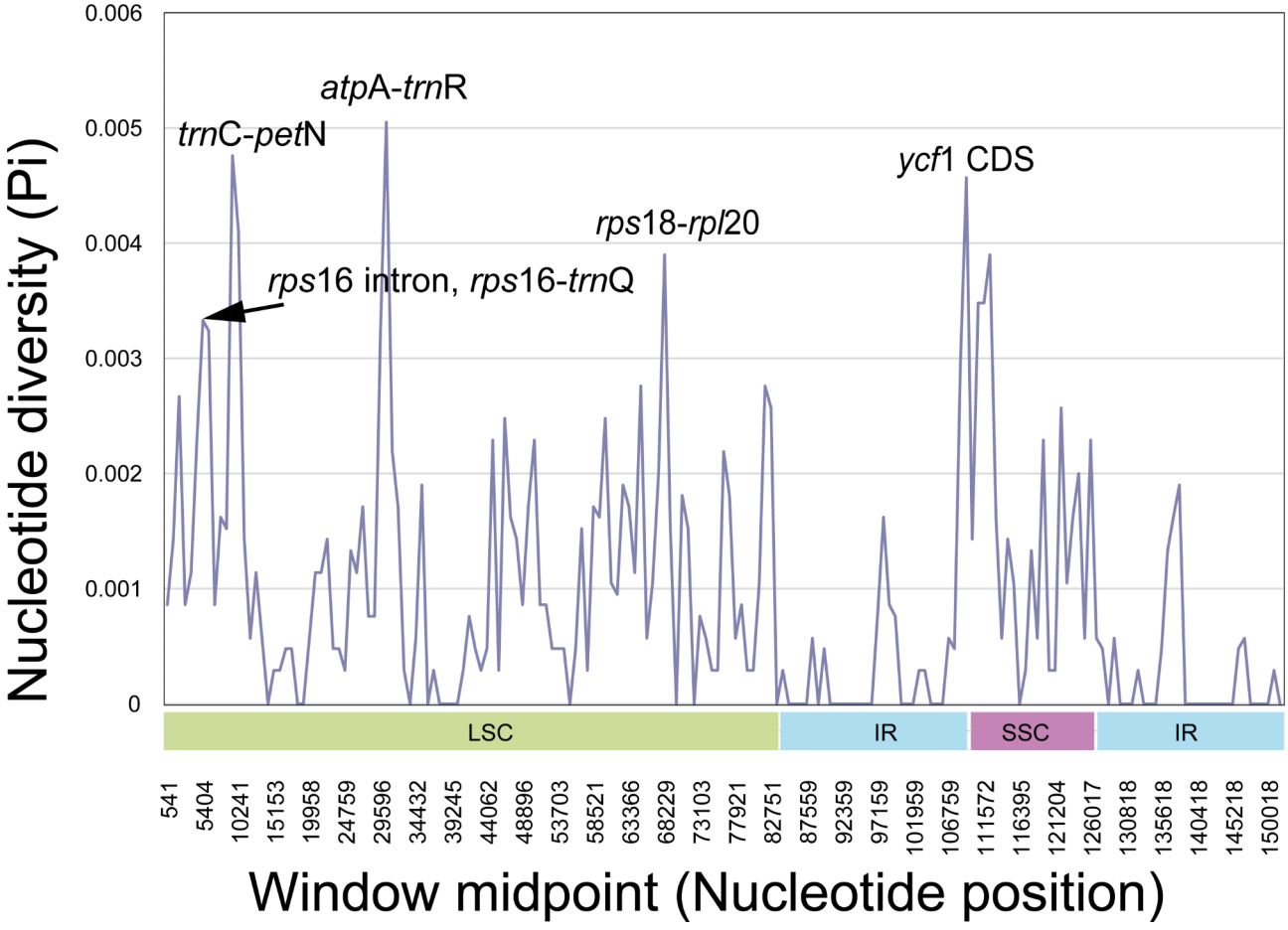

Fig. 6 Five hotspot regions in the seven *Robisonia* plastomes

[76], internal transcribed spacer (ITS) phylogeny supported the monophyly of *Robinsonia* species (including *R. berteroi*) [22], implying a single introduction of the ancestor of *Robinsonia* to the Islands. However, later studies [35, 77] which included more extensive sampling of *Senecio* species, reported that *R. berteroi* appeared to be more closely related to *Senecio* species than to the other *Robinsonia* species on ITS or ITS-the external transcribed spacer (ETS) combined phylogenies. However, in Pelser et al.'s [77], the combined plastid data (*ndh*F gene; *trn*L intron; *psb*A-*trn*H, *psb*J-*pet*A, 5' and 3' *trn*K, and *trn*L-F intergenic spacers) supported the monophyly of *Robinsonia* species (including *R. berteroi*), but the ITS-ETS combined data set failed to resolve *Robinsonia* as monophyletic, placing *R. berteroi* outside of the *Robinsonia* clade. Pelser et al. concluded that "Thus, the monophyly of *Robinsonia* remains inconclusive despite additional data and analyses." However, Pelser et al. [77] proposed merging *Robinsonia* into one of the largest genera of angiosperms *Senecio* because all species of the former were nested within the latter.

Although the results of the present study are highly concordant with those of previous molecular studies, the whole plastome sequences provided additional insights into questions that have remained unanswered despite several earlier studies. The sister lineages of *Robinsonia* and *Dendrosenecio* have yet to be determined due to the limited taxonomic sampling of the plastomes of the broadly occurring *Senecio*, however, the plastome phylogeny provided strong support for the monophyly of *Robinsonia*, with *R. berteroi* resolved within the same clade as other species (Fig. 7and Supplementary fig. S2). In addition, *R. berteroi* was resolved as a sister to a clade consisting of *R. thurifera*, *R. gayana* and *R. saxatilis*, rather than a sister to all other *Robinsonia* species, where it had commonly been placed in other molecular phylogenies, supporting its recognition as subg. *Rhetinodendron* [22, 77]. To the best of our knowledge, this is the first time that this relationship has been suggested for *R. berteroi.* Regarding the taxonomic treatment of *R. berteroi*, we continue to support its recognition as a separate subgenus (subg. *Rhetinodendron*), particularly because of its smaller flowering heads and narrowly elongated, thyrsoid capitulescences (Fig. 1A). *Robinsonia saxatilis* was initially viewed as being closely related to *R. evenia* of sect. *Eleutherolepis* [20]. However, Takayama et al. [21]

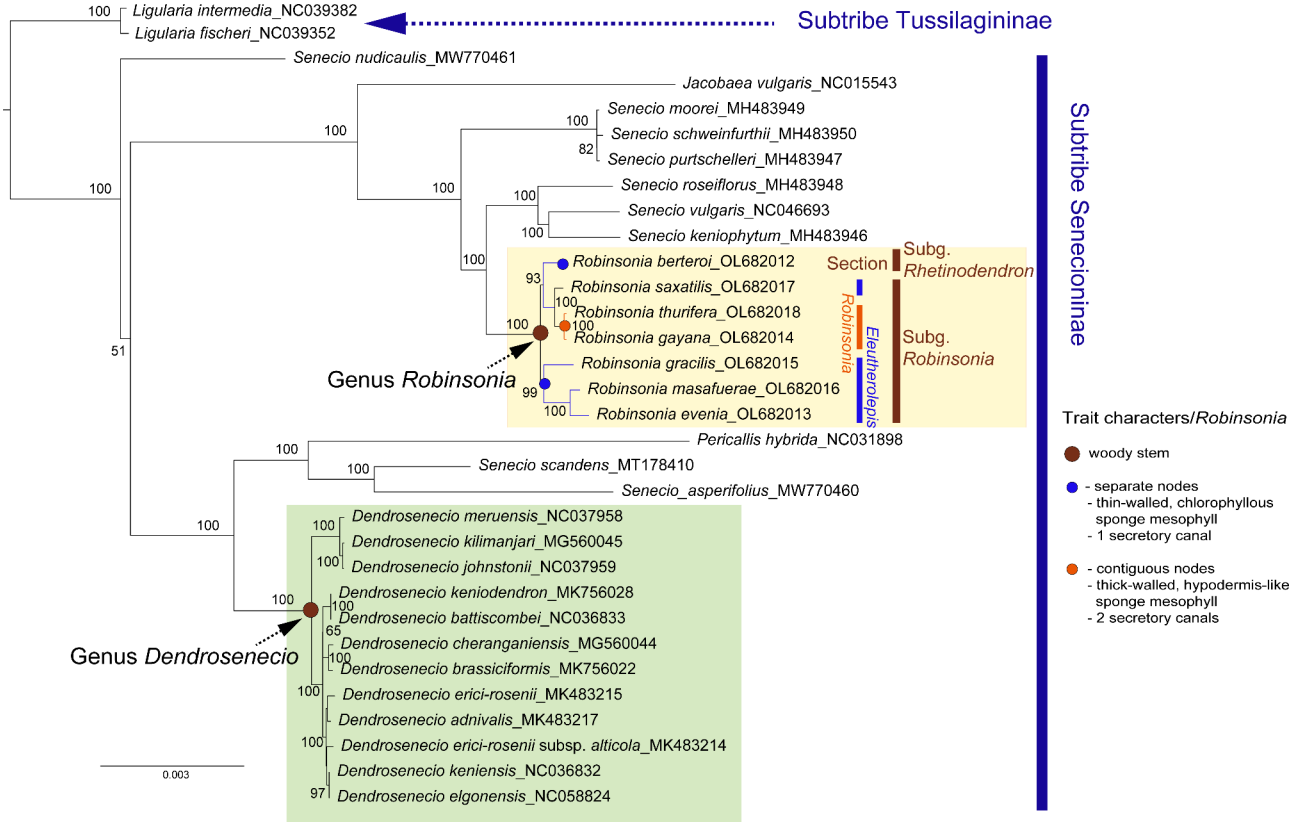

Fig. 7 Maximum likelihood tree based on the complete plastome sequences of 32 representatives of Asteraceae including species of *Robinsonia*, *Dendrosenecio* and *Senecio*. Numbers above nodes are bootstrap values with 1000 replicates. Trait characters are marked with brown circle representing woody stem; blue, separate nodes spacing, typically thin-walled, chlorophyllous leaf sponge mesophyll, and one secretory canal per vascular bundle; and orange, nearly contiguous nodes, thick-walled, strongly hypodermis-like mesophyll, and two secretory canals. Trait data taken from Skottsberg (1921) and Carlquist (1974) [1, 26], but not available for *R. saxatilis*

reported that this new species was more properly placed in section *Robinsonia* near *R. gayana* or possibly *R. thurifera* based on amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) analyses. The complete plastome phylogeny in the current study also suggests that *R. saxatilis* shares its most recent common ancestor with a common ancestor of *R. gayana-R. thurifera*, further corroborating earlier AFLP and SSR results.

Plastome structure and evolution

The whole cp. genomes of the seven *Robinsonia* species reported for the first time in this study, were highly conserved, sharing common genomic features such as sequence similarity, gene content, and gene number (Table 1). We compared their genomic features with the plastomes of *Dendrosenecio* and *Senecio* species previously reported by Gichira et al. [53] and found that the plastomes of the three genera shared similar genomic features despite their morphological and distributional divergences (i.e., the woody insular endemics of *Robinsonia* in the Juan Fernández Islands, the woody highland endemics of *Dendrosenecio* in East Africa, and the herbaceous endemics of *Senecio* in East Africa). Minor differences were found in their total lengths; with *Dendrosenecio* (150 kb) being slightly shorter than *Robinsonia* and *Senecio* (151 kb) with differences mainly in SSC sizes. Generally, the lengths of the chloroplast genome and its quadripartite regions vary among plant lineages because of the contraction and expansion of IR regions. Evaluating their contraction and expansion by comparing the location of the boundaries among the four chloroplast regions (two IRs, LSC and SSC) can provide insights into plastome evolution [78]. All contained the functional protein-coding gene of *ycf*1 at IR/SSC with its pseudogene copy, *ycf*1$^{\Psi}$ at SSC/IR, and functional *rps*19 at LSC/IR with pseudogene copy *rps*19$^{\Psi}$ at IR/LSC endpoints (Fig. 3).

Codon usage bias and positive selection were compared to investigate selective pressure on protein-coding genes in the plastomes of *Robinsonia*, *Dendrosenecio*, and *Senecio*. The patterns of frequently used codons and the average number of codon usage were very similar, with the highest RSCU value indicated in the usage of UUA(L) and AGA(R), whereas the lowest were AGC(S), CUC(L), UAC(Y), and CUG(L). Additionally, codons AUG (M) and UGG (W) showed no bias (RSCU=1) in any of the

plastomes of the three genera (Supplementary table S1). However, several genes that potentially evolved under positive selection were identified in the woody plastomes of *Robinsonia* and *Dendrosenecio* (Table 3), whereas no positive selection sites were detected in the herbaceous *Senecio* plastomes. Seven *Robinsonia* plastomes contained five coding genes, *acc*D, *ndh*F, *rpo*A, *ycf*1, and *ycf*2, under positive selection, however, only two genes, *atp*A and *rpoC*2, were detected in the six *Dendrosenecio* plastomes. Most of these genes encode proteins related to photosynthesis and self-replication of plastids, which are extremely important for plant survival, including the NADH dehydrogenase gene (*ndh*F), DNA-dependent RNA polymerase genes (*rpo*A and *rpo*C2), ATP subunit gene (*atp*A), Acetyl-CoA carboxylase gene (*acc*D), and genes of unknown function (*ycf*1 and *ycf*2). These findings suggest that a substantial amount of positive selection in these gene regions may play an important role in the adaptation strategies of woody endemics on oceanic islands (*Robinsonia*) and the tropical mountains of East Africa (*Dendrosenecio*). The equatorial highlands contain an ecological spectrum similar to that of oceanic islands, and woodiness increases in both regions. However, typical oceanic islands are distinct from equatorial highlands in that the former contain a wider gamut of habitats (shore to alpine, aquatic to xeric) in contrast to the latter with a single extreme habitat and relatively uniform climates throughout the year ('summer every day, winter every night') [1]. Plastid genes broadly adapt to changing ecological conditions [79, 80], and the positive selection of different sets of genes may have resulted in the adaptive radiation of *Robinsonia* and *Dendrosenecio* in their specific habitats.

Seven *Robinsonia* plastomes were highly conserved in the mVISTA and DnaSP analyses, but diverged from six *Dendrosenecio* and five *Senecio* plastomes, revealing differences in the pattern of polymorphic sites and overall nucleotide diversity. The nucleotide diversity (Pi) of *Robinsonia* (average 0.00092) was higher than that of *Dendrosenecio* (0.00058), but lower than that of *Senecio* (0.00357). *Robinsonia*'s plastome diversity was comparable to that of other insular woody endemics such as *Dendroseris* (0.00061) on the Juan Fernández Islands and *Dendrosonchus* (0.00090) on the Canary Islands [47, 81]. Furthermore, the overall patterns for highly variable regions were similar among the three genera with the highest diversity in the SSC region and the lowest in the IR regions. Although the chloroplast genomes were highly conserved among the seven *Robinsonia* species, we identified five divergence hotspots from the LSC and SSC regions as potential plastid markers for phylogenetic studies of *Robinsonia* and closely related taxa (Fig. 6): four intergenic hotspot regions (*rps*16-*trn*Q, *trn*C-*pet*N, *atp*A-*trn*R, and *rps*18-*rpl*20), and one protein coding region (*ycf*1).

Adaptive radiation of *Robinsonia*

The Juan Fernández Islands seems too limited to promote adaptive radiation due to its small size of two islands (Robinson Crusoe Island and Alejandro Selkirk Island), and outlying islets (Santa Clara Island). Nevertheless, *Robinsonia* represents early or limited adaptive radiation, and the species occupy very specific discontinuous niches over a range of mesophytic to xerophytic habitats, indicating a remarkable degree of correlation between habits and habitats [1]. Their habitats vary from obligate epiphytism on tree ferns (*R. evenia*) in very moist forest to growth on montane scrubland and open cliff faces (*R. gayana*). Carlquist (1974) has commented that the adaptive radiation on the Juan Fernández Islands seems to be in response to intense niche diversity and separation and that the species originated by in situ adaptation to local conditions [1]. Selection for adaptation to different ecological conditions drives adaptive radiation, accelerating speciation through the processes of genetic differentiation among populations within species, acquisition of reproductive isolation among populations, and the rise of ecological differentiation among such populations [82]. Consequently, oceanic island flora displays the differences in traits representation of dispersal capacity, pollination syndrome and life history strategy between island and mainland floras [83]. The phylogenetic inference in this study also suggests that the diversification of *Robinsonia* species could be the results of adaptive radiation associated with correlated habits (i.e., functional traits) and habitats on the Juan Fernández Islands.

The clade composing three species of *R. evenia*, *R. gracilis*, and *R. masafuerae* on the plastome phylogenetic tree (Fig. 7and Supplementary fig. S2) supports the section *Eleutherolepis* of subgenus *Robinsonia*, and those three species share the trait characters of separate nodes spacing, typically thin-walled, chlorophyllus leaf mesophyll, and one secretory canal per vascular bundle. On the other hand, *R. gayana* and *R. thurifera* (section *Robinsonia*) display nearly contiguous nodes, thick-walled hypodermis-like mesophyll, and two secretory canals [1, 19, 26]. Two groups of *Robinsonia* species are ecologically differentiated with habitats being mesophytic (section *Eleutherolepis*) or xerophytic (section *Robinsonia*). Hypodermis, a characteristic of brightly illuminated situations, tends to increase with exposed situation, and there is a relative tendency of conversion of spongy tissue to hypodermis observed in section *Robinsonia*. Increased presence of secretory canal with greater illumination and xeromorphy, although not uniform, seems evident as seen in Asteraceae plants of dry localities [1].

Crawford et al. (2018) synthesized information from several studies [1 pp. 206–211, 19, 84] and interpreted the possible factors driving the radiation of *Robinsonia* in the Juan Fernández Islands and maintaining species distinctions [85]. The mesomorphy in leaf anatomy of *R. evenia*, *R. gracilis*, and *R. masafuerae* (section *Eleutherolepis* of *Robinsonia*) is attributed to their epiphytism on cloud-forest tree fern; i.e., *R. evenia* (obligate epiphytism), *R. gracilis* (facultative epiphytism), and *R. masafuerae* (associated with ferns), all within mesic habitats of cloud forest. *R. evenia* is considered as an obligate epiphyte found on tree ferns [1, p. 206], although some specimens are known to occur on rock surfaces, i.e., eplithic lithophytes (Patricio López-Sepúlveda, personal observation). *R. gracilis* is not often epiphytic, but seedlings may start as epiphytes. *R. gracilis* may be found near to *R. gayana* (section *Robinsonia*), but the former occurs on ridges with open scrub, whereas the latter, often in crevices of cliffs in montane scrub. *R. masafuerae*, the only *Robinsonia* species found on Alejandro Selkirk Island and closely related with *R. evenia* on Robinson Crusoe Island, prefers fern-rich gullies and is sometimes epiphytic.

Contrastingly, the xeromorphy of *R. gayana* and *R. thurifera* (section *Robinsonia* of *Robinsonia*) is correlated with the growth on cliff faces and emergence in large size (to 5–6 m tall) from the fern-forest canopy, respectively [1, p. 210]. The closely related *R. gayana* and *R. thurifera* appeared to prefer different habitats, although they may occasionally occur together [19]. The former species grows primarily on exposed, rocky, xerophytic habitats, while the latter occurs primarily on forest edges in more mesic environments. Another factor isolating the two species is the later flowering of *R. thurifera* than its sympatric sister species, *R. gayana* [19]. *R. saxatilis*, which has been suggested to have evolved from a common ancestor of *R. gayana-R. thurifera* in this study, also exhibits strictly rock and non-forest ecology without epiphytic seedlings on tree fern trunks [20]. *R. berteroi* currently classified under monotypic subg. *Rhetinodendron* exhibits similar trait characters as section *Eleutherolepis* of *Robinsonia* species, however, it occurs from less mesomorphic habitats in open brushwood and forest along the ridge [1, 19]. The plastome phylogeny in this study resolved *R. berteroi* closer to the clade of xeromorphic *Robinsonia* species rather than to the clade of mesomorphic *Eleutherolepis* species with similar trait characters (Fig. 7).

Conclusions

This study, for the first time, assembled the complete plastome sequences of seven *Robinsonia* species, woody insular endemics on the Juan Fernández Islands in Chile, and performed phylogenetic and phylogenomic analyses to better understand the plastome evolution of *Robinsonia*. The analyses included woody *Dendrosenecio* and herbaceous *Senecio* from the tropical mountains in eastern Africa. Despite their morphological and distributional divergence, the whole cp genomes of the three genera were highly conserved, sharing common genomic features such as sequence similarity, gene content, and gene numbers. However, plastome sequences provide high resolution and strong support for the phylogenetic relationships among *Robinsonia* and closely related taxa including several *Senecio* and *Dendrosenecio* species. The monophyly of *Robinsonia* was robustly confirmed based on both ML trees of the complete plastome sequences and 80 concatenated coding genes. *Robinsonia* is divided into two major lineages with *R. berteroi* included within the same clade as other species, but nested within *Senecio*. *Robinsonia berteroi* currently classified under subg. *Rhetinodendron* was unexpectedly resolved as a sister to the clade consisting of *R. thurifera*, *R. gayana* and *R. saxatilis* (section *Robinsonia* of subg. *Robinsonia*) rather than being a sister to all remaining *Robinsonia* species (subg. *Robinsonia*). However, we support the current taxonomic treatment of *R. berteroi* as a separate subgenus (subg. *Rhetinodendron*), particularly because of its morphological distinction from the other *Robinsonia* species. *Robinsonia saxatilis*, which was initially described as being closely related to *R. evenia* of sect. *Eleutherolepis*, has been suggested to have evolved from a common ancestor of *R. gayana-R. thurifera*, supporting the findings based on AFLP and SSR analyses. Given the limited taxonomic sampling of the broadly occurring *Senecio* in this study, the sister lineages of *Robinsonia* and *Dendrosenecio* have yet to be determined. In the adaptive radiation procedure of *Robinsonia* species in the Juan Fernández Islands, the possible factors driving their radiation and maintaining species distinction could be their preference for different habitats and different flowering times even between species occasionally occurring together. The results of comparative phylogenomic analyses of the plastomes of *Robinsonia*, *Dendrosenecio* and *Senecio* species indicated that different sets of positively selected chloroplast genes, five in *Robinsonia* and two in *Dendrosenecio*, may have contributed to the adaptive radiation of these fascinating woody species in insular and continental sky island habitats. The overall patterns for the highly variable regions were similar among the three genera with the highest diversity in the SSC region and the lowest in the IR regions. Five mutation hotspots (*rps*16-*trn*Q, *trn*C-*pet*N, *atp*A-*trn*R, *rps*18-*rpl*20, and *ycf*1) in the LSC and SSC regions were identified as potential chloroplast markers for future phylogenetic and phylogeographic studies on *Robinsonia* and related *Senecio* groups.

Abbreviations

AFLP	Amplified fragment length polymorphic
BS	Bootstrap

cp	Chloroplast
cpDNA	Chloroplast DNA
DnaSP	DNA sequence polymorphism
ETS	External transcribed spacer
IGS	Intergenic spacer
ITS	Internal transcribed spacer
IR	Inverted repeat
LSC	Large single copy
ML	Maximum likelihood
Pi	Nucleotide diversity
Plastome	Plastid genome
RSCU	Relative synonymous codon usage
SSC	Small single copy
SSR	Simple sequence repeat

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-024-05711-3.

Supplementary Material 1: Supplementary Figure S1. Comparison of the plastid genomes of seven *Robinsonia*, six *Dendrosenecio* and five *Senecio* species, against *R. berteroi* by mVISTA.

Supplementary Material 2: Supplementary Figure S2. Maximum likelihood tree based on the protein-coding gene sequences of 32 representative Asteraceae species in *Robinsonia*, *Dendrosenecio* and *Senecio*. Trait characters are marked with brown circle representing woody stem; blue, separate nodes spacing, typically thin-walled, chlorophyllous leaf sponge mesophyll, and one secretory canal per vascular bundle; and orange, nearly contiguous nodes, thick-walled, strongly hypodermis-like mesophyll, and two secretory canals. Trait data taken from Skottsberg (1921) and Carlquist (1974) [1, 26], but not available for *R. saxatilis*

Supplementary Material 3: Supplementary Table S1. Relative synonymous codon usage (RSCU) of protein-coding genes in the chloroplast genomes of seven *Robinsonia* species

Acknowledgements
We thank the anonymous reviewers for their insightful comments and suggestions. We also thank the Cambridge University Press for the permission of using the photographs in Fig. 1, originally published by Stuessy et al. (Plants of Oceanic Islands: Evolution, Biogeography, and Conservation of the Flora of the Juan Fernández (Robinson Crusoe) Archipelago, 2018: C58, C59, C61-C67).

Author contributions
Conceptualization, M-S.C., JY.Y., S-C.K.; Methodology, M-S.C., JY.Y., S-H.K.; Software and analysis, M-S.C., JY.Y., S-H.K.; Resources, T.F.S., D.J.C., P.L-S.; Data curation, M-S.C., JY.Y.; Writing–original draft preparation by M-S.C., and review and editing by T.F.S., D.J.C., S-C.K.; Visualization, M-S.C., JY.Y., S-H.K.; Supervision and project administration, S-C.K.; Funding acquisition, M-S.C. All the authors have read and approved the final version of the manuscript.

Funding
This study was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education to M-S Cho (2022R1I1A2063355).

Data availability
The datasets generated and/or analyzed in the current study are available from the NCBI GenBank repository [https://www.ncbi.nlm.nih.gov/].

Declarations

Competing interests
The authors declare no competing interests.

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Received: 26 April 2024 / Accepted: 15 October 2024
Published online: 28 October 2024

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