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Characterization of the complete chloroplast genome of *Prosopis tamarugo* (*Prosopis, Leguminosae*), an endangered endemic tree species from the Atacama Desert

Caracterización del genoma completo del cloroplasto de *Prosopis tamarugo* (*Prosopis, Leguminosae*), una especie arbórea endémica en peligro del Desierto de Atacama

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SUMMARY

Prosopis tamarugo (*Prosopis*, Sect. Strombocarpa) is an important endangered tree species from the Atacama Desert (Chile). However, this species requires urgent conservation measures, for which it is necessary to evaluate their genetic diversity. Here, we present the characterization of the complete chloroplast genome of *P. tamarugo*; the first complete chloroplast of a species from the Strombocarpa section, obtained by next generation sequencing (NGS) methods. The complete chloroplast contains 161,575 bp and a total of 129 genes. A phylogenetic analysis of four *Prosopis* plastomes revealed that *P. tamarugo* is a sister species of the other *Prosopis* species albeit having smaller chloroplast sequence compared to those of other *Prosopis* species. Nine DNAcp markers were detected to distinguish between haplotypes. Therefore, the chloroplast sequence of *P. tamarugo* could be highly valuable for upcoming phylogenetic studies.

Key words: Strombocarpa section, NGS, plastome genome, variability.

RESUMEN

Prosopis tamarugo (*Prosopis*, Sect. Strombocarpa) es una importante especie arbórea en peligro de extinción del desierto de Atacama (Chile). Sin embargo, esta especie requiere medidas de conservación urgentes, para lo cual es necesario evaluar su diversidad genética. Aquí presentamos la caracterización del genoma completo del cloroplasto de *P. tamarugo*, el primer cloroplasto completo de una especie de la sección Strombocarpa, obtenido por métodos NGS. El cloroplasto completo comprende 161.575 pb y un total de 129 genes. El análisis filogenético de cuatro plastomas de *Prosopis* reveló que *P. tamarugo* es una especie hermana de las restantes especies de *Prosopis*. La secuencia completa de cloroplasto de *P. tamarugo* fue más pequeña que la de otras especies de *Prosopis*. Se detectaron nueve marcadores DNAcp para distinguir entre haplotipos. Por lo tanto, la secuencia de cloroplasto de *P. tamarugo* podría ser valiosa para los próximos estudios filogenéticos.

Palabras clave: Sección Strombocarpa, NGS, genoma de plastidios, variabilidad.

INTRODUCTION

The endangered endemic species *Prosopis tamarugo* Phil (*Prosopis*, Leguminosae) is a tree that survives in the most extreme area of Atacama Desert, inhabiting Pampa del Tamarugal (Altamirano 2006). *P. tamarugo* is a strict phreatophyte that lifts the groundwater to the surface through its roots and is adapted to high temperatures, extreme solar radiation and water stress (Lehner *et al.* 2001, Garrido *et al.* 2020). *P. tamarugo* is an important resource for livestock, people and the ecosystem (Barros 2010, Contreras *et al.* 2020a). The number of *P. tamarugo* individuals has been declining, mainly by over-exploitation of the underground aquifer, which makes it harder for the roots to reach the water level (Carevic *et al.* 2012). Even though molecular genetic methods based on nuclear (Zhang and Hewitt 2003) and organelle genomes have proven to be essential tools for species conservation (Daniell *et al.* 2016), there was no information for *P. tamarugo* available until now. Various aspects of genetic diversity play an important role in future conservation planning and management (Decuyper *et al.* 2016). Whole plastid analyses can offer valuable information of species and populations to aid biodiversity studies and develop conservation strategies (Liu *et al.* 2019). For this reason, we used NGS and assembled the complete chloroplast genome of *P. tamarugo*. We analyzed the complete chloroplast of *P. tamarugo* with regard to i) the structure, ii) gene

composition and iii) phylogeny, compared to other species of the Mimoseae tribe.

METHOD

Fresh leaves of a *P. tamarugo* individual were collected in the Tamarugal Province, Chile (20°21'03.6"S 69°39'47.9"W). Plant material was collected by the corresponding author according to the taxonomic criteria described by Burkart (1976). Plant material was deposited in the Departamento de Silvicultura y Conservación de la Naturaleza herbarium of Universidad de Chile (EIF, Index Herbariorum Code; voucher EIF13334). DNA was isolated from the fresh leaves with the modified cetyltrimethylammonium bromide (CTAB) protocol (Contreras *et al.* 2020b). The DNA extracted from *P. tamarugo* was quantified with a Qubit™ 3.0 fluorometer and a Qubit™ dsDNA HS Assay Kit. DNA integrity was verified with an Agilent 2100 Bioanalyzer prior to sequencing. Sequencing libraries were generated with a TruSeq Nano DNA LT Kit. The final libraries were run on an Agilent 2100 Bioanalyzer to verify the fragment size distribution and concentration. Sequencing was performed at Genoma Mayor (Universidad Mayor) with the Illumina sequencing platform. Paired-end sequences of 150 bp were generated for each read (R1 and R2). The filtered reads were assembled using SPAdes 4 software, version 3.13.0 (Bankevich *et al.* 2012). The chloroplast was annotated using DOGMA software (Wyman *et al.* 2004) and CPGAVAS2 (Shi *et al.* 2019), and then manually corrected. The graphical map of the chloroplast was generated with Organellar Genome DRAW (OGDRAW) (Greiner *et al.* 2019), and the complete nucleotide sequence of the chloroplast of *P. tamarugo* (MW582314.1) was deposited in the GenBank database. The complete chloroplast structures (LSC/IR, IR/SSC) of six other species (i.e. *Prosopis glandulosa* Torr., *Prosopis cineraria* (L.) Druce, *Prosopis juliflora* (Sw.) DC., *Leucaena trichandra* (Zucc.) Urb., *Piptadenia communis* var. *stipulacea* Benth. and *Stryphnodendron adstringens* (Mart.) Coville) of the Mimoseae tribe (family Fabaceae) were visualized for comparison using IRScope (Amiryousefi *et al.* 2018). The three *Prosopis* species were chosen because they are closely related to *P. tamarugo*. The other species showed a high percentage of similarity in GenBank (BLASTn). The genomes of 10 species were used for phylogenetic tree analysis: *P. tamarugo*, the six Mimoseae species mentioned above and three additional species of the Acaciae tribe (*Acacia murrayana* F.Muell. ex Benth., *Senegalia laeta* (R. Br. ex Benth.) Seigler & Ebinger and *Vachellia flava* (Forssk.) Kyal. & Boatwr.) as out-group. The sequences were aligned with MEGA6 (Tamura *et al.* 2013), using the maximum-likelihood (ML) method to construct the phylogenetic tree (Kumar *et al*. 2013); the nucleotide substitution model was the Kimura 2-parameter (K2P) model with branch support and 1000 bootstrap replicates. In addition, a sliding window analysis (window length: 600 pb, step size: 200 bp) was performed to assess the variability (*Pi*) between *P. tamarugo* and *P. glandulosa* chloroplasts with DnaSP version 5 software (Librado and Rozas 2009). *P. glandulosa* (one of the few *Prosopis* species available) was used as there was no complete chloroplast sequence data available for any species in the Strombocarpa section in the GeneBank database, and it had the highest percentage of similarity to *P. tamarugo*.

RESULT

The chloroplast of *P. tamarugo* comprises 161,575 bp and its structure contains two inverted repeat regions (IRs; 25,935 bp) separated by a large single copy region (LSC; 91,062 bp) and a small single copy region (SSC; 18,643 bp) (figure 1, figure 2). A total of 129 genes were identified: 82 protein-coding genes, 8 rRNA genes, 37 tRNA genes and 2 pseudogenes (*ycf1* and *infA*) with truncated reading frames. Six protein-coding genes, 4 rRNA genes and 7 tRNA genes of the IR regions contained duplicated genes (figure 1). Eighteen of the 129 genes contained at least one intron (figure 1).

The complete chloroplast sequence was smaller in *P. tamarugo* than in *P. glandulosa*, *P. juliflora* and *P. cineraria*, (1,465 bp; 1,662 bp and 2,102 bp less respectively) (figure 2). The GC content was similar among *Prosopis* species; 36 % in *P. tamarugo* and 35.9 % in the other *Prosopis* species. The LSC length of the *P. tamarugo* was smaller (~1,260 bp) than in other *Prosopis* species (figure 2). The chloroplast structure of the seven species of the Mimoseae tribe fluctuated in the IR regions between 25,919 bp and 26,062 bp; in the LSC regions between 91,044 bp and 93,690 bp and in the SSC regions between 18,643 bp and 19,001 bp (figure 2). Phylogenetic analysis revealed four clades with high support value, of which one was formed by the four *Prosopis* species and *Leucaena trichandra* (BP = 100), the second clade contained *Piptadenia communis* and *Stryphnodendron adstringens* (BP = 100), the third clade contained *Acacia murrayana* (BP = 100) and the fourth clade (outgroup) was formed by *Senegalia laeta* and *Vachellia flava* (figure 3). *Prosopis tamarugo* was found to be the sister species of the clade formed by the remaining *Prosopis* species (high support: BS = 100) (figure 3).

The level of divergence in the chloroplast genome sequences (i.e. nucleotide variability values (*Pi*)) between *P. tamarugo* (Section Strombocarpa) and *P. glandulosa* (Section Algarobia) ranged from 0 to 0.15167 with an average of 0.01041 (figure 4). We found nine loci with a high level of variation (*Pi* > 0.05333): *psbI-trnG* (*Pi* = 0.10333), *petN* (*Pi* = 0.07333), *trnfM-rps14* (*Pi* = 0.07167), *ycf3* (*Pi* = 0.05333), *trnL-trnF* (*Pi* = 0, 15167), *trnV-trnM* (*Pi* = 0.11500), *ycf4-cemA* (*Pi* = 0.08333), *psB-petL* (*Pi* = 0.09333) and *rps15-ycf1* (*Pi* = 0.10833) (figure 4). Eight of these loci are situated in the LSC region and the other in the SSC region. Additionally, a comparison among *Prosopis* species indicated 1,668 SNPs between *P. tamarugo* and

P. glandulosa, 172 SNPs between *P. juliflora* and *P. cineraria*, and 166 SNPs between *P. glandulosa* and *P. juliflora*.

DISCUSSION

Prosopis tamarugo is the key species in the fragile ecosystem of Pampa del Tamarugal and offers valuable products and services for livestock. However, *P. tamarugo* populations are decreasing in Pintados and Bellavista salt flats (Pampa del Tamarugal, Chávez *et al.* 2016), threatening the survival of the species as well as the ecosystem. Therefore, it is urgently required to find measures to enforce its conservation. Molecular differences in the complete chloroplast genome offer detailed genetic information about species and population differentiation (Yang *et al.* 2013). Moreover, chloroplast haplotypes can provide consistent information about the origin and history of the species (Laricchia *et al.* 2015). Here, we characterized the complete chloroplast genome sequence of *P. tamarugo*, a species from the Strombocarpa section of the genus *Prosopis*. We compared its chloroplast genome with chloroplast genomes of *P. cineraria (Sect. Prosopis)*, *P. juliflora* and *P. glandulosa (Sect. Algarobia, ser. Chilenses*), which were previously described by Asaf *et al.* (2020). We found a total of 129 genes in *P. tamarugo*, whereas Asaf *et al.* (2020) found 131, 132 and 128 genes in the chloroplast of *P. cineraria*, *P. juliflora* and *P. glandulosa*, respectively. However, we observed derangements in the sequence of *psbL* and *rpl22* genes of *P. tamarugo*, explaining why these genes were not included in the gene annotation. According to Lehner *et al.* (2001), *P. tamarugo* is photosynthetically highly adapted to solar radiation. As photosynthesis depends on the chloroplast gene expression (Pesaresi *et al.* 2006), this indicates that the genes of the *P. tamarugo* chloroplast, which were sequenced in this study, may potentially reveal important insights on this adaptation.

The length of the *P. tamarugo* chloroplast sequence is the smallest of the *Prosopis* species evaluated in this study.

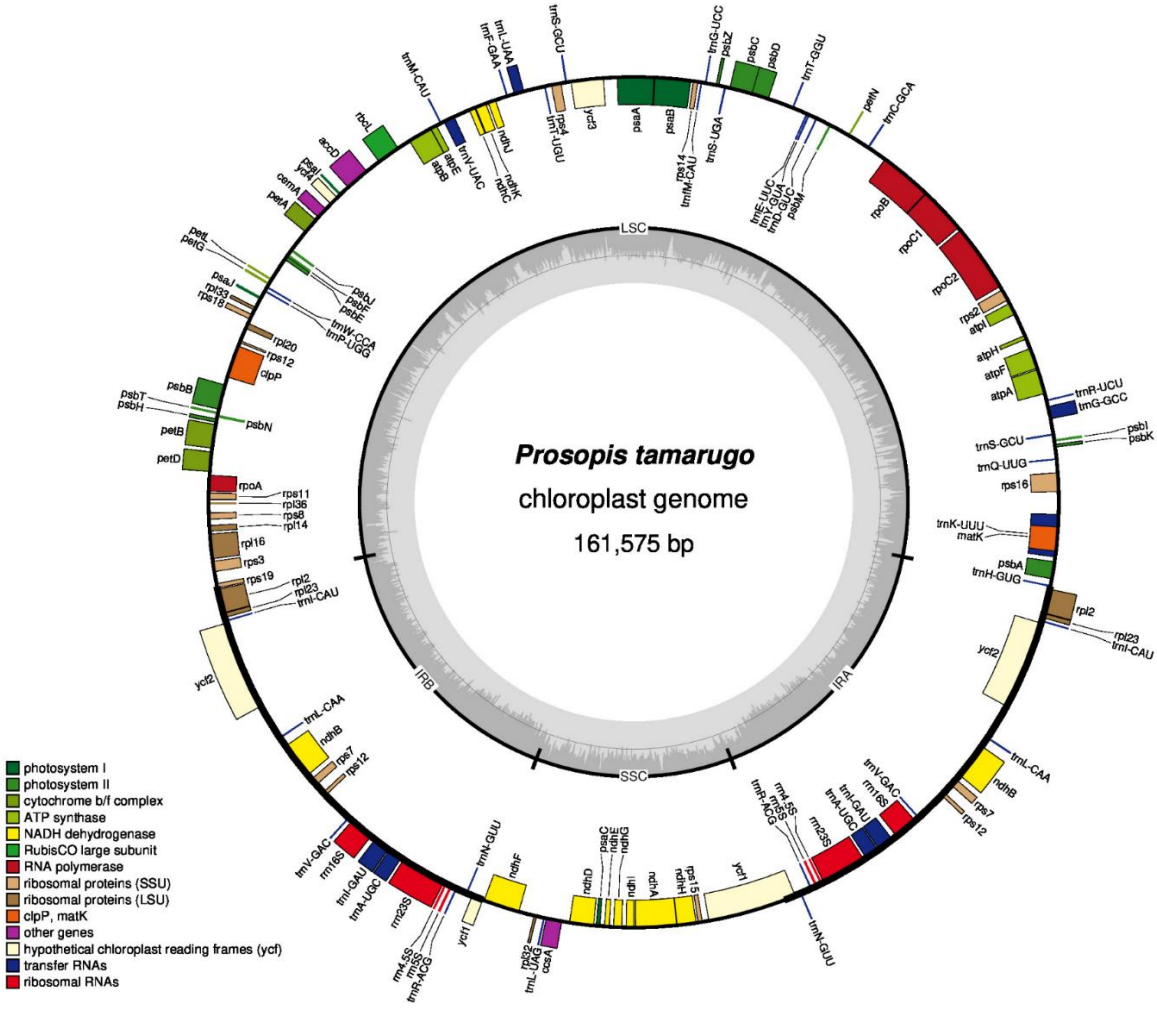

Figure 1. Circular gene map of the chloroplast genome of *Prosopis tamarugo*.

Mapa circular de genes del genoma del cloroplasto de *Prosopis tamarugo*.

Moreover, the LSC region of *P. tamarugo* is one of the smallest in Mimoseae species, similar in size to *S. adstringens*. In general, the rest of the structures of *P. tamarugo* (IRs and SSC) were comparable to the other *Prosopis* species. The phylogenetic analysis placed *P. tamarugo* (Section Strombocarpa) as sister to the rest genus *Prosopis* (section *Algarobia* and section *Prosopis*). This is in accordance with Saidman *et al.* (1996) who showed that there is an important difference in genetic variability among species of the Strombocarpa and Algarobia sections, and Catalano *et al.* (2008), who found that these two sections are sisters, that diverged in the Oligocene (Catalano *et al.* 2008).

We compared the nucleotide variability of the chloroplast of *P. tamarugo* (sect. Strombocarpa) and *P. glan-*

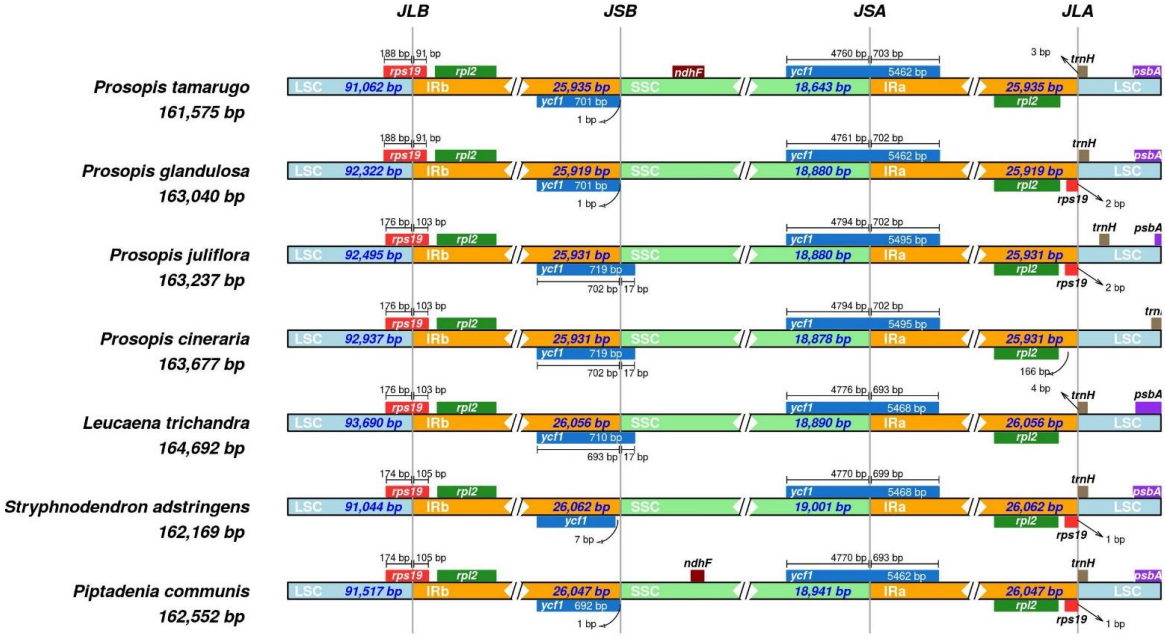

Figure 2. Comparison of chloroplast genomes among LSC, SSC and IRs junction sites regions among the seven species of Tribu Mimoseae. JLA, junction IRa/LSC; JLB, junction IRb/LSC; JSA, junction IRa/SSC; JSB, junction IRb/SSC.

Comparación de genomas de cloroplasto entre regiones de sitios de unión LSC, SSC e IR entre las siete especies de Tribu Mimoseae. JLA, unión IRa/LSC; JLB, unión IRb/LSC; JSA, unión IRa/SSC; JSB, unión IRb/SSC.

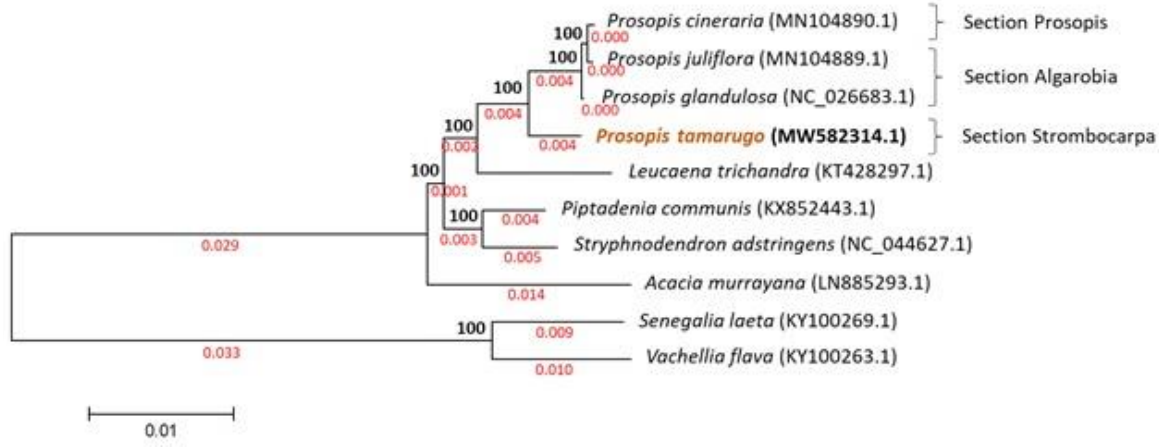

Figure 3. Molecular phylogenetic analysis (maximum likelihood method). Bootstrap values are placed on the nodes and values of the substitutions/sites in red color.

Análisis filogenético molecular (método de máxima verosimilitud). Los valores de Bootstrap se colocan en los nodos y valores de las sustituciones/sitios en color rojo.

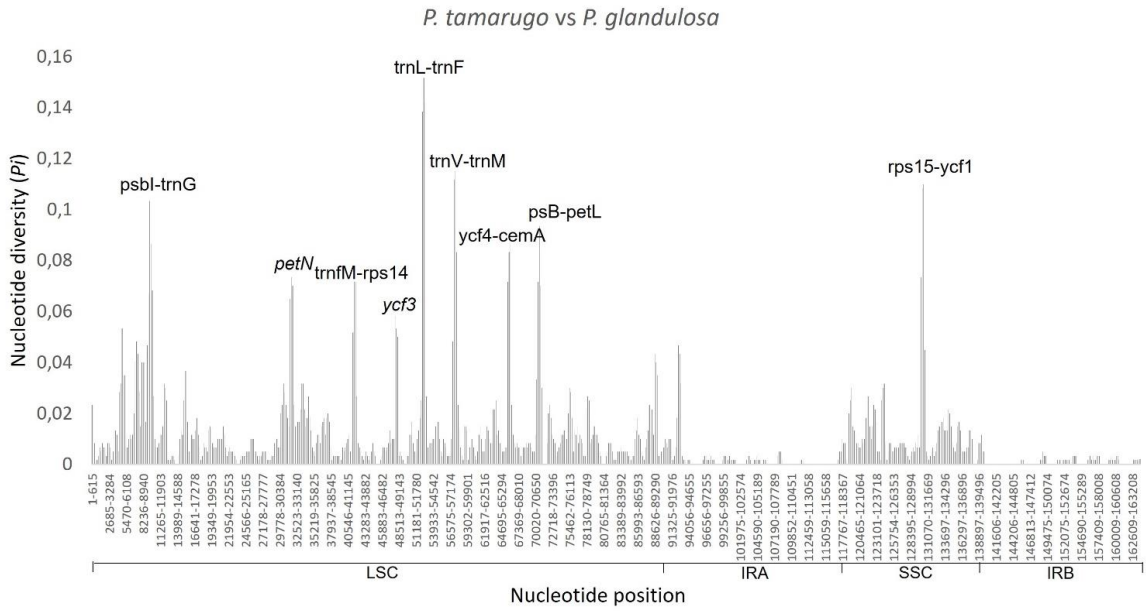

Figure 4. Sliding window analysis of the complete chloroplast genomes of two *Prosopis* species. Nucleotide diversity (*Pi*) between *P. tamarugo* and *P. glandulosa*.

Análisis de ventana deslizante de los genomas completos de cloroplasto de dos especies de *Prosopis*. Diversidad de nucleótidos (*Pi*) entre *P. tamarugo* y *P. glandulosa*.

dulosa (sect. Algarobia) and found large differences (*Pi* = 0.01041) among the chloroplast genomes compared to the average nucleotide variability between two species of the genus *Cercis* (Fabaceae) of *Pi* = 0.0006 (Liu *et al.* 2018), and two species of the genus *Lespedeza* (Fabaceae) of *Pi* = 0.00147 (Somaratne *et al.* 2019). Moreover, we detected nine DNAcp markers, which could be used to distinguish haplotypes. The number of SNPs between the plastomes of *P. tamarugo* and *P. glandulosa* was high (1,668 SNPs), compared to species of the same Algarobia section (166 SNPs). These results show that the differences found in the *P. tamarugo* plastome, compared to the other species, could be used for research that evaluates genotypes and population diversity of the species from the Strombocarpa section.

CONCLUSION

The comparison of the genomic structure and gene numbers of chloroplasts of *P. tamarugo*, *P. glandulosa*, *P. cineraria*, *P. juliflora*, *Leucaena trichandra*, *Stryphnodendron. adstringens* and *Piptadenia communis* showed that there are moderate differences among them. The ML phylogenetic analysis including chloroplast DNA indicated that *P. tamarugo* (sect. Strombocarpa) can be considered a sister species of the other three *Prosopis* species. The comparison of the cpDNA of *P. tamarugo* (sect. Strombocarpa) and *P. glandulosa* (sect. Algarobia) indicated large differences among the chloroplast genomes, which encourages the use of the complete chloroplast genome to determine haplotype diversity and evolutionary paths in the genus.

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