



## SHORT RESEARCH ARTICLE

# The complete chloroplast genome of the endangered species garra de león [*Bomarea ovallei* (Phil.) Ravenna] from Chile

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## Abstract

*Bomarea ovallei* (Phil.) Ravenna ( $2n=2x=18$ ) is an endangered endemic species that inhabits only a small part of the coast of the Atacama region. We describe the structure, gene composition and phylogeny of the complete chloroplast sequence of this elusive species. The chloroplast genome consists of 155,018 bp, with typical quadripartite structures: a large single copy region (LSC) of 84,132 bp, a small single copy region (SSC) of 17,794 bp, and two inverted repeat regions (IRs) contain 26,546 bp. One hundred and thirty-four genes were identified out of which 84 coding genes, 8 rRNA, 38 tRNA and 4 pseudogenes. *B. ovallei* chloroplast resembles chloroplasts from seven species of the order Liliales in length and structure and is most similar to *Bomarea edulis* (BP=100). The average nucleotide variability ( $P_i$ ) of 0.00254 between these two *Bomarea* species is moderate. Nine loci with increased variability were identified: *rps16-trnQ*, *atpF*, *trnL*, *ndhC-trnV*, *rbcL*, *psbJ*, *rpl32-trnL*, *ndhD* and *ycf1*. These loci could be used as DNA markers for classification and evaluation studies in *Bomarea* populations.

**Keywords:** *Bomarea ovallei*, chloroplast structure, cpDNA, flowering desert

## Introduction

*Bomarea ovallei*, belonging to the family Alstroemeriaceae, is commonly known as "garra de león (lion's claw)" (Fig. 1.) and is the most iconic flower of the flowering desert of Atacama (Vargas et al. 2018). The Alstroemeriaceae family consists of approximately 200 species classified in four genera: *Bomarea* Mirb., *Alstroemeria* L., *Luzuriaga* Ruiz & Pav. and *Drymophila* R. Br. The species are mainly distributed in Central America and South America (Aagesen L. and Sanso A. M. 2003). The phylogeny of the Alstroemeriaceae, based on morphology, *rps16* intron, and *rbcL* sequence data. Syst. Bot., 28(1): 47-69. and Sanso 2003; Chacón et al. 2012). *Bomarea* are popular and important flowering species because they are available in various colours. The genus *Bomarea* contains approximately 120 species and has a neotropical distribution from Mexico (24 °N) to Chile (40 °S) (Guarin, 2007; Chacón et al., 2012). In Chile, four species of the genus occur: *Bomarea involucrosa* (Herb.) Baker, *Bomarea dulcis* (Hook.) Beauverd, *Bomarea salsilla* (L.) Mirb. and *B. ovallei* (Phil.) Ravenna. These species have the same number of chromosomes ( $2n = 2x = 18$ ) with almost identical asymmetry (Palma-Rojas et al. 2007). However, geographically the species do not overlap in their distribution.

*B. ovallei* flowers between October and November

when the flowering desert phenomenon or Desierto Florido occurs (Contreras et al. 2020). It used to be called *Leontochir ovallei* Phil. but was placed in the *Bomarea* genus, rather than considering it to be a monotypic genus (Ravenna, 2000; Guarín, 2007). Phylogenetic analysis confirmed

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the placement of *L. ovallei* with the species of the genus *Bomarea*, leading to the renaming of *L. ovallei* to *B. ovallei* (Aagesen and Sanso 2003; Guarín 2007). However, [Baeza et al. \(2012\)](#) revived the genus *Leontochir* as an independent genus, based on the higher asymmetry index of the *L. ovallei* karyotype compared to the *Bomarea* species karyotype.

Evolutionary studies with nuclear and chloroplast DNA (cpDNA) markers can help resolve this debate. They can be used to analyze phylogenetic diversity, community structure and evaluate taxa and ecosystems to aid the conservation of rare species ([Scherson et al. 2014](#)). The cpDNA contains two identical “inverted repeats” (IRs), which are separated by a large single copy region (LSC) and a small single copy region (SSC) ([Kim et al. 2016](#)). Until now, only the chloroplast of *Bomarea edulis*, of the genus *Bomarea* has been completely sequenced (Kim et al. 2016). In this study, we sequenced and assembled the complete chloroplast of *B. ovallei* and analyzed its structure, gene composition and phylogeny compared to other species of the order *Liliales*.

Leaves were collected from an individual of *B. ovallei* located in Llanos de Challe National Park, Chile (28° 6'56.53 "S, 71° 5'53.99 "W; research permit CONAF N°106/2017 and

N° 122/2019). DNA was extracted according to the method described by (Contreras et al. 2021). The DNA was then quantified using Qubit™ 3.0 fluorometer. The sequencing library was prepared with the TruSeq Nano DNA LT Kit. Sequencing was performed on an Illumina sequencing platform by Genoma Mayor (Universidad Mayor, Chile). The chloroplast was then annotated with DOGMA software ([Wyman et al. 2004](#)). The graphical map of the chloroplast was generated by Organellar Genome DRAW (OGDRAW) ([Greiner et al. 2019](#)), and the complete nucleotide sequence of the chloroplast of *B. ovallei* (MW345247.1) was deposited in the GenBank database. The chloroplast structures (LSC/IR, IR/SSC) of *B. ovallei* and six other species of the order *Liliales* were visualized and compared using IRScope ([Amiryousefi et al. 2018](#)). The phylogenetic analysis (maximum likelihood) was performed in MEGA6 software ([Tamura et al. 2013](#)). A sliding window analysis was performed to assess the variability (*Pi*) between *B. edulis* and *B. ovallei* chloroplasts with DnaSP v5 software ([Librado and Rozas 2009](#)).

The chloroplast of *B. ovallei* comprises 155,018 bp, two inverted repeat regions (IRs) contain 26,546 bp and are separated by a large single copy region (LSC) of 84,132 bp

**Table 1. Gene composition in the chloroplast genome of *Bomarea ovallei***

Category of genes	Group of genes	Name of genes	N°
Photosynthesis	Photosystem I	<i>psaA, psaB, psaC, psal, psaj</i>	5
	Photosystem II	<i>psbA, psbB, psbC, psbD, psbE, psbF, psbH, psbl, psbJ, psbK, psbL, psbM, psbN, psbT, psbZ</i>	15
	ATP synthase	<i>atpA, atpB, atpE, atpF<sup>b</sup>, atpH, atpI</i>	6
	NADH-dehydrogenase	<i>ndhA<sup>b</sup>, ndhB<sup>ab</sup>, ndhC, ndhD, ndhE, ndhF, ndhG, ndhH, ndhI, ndhJ, ndhK</i>	12
	cytochrome b/f complex	<i>petA, petB, petD<sup>b</sup>, petG, petL, petN</i>	6
	Large subunit RUBISCO	<i>rbcl</i>	1
Protein synthesis and DNA replication	Transfer RNAs	<i>trnA-UGC<sup>ab</sup>, trnC-GCA, trnD-GUC, trnE-UUC, trnF-GAA, trnG-CAU, trnG-UCC<sup>b</sup>, trnG-GCC, trnH-GUG<sup>a</sup>, trnI-GAU<sup>ab</sup>, trnI-CAU<sup>a</sup>, trnK-UUU<sup>b</sup>, trnL-UAA<sup>b</sup>, trnL-CAA<sup>a</sup>, trnL-UAG, trnM-CAU, trnN-GUU<sup>a</sup>, trnP-UGG, trnQ-UUG, trnR-ACG<sup>a</sup>, trnR-UCU, trnS-GGA, trnS-UGA, trnS-GCU, trnT-GGU, trnT-UGU, trnV-UAC<sup>b</sup>, trnV-GAC<sup>a</sup>, trnW-CCA, trnY-GUA</i>	38
	Ribosomal RNAs	<i>rrn16S<sup>a</sup>, rrn23S<sup>a</sup>, rrn4.5S<sup>a</sup>, rrn5S<sup>a</sup></i>	8
	Ribosomal Protein large-subunit	<i>rpl14, rpl16, rpl2<sup>ab</sup>, rpl20, rpl22, rpl23<sup>a</sup>, rpl32, rpl33, rpl36</i>	11
	DNA dependent RNA polymerase	<i>rpoA, rpoB, rpoC1<sup>b</sup>, rpoC2</i>	4
	Ribosomal Protein Small-subunit	<i>rps11, rps12<sup>ab</sup>, rps14, rps15, rps16, rps18, rps19, rps2, rps3, rps4, rps7<sup>a</sup>, rps8</i>	14
Other functions	Subunit of Acetyl-CoA-carboxylase	<i>accD</i>	6
	c-type cytochrome synthesis gene	<i>ccsA</i>	
	Envelop membrane protein	<i>cemA</i>	
	Protease	<i>clpP</i>	
	Maturase	<i>matK</i>	
Unknown function	Initiation Factor	<i>infA</i>	
	Conserved open reading frames	<i>ycf1<sup>a</sup>, ycf2<sup>a</sup>, ycf3<sup>b</sup>, ycf4, ycf15<sup>a</sup></i>	8

<sup>a</sup>Duplicated genes; <sup>b</sup> Genes containing introns



Fig. 1. *Bomarea ovallei* in Llanos de Challe National Park

and a small single copy region (SSC) of 17,794 bp (Fig. 2.). A total of 134 genes were identified of which, 84 are coding genes, 8 rRNA genes, 38 tRNA genes and 4 pseudogenes (Table 1.). All five coding genes, 4 rRNA genes, 8 tRNA genes, and a pseudogene (ycf15) belong to IR regions containing duplicated genes (Table 1). The 4 pseudogenes contained a short pseudocopy ycf1, an *infA* copy and two *ycf15* copies. *B. ovallei* chloroplasts had a similar length and structure as other species of the order *Liliales* (Fig.3.A). The pseudogene *ycf68*, which is present in *B. edulis* (Kim et al. 2016), was absent in *B. ovallei*. The complete chloroplast sequence of *B. ovallei* was 93 bp larger than *B. edulis*. The GC content

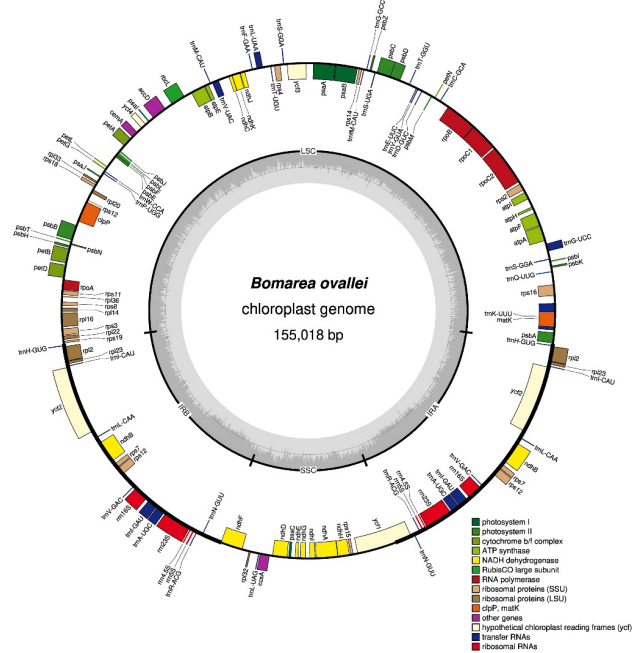


Fig. 2. Circular gene map of the chloroplast genomes of *Bomarea ovallei*

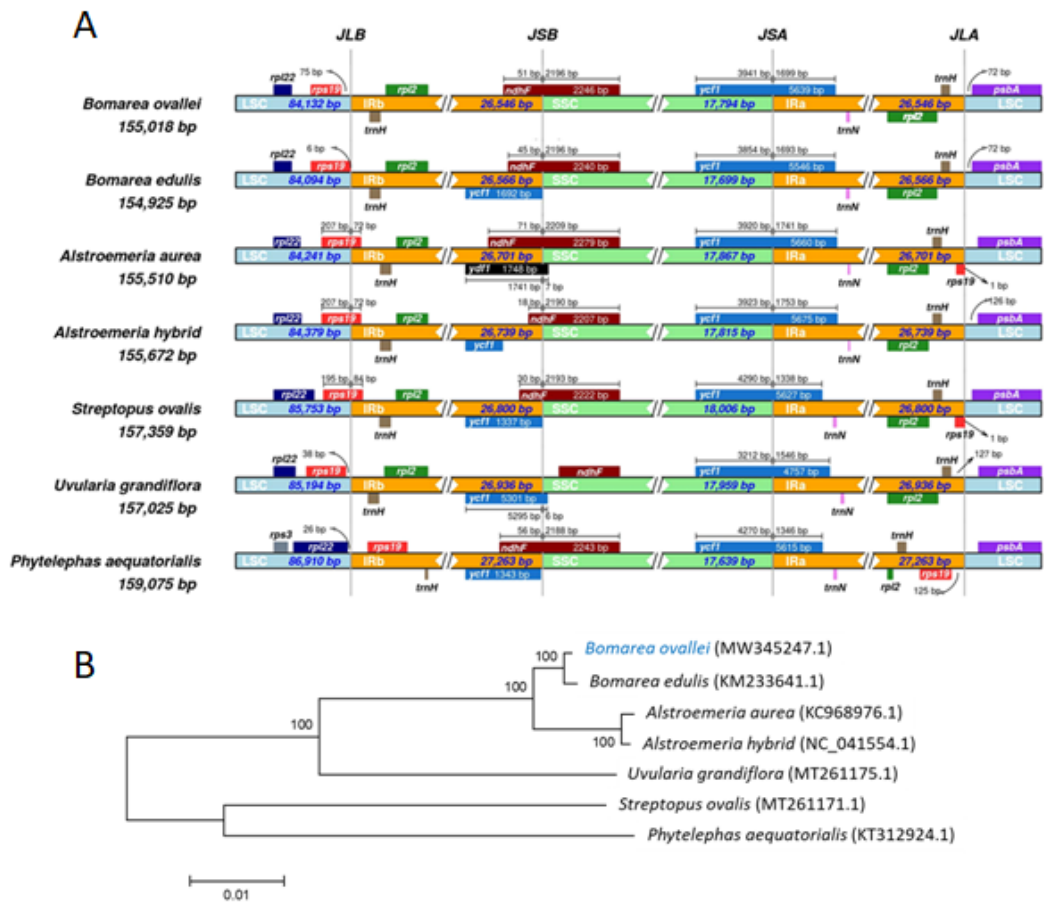


Fig. 3. Comparison of chloroplast genomes between the Large Single Copy region (LSC in blue), Small Single Copy region (SSC in green) and Inverted Repeat regions (IRa and IRb in orange) junction sites regions within the orden *Liliales* (A). Molecular phylogenetic analysis. Bootstrap values are place on the nodes (B)



was very similar, 38.1 and 38.2%, respectively (Fig. 2), and so were the lengths of the IR, LSC and SSC regions (Fig. 3A). Phylogenetic analysis of *B. ovallei* and six other species form the *Liliales* revealed four clades: one was formed by *B. ovallei* and *B. edulis* (BP=100), the second clade contained *Alstroemeria aurea* and *Alstroemeria* hybrid (BP=100), the third clade contained *Uvularia grandiflora* (BP=100) and the fourth clade (outgroup) was formed by *Streptopus ovalis* and *Phytelephasa equatorialis* (Fig. 3B).

The parentage analysis of *B. ovallei* has been controversial. Even though the studies show a high asymmetry index between *B. ovallei* and other species from the same genus (Baeza et al. 2012), phylogenetic analysis placed this species, with high support, with *B. involucrosa* (Alzate et al. 2008) and with *B. bolivariana* and *B. multiflora* (Chacón et al. 2012), placing this species firmly within the *Bomarea* genus.

Between *B. edulis* and *B. ovallei* the nucleotide variability ( $P_i$ ) indicated moderate differences (ranging from 0 to 0.03333, with an average of 0.00254). We found nine loci with higher variability that could be used as DNA markers to classify and evaluate the *Bomarea* taxon, and distinguish between its species: *rps16-trnQ* ( $P_i = 0.03333$ ), *atpF* ( $P_i = 0.01600$ ), *trnL* ( $P_i = 0.01167$ ), *ndhC-trnV* ( $P_i = 0.01333$ ), *rbcl* ( $P_i = 0.01000$ ), *psbJ* ( $P_i = 0.02500$ ), *rpl32-trnL* ( $P_i = 0.01167$ ), *ndhD* ( $P_i = 0.02167$ ) and *ycf1* ( $P_i = 0.01500$ ). Six of these loci are in the LSC region and three in the SSC region, which are highly variable in angiosperms (Souza et al. 2019). Additionally, 393 substitution events (SNPs) and 100 Indels were detected between the chloroplasts of *B. ovallei* and *B. edulis*. This amount is comparable to the 403 SNPs between *A. aurea* and *A. hybrid*, but higher than between *Machilus yunnanensis* and *Machilus balansae* (231 SNPs and 65 Indels) (Song et al. 2015). Despite the high geographic isolation of *B. ovallei*, caused by the natural barrier of the Atacama Desert, our results showed that *B. ovallei* presents a conserved level of chloroplast evolution due to a moderate degree of mutation events (SNP and Indels), compared to *B. edulis*. Due to the information obtained during this study, we could reinforce the placement of *B. ovallei* in the genus *Bomarea* and determine their chloroplast's structure and gene composition.

### Author's contribution

Conceptualization of research (RCD, WHM, LVDB, MAA); Designing of the experiments (RCD); Contribution of experimental materials (RCD, MAA); Execution of field/lab experiments and data collection (RCD, LVDB, MNF, MAA); Analysis of data and interpretation (RCD, WHM, LVDB, MAA); Preparation of the manuscript (RCD, WHM, LVDB, MNF, MAA).

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