

# Quantity, organization, and distribution of chloroplast microsatellites in all species of *Eucalyptus* with available plastome sequence

Mateus Chagas Andrade<sup>1</sup>, Matheus Perek<sup>1</sup>, Fernanda Bortolanza Pereira<sup>2</sup>, Michele Moro<sup>1</sup> and Evandro Vagner Tambarussi<sup>1,2\*</sup>

Crop Breeding and Applied Biotechnology  
18: 97-102, 2018  
Brazilian Society of Plant Breeding.  
Printed in Brazil  
<http://dx.doi.org/10.1590/1984-70332018v18n1a13>

**Abstract:** In this study, we quantify and document the distribution and organization of cpSSRs in the chloroplast genome of 31 *Eucalyptus* species. Our sample included all previously sequenced plastomes of *Eucalyptus* species available from the NCBI online database. We processed the complete cpDNA sequences and identified mono-, di-, tri-, tetra-, penta-, and hexanucleotide cpSSRs, with the majority of cpSSRs classified as mononucleotide. After genome microsatellite selection, we evaluated the microsatellites for coding and non-coding regions and cpSSRs were predominantly identified in non-coding regions of cpDNA for all nucleotide types. Penta- and hexanucleotide cpSSRs were the least frequent types of microsatellites. We also developed and virtually amplified 60 primer pairs that can be used in studies of *Eucalyptus* species. Thus, these cpSSR regions can be used in studies assessing the ecology, breeding, and conservation of the genus.

**Key words:** Chloroplast DNA, DNA fingerprint, genetic diversity, species differentiation.

## INTRODUCTION

*Eucalyptus* L'Hér. is the most dominant genus in Australian flora and with approximately 700 species, it is the largest genus in the Myrtaceae family (Smith et al. 2003). The genus originates from the region in and around Australia, including Timor, Indonesia, Papua New Guinea, the Molucas Islands, Irian Jaya, and the Southern Philippines, between 9° N and 44° S latitude (Eldridge et al. 1993). In 2015, *Eucalyptus* forest plantations represented about 10% of the approximately 291 million hectares of planted forests worldwide (FAO 2016, Sands 2005).

Because of its extensive use in forestry, plant ecologists, breeders and geneticists have significant interest in establishing methods for fast and robust *Eucalyptus* identification or species distinction. Currently, the use of marker-assisted approaches in *Eucalyptus* breeding programs is common (Mahajan and Gupta 2012, Fuchs et al. 2015). These approaches are powerful tools for phylogenetic and population research (Doorduyn et al. 2011) and can be used to assist ecologists and breeders in their analyses (Steane et al. 1998, Freeman et al. 2001, McKinnon et al. 2001, McKinnon et al. 2010, Melotto-Passarin et al. 2011).

\*Corresponding author:

E-mail: [tambarussi@gmail.com](mailto:tambarussi@gmail.com)

Received: 09 May 2017

Accepted: 21 June 2017

<sup>1</sup> Universidade Estadual do Centro-Oeste (UNICENTRO), PR 153, km 7, Riozinho, 84.500-000, Irati, PR, Brazil

<sup>2</sup> Universidade Estadual Paulista (UNESP), Faculdade de Ciências Agrônômicas, Rua José Barbosa de Barros, 1780, Portaria II, Rodovia Alcides Soares, km 3, 18.610-307, Botucatu, SP, Brazil

The development of molecular markers for *Eucalyptus* species improved dramatically after the complete sequencing of its nuclear genome (nDNA) (Myburg et al. 2014, Fuchs et al. 2015). Currently, microsatellite markers (SSRs) are widely used in. Although many nSSRs have been developed for the *Eucalyptus* genus (Kirst et al. 2005), the use of cpSSRs is still limited. Nevertheless, as with nDNA, cpDNA (i.e., cpSSR) has demonstrated great potential for use in phylogeography, analyses of DNA fingerprints, hybridization and progeny analyses in tree species (Steane et al. 2005, Delgado et al. 2007, McKinnon et al. 2010). Because the majority of cpDNA evolves slowly, its overall utility for evolutionary and population genetic studies can be limited, particularly at lower taxonomic levels (Steane et al. 1991, Steane et al. 1998, Freeman et al. 2001, Shaw et al. 2005). As a result, many researchers have sought to use hypervariable regions of cpDNA to characterize the genetic variation of species (Shaw et al. 2007). In this context, cpSSRs have become a significant resource for genetic analyses (Weising and Gardner 1999) focused on ecology, systematics, conservation, phylogenetic studies, and in breeding programs. As such, chloroplast genomes (cpDNA) can provide important data that can be used in ecological (Latouche-Hallé et al. 2003) and breeding studies of *Eucalyptus* species, such as estimating the phylogenetic relationships within and between species (Whitlock et al. 2003).

In this study, we aim to quantify and document the distribution and organization of cpSSRs in all 31 species of *Eucalyptus* that have an available plastome sequence. We also developed 60 potential primer pairs that can be used by researchers in a variety of different fields of study.

## MATERIAL AND METHODS

### In silico identification of microsatellite

This study is based on complete plastid genome sequences available from the National Center for Biotechnology Information (NCBI; <https://www.ncbi.nlm.nih.gov/genomes/GenomesGroup.cgi?opt=plastid&taxid=2759>). From this database, all 31 chloroplast genomes of *Eucalyptus* spp. were used (Table 1). The complete cpDNA sequences were collected from NCBI and processed using the FastPCR 6.5.40.0 software (Kalendar et al. 2016) to identify the microsatellite regions (cpSSR) as mono-, di-, tri-, tetra-, penta-, or hexanucleotide. We considered only those repeats in which the motifs repeated as follows: mononucleotide repeats with a repeat length  $\geq 8$ ; dinucleotide with a repeat length  $\geq 6$ ; and tri-, tetra-, penta-, and hexanucleotide with a repeat length  $\geq 3$ . After identification, we assessed microsatellites for coding (gene) and non-coding (intergenic) regions according the information available in the NCBI database for each species.

**Table 1.** Plastome sequence and length for 31 *Eucalyptus* species

Plant Species	Plastome Size (bp)	Accession Number	Plant Species	Plastome Size (bp)	Accession Number
<i>E. aromaphloia</i> L.D.Pryor & J.H.Willis	160,149	NC_022396	<i>E. melliodora</i> A. Cunn	160,386	NC_022392
<i>E. baxteri</i> (Benth.) Donzela & Blakely	160,032	NC_022382	<i>E. microcorys</i> F. Muell	160,225	NC_022404
<i>E. camaldulensis</i> Dehnh	160,164	NC_022398	<i>E. nitens</i> H.Deane & Maiden	160,271	NC_022395
<i>E. cladocalyx</i> F. Muell	160,213	NC_022394	<i>E. obliqua</i> L'Hér	159,527	NC_022378
<i>E. cloeziana</i> F. Muell	160,015	NC_022388	<i>E. patens</i> Benth.	160,187	NC_022389
<i>E. curtisii</i> Blakely & C.T. White	160,038	NC_022391	<i>E. polybractea</i> R. Baker	160,268	NC_022393
<i>E. deglupta</i> Blume	160,177	NC_022399	<i>E. radiata</i> Sieber	159,529	NC_022379
<i>E. delegatensis</i> R.T.Baker	159,724	NC_022380	<i>E. regnans</i> F. Muell.	160,031	NC_022386
<i>E. diversicolor</i> F. Muell	160,214	NC_022402	<i>E. saligna</i> Smith	160,015	NC_022397
<i>E. diversifolia</i> Bonpl	159,954	NC_022383	<i>E. salmonophloia</i> F. Muell	160,413	NC_022403
<i>E. elata</i> Dehnh	159,899	NC_022385	<i>E. sieberi</i> L.A.A. Johnson	159,985	NC_022384
<i>E. erythrocorys</i> F. Muell	159,742	NC_022406	<i>E. spathulata</i> Hook	161,071	NC_022400
<i>E. globulus</i> Labill	160,286	NC_008115	<i>E. torquata</i> Luehm	160,223	NC_022401
<i>E. grandis</i> W. Hill ex Maiden	160,137	NC_014570	<i>E. umbra</i> R. Baker	159,576	NC_022387
<i>E. guilfoylei</i> Maiden	160,520	NC_022405	<i>E. verrucata</i> P.Y. Lagiges	160,109	NC_022381
<i>E. marginata</i> Donn ex Sm.	160,076	NC_022390	-	-	-

## Primer design and in silico PCR amplification

The complete chloroplast genome of *Eucalyptus grandis* (GenBank NC\_014570) was screened for cpSSRs and 60 cpSSR flanking hypervariable regions were used to develop primer pairs. Primer pairs were designed using Primer3 (v. 0.4.0) (Untergasser et al. 2012) based on the following criteria: annealing temperature between 57 and 63 °C; final amplification product of 100 – 350bp; GC content of 20 – 80%; and primer size of 18 – 27bp.

Virtual amplification was performed using the Sequence Manipulation Suite ([http://www.bioinformatics.org/sms2/pcr\\_products.html](http://www.bioinformatics.org/sms2/pcr_products.html); Stothard 2000) for all 31 *Eucalyptus* species. The Suite determines the expected product size for each primer pair.

## RESULTS AND DISCUSSION

All microsatellite regions in the cpDNA for the 31 studied *Eucalyptus* species were evaluated (Table 2). The total number of microsatellites observed for each species varied from 71 for *E. melliodora* to 135 for *E. aromaphloia*.

While we identified mono-, tri-, and tetranucleotide microsatellites for all species (Table 2), the majority of *Eucalyptus* cpSSRs were classified as mononucleotide. Currently there are no other studies that assess the distribution and organization

**Table 2.** Frequency (%) of the genic and intergenic cpSSRs based on motif size for each species

Species	Mono		Di		Tri		Tetra		Penta		Hexa		Total
	Intergenic %	Genic %	Intergenic %	Genic %	Intergenic %	Genic %	Intergenic %	Genic %	Intergenic %	Genic %	Intergenic %	Genic %	
<i>E. aromaphloia</i>	83.6 (92)	16.3 (18)	100 (1)	0.0	72.2 (13)	27.7 (5)	83.3 (5)	16.6 (1)	0.0	0.0	0.0	0.0	135
<i>E. baxteri</i>	83.0 (83)	17.0 (17)	0.0	0.0	100 (4)	0.0	75.0 (3)	25.0 (1)	100 (1)	0.0	0.0	0.0	109
<i>E. camaldulensis</i>	83.3 (90)	16.6 (18)	100 (1)	0.0	100 (4)	0.0	75.0 (6)	25.0 (2)	0.0	0.0	0.0	0.0	121
<i>E. cladocalyx</i>	85.7 (96)	14.3 (16)	0.0	0.0	100 (4)	0.0	85.7 (6)	14.2 (1)	0.0	0.0	0.0	0.0	123
<i>E. cloeziana</i>	85.9 (92)	14.0 (15)	100 (1)	0.0	100 (3)	0.0	80 (4)	20 (1)	0.0	0.0	0.0	0.0	116
<i>E. curtisii</i>	80.9 (89)	19.09 (21)	0.0	0.0	100 (3)	0.0	80 (4)	20 (1)	0.0	0.0	0.0	0.0	118
<i>E. deglupta</i>	78.8 (86)	21.1 (23)	0.0	0.0	100 (4)	0.0	66.6 (4)	33.3 (2)	0.0	0.0	0.0	0.0	119
<i>E. delegatensis</i>	83.7 (93)	16.2 (18)	0.0	0.0	100 (4)	0.0	100 (3)	0.0	0.0	0.0	0.0	0.0	118
<i>E. diversicolor</i>	83.8 (83)	16.1 (16)	0.0	0.0	100 (4)	0.0	75 (3)	25 (1)	0.0	0.0	100 (1)	0.0	108
<i>E. diversifolia</i>	85.0 (97)	14.9 (17)	0.0	0.0	100 (4)	0.0	50 (2)	50 (2)	0.0	0.0	0.0	0.0	122
<i>E. elata</i>	87.7 (93)	12.26 (16)	100 (1)	0.0	100 (3)	0.0	100 (4)	0.0	0.0	0.0	0.0	0.0	117
<i>E. erythrocorys</i>	81.0 (90)	18.9 (21)	100 (1)	0.0	100 (3)	0.0	40 (2)	60 (3)	0.0	0.0	0.0	0.0	120
<i>E. glóbulos</i>	78.3 (87)	21.6 (24)	100 (1)	0.0	100 (3)	0.0	100 (2)	0.0	0.0	0.0	0.0	0.0	117
<i>E. grandis</i>	84.1 (92)	15.5 (17)	0.0	0.0	100 (4)	0.0	57.1 (4)	42.8 (3)	0.0	0.0	0.0	0.0	120
<i>E. guilfoylei</i>	84.1 (90)	15.8 (17)	0.0	0.0	100 (3)	0.0	60 (3)	40 (2)	0.0	0.0	0.0	0.0	115
<i>E. marginata</i>	86.3 (95)	13.6 (15)	100 (2)	0.0	100 (2)	0.0	83.3 (5)	16.6 (1)	0.0	0.0	0.0	0.0	120
<i>E. melliodora</i>	80.6 (50)	19.3 (12)	0.0	0.0	100 (4)	0.0	40 (2)	60 (3)	0.0	0.0	0.0	0.0	71
<i>E. microcorys</i>	82 (82)	18 (18)	0.0	0.0	100 (3)	0.0	50 (3)	50 (3)	0.0	0.0	0.0	0.0	109
<i>E. nitens</i>	58 (57)	42 (42)	0.0	0.0	66.6 (3)	0.0	66.6 (3)	33.3 (3)	0.0	0.0	0.0	0.0	108
<i>E. obliqua</i>	87.6 (71)	12.3 (10)	0.0	0.0	75 (6)	25 (2)	75 (3)	25 (1)	0.0	0.0	0.0	0.0	93
<i>E. patens</i>	88 (66)	12 (9)	100 (1)	0.0	66.6 (2)	33.3 (1)	100 (5)	0.0	0.0	0.0	0.0	0.0	84
<i>E. polybractea</i>	84.2 (64)	15.7 (12)	100 (1)	0.0	75 (3)	25 (1)	71.4 (5)	28.5 (2)	0.0	0.0	0.0	0.0	88
<i>E. radiata</i>	83.6 (87)	16.3 (17)	0.0	0.0	100 (4)	0.0	50 (1)	51 (1)	0.0	0.0	0.0	0.0	110
<i>E. regnans</i>	77.6 (87)	22.3 (25)	100 (1)	0.0	100 (3)	0.0	71.4 (5)	28.5 (2)	0.0	0.0	0.0	0.0	123
<i>E. saligna</i>	86.9 (93)	13.8 (14)	100 (1)	0.0	100 (5)	0.0	83.3 (5)	16.6 (1)	0.0	0.0	0.0	0.0	119
<i>E. salmonophloia</i>	89.1 (98)	10.9 (12)	100 (2)	0.0	100 (3)	0.0	75 (3)	25 (1)	0.0	0.0	0.0	0.0	119
<i>E. sieberi</i>	92.5 (100)	7.4 (8)	100 (1)	0.0	100 (3)	0.0	100 (4)	0.0	0.0	0.0	0.0	0.0	116
<i>E. spathulata</i>	86.2 (94)	13.7 (15)	0.0	0.0	100 (1)	0.0	57.1 (4)	42.8 (3)	0.0	0.0	0.0	0.0	117
<i>E. torquata</i>	84.6 (99)	15.3 (18)	0.0	0.0	100 (4)	0.0	85.7 (6)	14.2 (1)	0.0	0.0	0.0	0.0	128
<i>E. umbra</i>	81 (81)	19 (19)	100 (1)	0.0	100 (4)	0.0	60 (3)	40 (2)	0.0	0.0	0.0	0.0	110
<i>E. verrucata</i>	82.3 (84)	17.6 (18)	100 (1)	0.0	100 (3)	0.0	33.3 (1)	66.6 (2)	0.0	0.0	0.0	0.0	109

of cpSSRs in *Eucalyptus* species;; therefore, we provide a general comparison between our results and published studies on other plant species. For example, Vieira et al. (2015) analyzed the occurrence, type, and distribution of cpSSRs for 20 Bambuseae species and their results showed an average total of 141.8 cpSSRs, with a predominance of mono- and dinucleotide repeats. Similarly, Vieira et al. (2016a), studying *Retrophyllum piresii* Silba C.N. Page, reported that 94.5% of total cpSSRs were mono- and dinucleotides. Furthermore, George et al. (2015), studying the abundance and distribution of SSRs in 164 sequenced plastomes from a wide range of plants, demonstrate that plant species predominantly present mononucleotide repeats, as observed herein for *Eucalyptus* species.

Chloroplasts are well understood to originate from endosymbiosis between cyanobacteria and higher plants (McFadden 2001, Raven and Allen 2003). The theory of endosymbiosis argues that cyanobacteria, also called blue-green algae, are the ancestors of chloroplasts, despite the fact that cyanobacteria are prokaryotes (Clegg et al. 1994). For the studied *Eucalyptus* species, most mononucleotide cpSSRs were found in non-coding regions of cpDNA, with the exception of *E. nitens* and *E. marginata* which present a more balanced proportion of mononucleotide microsatellites in coding and non-coding regions (Table 2). Gandhi et al. (2010) evaluated cpSSR dynamics in 12 species of the Brassicaceae family using data collected from GenBank. Their results revealed that, although 51% of cpDNA occurs in coding regions, the majority of the total number of SSRs was in non-coding regions that contain twice the number of SSRs than those in coding cpDNA regions.

Herein, dinucleotide microsatellites were found in cpDNA of 15 species, all in non-coding regions (Table 2). Tambarussi et al. (2009), Gandhi et al. (2010) and Melotto-Passarin et al. (2011) observed similar results for Solanaceae, Brassicaceae and Poaceae families, respectively. We also found that penta- and hexanucleotide microsatellites occur predominantly in non-coding regions and these two motifs were the least frequent types of microsatellites for all studied *Eucalyptus* species. Penta- and hexanucleotide cpSSRs were observed only in *E. baxteri* and *E. diversicolor*, respectively (Table 2), all in non-coding regions. Similar to the results presented herein, penta- and hexanucleotide repeats occurred at a low frequency in Bambuseae species, but in coding sequences (Vieira et al. 2015). For cpDNA of *R. piresii*, Vieira et al. (2016a) found no penta- or hexanucleotide cpSSRs, which is consistent with the results found for most of the studied *Eucalyptus* species.

Sixty primer pairs were developed and only 20% (12) of them occur in genic regions. All primers virtually amplified products around the expected size and displayed interspecific polymorphism. According to Sumathi and Yasodha (2014), until 2014 only 35 cpSSRs had been identified for different species of *Eucalyptus*. Therefore, our results indicate a wide range of regions in cpDNA that have yet to be explored (Doorduyn et al. 2011). As cpDNA markers are nonrecombinant, uniparentally inherited, and present low rates of mutation in the plastome sequence (Provan et al. 2001, Vieira et al. 2016b), they can be used in phylogenetic analyses and breeding and conservation programs for this genus.

Several studies have indicated that chloroplast genomes and cpSSRs have enormous potential (Zhang et al. 2017) and they are an important tool for plant biologists and breeders in assessing genetic diversity (Doorduyn et al. 2011), spatial genetic structure (Islam et al. 2015), evolutionary history, and hybridization in native and improved species (Ebert and Peakall 2009, Doorduyn et al. 2011). Thus, our analysis provides a rich database for researchers that can be used to study these *Eucalyptus* species across a wide range of scientific disciplines.

## ACKNOWLEDGEMENTS

The authors wish to thank CNPq, Fundação Arauácia and CAPES for scholarships attributed to the first, second and third author, respectively. The authors are very grateful to the anonymous referees for their important suggestions in the data analysis. We also thank Dr. Evelyn R. Nimmo for editing the English of the manuscript.

## DATA ARCHIVING STATEMENT

All cpSSRs are provided as additional files.

## REFERENCES

- Clegg MT, Gaut BS, Learn Jr BH and Morton BR (1994) Rates and patterns of 643 chloroplast DNA evolution. **Proceedings of the National Academy of Sciences** **91**: 6795-6801.
- Delgado P, Salas-Lizana R, Vázquez-Lobo A, Wegier A, Anzidei M, Alvarez Buylla ER, Vendramin GG and Piñero D (2007) Introgressive hybridization in *Pinus montezumae* Lamb and *Pinus pseudostrobus* Lindl. (Pinaceae): Morphological and molecular (cpSSR) evidence. **Journal of Plant Science** **168**: 861-875.
- Doorduyn L, Gravendeel B, Lammers Y, Ariyurek Y, Chin-A-Woeng T and Vrieling K (2011) The complete chloroplast genome of 17 individuals of pest species *Jacobaea vulgaris*: SNPs, microsatellites and barcoding markers for population and phylogenetic studies. **DNA Research** **18**: 93-105.
- Ebert D and Peakall R (2009) Chloroplast simple sequence repeats (cpSSRs): technical resources and recommendations for expanding cpSSR discovery and applications to a wide array of plant species. **Molecular Ecology Resources** **9**: 673-690.
- Eldridge K, Davidson J, Harwood C and Van Wyk G (1993) **Eucalypt domestication and breeding**. Clarendon Press, Oxford, 312p.
- FAO (2006) **Global planted forests thematic study: results and analysis**. FAO, Rome, 178p.
- FAO (2016) **Global forest resources assessment 2015**. 2<sup>nd</sup> edn, FAO, Rome, 54p.
- Freeman JS, Jackson HD, Steane DA, McKinnon GE, Dutkowski GW, Potts BM and Vaillancourt RE (2001) Chloroplast DNA phylogeography of *Eucalyptus globulus*. **Australian Journal of Botany** **49**: 585-596.
- Fuchs MCP, Tambarussi EV, Lourenção JC, Nogueira LM, Bortoloto TM, González ER, Oda S and Marino CL (2015) Molecular marker associated with a deleterious recessive anomaly in *Eucalyptus grandis* seedlings. **Annals of Forest Science** **72**: 1043-1052.
- Gandhi SG, Awasthi P and Bedi YS (2010) Analysis of SSR dynamics in chloroplast genomes of Brassicaceae family. **Bioinformation** **5**: 16-20.
- George B, Bhatt BS, Awasthi M, George B and Singh AK (2015) Comparative analysis of microsatellites in chloroplast genomes of lower and higher plants. **Current Genetics** **61**: 665-677.
- Islam MS, Lian C, Kameyama N and Hogetsu T (2015) Analysis of the mating system, reproductive characteristics, and spatial genetic structure in a natural mangrove tree (*Bruguiera gymnorhiza*) population at its northern biogeographic limit in the southern Japanese archipelago. **Journal of Forest Research** **20**: 293-300.
- Kalendar R, Lee D and Schulman AH (2016) FastPCR software for PCR primer and probe design and repeat search. **Genes, Genomes and Genomics** **3**. Available at <www.biocenter.helsinki.fi/bi/Programs/fastpccr.htm>. Accessed on Feb 16, 2016.
- Kirst M, Cordeiro CM, Rezende GDS and Grattapaglia D (2005) Power of microsatellite markers for fingerprinting and parentage analysis in *Eucalyptus grandis* breeding population. **Journal of Heredity** **96**: 1-6.
- Latouche-Hallé C, Ramboer A, Bandou E, Caron H and Kremer A (2003) Nuclear and chloroplast genetic structure indicate fine-scale spatial dynamics in a Neotropical tree population. **Heredity** **91**: 181-190.
- Mahajan R and Gupta P (2012) Molecular markers: their use in tree improvement. **Journal of Forest Science** **58**: 137-144.
- McFadden GI (2001) Chloroplast origin and integration. **Plant Physiology** **125**: 50-53.
- McKinnon GE, Smith JJ and Potts BM (2010) Recurrent nuclear DNA introgression accompanies chloroplast DNA exchange between two eucalypt species. **Molecular Ecology** **19**: 1367-1380.
- McKinnon GE, Vaillancourt RE, Jackson HD and Potts BM (2001) Chloroplast sharing in the Tasmanian eucalypts. **Evolution** **55**: 703-711.
- Melotto-Passarini DM, Tambarussi EV, Dressano K, De Martin VF and Carrer H (2011) Characterization of chloroplast DNA microsatellites from *Saccharum* spp. and related species. **Genetics and Molecular Research** **10**: 2024-2033.
- Myburg AA, Grattapaglia D, Tuskan GA, Hellsten U, Hayes RD, Jane Grimwood J, Jenkins J, Lindquist E, Tice H, Bauer D, Goodstein DM, Dubchak I, Poliakov A, Mizrachi E, Kullán ARK, Hussey SG, Pinard D, Merwe K, Singh P, Jaarsveld I, Silva-Junior OB, Togawa RC, Pappas MR, Faria DA, Sansaloni CP, Petroli CD, Yang X, Ranjan P, Tschaplinski TJ, Ye C-Y, Li T, Sterck L, Vanneste K, Murat F, Soler M, Clemente HS, Saidi N, Cassan-Wang H, Dunand C, Hefer CA, Bornberg-Bauer E, Kersting AR, Vining K, Amarasinghe V, Ranik M, Naithani S, Elser J, Boyd AE, Liston A, Spatafora JW, Dharmawardhana P, Raja R, Sullivan C, Romanel E, Alves-Ferreira M, Külheim C, Foley W, Carocha V, Paiva J, Kudrna D, Brommonschenkel SH, Pasquali G, Byrne M, Rigault P, Tibbits J, Spokevicius A, Jones RC, Steane DA, Vaillancourt RE, Potts BM, Joubert F, Barry K, Pappas GJ, Strauss SH, Jaiswal P, Grima-Pettenati J, Salse J, Peer YV, Rokhsar DS and Schmutz J (2014) The genome of *Eucalyptus grandis*. **Nature** **510**: 356-362.
- Provan J, Powell W and Hollingsworth PM (2001) Chloroplast microsatellites: new tools for studies in plant ecology and evolution. **Trends in Ecology & Evolution** **16**: 142-147.
- Raven JA and Allen JF (2003) Genomics and chloroplast evolution: what did cyanobacteria do for plants? **Genome Biology** **4**: 209.
- Sands R (2005) **Forestry in a global context**. CABI Publishing, United Kingdom, 233p.
- Shaw J, Lickey EB, Beck JT, Farmer SB, Liu W, Miller J, Siripun KC, Winder CT, Schilling EE and Small RL (2005) The torroise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. **American Journal of Botany** **92**: 142-166.
- Shaw J, Lickey EB, Schilling EE and Small RL (2007) Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III analysis. **American Journal of Botany** **94**: 275-288.
- Smith S, Hughes J and Wardell-Johnson G (2003) High population differentiation and extensive clonality in a rare mallee eucalypt: *Eucalyptus curtisii*. **Conservation Genetics** **4**: 289-300.

- Steane DA, Byrne M, Vaillancourt RE and Potts BM (1998) Chloroplast DNA polymorphism signals complex interspecific interactions in *Eucalyptus* (Myrtaceae). **Australian Systematic Botany** **11**: 25-40.
- Steane DA, Jones RC and Vaillancourt RE (2005) A set of chloroplast microsatellite primers for *Eucalyptus* (Myrtaceae). **Molecular Ecology Notes** **5**: 538-541.
- Steane DA, West AK, Potts BM, Ovenden JR and Reid JB (1991) Restriction fragment length polymorphisms in chloroplast DNA from six species of *Eucalyptus*. **Australian Journal of Botany** **39**: 399-414.
- Stothard P (2000) The Sequence Manipulation Suite: JavaScript programs for analyzing and formatting protein and DNA sequences. **Biotechniques** **28**: 1102-1104.
- Sumathi M and Yasodha R (2014) Micorsatellite resources of *Eucalyptus*: current status and future perspectives. **Botanical Studies** **55**: 73.
- Tambarussi EV, Melotto-Passarin DM, Dressano K, Guidetti-Gonzalez S, Brigati JB, Jesus FA, Barbosa, AL and Carrer H (2009) In silico analysis of Simple Sequence Repeats from chloroplast genomes of Solanaceae species. **Crop Breeding and Applied Biotechnology** **9**: 342-352.
- Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M and Rozen SG (2012) Primer3 - new capabilities and interfaces. **Nucleic Acids Research** **40**: e115.
- Vieira LN, Anjos KG, Faoro H, Fraga HPF, Greco TM, Pedrosa FO, Souza EM, Rogalski M, Souza RF and Guerra MP (2015) Phylogenetic inference and SSR characterization of tropical woody bamboos tribe Bambuseae (Poaceae: Bambusoideae) based on complete plastid genome sequences. **Current Genetics** **62**: 443-453.
- Vieira LN, Rogalski M, Faoro H, Fraga HPF, Anjos KG, Picchi GFA, Nodari RO, Pedrosa FO, Souza EM and Guerra MP (2016a) The plastome sequence of the endemic Amazonian conifer, *Retrophyllum piresii* (Silba) C.N.Page, reveals different recombination events and plastome isoforms. **Tree Genetics & Genomes** **12**: 10.
- Vieira MLCV, Santini L, Diniz AL and Munhoz CF (2016b) Microsatellite markers: what they mean and why they are so useful. **Genetics & Molecular Biology** **39**: 312-328.
- Weising K and Gardner RC (1999) A set of conserved PCR primers for the analysis of simple sequence repeat polymorphisms in chloroplast genomes of dicotyledonous angiosperms. **Genome** **42**: 9-19.
- Whitlock S, Steane SA, Vaillancourt RE and Potts BM (2003) Molecular evidence shows that the tropical boxes (*Eucalyptus* subgenus *Minutifructus*) are over-ranked. **Transactions of Royal Society of South Australian** **127**: 27-32.
- Zhang H, Hall N, McElroy JS, Lowe EK and Goertzen LR (2017) Complete plastid genome sequence of goosegrass (*Eleusine indica*) and comparison with other Poaceae. **Gene** **5**: 36-43.