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Supplementary material

Whole-chloroplast analysis as an approach for fine-tuning the preservation of a highly charismatic but critically endangered species, *Wollemia nobilis* (Araucariaceae)

Abigail Greenfield[A,B], *Hannah McPherson*[B,E], *Tony Auld*[C], *Sven Delaney*[A], *Catherine A. Offord*[D], *Marlien van der Merwe*[B], *Jia-Yee S. Yap*[B] *and Maurizio Rossetto*[B]

[A]School of Biotechnology and Biomolecular Biosciences, University of New South Wales, Kensington, NSW 2033, Australia.

[B]National Herbarium of NSW, Royal Botanic Garden Sydney, Mrs Macquaries Road, Sydney, NSW 2000, Australia.

[C]NSW Office of Environment and Heritage, 43 Bridge Street, Hurstville, NSW 2220, Australia.

[D]The Australian PlantBank, Australian Botanic Garden, Narellan Road, Mount Annan, NSW, 2567, Australia.

[E]Corresponding author. Email: hannah.mcpherson@rbgsyd.nsw.gov.au

DNA extraction method

120 mg of each leaf sample was removed from -80 °C, cut into 2 mm pieces and placed into 100 µl of CTAB buffer (2% CTAB, 1.4M NaCl, 0.2% 2-mercaptoethanol, 20 mM EDTA, 100 mM Tris-HCL adjusted to pH 8). Tissue was then disrupted using a TissueLyser (Qiagen) at 30 mHz for 1 min. 100 µl of CTAB buffer was added and the mechanical disruption was repeated. The disrupted tissue was split equally into three 2 ml microfuge tubes and total volume was made up to 400 µl in each tube with CTAB buffer. The samples were incubated at 60 °C for 45 min with periodic inversion.

After incubation one volume of chloroform-isoamyl alcohol (24:1) was added to each sample and mixed by inversion until homogeneous. The samples were centrifuged at 3000 x g for 10 min. The upper aqueous phase was kept and DNA was precipitated by the addition of two thirds the total volume of isopropanol and incubation overnight at -20 °C.

The DNA was then pelleted by centrifugation at 1000 x g for 1 to 10 min until a solid pellet was formed. The pellet was washed with 1 ml wash buffer (76% ethanol, 10 mM ammonium acetate) and incubated at room temperature for at least 20 min before centrifugation at 1000 x g. The supernatant was discarded and 400 µl 1 X TE was added. The samples were left at room temperature for 2 to 3 hrs or 4 °C overnight to allow re-suspension. RNase A was added to a final concentration of 10 µg/ml and the reaction was incubated for 10 min at room temperature. One fifteenth volume of 20% (w/v) sodium dodecyl sulfate (SDS) and one third volume of 5M potassium acetate were then added and the reaction was mixed gently and placed on ice for 30 min. Samples were then centrifuged at maximum speed and the supernatant was retained and purified further by phenol/chloroform extraction and ethanol precipitation before re-suspension in 60 µl 10 mM Tris-HCL (pH 8).

Table S1 Primers designed for Sanger sequencing using Primer3 on Geneious Pro v5.5.6 (Biomatters Ltd.). PCRs followed the protocol of Ebert and Peakall (2009) unless indicated otherwise. Sequences are available through DRYAD (doi: xxxxx) at http://datadryad.org/.

No.	Primer pair name	Variant position on chloroplast genome	Start	End	Target size	Cellular compartment	Cycling conditions	PCR Reaction successful	SNP confirmed
1	Wn14004RBG	14004	13830	14661	832	Chloroplast	Ebert and Peakall (2009)	Yes	Yes
2	Wn35316RBG	35316	35078	35925	848	Chloroplast	Ebert and Peakall (2009)	Yes	Yes
3	Wn48513RBG	48513	48103	48627	525	Chloroplast	Ebert and Peakall (2009)	Yes	Yes
4	Wn119691RBG	119691	119420	119940	521	Chloroplast	Ebert and Peakall (2009)	Yes	Yes
5	Wn144941RBG	144941	144860	145275	416	Chloroplast	Ebert and Peakall (2009)	Yes	Yes
6	Wn10712RBG	10712	10042	10858	817	Chloroplast	*	Yes	Yes
7	Wn141323RBG	141323	141166	141398	233	Chloroplast	Ebert and Peakall (2009)	Yes	No
8	Wn140921RBG	140921	141166	141398	829	Chloroplast	Ebert and Peakall (2009)	Yes	No
9	Wn105863RBG	105863	106466	106710	244	Chloroplast	Ebert and Peakall (2009)	Yes	No
10	Wn142576RBG	142576	142301	142927	627	Chloroplast	Ebert and Peakall (2009)	Yes	No
11	Wn144199RBG	144199	143754	144436	683	Chloroplast	Ebert and Peakall (2009)	Yes	No

*Reaction conditions were initial denaturation at 95°C for 10 minutes, the following for 30 cycles: denaturation at 95°C for 30s, annealing at 55°C for 30s for primer set 10712, extension was at 72°C for 30s and final extension was at 72°C for 10 minutes.

Table S2 Summary of reads (from all libraries combined) mapped to the chloroplast reference sequence

Total no. of trimmed reads mapped to reference	817129
% trimmed reads mapped to chloroplast reference sequence	3.33%

Table S3 Trim data from Illumina shotgun sequencing

Library	**No. of reads**	**Avg. length**	**No. of reads after trim**	**% Kept after trimmed**	**Avg. length after trim**
1	7657422	252.6	6814929	89.00%	256.7
2	5585082	257.5	4926740	88.21%	263.3
3	6576520	267.1	6020013	91.54%	265.9
4	7878506	248.1	6780243	86.06%	258.2
Pollen	9096656	254.8	8039005	88.37%	260.4
Total no. of reads	27697530		24541925		261