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Supplementary material

Morphological, phytochemical and molecular analyses define species limits in *Eucalyptus magnificata* (Myrtaceae) and lead to the discovery of a new rare species

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Methods – genotyping-by-sequencing protocols

Genomic DNA extraction

Field-collected leaves were frozen at –32°C, then freeze-dried in a Dynavac freeze-drier Model FD12 (Dynapumps, Belgrave, WA, Australia). Sections of leaf weighing 25–30 mg and showing no visible signs of pathogen or insect damage were placed into 2-mL microcentrifuge tubes (Sigma–Aldrich, Sydney, NSW, Australia) with a stainless-steel ball bearing. Tubes were immersed in liquid nitrogen for 60 s and reduced to fine powder by two 1-min runs in a Qiagen TissueLyser 2 (Qiagen Pty Ltd, Hilden, Germany), turning the sample caddy 180° between runs to ensure even homogenisation. Genomic DNA was extracted using the Stratec Invisorb Spin Plant Mini Kit (Stratec Biomedical AG, Berlin, Germany) following manufacturer's instructions, with digestion time extended to 2 h.

GBS library preparation

Each of 191 DNA samples was diluted to 200 ng of gDNA in 13 µL. These, plus a negative control (blank) sample, were transferred onto two 96-well plates pre-loaded with 3 µL of PstI barcode adaptors. A single-enzyme digestion reaction, based on a modified protocol from Poland *et al.* (2012), comprising 1.8 µL of H_2O, 2 µL of NEB 10× Cutsmart buffer (New England Biolabs (NEB), Ipswich, MA, USA), 0.2 µL of NEB *Pst*I–HF was added to each well and incubated at 37°C for 2 h, and then held at 4°C.

Immediately after digestion, a ligation reaction was added to each well comprising 21.5 µL of H_2O, 2.5 µL of NEB 10× Cutsmart buffer, 5 µL of 10 mM ATP, 1 µL of NEB T4 DNA ligase. The reaction was incubated on a GenePro thermal cycler (Hangzhou Bioer Technology Company Ltd, Tokyo, Japan) for three cycles of 30 min at 16°C and 2 min at 37°C (2 min), then one cycle of 80°C (30 min) before being kept at 4°C. Plates were stored overnight at –20°C.

Purification

A preliminary assay determined that Sera-Pure beads (Rohland and Reich 2012) should be used at 1.6× concentration (80 µL) to select DNA fragments 300 bp in size. The beads were added to each sample, incubated for 5 min and placed on a magnet to form a pellet. Supernatant was discarded and

the pellet washed three times with 80% ethanol, and once with 100 % ethanol. The pellet was air-dried at room temperature. Purified library fragments were eluted using 25 µL of 10 mM Tris–HCl.

Polymerase chain reaction

The polymerase chain reaction (PCR) was prepared by combining 0.5 µL of dH_2O, 12.5 µL of Bioline MyTaq HotStart (Bioline (Aust) Pty Ltd, Sydney, NSW, Australia) 2×, 1 µL of Primer F (10 µM), 1uL Primer R (10 µM) and 10 µL of DNA template, to make up a total reaction volume of 25 µL. The reaction was run over 24 cycles on the Genepro thermal cycler with temperature and time settings as in Table S1.

Table S1. Polymerase chain reaction thermocycler settings

Denaturation, annealing and extension steps were repeated 24 times

Temperature (°C)	Time (s)	Action
95	60	Initial incubation
95	30	Denaturation
65	30	Annealing
72	30	Extension
72	300	
10	hold	

Size selection and purification

PCR products were quantified on a Qubit Fluorometer 3.0 (ThermoFisher Scientific Inc., Melbourne, Vic., Australia) using a High Sensitivity Assay Kit (ThermoFisher Scientific Inc.). A multiplexed library was prepared by combining 25 ng of DNA per sample into a single 1.5-mL microcentrifuge tube.

Multiplex samples were run in five 25-µL lanes on a 1.5% agarose gel and bands 350–550 bp in size were excised. Gel clean-up was split between two columns and purified with Bioline Gel Purification Kit (Bioline (Aust) Pty Ltd), then eluted in 10 mM Tris–HCl to produce a 1.75 ng μL^{-1} sample.

Sequencing

The GBS library was sequenced at the Australian Cancer Research Foundation Biomolecular Research Facility at the John Curtin School of Medical Research, Canberra, using an Illumina NextSeq-500 (Illumina Australia, Melbourne, Vic., Australia) desktop sequencing system. Sequence length was 75 bp, with mid-output flow-cell density, and paired-end reads. Data were transferred to the UNE Linux Ubuntu 14.04.3 LTS (GNU/Linux 3.19.0-25-generic x86_64) from a cloud server.

Sequence alignment, filtering and SNP scoring

The sequence data were demultiplexed by matching the barcodes with the sample identification number using GBSX (ver. 2, https://github.com/GenomicsCoreLeuven/GBSX, accessed 17 January 2019; Herten *et al.* 2015). Low-quality reads and those shorter than 50 bp in length after trimming were removed. One mismatch was allowed in each barcode, and one in the restriction-enzyme cut site. The

restriction-enzyme cut site was retained, but both reads were trimmed when adaptor contamination was detected. One mismatch was allowed when detecting adaptor contamination, which was recognised using the sequence 'AGATCGGAAGAGCG'. Sequences were aligned with Burrows-Wheeler Aligner (ver. 0.7.12, http://bio-bwa.sourceforge.net/, accessed 17 January 2019; Li and Durbin 2009) to the *E. grandis* reference genome (BRASUZ1 genome assembly ver. 2.0 (Egrandis_297_v2.0)) scaffolds (Myburg *et al.* 2014) downloaded from Phytozome (https://phytozome.jgi.doe.gov/, accessed 17 January 2019) using BWA (ver, 0.7.12; Li and Durbin 2009).

Following alignment with bwa-mem (Li 2013), the Genome Analysis Toolkit (GATK) haplotype caller detected sequence variants. Filtering was performed using the *VariantAnnotation* package (Obenchain *et al.* 2014) in *R* (ver. 0.99.489, R Foundation for Statistical Computing, Vienna, Austria, see http://www.R-project.org/ , accessed 31 January 2018) through the RStudio Server (ver. 0.99.489) interface. Filtering excluded low-quality genotypes (GQ<20) and retained the 160 samples with the greatest number of scored genotypes (aside from the blank). Further filtering excluded invariant sites, indels, variants with more than two alleles, samples scored for <0.7 of the remaining sites and singleton variants (where the minor allele was represented by only one sample, either heterozygous or homozygous for the minor allele). The *basic.stats* function in the *hierfstat* package (ver. 0.04-22, J. Goudet and T. Jombart, see https://CRAN.R-project.org/package=hierfstat, accessed 17 January 2019), assuming putative taxa as populations, removed sites with global $F_{IS} < -0.4$ or $F_{IS} > 0.4$. The removal of SNPs with unusually high-observed heterozygosity excluded paralogs and the removal of those with unusually low observed heterozygosity relative to homozygosity excluded loci with high frequencies of null alleles. On the basis of these methods, 5493 variants from 144 individuals were used in further exploratory analyses. Random sampling was used to assemble two thinned data sets ensuring at least 500-bp separation between SNPs to reduce the effects of linkage disequilibrium. Results from only one thinned dataset are shown, as the two sets of results were consistent.

Table S2. Comprehensive list of gatherings

Specimen code, type of analysis, collector number, entity and location for all gatherings used in this study. Analyses: M, morphology; P, phytochemistry; GBS, genotyping by sequencing; TLC, T. L. Collins; NSW, New South Wales; Qld, Queensland; OWRNP, Oxley Wild Rivers National Park

Specimen code	Analysis M	P	GBS	Voucher	Entity	Location
B1	✓	✓	✓	TLC 832	*E. baueriana*	St Marys, Sydney
B2		✓	✓	TLC 833	*E. baueriana*	St Marys, Sydney
B3		✓	✓	TLC 834	*E. baueriana*	St Marys, Sydney
B4		✓	✓	TLC 835	*E. baueriana*	St Marys, Sydney
B5		✓	✓	TLC 836	*E. baueriana*	St Marys, Sydney
B6		✓		TLC 837	*E. baueriana*	St Marys, Sydney
B7		✓	✓	TLC 838	*E. baueriana*	St Marys, Sydney
B8		✓	✓	TLC 839	*E. baueriana*	St Marys, Sydney
B9		✓	✓	TLC 840	*E. baueriana*	St Marys, Sydney
B10	✓	✓	✓	TLC 841	*E. baueriana*	St Marys, Sydney
B11	✓	✓		TLC 842	*E. baueriana*	St Marys, Sydney
B12		✓		TLC 843	*E. baueriana*	Panania, Sydney
B13		✓		TLC 844	*E. baueriana*	Panania, Sydney
B14		✓	✓	TLC 845	*E. baueriana*	Panania, Sydney
B15		✓	✓	TLC 846	*E. baueriana*	Panania, Sydney
B16		✓		TLC 847	*E. baueriana*	Panania, Sydney
B17		✓	✓	TLC 848	*E. baueriana*	Panania, Sydney
B18		✓	✓	TLC 849	*E. baueriana*	Panania, Sydney
B19		✓		TLC 850	*E. baueriana*	Bendalong, NSW
B20		✓	✓	TLC 851	*E. baueriana*	Bendalong, NSW
B21	✓	✓	✓	TLC 852	*E. baueriana*	Bendalong, NSW
B22	✓	✓	✓	TLC 853	*E. baueriana*	Bendalong, NSW
B23	✓	✓		TLC 854	*E. baueriana*	Bendalong, NSW
B24		✓	✓	TLC 855	*E. baueriana*	Bendalong, NSW

Specimen code	Analysis M	P	GBS	Voucher	Entity	Location
B25		✓	✓	TLC 856	*E. baueriana*	Bendalong, NSW
B26		✓		J.Linney s.n.	*E. baueriana*	Moruya, NSW
C1	✓	✓	✓	TLC 715	*E. conica*	Stanthorpe, Qld
C2		✓	✓	TLC 716	*E. conica*	Stanthorpe, Qld
C3		✓	✓	TLC 719	*E. conica*	Tenterfield, NSW
C4		✓	✓	TLC 720	*E. conica*	Tenterfield, NSW
C5		✓	✓	TLC 721	*E. conica*	Tenterfield, NSW
C6	✓	✓		TLC 724	*E. conica*	Dundee, NSW
C7		✓	✓	TLC 725	*E. conica*	Dundee, NSW
C8	✓	✓	✓	TLC 726	*E. conica*	Abington Creek, NSW
C9	✓	✓	✓	TLC 741	*E. conica*	Bingara, NSW
C10	✓	✓	✓	TLC 768	*E. conica*	Cherry Gully, Qld
C11	✓	✓	✓	TLC 769	*E. conica*	Cherry Gully, Qld
C12		✓		TLC 770	*E. conica*	Cherry Gully, Qld
C13		✓	✓	TLC 772	*E. conica*	Cherry Gully, Qld
C14		✓		TLC 773	*E. conica*	Cherry Gully, Qld
C15	✓	✓	✓	TLC 775	*E. conica*	Inglewood, Qld
C16		✓	✓	TLC 776	*E. conica*	Inglewood, Qld
C17		✓	✓	TLC 780	*E. conica*	Inglewood, Qld
C18	✓	✓	✓	TLC 804	*E. conica*	Tenterfield, NSW
C19	✓	✓	✓	TLC 805	*E. conica*	Tenterfield, NSW
C20		✓	✓	TLC 806	*E. conica*	Tenterfield, NSW
D1		✓	✓	TLC 785	*E.* sp. Dalveen	Dalveen, Qld
D2	✓	✓	✓	TLC 786	*E.* sp. Dalveen	Dalveen, Qld
D3		✓	✓	TLC 787	*E.* sp. Dalveen	Dalveen, Qld
D4		✓		TLC 792	*E.* sp. Dalveen	Dalveen, Qld
D5		✓	✓	TLC 793	*E.* sp. Dalveen	Dalveen, Qld
D6	✓	✓	✓	TLC 794	*E.* sp. Dalveen	Dalveen, Qld
D7		✓	✓	TLC 795	*E.* sp. Dalveen	Dalveen, Qld

Specimen code	Analysis M	P	GBS	Voucher	Entity	Location
D8	✓	✓	✓	TLC 796	*E.* sp. Dalveen	Dalveen, Qld
D9		✓	✓	TLC 797	*E.* sp. Dalveen	Dalveen, Qld
D10		✓	✓	TLC 798	*E.* sp. Dalveen	Dalveen, Qld
D11		✓	✓	TLC 799	*E.* sp. Dalveen	Dalveen, Qld
D12		✓	✓	TLC 800	*E.* sp. Dalveen	Dalveen, Qld
D13	✓	✓	✓	TLC 801	*E.* sp. Dalveen	Dalveen, Qld
D14	✓	✓	✓	TLC 789	*E.* sp. Dalveen	Dalveen, Qld
M1		✓	✓	TLC 714	*E. magnificata*	Hillgrove, NSW
M2	✓	✓		TLC 730	*E. magnificata*	Hillgrove, NSW
M3		✓	✓	TLC 731	*E. magnificata*	Hillgrove, NSW
M4		✓	✓	TLC 732	*E. magnificata*	Hillgrove, NSW
M5		✓	✓	TLC 733	*E. magnificata*	Hillgrove, NSW
M6		✓	✓	TLC 734	*E. magnificata*	Hillgrove, NSW
M7		✓	✓	TLC 750	*E. magnificata*	Enmore, NSW
M8		✓		TLC 751	*E. magnificata*	Enmore, NSW
M9		✓	✓	TLC 752	*E. magnificata*	Enmore, NSW
M10		✓	✓	TLC 753	*E. magnificata*	Enmore, NSW
M11		✓	✓	TLC 754	*E. magnificata*	Enmore, NSW
M12		✓	✓	TLC 755	*E. magnificata*	Enmore, NSW
M13		✓	✓	TLC 756	*E. magnificata*	Enmore, NSW
M14	✓	✓		TLC 757	*E. magnificata*	Enmore, NSW
M15		✓	✓	TLC 758	*E. magnificata*	Enmore, NSW
M16	✓	✓		TLC 760	*E. magnificata*	Metz Gorge, NSW
M17		✓	✓	TLC 761	*E. magnificata*	Metz Gorge, NSW
M18		✓		TLC 762	*E. magnificata*	Metz Gorge, NSW
M19		✓		TLC 763	*E. magnificata*	Metz Gorge, NSW
M20		✓		TLC 764	*E. magnificata*	Metz Gorge, NSW
M21		✓	✓	TLC 766	*E. magnificata*	Hillgrove, NSW
M22		✓	✓	TLC 767	*E. magnificata*	Hillgrove, NSW

Specimen code	Analysis M	P	GBS	Voucher	Entity	Location
M23	✓	✓	✓	TLC 807	*E. magnificata*	Hillgrove, NSW
M24		✓	✓	TLC 808	*E. magnificata*	Hillgrove, NSW
M25		✓	✓	TLC 809	*E. magnificata*	Hillgrove, NSW
M26		✓		TLC 810	*E. magnificata*	Hillgrove, NSW
M27		✓		TLC 811	*E. magnificata*	Hillgrove, NSW
M28		✓	✓	TLC 812	*E. magnificata*	Hillgrove, NSW
M29		✓	✓	TLC 813	*E. magnificata*	Long Point Road, NSW
M30		✓	✓	TLC 814	*E. magnificata*	Long Point Road, NSW
M31		✓	✓	TLC 815	*E. magnificata*	Long Point Road, NSW
M32		✓	✓	TLC 816	*E. magnificata*	Long Point Road, NSW
M33		✓		TLC 817	*E. magnificata*	Long Point Road, NSW
M34		✓		TLC 818	*E. magnificata*	Long Point Road, NSW
M35		✓	✓	TLC 819	*E. magnificata*	Long Point Road, NSW
M36		✓	✓	TLC 820	*E. magnificata*	Long Point Road, NSW
M37		✓	✓	TLC 821	*E. magnificata*	Long Point Road, NSW
M38		✓	✓	TLC 822	*E. magnificata*	Long Point Road, NSW
M39		✓	✓	TLC 823	*E. magnificata*	Long Point Road, NSW
M40		✓	✓	TLC 824	*E. magnificata*	Long Point Road, NSW
M41		✓		TLC 825	*E. magnificata*	Long Point Road, NSW
M42		✓	✓	TLC 826	*E. magnificata*	Long Point Road, NSW
M43	✓	✓		TLC 827	*E. magnificata*	Long Point Road, NSW
M44		✓		TLC 828	*E. magnificata*	Long Point Road, NSW
M45		✓		TLC 829	*E. magnificata*	Long Point Road, NSW
M46		✓		TLC 830	*E. magnificata*	Long Point Road, NSW
M47		✓	✓	TLC 831	*E. magnificata*	Long Point Road, NSW
M48		✓	✓	TLC 866	*E. magnificata*	Long Point Road, NSW
M49		✓	✓	TLC 868	*E. magnificata*	Long Point Road, NSW
M50		✓	✓	TLC 869	*E. magnificata*	Long Point Road, NSW
M51		✓		TLC 870	*E. magnificata*	Long Point Road, NSW

Specimen code	Analysis M	P	GBS	Voucher	Entity	Location
M52		✓		TLC 871	*E. magnificata*	Long Point Road, NSW
M53		✓		TLC 872	*E. magnificata*	Long Point Road, NSW
M54		✓		TLC 873	*E. magnificata*	Long Point Road, NSW
M55		✓		TLC 874	*E. magnificata*	Long Point Road, NSW
M56		✓	✓	TLC 875	*E. magnificata*	Long Point Road, NSW
M57		✓		TLC 876	*E. magnificata*	Long Point Road, NSW
M58		✓		TLC 877	*E. magnificata*	Long Point Road, NSW
M59		✓		TLC 878	*E. magnificata*	Long Point Road, NSW
M60		✓	✓	TLC 879	*E. magnificata*	Long Point Road, NSW
M61		✓	✓	TLC 880	*E. magnificata*	Long Point Road, NSW
M62		✓	✓	TLC 881	*E. magnificata*	Long Point Road, NSW
M63		✓	✓	TLC 882	*E. magnificata*	Long Point Road, NSW
M64		✓		TLC 883	*E. magnificata*	Long Point Road, NSW
M65		✓		TLC 884	*E. magnificata*	Long Point Road, NSW
M66		✓	✓	TLC 885	*E. magnificata*	Long Point Road, NSW
M67		✓		TLC 886	*E. magnificata*	Long Point Road, NSW
M68		✓		TLC 887	*E. magnificata*	Long Point Road, NSW
M69		✓	✓	TLC 888	*E. magnificata*	Long Point Road, NSW
M70		✓	✓	TLC 889	*E. magnificata*	Long Point Road, NSW
M71		✓	✓	TLC 890	*E. magnificata*	Long Point Road, NSW
M72		✓	✓	TLC 891	*E. magnificata*	Long Point Road, NSW
M73		✓	✓	TLC 892	*E. magnificata*	Long Point Road, NSW
M74		✓	✓	TLC 893	*E. magnificata*	Long Point Road, NSW
M75		✓	✓	TLC 894	*E. magnificata*	Long Point Road, NSW
M76		✓	✓	TLC 895	*E. magnificata*	Long Point Road, NSW
M77		✓	✓	TLC 896	*E. magnificata*	Long Point Road, NSW
M78		✓		TLC 897	*E. magnificata*	Long Point Road, NSW
M79		✓	✓	TLC 898	*E. magnificata*	Long Point Road, NSW
M80		✓	✓	TLC 899	*E. magnificata*	Hillgrove, NSW

Specimen code	Analysis M	P	GBS	Voucher	Entity	Location
M81		✓		TLC 900	*E. magnificata*	Hillgrove, NSW
M82		✓	✓	TLC 901	*E. magnificata*	Hillgrove, NSW
M83		✓		TLC 902	*E. magnificata*	Hillgrove, NSW
M84		✓	✓	TLC 903	*E. magnificata*	Hillgrove, NSW
M85		✓	✓	TLC 904	*E. magnificata*	Hillgrove, NSW
M86		✓	✓	TLC 905	*E. magnificata*	Hillgrove, NSW
M87		✓		TLC 906	*E. magnificata*	Hillgrove, NSW
M88	✓	✓	✓	TLC 907	*E. magnificata*	Hillgrove, NSW
M89		✓	✓	TLC 916	*E. magnificata*	McDirtys Lookout, OWRNP, NSW
M90		✓		TLC 917	*E. magnificata*	McDirtys Lookout, OWRNP, NSW
M91		✓	✓	TLC 918	*E. magnificata*	McDirtys Lookout, OWRNP, NSW
M92		✓	✓	TLC 939	*E. magnificata*	McDirtys Lookout, OWRNP, NSW
M93		✓	✓	TLC 940	*E. magnificata*	McDirtys Lookout, OWRNP, NSW
M94		✓		TLC 941	*E. magnificata*	McDirtys Lookout, OWRNP, NSW
M95		✓	✓	TLC 727	*E. magnificata*	Hillgrove, NSW
M96		✓	✓	TLC 728	*E. magnificata*	Hillgrove, NSW
O1		✓		TLC 737	*E.* sp. Oxley	Tabletop, NSW
O2	✓	✓	✓	TLC 739	*E.* sp. Oxley	Tabletop, NSW
O3	✓	✓	✓	TLC 740	*E.* sp. Oxley	Tabletop, NSW
O4		✓	✓	TLC 908	*E.* sp. Oxley	Tabletop, OWRNP, NSW
O5	✓	✓	✓	TLC 909	*E.* sp. Oxley	Tabletop, OWRNP, NSW
O6		✓		TLC 910	*E.* sp. Oxley	Tabletop, OWRNP, NSW
O7		✓	✓	TLC 911	*E.* sp. Oxley	Tabletop, OWRNP, NSW
O8	✓	✓		TLC 912	*E.* sp. Oxley	Tabletop, OWRNP, NSW
O9		✓	✓	TLC 913	*E.* sp. Oxley	Tabletop, OWRNP, NSW
O10		✓	✓	TLC 914	*E.* sp. Oxley	Cheyenne, NSW
O11		✓	✓	TLC 915	*E.* sp. Oxley	Cheyenne, NSW
O12	✓	✓		TLC 738-1	*E.* sp. Oxley	Tabletop, NSW
O13		✓	✓	TLC 738-2	*E.* sp. Oxley	Tabletop, NSW

Specimen code	Analysis M	P	GBS	Voucher	Entity	Location
O14		✓	✓	TLC 738-3	*E.* sp. Oxley	Tabletop, NSW
O15		✓	✓	TLC 738-4	*E.* sp. Oxley	Tabletop, NSW
O16	✓	✓	✓	TLC 922-1	*E.* sp. Oxley	Wollomombi, NSW
O17		✓	✓	TLC 922-2	*E.* sp. Oxley	Wollomombi, NSW
O18		✓	✓	TLC 922-3	*E.* sp. Oxley	Wollomombi, NSW
O19		✓		TLC 922-4	*E.* sp. Oxley	Wollomombi, NSW
O20		✓		TLC 922-5	*E.* sp. Oxley	Wollomombi, NSW
O21		✓	✓	TLC s.n.	*E.* sp. Oxley	Stockton, NSW
O22	✓	✓	✓	TLC 946	*E.* sp. Oxley	Henry River Falls, NSW
O23		✓	✓	TLC 947	*E.* sp. Oxley	Henry River Falls, NSW
O24	✓	✓	✓	TLC 948	*E.* sp. Oxley	Henry River Falls, NSW
O25	✓	✓	✓	TLC 949	*E.* sp. Oxley	Henry River Falls, NSW
O26		✓	✓	TLC 950	*E.* sp. Oxley	Henry River Falls, NSW
P1		✓	✓	TLC 857	*E. polyanthemos*	Capertee, NSW
P2		✓	✓	TLC 858	*E. polyanthemos*	Capertee, NSW
P3		✓	✓	TLC 859	*E. polyanthemos*	Capertee, NSW
P4		✓	✓	TLC 861	*E. polyanthemos*	Capertee, NSW
P5		✓	✓	TLC 862	*E. polyanthemos*	Capertee, NSW
P6		✓	✓	TLC 863	*E. polyanthemos*	Rhylstone, NSW
P7		✓	✓	TLC 864	*E. polyanthemos*	Rhylstone, NSW
P8		✓	✓	TLC 865	*E. polyanthemos*	Rhylstone, NSW
P9		✓	✓	TLC 945	*E. polyanthemos*	Canberra, ACT
TYPE	✓			R.H.Cambage 3776	*E. magnificata*	Enmore, NSW
			✓	TLC 788	*E.* sp. Dalveen	Dalveen, Qld
			✓	TLC 791	*E.* sp. Dalveen	Dalveen, Qld

Table S3. Phytochemistry data matrix

Arithmetic retention index (AI), specimen code (SC; see Table S2) and percentage concentration determined by gas chromatography–mass spectrometry for 23 compounds used in phenetic analysis of leaf-oil volatiles from some species of *Eucalyptus*

AI	942	983	1018	1020	1022	1201	1425	1435	1447	1464	1464	1487	1606	1609	1614	1633	1646	1651	1654	1803	1814	1842	1879
SC																							
M1	5.99			4.56	48.18	3.73		7.97		1.33				4.83	2.15							3.31	1.03
M2	9.65			2.12	48.28	2.56		10.26		0.00				5.90	2.43							0.00	0.00
M3	5.44			4.04	47.62	5.25		8.30		0.00				5.50	2.78							1.67	0.41
M4	7.35			3.20	46.66	3.62		6.31		0.50				4.25	2.68							2.95	0.99
M5	6.53			4.31	45.01	2.77		9.36		0.00				5.28	2.08							2.68	0.72
M6	11.91			5.75	53.14	7.20		3.82		4.73				2.79	1.30							1.14	1.10
M7	4.89			14.61	9.31	0.84		0.71		19.89				1.15	1.00							0.00	0.00
M8	3.59			4.29	47.63	6.59		10.59		1.99				5.51	2.26							3.25	1.10
M9	3.29			20.10	0.64	0.00		0.00		23.66				0.94	1.29							0.00	0.90
M10	6.09			4.36	44.04	7.46		12.12		2.84				6.09	2.10							2.83	0.93
M11	5.94			3.82	40.63	6.25		7.39		1.20				4.16	1.69							3.68	0.64
M12	3.89			4.66	42.44	4.29		4.95		2.63				3.90	1.52							1.67	0.00
M13	3.76			4.41	42.10	7.06		6.49		0.99				4.47	2.30							3.26	0.48
M14	4.42			14.74	0.38	0.00		0.49		48.18				1.69	2.96							0.00	0.00
M15	3.50			3.92	41.09	5.03		8.20		1.73				5.08	2.53							3.78	0.86
M16	9.17			4.15	61.23	5.98		4.17		0.48				2.44	1.08							3.64	
M17	6.84			5.27	62.75	8.08		5.61		1.69				2.51	0.95							0.00	0.00
M18	8.98			5.51	58.37	9.34		5.73		1.70				2.49	0.88							0.00	0.00
M19	8.02			5.29	55.61	8.07		7.64		2.04				3.92	1.20							0.00	0.00
M20	10.17			4.50	49.68	7.88		5.19		0.91				3.48	1.78							4.03	0.78
M21	6.35			2.79	34.92	4.56		0.65		0.00				1.10	0.00							1.82	0.98
M22	12.06			2.53	54.19	1.75		3.92		0.00				2.87	0.00							3.85	0.00
M23	6.19			3.60	49.62	5.53		6.24		0.48				4.43	2.14							4.19	0.72
M24	6.54			2.85	52.88	4.88		6.15		0.36				4.19	1.90							1.90	0.00
M25	6.30			5.71	44.70	2.54		5.67		0.41				3.79	1.87							1.93	0.34
M26	7.99			4.35	50.77	5.73		5.72		0.76				3.77	1.89							4.79	0.82
M27	6.88			4.12	49.18	7.80		6.35		0.72				4.44	2.00							0.75	0.68
M28	9.71			4.27	46.39	6.58		4.71		0.60				2.84	1.64							6.02	1.11
M29	5.63			4.81	63.31	7.86		7.17		1.55				2.87	0.00							0.00	0.00
M30	11.36			4.25	64.16	6.79		6.61		0.00				3.08	0.00							0.00	0.00
M31	8.34			4.48	56.50	5.47		4.66		0.80				2.33	0.86							9.36	0.00
M32	6.59			5.16	57.47	7.75		5.32		1.12				3.23	1.79							2.96	0.00
M33	10.04			4.74	51.72	8.65		4.97		0.00				3.21	1.56							3.22	0.00
M34	8.23			4.80	52.16	7.06		4.27		0.61				3.08	1.37							0.00	0.28
M35	3.05			3.82	42.72	5.89		7.90		1.08				5.07	2.03							2.81	1.05

AI	942	983	1018	1020	1022	1201	1425	1435	1447	1464	1464	1487	1606	1609	1614	1633	1646	1651	1654	1803	1814	1842	1879
M36	5.23			3.00	39.04	5.68		4.16		0.56				2.91	1.62							3.38	0.53
M37	4.21			4.10	44.27	7.48		6.89		0.81				4.68	1.95							0.00	0.36
M38	4.51			5.77	47.49	6.18		5.64		0.94				3.48	1.56							3.31	0.51
M39	3.51			3.91	50.90	3.90		8.38		0.76				5.68	2.30							1.36	0.56
M40	14.76			3.82	43.75	5.58		6.35		0.89				3.91	1.58							0.00	0.72
M41	7.64			4.36	57.60	7.10		8.24		0.76				5.41	1.88							0.00	0.00
M42	5.14			4.69	44.70	5.43		11.87		3.04				6.46	2.57							1.16	0.00
M43	6.94			4.35	50.86	6.77		7.60		0.99				5.01	2.02							2.22	0.70
M44	8.77			5.55	45.23	6.47		6.16		0.73				4.01	1.77							4.14	0.44
M45	14.68			3.60	52.91	5.76		4.46		0.00				2.59	1.25							0.00	0.00
M46	7.58			3.59	43.79	4.50		7.82		0.73				5.24	2.13							0.96	0.78
M47	7.36			4.10	47.32	5.83		7.57		0.85				5.08	2.21							0.63	0.00
M48	5.65			4.53	49.33	6.87		7.20		1.06				4.34	2.14							4.77	0.00
M49	4.47			3.93	57.65	6.44		6.14		0.55				4.29	2.22							2.37	0.00
M50	6.01			4.30	50.73	6.34		5.34		0.79				3.52	1.87							2.29	0.00
M51	6.99			4.56	56.92	3.92		5.81		0.59				3.56	1.83							1.81	0.00
M52	2.89			4.51	49.25	6.01		6.48		0.49				4.27	1.99							4.61	0.00
M53	8.26			4.47	55.22	8.54		5.26		0.65				3.24	1.57							0.00	0.52
M54	10.41			5.04	56.89	7.23		5.19		0.66				3.19	1.60							0.00	0.00
M55	5.07			5.18	55.27	8.91		5.00		0.63				3.28	1.79							2.00	0.00
M56	6.99			4.23	53.68	7.58		6.18		0.58				3.99	1.70							3.78	0.00
M57	4.93			4.61	47.55	5.44		4.72		0.78				3.11	1.95							5.76	0.42
M58	5.51			4.01	47.44	3.15		7.16		0.62				4.63	1.87							3.08	0.43
M59	6.23			3.52	43.31	6.37		7.74		0.62				4.12	1.93							3.09	0.59
M60	4.08			3.78	42.16	4.19		11.57		1.54				6.24	3.08							0.00	0.00
M61	5.14			3.42	37.90	2.58		9.40		1.01				5.67	2.44							0.00	0.57
M62	6.11			3.92	53.03	5.85		7.95		0.58				5.15	2.43							0.00	0.55
M63	10.04			3.86	49.29	6.61		6.86		0.61				4.52	1.82							4.61	0.00
M64	8.96			3.90	52.14	5.97		5.87		0.49				3.69	2.10							1.98	0.58
M65	12.91			4.22	51.48	6.91		4.83		0.45				3.18	1.52							4.58	0.35
M66	9.61			3.66	42.46	5.40		6.66		0.60				4.40	2.19							1.95	0.87
M67	9.83			3.03	37.04	4.38		8.59		0.67				5.38	2.18							1.49	0.32
M68	12.35			3.94	46.24	6.54		6.43		0.53				4.07	1.98							3.61	0.48
M69	10.40			4.40	52.77	6.84		5.46		0.48				3.80	1.77							4.83	0.00
M70	4.27			4.71	53.08	4.18		4.78		0.72				3.40	1.53							2.69	0.51
M71	7.40			4.40	53.19	6.56		7.19		0.75				4.43	2.04							1.22	0.00
M72	8.00			3.89	49.67	5.74		7.82		0.84				4.82	2.02							5.36	0.76
M73	7.92			4.27	53.06	3.58		4.26		0.00				2.92	1.43							3.80	0.00
M74	6.25			3.37	50.39	5.32		5.37		0.00				3.70	2.00							3.21	0.00
M75	7.13			3.83	46.43	6.02		8.13		0.96				4.66	2.14							2.52	0.00
M76	6.85			4.73	51.13	7.10		6.08		0.89				4.52	2.09							3.07	0.65
M77	4.66			5.15	54.37	6.33		5.47		0.00				3.73	2.09							3.19	0.00
M78	7.17			5.06	52.51	6.05		6.30		1.39				4.11	1.92							3.50	0.00
M79	5.58			4.56	49.65	7.38		6.64		0.88				4.07	1.90							5.83	0.00

AI	942	983	1018	1020	1022	1201	1425	1435	1447	1464	1464	1487	1606	1609	1614	1633	1646	1651	1654	1803	1814	1842	1879
M80	7.85			2.62	24.81	3.66		10.43		1.59				5.42	1.84							3.62	0.73
M81	10.22			5.00	51.52	7.54		4.26		1.06				2.69	1.16							4.60	0.99
M82	12.27			3.86	40.45	5.61		6.40		1.14				3.58	1.55							4.67	0.86
M83	4.25			4.19	48.95	6.37		5.35		0.47				3.67	2.02							4.38	0.56
M84	10.65			3.29	39.27	3.01		4.40		0.49				2.69	1.29							2.31	0.65
M85	5.55			4.50	55.47	6.31		4.05		0.00				2.77	1.29							3.11	0.00
M86	4.60			3.20	43.16	4.44		8.56		0.60				5.75	2.64							0.00	0.00
M87	11.84			3.06	40.49	5.30		9.62		0.75				5.84	2.24							2.24	0.74
M88	3.94			3.72	56.73	4.72		5.35		0.46				3.38	1.48							3.05	0.62
M89	3.31			3.65	49.30	8.30		4.09		0.54				2.48	1.15							2.37	0.42
M90	5.40			4.27	41.38	5.36		6.23		0.78				3.77	1.55							3.33	0.13
M91	4.78			3.14	42.14	5.36		5.19		0.52				3.09	1.35							3.25	0.29
M92	6.23			3.40	42.70	5.59		5.97		0.52				4.02	1.75							1.81	0.25
M93	4.51			3.47	45.19	5.84		4.38		0.50				2.99	1.42							2.89	0.25
M94	3.91			2.86	40.19	5.49		5.99		0.46				4.20	2.17							0.30	0.48
M95	7.61			4.19	45.77	5.76		6.13		1.25				4.58	2.23							3.11	
M96	3.35			4.32	54.68	6.78		7.15		1.31				4.23	1.63							3.00	
C1	4.70	7.36			53.97		1.86	1.41	1.75	18.83		0.00		0.00	0.00								
C2	14.58	4.67			24.89		2.78	4.17	2.67	34.73		0.00		1.22	0.00								
C3	4.12	4.22			44.42		0.00	8.19	3.79	1.65		6.37		3.74	1.82								
C4	8.01	12.18			35.57		1.61	1.68	1.90	28.26		0.00		0.00	0.00								
C5	39.37	0.00			0.00		2.86	21.17	2.99	22.95		0.68		4.27	0.88								
C6	3.48	5.08			2.49		2.89	10.54	1.80	41.02		0.47		1.59	0.66								
C7	3.43	3.80			9.91		1.62	25.40	2.79	10.08		0.96		6.82	2.19								
C8	0.00	0.00			0.00		9.13	0.00	0.00	4.47		13.83		7.46	0.00								
C9	0.00	0.00			3.67		4.37	0.00	0.00	0.00		1.75		2.67	0.00								
C10	16.58	0.00			50.79		0.00	2.56	1.97	12.99		3.77		1.95	0.00								
C11	0.00	0.00			58.73		3.17	9.50	3.20	8.23		2.22		3.40	1.77								
C12	20.33	0.00			33.84		3.74	0.00	0.00	6.50		0.00		0.00	0.00								
C13	31.66	0.00			32.68		0.00	7.05	2.41	4.36		0.00		2.47	0.00								
C14	9.03	12.15			51.85		0.00	2.07	1.65	7.29		1.98		1.10	0.00								
C15	0.00	0.00			3.91		9.81	0.00	0.00	33.80		0.00		0.00	0.00								
C16	9.20	11.20			47.99		0.00	5.51	2.46	7.03		3.35		2.36	1.13								
C17	14.69	18.89			46.45		0.00	7.65	0.00	0.00		7.43		0.00	0.00								
C18	0.00	6.01			0.00		0.00	28.09	4.08	10.09		3.20		5.86	0.00								
C19	15.43	18.99			30.64		0.00	7.92	3.61	0.00		8.26		3.75	1.25								
C20	9.52	12.45			0.00		0.00	17.72	0.00	12.65		12.28		6.13	2.20								
B1	0.00		0.00	3.37	31.96	5.19	0.00	16.93	3.11	3.00	0.00			8.22	2.61			0.00	0.00				0.00
B2	0.00		0.00	8.38	9.25	0.00	16.38	7.18	0.00	0.00	9.46			4.63	0.00			0.00	0.00				5.24
B3	0.00		3.98	0.00	0.00	0.00	0.00	29.76	3.59	2.98	0.00			11.97	2.18			0.00	0.00				0.72
B4	9.24		3.36	1.20	0.00	0.00	3.85	21.24	2.96	1.85	0.00			9.11	2.30			0.00	0.00				0.00
B5	0.00		0.00	5.61	47.38	6.51	0.00	11.39	2.62	2.12	0.00			6.03	2.17			0.00	0.00				0.99
B6	4.82		7.38	20.87	0.00	0.00	8.59	0.00	1.45	12.72	14.47			3.58	2.31			0.00	0.00				2.34
B7	4.85		15.87	0.00	0.00	0.00	15.00	0.00	1.56	12.95	14.95			3.74	3.32			0.00	0.00				2.76

AI	942	983	1018	1020	1022	1201	1425	1435	1447	1464	1464	1487	1606	1609	1614	1633	1646	1651	1654	1803	1814	1842	1879
B8	10.11		0.00	4.93	43.81	5.85	0.00	10.67	2.51	2.13	0.00			5.82	2.05			0.00	0.00				0.00
B9	2.12		11.65	24.33	0.53	0.00	12.63	0.00	0.52	0.63	4.51			2.78	1.45			0.00	1.93				0.53
B10	0.00		0.00	2.64	27.76	0.00	5.78	27.20	3.36	6.75	5.64			9.08	3.78			0.00	0.00				0.00
B11	0.00		2.32	2.77	23.83	3.33	2.16	13.60	2.49	1.16	0.38			5.96	2.05			1.50	1.86				0.67
B12	0.00		0.00	0.00	0.00	0.00	3.89	0.00	0.00	0.00	0.00			0.00	0.00			0.00	0.00				1.53
B13	8.54		0.00	0.75	0.00	0.69	16.92	1.92	2.04	0.51	4.26			5.59	7.90			2.17	2.78				0.92
B14	11.13		0.00	0.82	0.00	0.00	10.61	1.17	2.07	0.00	6.11			4.31	5.28			0.00	2.49				0.87
B15	16.93		0.00	1.32	0.00	0.00	14.20	0.00	0.00	0.00	5.90			3.86	1.06			6.88	11.59				3.11
B16	8.53		6.70	17.81	13.13	2.15	2.81	6.40	1.46	0.00	5.11			4.45	3.42			7.06	7.06				1.62
B17	16.10		0.52	0.94	1.48	0.97	9.68	1.76	1.49	0.00	12.58			4.40	4.25			3.02	3.02				1.42
B18	11.37		0.00	0.60	0.00	0.77	7.84	1.54	2.05	0.62	12.79			4.47	4.95			9.49	9.49				1.44
B19	4.92		5.17	2.28	27.70	2.98	2.22	14.25	2.42	0.65	0.42			6.60	1.86			2.68	5.22				0.82
B20	14.93		0.00	1.98	34.62	4.65	0.00	5.53	1.37	0.61	0.00			3.42	1.14			2.34	2.43				0.54
B21	8.90		0.00	2.72	37.96	6.37	1.19	11.54	2.37	1.47	0.00			5.58	1.76			1.64	2.20				0.12
B22	7.67		0.00	3.33	32.58	5.39	2.15	6.96	1.54	0.82	0.00			3.23	1.17			2.27	2.54				0.68
B23	5.89		0.00	3.45	34.15	5.86	0.00	11.85	2.13	1.65	0.00			6.36	2.26			3.02	6.17				0.76
B24	5.11		0.00	3.87	34.16	6.01	2.19	9.13	2.08	0.82	0.00			4.44	1.62			1.45	1.95				0.59
B25	4.53		1.11	3.01	22.05	4.08	2.19	15.22	2.37	1.26	0.00			6.50	1.62			0.00	13.56				1.42
B26	3.94		0.00	2.80	44.78	1.99	0.00	8.87	2.46	0.84	0.00			4.88	2.87			1.40	3.10				0.49
P1			0.34	1.60	22.67	2.07	4.49			0.41				2.41									0.32
P2			0.72	3.11	0.00	1.68	1.67			0.00				0.95									0.95
P3			10.49	25.28	9.05	0.00	7.55			6.04				2.34									0.00
P4			0.00	31.12	5.76	0.00	3.26			31.54				0.00									1.57
P5			0.00	4.55	78.75	3.48	0.00			0.00				1.06									0.00
P6			1.44	5.09	62.97	6.93	0.00			2.30				0.00									0.00
P7			0.00	7.13	51.80	2.52	3.25			11.02				0.00									1.91
P8			3.49	3.03	76.38	1.19	5.30			2.80				1.44									1.51
P9			10.25	5.45	18.13	0.00	0.00			0.00				4.86									1.02
D1							1.16			0.75	11.13		12.60			0.00	41.60			2.77	23.25		
D2							1.41			11.04	15.92		9.71			1.39	32.87			2.39	18.79		
D3							1.54			0.67	15.99		11.93			1.73	38.06			2.77	20.53		
D4							2.85			0.00	2.31		8.94			1.70	31.21			3.09	21.44		
D5							1.16			1.08	27.99		10.05			0.00	32.67			2.36	17.84		
D6							1.59			1.30	42.35		6.88			0.96	22.96			1.58	12.17		
D7							2.14			13.08	7.93		7.99			1.27	29.37			2.45	17.92		
D8							1.19			13.69	25.87		7.42			0.96	25.86			1.94	15.63		
D9							1.42			0.73	30.21		9.48			1.33	32.38			2.19	16.68		
D10							1.46			0.67	26.60		10.07			1.45	32.83			2.37	17.77		
D11							1.53			0.81	21.30		11.00			1.56	34.88			2.71	19.40		
D12							1.33			13.86	17.70		8.24			1.36	26.84			2.38	16.34		
D13							1.45			0.98	26.88		9.83			1.43	32.68			2.37	17.78		
O1	0.00			0.00	0.00	0.00		23.56	3.14	0.00				9.16	2.07								1.51
O2	3.21			3.66	57.20	4.06		6.33	1.66	0.83				4.66	2.26								0.00
O3	3.84			5.74	61.26	6.01		6.03	1.73	1.17				3.44	2.24								0.63

AI	942	983	1018	1020	1022	1201	1425	1435	1447	1464	1464	1487	1606	1609	1614	1633	1646	1651	1654	1803	1814	1842	1879
O4	3.14			3.18	45.18	2.89		6.52	1.64	2.01				3.90	1.91								2.71
O5	0.00			12.81	39.30	3.42		5.28	1.79	14.78				4.24	1.51								2.92
O6	2.84			2.54	45.51	3.39		10.49	2.37	0.00				6.23	2.61								0.86
O7	2.13			2.70	58.03	3.98		4.59	1.16	0.25				2.95	1.29								0.00
O8	1.57			2.68	44.96	4.35		6.65	1.80	0.58				4.23	1.91								0.00
O9	4.30			2.98	44.06	3.43		6.72	1.67	0.64				4.01	1.86								0.93
O10	3.59			1.91	39.96	2.72		6.97	1.70	0.50				3.68	1.58								0.56
O11	5.47			1.49	24.76	1.63		4.47	1.38	1.75				2.30	1.72								0.94
O12	2.30			5.09	58.60	6.65		3.92	1.03	0.00				2.81	1.17								0.00
O13	7.51			3.81	42.63	1.55		10.02	2.13	1.10				6.22	2.83								0.00
O14	5.77			5.15	54.73	8.74		3.45	1.15	0.35				2.74	1.78								0.00
O15	4.15			5.15	62.58	7.08		4.98	1.51	0.47				3.49	2.08								0.38
O16	0.00			3.91	56.71	4.30		5.13	1.47	0.98				3.08	1.73								1.06
O17	3.33			3.63	60.16	1.83		4.49	1.37	0.95				2.69	1.10								1.62
O18	19.28			3.58	33.74	3.65		6.63	1.99	1.05				3.14	1.41								0.88
O19	3.35			3.59	52.11	2.98		6.63	1.85	0.97				4.19	2.02								1.73
O20	4.36			2.60	40.62	1.42		16.37	2.97	1.56				8.28	2.66								2.33
O21	4.29			2.85	41.84	2.76		16.06	2.88	1.27				8.34	3.03								0.86
O22	3.18			3.68	78.13	4.42		6.50	0.00	0.00				4.09	0.00								
O23	1.26			3.76	63.01	5.22		11.13	2.67	0.00				7.54	2.42								
O24	1.91			3.77	62.33	6.37		10.61	2.38	0.00				7.21	2.48								
O25	3.57			2.34	30.45	2.30		17.81	2.64	0.76				9.01	2.68								
O26	2.92			4.34	64.57	3.95		13.24	2.54	0.00				6.52	1.91								

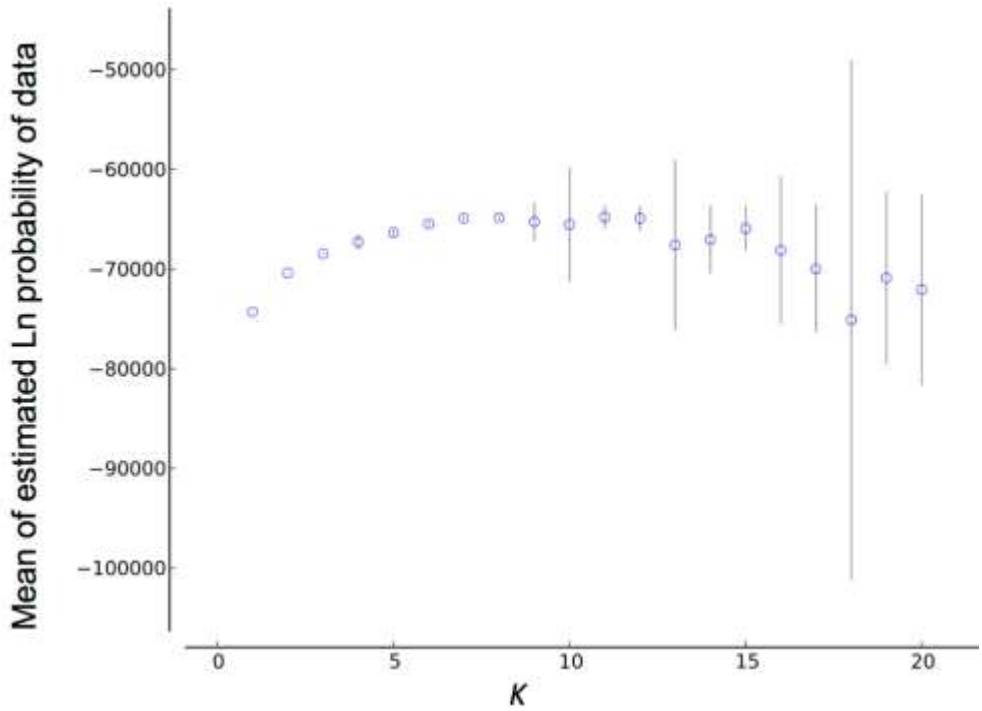

Fig. S1. Changes in mean of estimated log probability *v. K* for STRUCTURE analysis of all samples; $n = 141$.

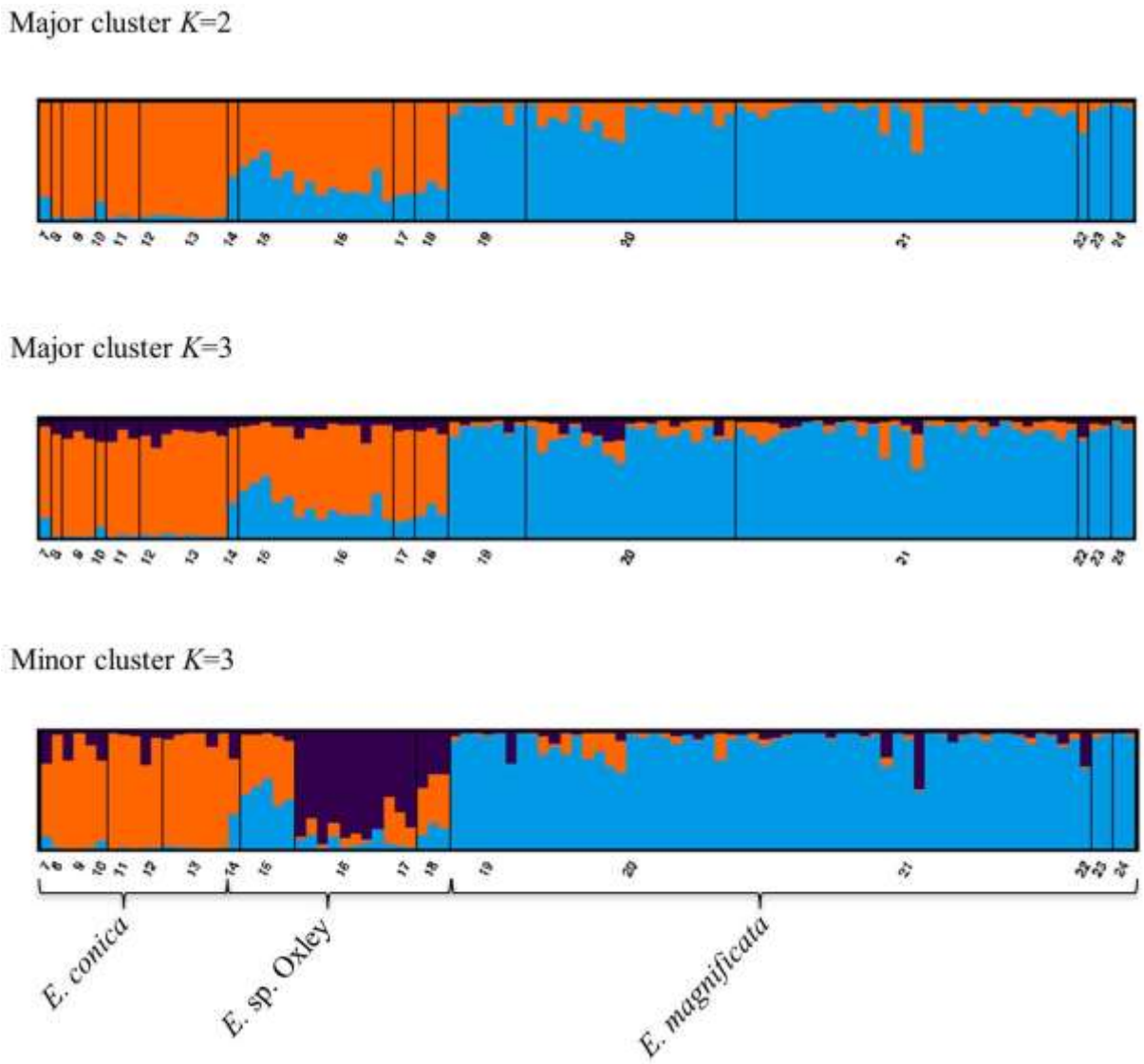

Fig. S2. CLUMPAK major and minor clustering modes for *Eucalyptus conica*, *E.* sp. Oxley and *E. magnificata* data set. STRUCTURE bar plots of *E. conica*, *E.* sp. Oxley and *E. magnificata* samples including major and minor clustering modes K = 2–3. Bar plots show each individual as a vertical bar divided into segments on the basis of the proportion of ancestry suggested for two or three subpopulations. Minor clustering modes represent alternative clustering hypotheses across the 10 runs and occurred at K = 3: 3 of 10 runs; n = 99. Fine black lines delineate sampling locations, numbered as in Table 1.

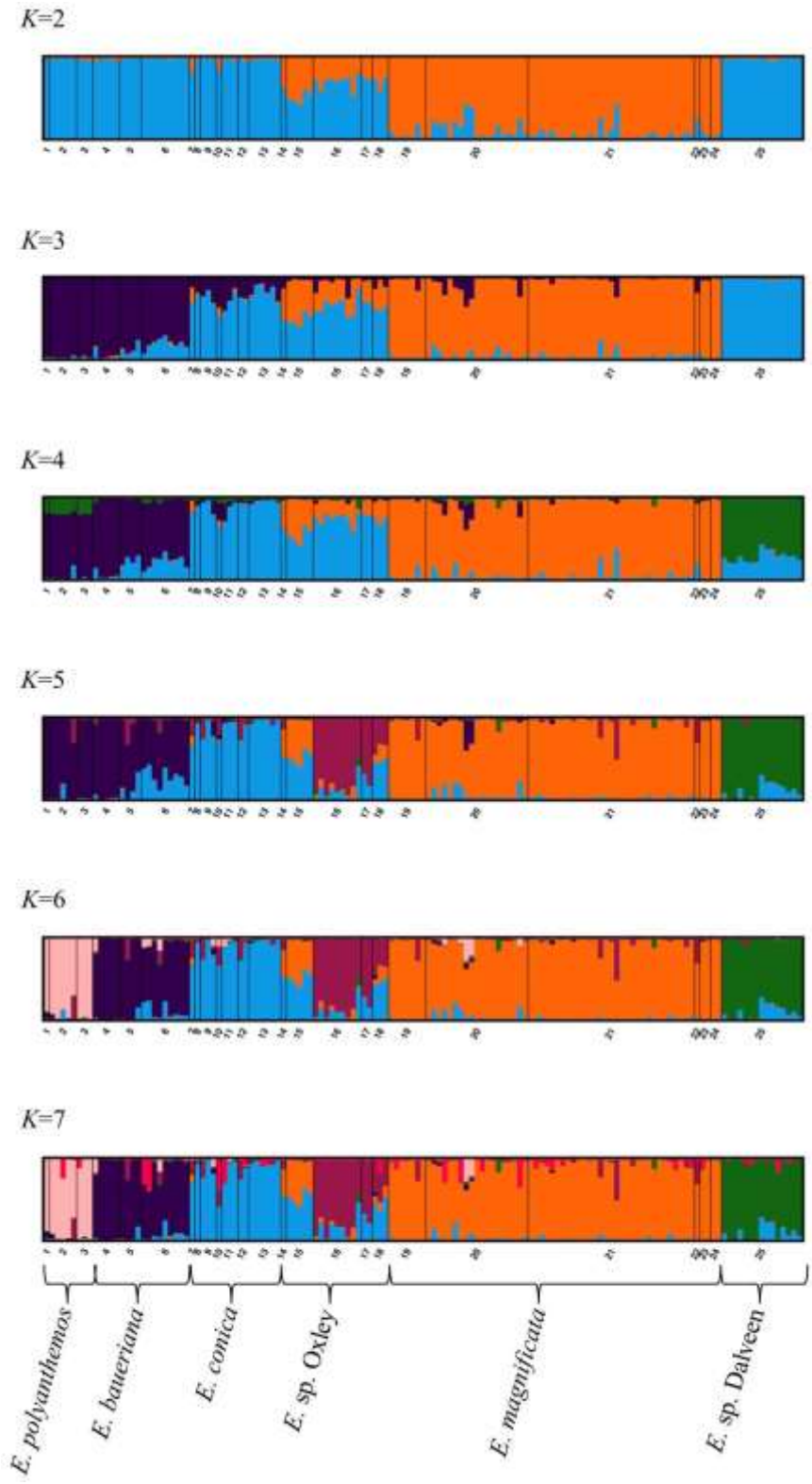

Fig. S3. CLUMPAK major clustering modes for main data set STRUCTURE bar plots of all samples major clustering modes K = 2–7. Bar plots show each individual as a vertical bar divided into segments on the basis of the proportion of ancestry suggested for 2–7 subpopulations; n = 141. Fine black lines delineate sampling locations, numbered as in Table 1.

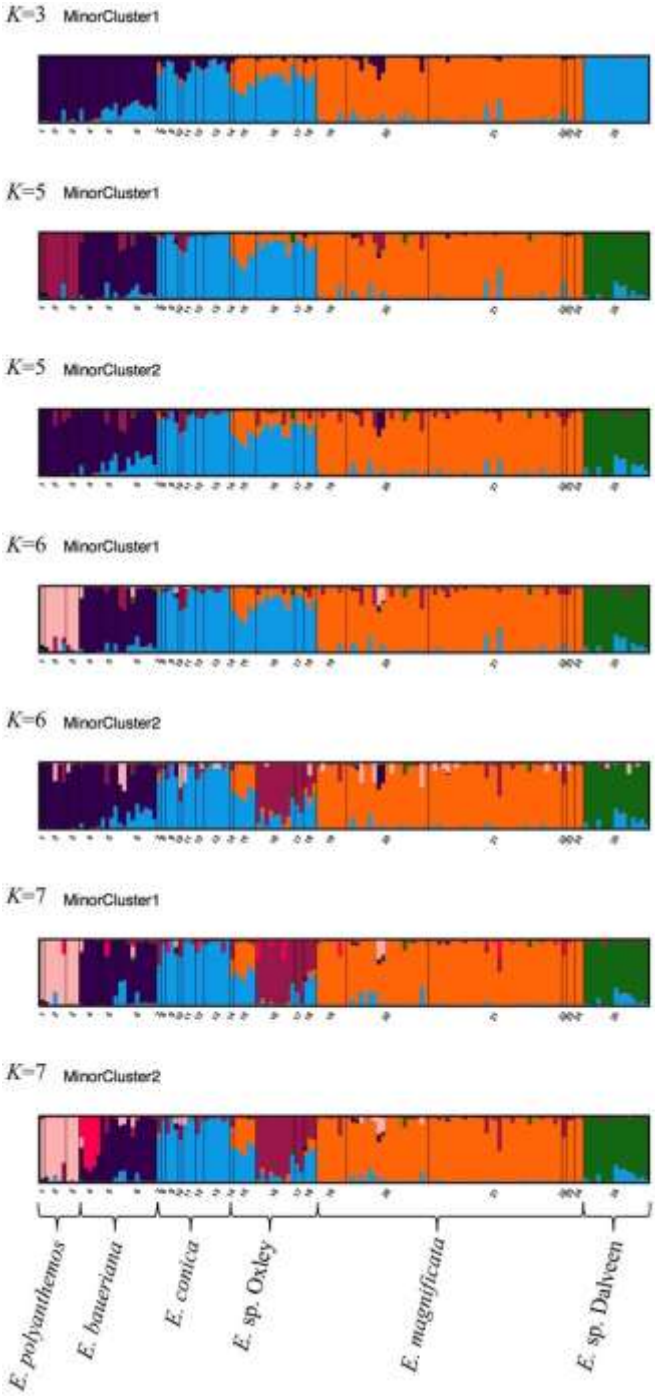

Fig. S4. CLUMPAK minor clustering modes for main data set STRUCTURE bar plots of all samples minor clustering modes K = 2–7. Bar plots show each individual as a vertical bar divided into segments on the basis of the proportion of ancestry suggested for 2–7 subpopulations. Minor clustering modes represent alternative clustering hypotheses across the 20 runs and occurred at K = 3: 7 of 20 runs; K = 5 minor clustering mode 1: 6 of 20 runs; minor clustering mode 2: 3 of 20 runs; K = 6 minor clustering mode 1: 7 of 20 runs; minor clustering mode 2: 3 of 20 runs; K = 7 minor clustering mode 1: 2 of 20 runs; minor clustering mode 2: 2 of 20 runs; n = 141. Fine black lines delineate sampling locations, numbered as in Table 1.

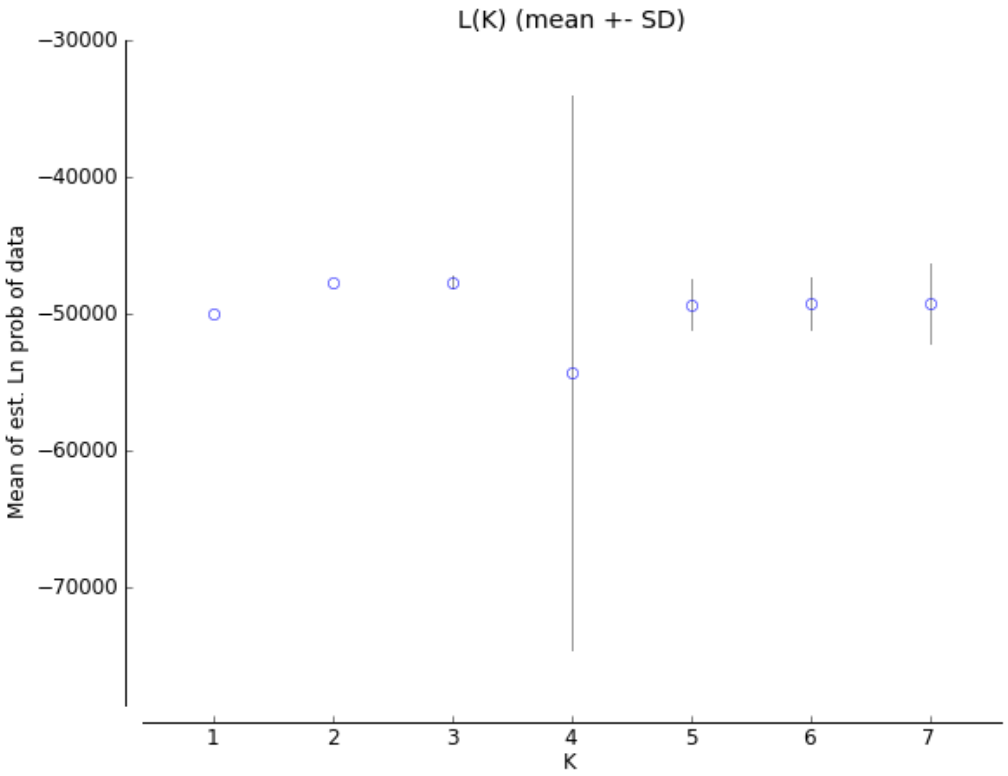

Fig. S5. Changes in mean of estimated log probability *v. K* for STRUCTURE analysis of *Eucalyptus conica*, *E*. sp. Oxley and *E. magnificata* samples; *n* = 99.

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