

Supplementary material

**Identifying evolutionary lineages in the *Elaeocarpus obovatus* complex: population genetics and morphometric analyses support a new subspecies, *Elaeocarpus obovatus* subsp. *umbratilis*, from northern Queensland, Australia**

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**Table S1. Nuclear microsatellite markers, repeats, sequences and annealing temperature employed in the polymerase chain reaction assays in this study**

Size given in the length excluding the 5'M13 universal primer sequences

Marker	Repeat	5'	3'	Size	Annealing temperature (°C)
<i>scu01Eg</i>	(AG) <i>n</i>	CCAAATGAAGAATACCTCCA	AGTGGCTTGGTCAGAGATTA	292–332	56
<i>scu20Eg</i>	(AG) <i>n</i>	TACGCCATCACTGTCTTCACCACC	ACACTCACCATCCTGTGCTCTATCC	144–168	61
<i>scu22Eg</i>	(AG) <i>n</i>	CGCTTCTTACGGTTCTTCTTGAAA	TTCGCTGCTCCCCCTGATAA	99–130	61
<i>scu25Eg</i>	(TGG) <i>n</i>	TTTGAGTAGCTCACTCTGCTCTTGG	CGTTGGATTGCCTCCCGATT	282–343	55
<i>scu31Eg</i>	(GA) <i>n</i> ...(GT) <i>n</i>	GCAAAGCAAGGGCAAGTTCTCTT	CGGCTTCCTAAATTCAGTGTATGGA	316–406	61
<i>scu33Eg</i>	(AG) <i>n</i>	GCTTTACACCAAGTAGGAACTACCA	CTAGCTTCTCGGTCGTTATCATTT	287–307	55

**Table S2. Error rate for each locus across all the entities**

Polymearase chain reaction (PCR) amplification failure is of 181 samples. The numbers in the parentheses indicate the numbers of samples that showed more than four peaks

Locus	Success number/repeat samples	Error rate (%)	PCR failure	PCR failure rate (%)
<i>scu01Eg</i>	13/13	0	4	2
<i>scu20Eg</i>	91/93	2	17 (12)	17
<i>scu22Eg</i>	18/19	5	2	1
<i>scu25Eg</i>	15/15	0	14 (2)	9
<i>scu31Eg</i>	95/100	5	6	3
<i>scu33Eg</i>	107/111	4	13	7
Total	339/351	3	56 of 1086 overall samples	5

**Table S3. Evidence of polyploidy based on allele peak counts of the microsatellites for each locus and POLYSAT ploidy assessment**

‘-9’ indicates missing data. See Table 1 in the main paper for the population codes

Sample	Manual allele count						POLYSAT		
	Locus						Allele		
	<i>scu01Eg</i>	<i>scu20Eg</i>	<i>scu22Eg</i>	<i>scu25Eg</i>	<i>scu31Eg</i>	<i>scu33Eg</i>	Max.	Mean	Ploidy
ACO1	2	3	3	2	3	2	3	2.5	3
ACO2	3	3	4	1	3	2	4	2.7	4
ACO3	2	3	3	2	3	2	3	2.5	3
ACO4	3	1	3	-9	2	3	3	2.4	3
ACO5	2	2	2	-9	3	2	3	2.2	3
ALF1	1	1	3	-9	1	3	3	1.8	3
ALF2	2	1	2	-9	4	2	4	2.2	4
ALF3	2	3	4	-9	2	2	4	2.6	4
ALF4	1	1	4	1	2	4	4	2.2	4
ALF5	2	1	2	1	2	4	4	2	4
ALF6	2	1	-9	3	2	3	3	2.2	3
ALF7	2	3	3	3	2	3	3	2.7	3
ALF8	3	2	3	1	3	2	3	2.3	3
ALF9	3	3	2	2	2	4	4	2.7	4
ALF10	2	2	3	1	2	4	4	2.3	4
ALF11	2	2	3	-9	2	2	3	2.2	3
ALF12	3	2	3	2	2	4	4	2.7	4
ALF13	3	1	3	2	4	2	4	2.5	4
ALF14	2	1	2	3	3	3	3	2.3	3
ALF15	2	2	2	2	1	2	2	1.8	2
ALF16	3	2	3	2	2	3	3	2.5	3
ALF17	3	2	3	2	2	4	4	2.7	4
AIR1	3	2	3	2	1	3	3	2.3	3
AIR2	3	2	3	2	1	3	3	2.3	3
AIR3	3	2	3	2	1	3	3	2.3	3
APP1	2	2	2	2	3	3	3	2.3	3
APP2	1	4	2	3	2	4	4	2.7	4
APP3	1	4	3	3	2	2	4	2.5	4
APP4	2	1	2	1	2	4	4	2	4

Sample	Manual allele count						POLYSAT		
	Locus						Allele		
	<i>scu01Eg</i>	<i>scu20Eg</i>	<i>scu22Eg</i>	<i>scu25Eg</i>	<i>scu31Eg</i>	<i>scu33Eg</i>	Max.	Mean	Ploidy
APP5	1	1	2	3	4	4	4	2.3	4
APP6	2	3	3	2	4	2	3	2.5	3
APP7	2	3	3	2	4	2	3	2.5	3
APP8	1	2	2	3	4	4	4	2.5	4
APP9	3	2	3	2	4	3	3	2.7	3
ANPA1	2	1	2	1	2	3	3	1.8	3
ANPA2	3	2	3	2	3	3	3	2.5	3
ANPA3	3	2	2	1	2	3	3	2	3
ANPA4	1	2	2	2	1	3	3	1.8	3
ANPA5	3	4	2	2	2	3	4	2.7	4
ANPA6	1	2	3	2	3	3	3	2.3	3
ANPA7	2	2	3	2	3	3	3	2.5	3
ANPA8	1	2	2	2	2	3	3	2	3
ANPA9	1	1	3	2	2	3	3	2	3
ANPA10	1	1	1	2	1	1	2	1.2	2
ABS1	2	3	2	1	3	3	3	2.3	3
ABS2	1	3	2	1	3	4	4	2.3	4
ABS3	3	3	2	2	3	1	3	2.3	3
ABS4	1	3	2	2	2	3	3	2.2	3
ABS5	1	-9	3	3	2	3	3	2.4	3
ABS6	3	3	2	3	2	3	3	2.7	3
ABD1	4	3	3	2	3	3	4	3	4
ABD2	1	4	3	2	3	3	4	2.7	4
ABD3	2	4	3	3	3	3	4	3	4
ABD4	1	4	4	3	1	3	4	2.7	4
ABD5	4	2	3	2	3	3	4	2.8	4
ABD6	2	2	2	3	3	4	4	2.7	4
ABD7	2	4	2	3	3	4	4	3	4
ABD8	1	3	3	2	2	2	3	2.2	3
AMK1	2	3	3	2	3	2	3	2.5	3
AMK2	-9	2	3	2	2	4	4	2.6	4
AMK3	3	1	2	2	4	4	4	2.7	4
AMK4	3	1	2	2	2	2	3	2	3
AMK5	1	2	3	2	2	2	3	2	3
AMK6	2	3	2	2	1	3	3	2.2	3
AMK7	3	3	3	2	2	4	4	2.8	4
AMK8	1	2	3	2	2	3	3	2.2	3
AMK9	2	3	4	3	3	2	4	2.8	4
AMK10	1	2	4	2	4	3	4	2.7	4
AMK11	2	2	4	2	1	3	4	2.3	4
AMK12	1	2	2	2	2	4	4	2.2	4
AMK13	3	2	3	2	2	3	3	2.5	3
AMK14	2	2	4	2	2	2	4	2.3	4
AMK15	1	3	4	2	2	3	4	2.5	4
AMK16	1	4	3	4	2	2	4	2.7	4
AMK17	1	2	3	1	3	2	3	2	3
AMK18	2	3	3	2	4	3	4	2.8	4
AMK19	1	2	1	3	3	2	3	2	3
AMK20	2	3	3	3	3	2	3	2.7	3
ALL1	3	2	3	3	3	3	3	2.8	3
ALL2	1	3	2	2	3	4	4	2.5	4
ALL3	1	3	3	2	3	2	3	2.3	3
ALL4	2	3	2	2	2	2	3	2.2	3
ALL5	2	3	3	1	2	-9	3	2.2	3
ALL6	1	4	2	1	2	3	4	2.2	4
ALL7	1	3	2	2	2	3	3	2.2	3
AMR1	2	2	2	2	3	2	3	2.2	3
AMR2	2	1	2	1	2	3	3	1.8	3

Sample	Manual allele count						POLYSAT		
	Locus						Allele		
	<i>scu01Eg</i>	<i>scu20Eg</i>	<i>scu22Eg</i>	<i>scu25Eg</i>	<i>scu31Eg</i>	<i>scu33Eg</i>	Max.	Mean	Ploidy
AMR3	3	2	3	3	2	2	3	2.5	3
AMR4	2	2	3	2	4	2	4	2.5	4
AMR5	2	2	4	1	3	3	4	2.5	4
ANT1	2	2	3	1	3	3	3	2.3	3
ANT2	1	2	3	1	1	2	3	1.7	3
ATS1	2	2	3	2	3	4	4	2.7	4
OUC1	2	3	1	1	2	2	3	1.8	3
OUC2	1	2	1	1	1	2	2	1.3	2
OUC3	1	2	1	1	1	2	2	1.3	2
OUC4	2	3	1	1	1	1	3	1.5	3
OUC5	1	4	1	1	2	1	4	1.7	4
OUC6	2	4	2	1	1	1	4	1.8	4
OUC7	-9	4	-9	-9	1	2	4	2.3	4
OUC8	1	3	2	1	1	2	3	1.7	3
OUC9	1	4	2	1	1	2	4	1.8	4
OTVE1	2	3	2	1	1	2	3	1.8	3
OTVE2	1	4	1	1	2	1	4	1.7	4
OMA1	1	3	1	2	1	1	3	1.5	3
OMA2	1	4	2	1	2	1	4	1.8	4
OMA3	1	4	2	1	2	1	4	1.8	4
OMA4	1	3	3	2	4	1	4	2.3	4
OMA5	1	-9	1	1	3	1	3	1.4	3
OPR1	1	4	2	2	2	2	4	2.2	4
OPR2	2	4	2	2	2	2	4	2.3	4
OPR3	1	4	1	1	1	1	4	1.5	4
OPR4	1	-9	1	1	1	1	1	1	2 <sup>A</sup>
OPR5	1	-9	2	1	2	2	2	1.6	2
OBR1	4	4	4	4	2	2	4	3.3	4
OBR2	2	-9	4	1	2	1	4	2	4
OBR3	1	-9	2	1	2	1	2	1.4	2
OBR4	3	-9	3	2	1	1	3	2	3
OBR5	3	4	1	2	2	2	4	2.3	4
OBR6	3	-9	3	2	2	1	3	2.2	3
OMC1	3	4	4	1	2	3	4	2.8	4
OMC2	3	4	4	1	3	2	4	2.8	4
OMC3	3	4	3	3	3	3	4	3.2	4
OMC4	2	-9	3	3	3	2	3	2.6	3
OMC5	2	-9	4	2	3	3	4	2.8	4
OML1	3	4	3	2	1	3	4	2.7	4
OML2	3	-9	4	-9	2	3	4	3	4
OML3	4	4	3	2	4	2	4	3.2	4
OML4	3	-9	4	2	3	3	4	3	4
OMtB1	3	3	3	-9	2	3	3	2.8	3
OMtB2	4	4	4	3	3	3	4	3.5	4
OMtB3	4	4	4	3	2	4	4	3.5	4
OMtB4	4	3	4	3	1	3	4	3	4
OMtB5	4	4	4	3	2	3	4	3.3	4
OMtB6	4	4	4	3	2	3	4	3.3	4
OMtB7	4	-9	3	1	3	2	4	2.6	4
OMtB8	4	4	4	3	1	2	4	3	4
OMB1	2	3	2	2	3	3	3	2.5	3
OMB2	3	-9	3	3	1	3	3	2.6	3
OMB3	4	4	4	1	3	1	4	2.8	4
OMB4	3	-9	2	2	1	2	3	2	3
OBB1	2	2	3	2	1	-9	3	2	3
OBB2	3	-9	4	1	3	1	4	2.4	4
OBB3	3	-9	-9	1	4	1	4	2.3	4
OBB4	2	-9	3	2	2	1	3	2	3

Sample	Manual allele count						POLYSAT		
	Locus						Allele		
	<i>scu01Eg</i>	<i>scu20Eg</i>	<i>scu22Eg</i>	<i>scu25Eg</i>	<i>scu31Eg</i>	<i>scu33Eg</i>	Max.	Mean	Ploidy
OBH1	2	4	2	2	2	-9	4	2.4	4
OBH2	1	1	3	1	1	-9	3	1.4	3
OBH3	2	-9	3	2	1	-9	3	2	3
OBH4	3	-9	4	1	2	-9	4	2.5	4
OBH5	2	-9	3	2	2	1	3	2	3
BCL1	2	2	2	2	2	1	2	1.8	2
BCL2	1	1	2	2	2	1	2	1.5	2
BCL3	1	2	2	1	2	1	2	1.5	2
BCL4	1	1	2	1	2	1	2	1.3	2
BPR1	1	1	2	1	2	1	2	1.3	2
BPR2	2	1	2	2	2	1	2	1.7	2
BPR3	2	1	2	2	2	1	2	1.7	2
BPR4	2	-9	2	-9	2	1	2	1.8	2
BPR5	1	-9	2	1	2	1	2	1.4	2
BWT1	-9	3	1	-9	-9	-9	3	2	3
BWT2	2	3	2	1	1	1	3	1.7	3
BWT3	1	4	2	3	2	1	4	2.2	4
BWT4	2	-9	1	-9	2	-9	2	1.7	2
BWT5	1	-9	2	2	2	1	2	1.6	2
BMtL1	2	2	2	1	1	2	2	1.7	2
BMtL2	1	1	2	1	2	1	2	1.3	2
BMtL3	1	1	2	1	1	1	2	1.2	2
BMtL4	1	-9	2	-9	2	-9	2	1.7	2
BTR1	1	-9	1	1	2	-9	2	1.3	2
BTR2	1	-9	2	1	1	1	2	1.2	2
BTR3	1	-9	2	-9	2	1	2	1.5	2
BTR4	1	-9	2	-9	2	1	2	1.5	2
BTR5	-9	-9	2	-9	1	1	2	1.3	2
BDC1	1	2	2	2	2	1	2	1.7	2
BDC2	1	1	2	2	2	2	2	1.7	2
BDC3	2	1	2	1	2	1	2	1.5	2
ADC1	2	1	2	1	1	1	2	1.3	2
ADC2	2	2	1	2	-9	2	2	1.8	2
ADC3	1	1	1	2	1	1	2	1.2	2
COO1	2	3	3	1	-9	-9	3	2.3	3
COO2	2	1	2	2	-9	2	2	1.8	2

<sup>A</sup>Manipulated to adjust to the minimum ploidy level of two.

**Table S4. Characteristics of the six nuclear microsatellite loci used in this study for *Elaeocarpus arnhemicus*, *E. obovatus* and *E. sp. Mt Bellenden Ker***

Results for *E. coorangooloo*, ADC and BDC are not shown, because samples sizes are smaller than five. s.d., standard deviation. *N*, sample size; *S*, range of allele sizes (bp);  $uA_I$ , unbiased mean number of alleles per locus (mean number of alleles per locus  $A_I$ , averaged by the number of samples in the population, *N*);  $uA_R$ , unbiased mean number of unique alleles per locus (mean number of unique alleles per locus  $A_R$ , averaged by the number of samples in the population, *N*);  $P \geq 2A$ , proportion of individuals with two or more alleles per locus; *R*, range of alleles per individual

Locus	<i>E. arnhemicus</i>					<i>E. obovatus</i>					<i>E. sp. Mt Bellenden Ker</i>				
	<i>N</i>	<i>S</i>	$uA_I (P \geq 2A)$	$uA_R$	<i>R</i>	<i>N</i>	<i>S</i>	$uA_I (P \geq 2A)$	$uA_R$	<i>R</i>	<i>N</i>	<i>S</i>	$uA_I (P \geq 2A)$	$uA_R$	<i>R</i>
<i>scu01Eg</i>	92	314–335	2.0 (0.67)	8	1–4	56	310–341	2.3 (0.70)	11	1–4	21	316–335	1.3 (0.33)	5	1–2
<i>scu20Eg</i>	92	160–184	2.3 (0.83)	12	1–4	37	160–186	2.4 (0.97)	13	1–4	13	164–172	1.7 (0.46 <sup>A</sup> )	6	1–3 <sup>A</sup>
<i>scu22Eg</i>	92	120–144	2.7 (0.98)	13	1–4	55	116–148	2.6 (0.80)	15	1–4	23	128–136	2.5 (0.82)	6	1–2
<i>scu25Eg</i>	87	300–340	2.2 (0.80)	9	1–4	54	300–343	1.7 (0.52)	7	1–4	16	300–340	1.6 (0.38 <sup>A</sup> )	6	1–3 <sup>A</sup>
<i>scu31Eg</i>	93	372–390	2.5 (0.88)	7	1–4	57	374–420	2.0 (0.65)	15	1–4	22	372–424	2.0 (0.77)	9	1–2
<i>scu33Eg</i>	92	305–325	2.8 (0.98)	9	1–4	52	309–325	1.9 (0.62)	7	1–4	19	319–325	1.1 (0.05)	3	1
Total	93	–	2.4 (s.d. = 0.31) (0.86; s.d. = 0.12)	58	–	57	–	2.2 (s.d. = 0.34) (0.71; s.d. = 0.16)	68	–	23	–	1.7 (s.d. = 0.50) (0.47; s.d. = 0.29)	35	–

<sup>A</sup>Three alleles were found in individuals from BWT at *scu20Eg* & *scu25Eg*.