

Supplementary Material

Molecular and morphological analyses support recognition of *Prostanthera volucris* (Lamiaceae), a new species from the Central Tablelands of New South Wales

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Table S1. Samples of *Prostanthera* that were sequenced and co-analysed to produce DArTseq SNP data, arranged alphabetically by species.

Species	Locality	OTU Code	Voucher	Herbarium and accession number	A	B
<i>P. densa</i> A.A. Ham.	Gaan Gaan Hill, N.S.W.	-	Wood s.n. ^A	NSW1040587	-	✓
	Beecroft Peninsula, N.S.W	-	Conn 2571 ^A	NSW203752	-	✓
<i>P. gilesii</i> G.W.Althofer ex B.J.Conn & T.C.Wilson	Towac Creek, Mount Canobolas SCA, NSW	HZ1	Zimmer 1	-	✓	✓
		HZ1.1	Zimmer 1.1 ^B	-	✓ ^C	-
		HZ2	Zimmer 2	-	✓	-
		HZ3	Zimmer 3	-	✓	-
		HZ4	Zimmer 4	-	✓	-
		HZ75	Zimmer 75	-	✓	-
		HZ76	Zimmer 76	-	✓	-
		HZ77	Zimmer 77	-	✓	-
		HZ79	Zimmer 79	-	✓	-
		HZ81	Zimmer 81	-	✓	-
		HZ82	Zimmer 82	-	✓	-
		HZ83	Zimmer 83	-	✓	-
		HZ90	Zimmer 90	-	✓	-
		HZ91	Zimmer 91	-	✓	-
		HZ92	Zimmer 92	-	✓	-
		HZ93	Zimmer 93	-	✓	-
		HZ93.1	Zimmer 93.1 ^B	-	✓	-
		HZ94	Zimmer 94	-	✓	-
		HZ95	Zimmer 95	-	✓	-
		HZ96	Zimmer 96	-	✓	-
		HZ97	Zimmer 97	-	✓	-
		HZ101	Zimmer 101	-	✓	-
		HZ107	Zimmer 107	-	✓	-
		HZ109	Zimmer 109	-	✓	-
		HZ111	Zimmer 111	-	✓ ^C	-
		HZ113	Zimmer 113	-	✓	-
		HZ115	Zimmer 115	-	✓	-
		JJB3602a	Bruhl 3602a	NE108967	✓	✓
		JJB3602aa	Bruhl 3602aa	NE108967	✓	-
		JJB3602b	Bruhl 3602b	NE108967	✓	-
		JJB3602bb	Bruhl 3602bb	NE108967	✓	-
		JJB3602c	Bruhl 3602c	NE108967	✓	-
		JJB3602cc	Bruhl 3602cc	NE108967	✓	-
		JJB3602d	Bruhl 3602d	NE108967	✓	-
		JJB3602dd	Bruhl 3602dd	NE108967	✓	-
		JJB3602e	Bruhl 3602e	NE108967	✓	-
		JJB3602ee	Bruhl 3602ee	NE108967	✓	-
		JJB3602ee.1	Bruhl 3602ee.1 ^B	NE108967	✓ ^C	-
		JJB3602f	Bruhl 3602f	NE108967	✓	-
		JJB3602g	Bruhl 3602g	NE108967	✓	-
		JJB3602h	Bruhl 3602h	NE108967	✓	-
		JJB3602i	Bruhl 3602i	NE108967	✓	-
		JJB3602j	Bruhl 3602j	NE108967	✓	-
		JJB3602k	Bruhl 3602k	NE108967	✓	-
		JJB3602l	Bruhl 3602l	NE108967	✓	-
		JJB3602m	Bruhl 3602m	NE108967	✓	-
		JJB3602n	Bruhl 3602n	NE108967	✓	-
		JJB3602o	Bruhl 3602o	NE108967	✓	-
		JJB3602p	Bruhl 3602p	NE108967	✓	-
		JJB3602q	Bruhl 3602q	NE108967	✓	-
		JJB3602r	Bruhl 3602r	NE108967	✓	-
		JJB3602s	Bruhl 3602s	NE108967	✓	-
		JJB3602t	Bruhl 3602t	NE108967	✓	-
		JJB3602u	Bruhl 3602u	NE108967	✓	-
		JJB3602v	Bruhl 3602v	NE108967	✓	-
		JJB3602w	Bruhl 3602w	NE108967	✓	-

Species	Locality	OTU Code	Voucher	Herbarium and accession number	A	B
	The Walls, Mount Canobolas SCA, NSW	JJB3602x	Bruhl 3602x	NE108967	✓	-
		JJB3602y	Bruhl 3602y	NE108967	✓	-
		JJB3602z	Bruhl 3602z	NE108967	✓	-
		JJBsnf1	Bruhl snf1	-	✓	-
		JJBsnf2	Bruhl snf2	-	✓	-
		JJBsnf3	Bruhl snf3	-	✓	-
		JJBsng	Bruhl sng	-	✓	-
		TCW581	Wilson 581	-	✓	-
		TCW577	Wilson 577	NSW998442	✓ ^C	✓
		RWM7111	Medd 7111 ^D	-	✓ ^C	✓
<i>P. granitica</i> Maiden & Betcher	Warrumbungles NP, NSW	-	Tuft 162 (1) ^A	NSW1057815	-	✓
		-	Tuft 162 (2) ^D	NSW844192	-	✓
<i>P. marifolia</i> R.Br.	Seaforth, NSW	-	Conn 5676 ^A	NSW841013	-	✓
	Wakehurst, NSW	-	McVickar s.n. ^A	NSW1038335	-	✓
	Manly, NSW	-	Wilson 578 ^A	NSW1057839	-	✓
<i>P. phyllicifolia</i> F.Muell.	Deua NP, NSW	NSW1057814	Liney 2039 ^A	NSW1057814	✓ ^E	✓
	Kosciuszko NP, NSW	RWM2170961a	Medd 2170961a	NE109821	✓ ^C	✓
		RWM2170961b	Medd 2170961b	NE109821	✓ ^C	-
		RWM2170962a	Medd 2170962a	NE109821	✓	✓
		RWM2170962b	Medd 2170962b	NE109821	✓	-
	Dangelong NR, NSW	RWM217099a1	Medd 217099a1	NE109826	✓ ^C	✓
		RWM217099b1	Medd 217099b1	NE109826	✓ ^C	✓
		RWM217099c1	Medd 217099c1	NE109826	✓ ^C	-
		RWM217099d1	Medd 217099d1	NE109826	✓ ^C	-
		RWM217099e1	Medd 217099e1	NE109826	✓ ^C	-
	Adaminaby, NSW	RWM217100a1	Medd 217100a1	NE109825	✓ ^C	✓
		RWM217100b1	Medd 217100b1	NE109825	✓ ^C	✓
		RWM217100c1	Medd 217100c1	NE109825	✓ ^C	-
		RWM217100d1	Medd 217100d1	NE109825	✓ ^C	-
		RWM217100e1	Medd 217100e1	NE109825	✓ ^C	-
	Nullica SF, NSW	GPP219	Phillips 219 ^D	NSW992045	✓ ^E	✓
		MP9561	Parris 9561 ^D	NSW456970	✓ ^E	✓ ^E
	Cobruna, Vic.	NGW8979	Walsh 8979 ^D	MEL2470086	✓ ^C	✓
		NGW9013	Walsh 9013 ^D	MEL2470120	✓ ^C	✓
	Gelantipy, Vic.	ROM984	Makinson 984 ^D	NSW518048	✓ ^E	✓ ^E
	Tinderry, NSW	RPO61	O'Donnell 61 ^D	NSW100407	✓	✓
		RPO62	O'Donnell 62 ^D	NSW1100408	✓ ^C	✓
<i>P. scutellarioides</i> (R.Br.) Briq.	Castlereagh NR NSW	-	Wilson 215 ^A	NSW799702	-	✓
	Myall River, NSW	-	Bell s.n. ^D	NSW847837	-	✓ ^E
<i>P. sp.</i> Evans Crown	Evans Crown NR, NSW	GMT853	Taseski 853	NSW1055966	✓ ^E	-
		GMT853a	Taseski 853a	NSW1055966	✓	-
		GMT853b	Taseski 853b	NSW1055966	✓ ^C	-
		GMT853c	Taseski 853c	NSW1055966	✓ ^C	✓
		GMT853d	Taseski 853d	NSW1055966	✓	-
		GMT853e	Taseski 853e	NSW1055966	✓	-
		GMT853f	Taseski 853f	NSW1055966	✓	-

Species	Locality	OTU Code	Voucher	Herbarium and accession number	A	B
		RPO28a	O'Donnell 28a ^D	NSW1100357	✓	-
		RPO28b	O'Donnell 28b ^D	NSW1100358	✓	-
		RPO28c	O'Donnell 28c ^D	NSW1100359	✓	-
		RPO28d	O'Donnell 28d ^D	NSW1100360	✓ ^C	-
		RPO28e	O'Donnell 28e ^D	NSW1100361	✓	-
		RPO28f	O'Donnell 28f ^D	NSW1100362	✓	-
		RPO28g	O'Donnell 28g ^D	NSW1100363	✓	-
		RPO28i	O'Donnell 28i ^D	NSW1100365	✓	-
		RPO28j	O'Donnell 28j ^D	NSW1100366	✓	-
		RPO29a	O'Donnell 29a ^D	NSW1100369	✓ ^C	✓
		RPO29b	O'Donnell 29b ^D	NSW1100370	✓ ^C	-
		RPO29d	O'Donnell 29d ^D	NSW1100412	✓ ^C	-
		RPO29e	O'Donnell 29e ^D	NSW1100373	✓	-
		RPO29f	O'Donnell 29f ^D	NSW1100374	✓	-
		RPO29g	O'Donnell 29g ^D	NSW1100375	✓	-
		RPO29h1	O'Donnell 29h1 ^D	NSW1100376	✓	-
		RPO29h2	O'Donnell 29h2 ^D	NSW1100376	✓	-
		RPO29i	O'Donnell 29i ^D	NSW1100377	✓ ^C	-
		RPO29j	O'Donnell 29j ^D	NSW1100378	✓ ^C	-
		RPO30b	O'Donnell 30b ^D	NSW1100380	✓	-
		RPO30c	O'Donnell 30c ^D	NSW1100381	✓ ^C	-
		RPO30d	O'Donnell 30d ^D	NSW1100382	✓	-
		RPO30e	O'Donnell 30e ^D	NSW1100383	✓	-
		RPO30f	O'Donnell 30f ^D	NSW1100384	✓ ^C	-
		RPO30g	O'Donnell 30g ^D	NSW1100385	✓ ^C	-
		RPO30h	O'Donnell 30h ^D	NSW1100386	✓	-
		RPO30i	O'Donnell 30i ^D	NSW1100387	✓	-
		RPO30j	O'Donnell 30j ^D	NSW1100388	✓ ^C	-
		RPO55	O'Donnell 55 ^D	NSW1100402	✓ ^C	✓
		RPO56	O'Donnell 56 ^D	NSW1100403	✓ ^C	-

NP, National Park; NR, Nature Reserve; SF, State Forest; SCA, State Conservation Area; NSW, New South Wales; Vic., Victoria. Category A, primary SNP dataset; category B, secondary phylogenetic dataset.

^AReCER samples.

^BTechnical duplicates.

^CIndividuals retained for genetic diversity calculations following removal of putative clones.

^DNewly sequenced for this study.

^ERemoved by filtering processes.

Table S2. Voucher specimens and OTU codes of *Prostanthera* measured for multivariate phenetic analysis in this study.

Taxon	OTU code	Locality	Voucher	Herbarium and accession number
<i>P.</i> Evans Crown	EC1	Evans Crown NR, NSW	Rodd 11009	NSW856887
	EC2	Evans Crown NR, NSW	McKee 7043	NSW237164
	EC3	Evans Crown NR, NSW	Taseski 853	NSW1055966
	EC4	Evans Crown NR, NSW	O'Donnell 55	NSW1100402
	EC5	Evans Crown NR, NSW	O'Donnell 28	NSW1100357
<i>P. gilesii</i>	gil1	Mt Canobolas SCA, NSW	Bruhl 3601b	NE108967
	gil2	Mt Canobolas SCA, NSW	Bruhl 3619	NE109460
	gil3	Mt Canobolas SCA, NSW	Bruhl 3601d	NE108967
	gil4	Mt Canobolas SCA, NSW	Giles s.n.	NSW128315
	gil5	Mt Canobolas SCA, NSW	Giles s.n.	NSW128313
<i>P. phyllicifolia</i>	phyCobb	Mt Cobberas #2, Vic.	Walsh 2039	NSW243340
	phyWul	Wulgulmerang, Vic.	Melville 3036	NSW1051719
	phyCoop	Mt Coopracambra, Vic.	Albrecht 3684	NSW1051502
	phyAda	Adaminaby, NSW	Medd 217100a	NE109830
	phyKNP	Kosciuszko NP, NSW	Medd 217096b	NE109822
	phyDang	Dangelong NR, NSW	Medd 217099b	NE109826
	phyDeua	Big Badja Hill, Deua NP, NSW	Liney 2039	NSW704841
	phyNam	Namadgi NP, ACT	Taws 315	NSW624610
	phyTur	Tuross River, NSW	Briggs s.n.	NSW275953
	phyTin	Tinderry Ranges, NSW	O'Donnell 61	NSW1100407

NP, National Park; NR, Nature Reserve; SF, State Forest; SCA, State Conservation Area; ACT, Australian Capital Territory; NSW, New South Wales; Vic., Victoria.

Table S3. Morphological characters used in multivariate phenetic analysis of selected specimens of *P. gilesii*, *P. phlycifolia* and the Evans Crown population.

Character number	Character (units)	Character number	Character (units)
1	Branch hair direction: trichomes absent (0); trichomes antrorse (1); trichomes retrorse (2); trichomes spreading (3)	16	Prophyll length (mm)
2	Branch hair density (trichomes mm ⁻²)	17	Prophyll width (mm)
3	Branch hair length (mm)	18	Prophyll L:W ratio
4	Leaf petiole hair direction: trichomes absent (0); trichomes antrorse (1); trichomes retrorse (2); trichomes spreading (3)	19	Prophyll trichome density (trichomes mm ⁻²)
5	Leaf lamina length (mm)	20	Prophyll hair direction: trichomes absent (0); trichomes antrorse (1); trichomes retrorse (2); trichomes spreading (3)
6	Leaf lamina width (mm)	21	Calyx outer surface hair direction: trichomes absent (0); trichomes antrorse (1); trichomes retrorse (2); trichomes spreading (3)
7	Leaf lamina L:W ratio	22	Calyx outer surface hair density (trichomes mm ⁻²)
8	Leaf lamina abaxial surface hair density (trichomes mm ⁻²)	23	Calyx abaxial lobe length (mm)
9	Leaf lamina abaxial surface hair length (mm)	24	Calyx abaxial lobe width (mm)
10	Leaf lamina abaxial surface midrib hair direction: trichomes absent (0); trichomes antrorse (1); trichomes retrorse (2); trichomes spreading (3)	25	Calyx abaxial lobe L:W ratio
11	Leaf lamina adaxial surface hair density (trichomes mm ⁻²)	26	Calyx adaxial lobe length (mm)
12	Leaf lamina adaxial surface hair length (mm)	27	Calyx adaxial lobe width (mm)
13	a ₁ axis length (mm)	28	Calyx adaxial lobe L:W ratio
14	a ₁ axis hair density (trichomes mm ⁻²)	29	Calyx abaxial:adaxial lobe length ratio
15	a ₁ axis hair direction: trichomes absent (0); trichomes antrorse (1); trichomes retrorse (2); trichomes spreading (3)		

Table S4. Morphological data matrix used in multivariate phenetic analysis of selected specimens of *P. gilesii*, *P. phyllicifolia* and *P. sp. Evans Crown*.

Character	OTU																			
	EC1	EC2	EC3	EC4	EC5	gil1	gil2	gil3	gil4	gil5	phyCobb	phyWul	phyCoop	phyMurr	phyKNP	phyDang	phyDeua	phyNam	phyTur	phyTin
#1	2	2	2	2	2	1	1	1	1	1	3	1	1	1	1	1	1	3	1	1
#2	51.333	29.667	40	43	33.333	21.333	19.667	16	26.667	26	0	11	18.333	4	9	7.333	25.333	12.667	10.333	16.333
#3	0.333	0.283	0.25	0.217	0.183	0.217	0.233	0.408	0.167	0.225	0.1	0.167	0.25	0.15	0.175	0.092	0.158	0.158	0.15	0.117
#4	2	2	2	2	2	1	1	1	1	1	0	0	0	0	0	0	1	0	1	0
#5	17	14.333	13.833	12.667	16	16.833	17.5	20	16.5	19.333	8	10.833	12.667	9	15	9	11.833	11	9.167	11.667
#6	4.167	3	3.5	4.5	4	5.667	5.167	6.667	4.167	5.667	2.167	1.833	3	2	2.667	2.333	2.833	3.667	2.333	2.333
#7	4.093	4.778	3.988	2.83	4	2.994	3.426	2.992	3.977	3.422	3.767	6.028	4.222	4.5	5.748	3.867	4.276	3	3.972	5.033
#8	27	29.333	22.667	17.333	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
#9	0.258	0.325	0.267	0.242	0.292	0.333	0.292	0.425	0.225	0.342	0	0	0.3	0	0	0	0.133	0	0	0.183
#10	3	3	3	3	3	1	1	1	1	1	0	0	1	0	0	0	1	0	0	1
#11	21.333	21.333	22.667	24	17.333	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
#12	0.117	0.167	0.208	0.15	0.192	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
#13	1	1.896	1.167	1.708	1.813	3.708	2.208	3.281	1.917	1.771	2.75	3.125	3.396	2.813	2.625	2.292	1.313	1.708	2.083	1.354
#14	60	42.667	48	49.333	41	10	0	16	0	0	0	3	0	0	0	0	0	3.667	0	0
#15	2	2	2	2	2	1	0	1	1	0	3	1	0	0	0	0	1	3	0	0
#16	4.521	4.313	4.708	5.125	8	5.667	3.521	6.979	3	4.396	2.771	3.479	2.563	2.333	2.688	2.604	3	3.417	3.021	2.813
#17	1.229	0.938	1.021	1.354	4.229	1.208	0.667	2.313	0.354	0.521	0.375	0.375	0.354	0.229	0.292	0.292	0.5	0.354	0.271	0.354
#18	4.055	4.611	4.601	3.847	1.892	5.73	5.324	3.02	8.533	8.444	7.603	9.633	8	10.306	9.361	8.917	6.241	11.12	11.25	8.254
#19	29.333	28	30.667	29.333	30.667	4	5.333	0	8	3	0	0	0	0	0	0	0	0	0	0
#20	2	2	2	2	2	1	1	1	1	1	3	0	0	0	0	0	0	0	0	0
#21	2	2	2	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
#22	42.667	42.667	49.333	49.333	40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10
#23	5.021	6.604	6.167	7.167	5.958	7.833	7.563	8.75	5.479	7.688	5.104	5.271	5.479	5.208	6.521	6.125	5.333	5.146	5.833	5
#24	3.708	4.042	5.167	4.333	4.292	4.833	4.063	5.344	3.313	5.375	2.583	2.167	2.542	2.375	2.396	3.563	2.646	2.479	3.375	2.688
#25	1.384	1.633	1.194	1.657	1.469	1.633	1.865	1.647	1.655	1.448	1.996	2.435	2.165	2.196	2.733	1.722	2.016	2.075	1.729	1.86
#26	6.75	7.792	7.833	8.167	6.229	9.667	8	9.75	7.646	9.167	5.938	6.5	6.417	5.875	7.396	7.438	6	5.854	6.375	5.313
#27	3.688	4.188	6	4.833	4.104	5.333	4.583	5.344	3.813	4.567	2.646	2.854	2.625	2.938	2.917	3.563	2.667	2.917	3.75	2.594
#28	1.848	1.865	1.306	1.7	1.525	1.815	1.768	1.835	2.041	2.056	2.259	2.321	2.449	2.038	2.579	2.093	2.274	2.024	1.7	2.047
#29	0.551	0.519	0.789	0.887	0.963	0.811	0.945	0.897	0.715	0.84	0.852	0.818	0.855	0.886	0.883	0.823	0.901	0.879	0.915	0.938

OTU codes follow those outlined in Table S2 and character codes are outlined in Table S3.

Table S5. Correlation of characters with ordination pattern (PCC) from semi-strong hybrid multidimensional scaling (SSH MDS) ordination for all selected specimens of *P. gilesii*, *P. phyllifolia*, and the Evans Crown population.

Character number	Character	<i>X</i>	<i>Y</i>	<i>Z</i>	<i>r</i> ²
10	Midrib hair direction	-0.386	-0.612	0.69	0.971
19	Prophyll hair density	-0.551	-0.112	0.827	0.966
22	Calyx outer surface hair density	-0.666	-0.164	0.727	0.929
11	Lamina ad hair density	-0.562	0.073	0.824	0.925
8	Lamina ab hair density	-0.553	0.264	0.79	0.923
12	Ad hair length	-0.691	-0.35	0.633	0.922
14	a ₁ axis hair density	-0.269	0.32	0.908	0.921
21	Calyx outer surface hair direction	-0.742	-0.279	0.61	0.918
4	Petiole hair direction	0.068	-0.11	0.992	0.912
26	Calyx ad lobe length	0.923	-0.036	0.383	0.873
24	Calyx ab lobe width	0.72	-0.388	0.575	0.835
27	Calyx ad lobe width	0.759	-0.208	0.617	0.827
23	Calyx ab lobe length	0.914	-0.27	0.303	0.816
2	Branch hair density	-0.14	-0.463	0.875	0.805
9	Ab hair length	0.415	-0.839	0.351	0.787
6	Lamina width	0.884	-0.173	0.435	0.771
18	Prophyll L:W ratio	-0.073	0.742	-0.666	0.732
5	Lamina length	0.749	-0.536	0.39	0.66
25	Calyx ab lobe L:W ratio	-0.281	0.122	-0.952	0.62
3	Branch hair length	0.768	0.156	0.621	0.614
28	Calyx ad lobe L:W ratio	-0.162	0.081	-0.984	0.587
1	Branch hair direction	-0.506	0.812	0.291	0.58
16	Prophyll length	0.454	-0.529	0.717	0.578
15	a ₁ axis hair direction	-0.371	0.831	0.416	0.552
20	Prophyll hair direction	-0.34	0.339	0.877	0.55
17	Prophyll width	-0.111	-0.842	0.528	0.431
29	Calyx abaxial:adaxial lobe length ratio	-0.069	-0.887	-0.457	0.43
13	a ₁ axis length	0.945	0.062	-0.322	0.416
7	Lamina L:W ratio	-0.734	-0.45	-0.508	0.326

Ad, adaxial surface; Ab, abaxial surface.

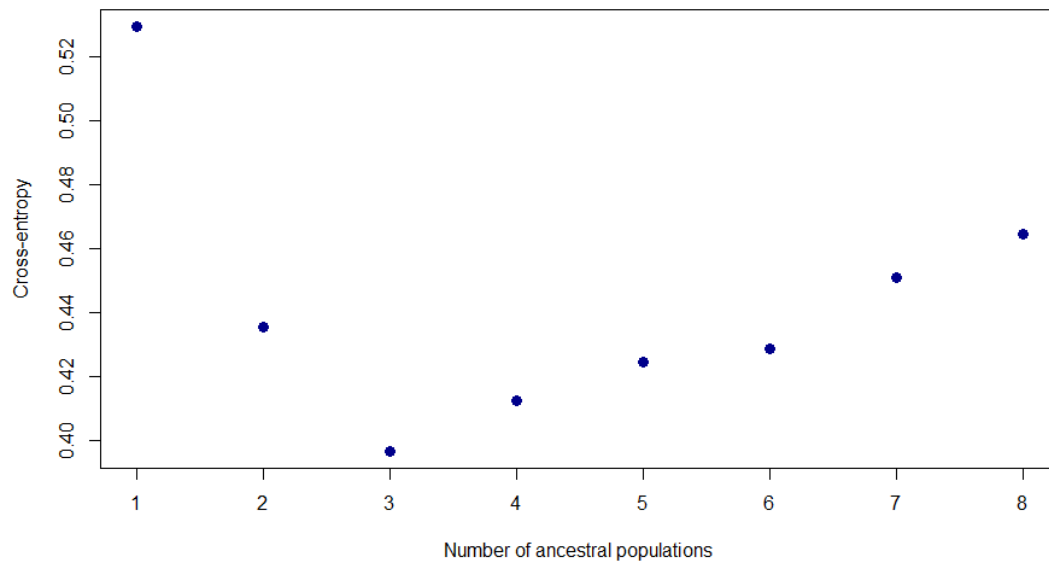


Fig. S1 Cross-entropy value plots with a stabilisation point of $K = 3$ indicating three probable ancestral populations represented in the data.

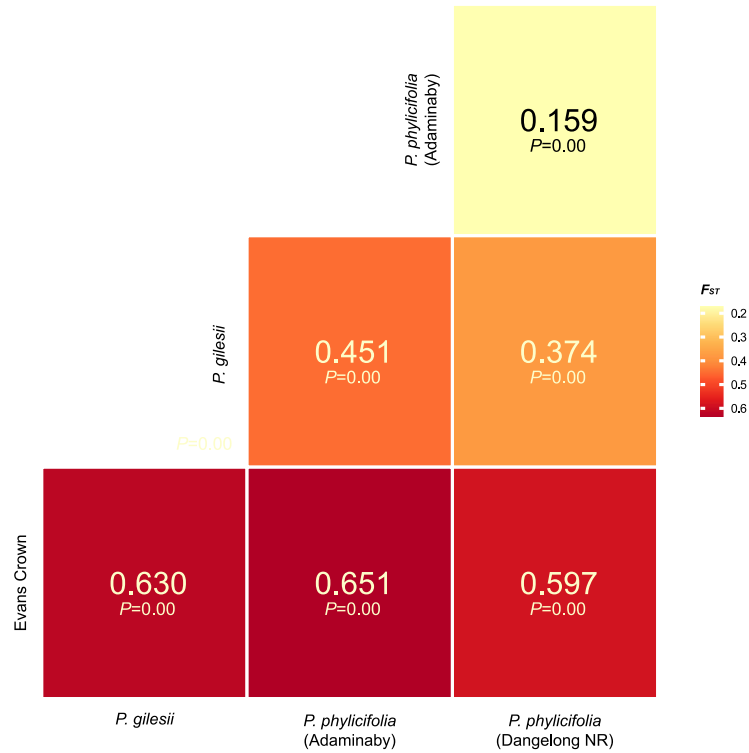


Fig. S2. Pairwise F_{ST} and P values between populations with ≥ 5 samples of *P. gilesii*, *P. phyllicifolia* and the Evans Crown population following removal of putative clones.

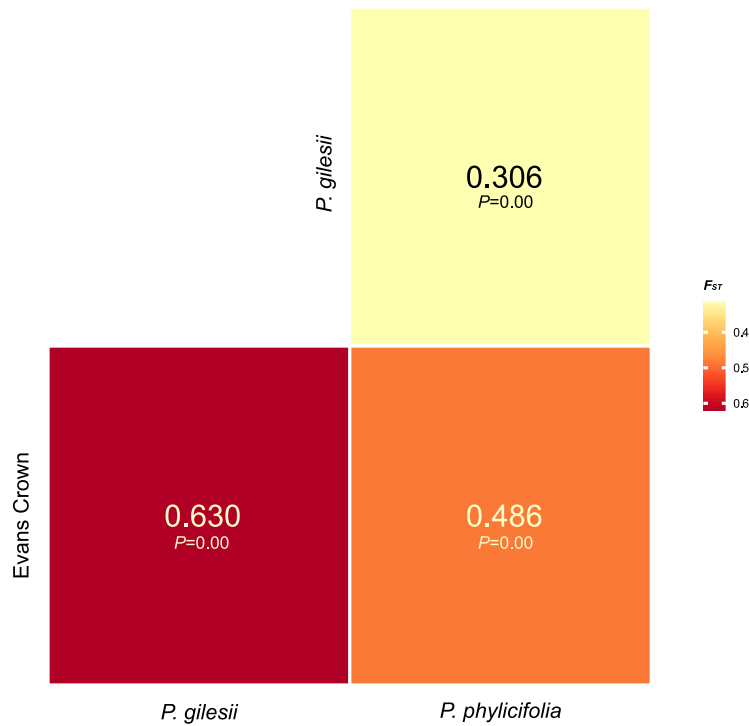


Fig. S3. Species-level pairwise F_{ST} and P values between *P. gilesii*, *P. phyllicifolia* and the Evans Crown population following removal of putative clones.