Conservation genomics of *Pittosporum sp. Coffs Harbour*.

2022

FINAL REPORT

Research Centre for Ecosystem Resilience

Australian Institute for Botanical Science

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Acknowledgements

We acknowledge the Traditional Custodians of the land on which the plant species in this study are found on, and pay respects to Elders past and present. We acknowledge all collectors who conducted the field sampling.

Citation

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Preliminary results of Stage 1 of Conservation genomic project on *Pittosporum sp. Coffs Harbour*

prepared by the Research Centre for Ecosystem Resilience

(Royal Botanic Garden Sydney)

Background

A pilot conservation genomic study of the newly discovered *Pittosporum sp. Coffs Harbour* was conducted to verify its taxonomic status, estimate its relatedness the potential to hybridise with co-occurring congeneric species.

Preliminary Results

We report results based on analyses of genomic data sourced from samples belonging to *Pittosporum sp. Coffs Harbour* and congeneric species presumably most related to it, *P. undulatum*, *P. revolutum*, *P. lancifolium*, *P. spinescens* and *P. multiflorum*. Sampling for *P.* sp. Coffs Harbour was undertaken across all known populations/patches (Fig. 1), which are near the Coffs Harbour bypass development.

- A high-quality genome scan was successfully obtained and analysed. It produced 77,324 genome-wide markers (SNPs) across 228 samples: 72 *P.* sp. Coffs Harbour, 78 *P. undulatum*, 14 *P. revolutum*, 4 *P. lancifolium*, 2 *P. spinescens* and 58 *P. multiflorum*;
- Principal Component Analysis and phylogenetic tree and network analyses (SVDQ and splitstree; Fig. 2) indicate all samples of *P.* sp. Coffs Harbour are genetically distinct from all congeneric species studied, including those that were sympatric with *P.* sp. Coffs Harbour, i.e. *P. lancifolium*, *P. revolutum* and *P. undulatum*;
- Kinship analysis for the *P.* sp. Coffs Harbour individuals (Fig. 3) detected high relatedness among individuals within each of the eight populations, with 24 unique genets observed among the 70 individuals sampled and genets belonging to the same population are siblings/half-siblings to each another (Table 1).
- Diversity calculations indicate overall genetic variability within *P.* sp. Coffs Harbour is comparable to other *Pittosporum* species (Table 2) despite high relatedness among individuals within populations, suggesting at least one representative from each population is needed to preserve the species' diversity.

Proposed Stage #2 implementation

Stage #1 of the project showed that our standard methodologies and analytical workflows are effective to test species concept of *Pittosporum* sp. Coffs Harbour, determine its relationship with other Pittosporum species and quantification of genetic diversity and relatedness between and within populations from selected individuals of *P.* sp. Coffs Harbour. To progress with a targeted conservation program for *Pittosporum* sp. Coffs Harbour, a genetic research program in Stage #2 will need to address the following questions:

- What is the extent of genetic diversity at the all known sub-sites of Pittosporum sp. 'Coffs Harbour'? Current sampling involved collecting a fixed number of individuals at each patch, this may have overlooked larger populations that are likely to harbour greater diversities than smaller populations. The current data identified more genets in selected populations suggests we have not sampled extensive enough to verify the extent of this species' diversity.
- What proportion of individuals from each of the sub-sites is required to maintain maximum genetic diversity (and fitness) of ex situ collections and translocated populations?
- What translocation design should be followed to maximise long term viability?

Once the relevant knowledge is acquired, then informed conservation and management actions can be developed as deemed necessary.

Figure 1. The 8 study sites of *Pittosporum* sp. Coffs Harbour. See Table 1 for more sampling information.

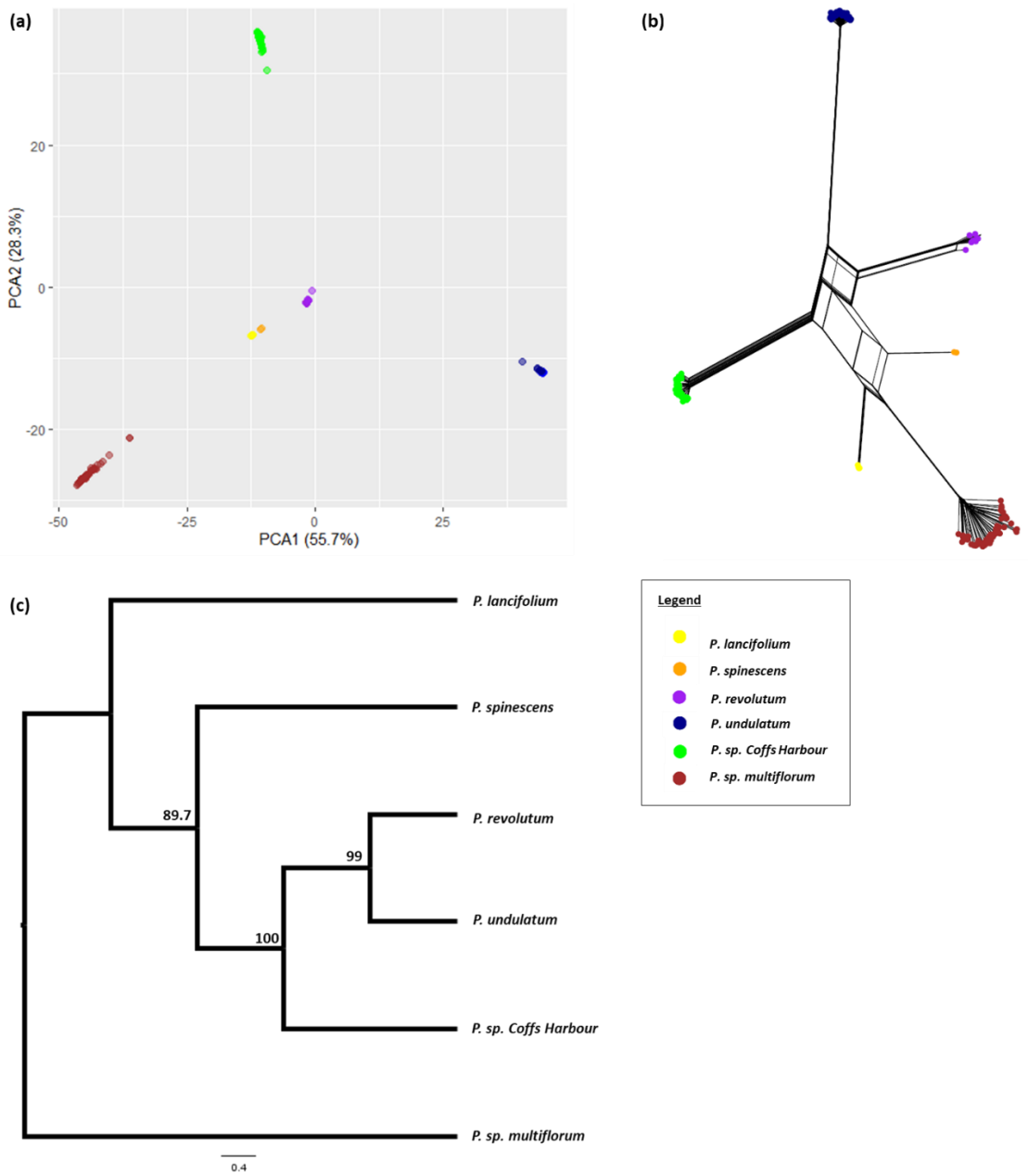

Figure 2. Principle Component analysis (a), Splits tree network (b) and SVDQuartets analysis of the Single Nucleotide Polymorphism (SNP) data from 228 specimens of *Pittosporum* sp. Coffs Harbour and relevant congeneric species.

Bootstrap support values above 50% are labelled in the SVDQ tree (c), and higher than 80% are considered strong support. The total weight of compatible quartets = 97964 (97.964%) was relatively higher than total weight of incompatible quartets = 2036 (2.036%), which means probability of lineage sorting is low, which supports that the *Pittosporum* sp. Coffs Harbour is a distinct species.

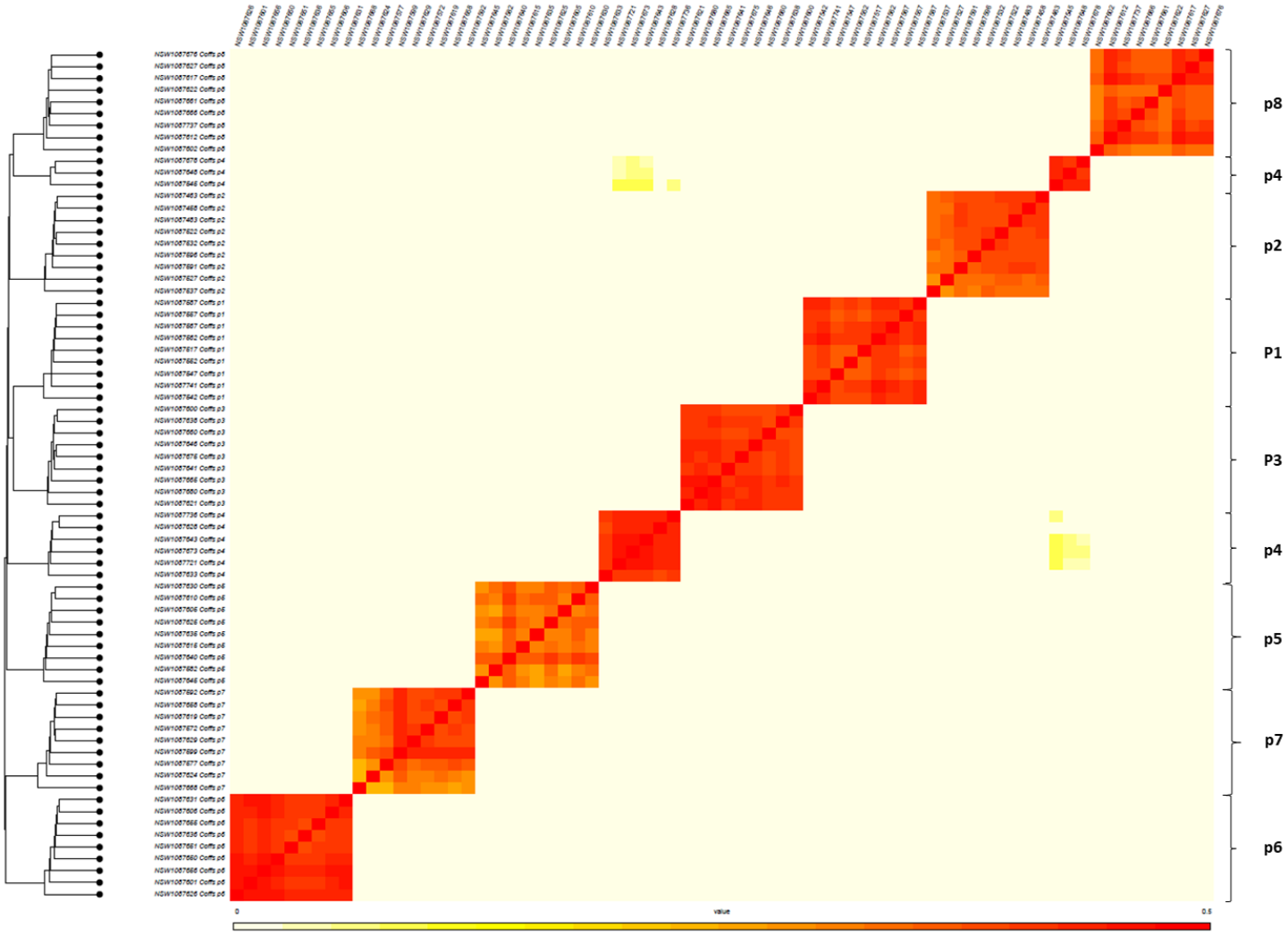

Figure 3. Composite UPGMA tree/Kinship heatmap analysed from the SNP data for 71 specimens of *Pittosporum* sp. Coffs Harbour.

All 72 specimens from the 8 study sites (labelled on the right of the pairwise matrix) were studied in a pairwise kinship analysis, and this resulted in the heatmap above consists of pairwise kinship coefficients displayed as colours: RED colouration corresponding to the highest pairwise kinship coefficients (0.4 or greater = clone), ORANGE-YELLOW colouration corresponding to medium pairwise kinship coefficients (less than 0.4 but greater than 0.25 = sibling) and EGGSHELL WHITE colouration corresponding to the lowest pairwise kinship coefficients (0). The descending red diagonal on the graph is the result of an individual matched with itself.

To the left of the heatmap is a UPGMA tree generated from a distance-based matrix of the SNP data. The tree tip labels include each individual's unique ID (i.e. NSW number) and site information (Table 1 summarises the kinship results here).

Table 1 Population sampling information and kinship results for *Pittosporum* sp. Coffs Harbour.

Population/patch ID	N samples analysed	Kinship		Kinship notes
		N unique genets	N genets of multiple ramets	
p1	9	1	1	
p2	9	3	1	1 genet has 7 ramets, and is siblings to the other 2 genets
p3	9	1	1	
p4	9	2	2	1 genet has 6 ramets, the other has 3 ramets
p5	9	9	0	all genets are siblings to each other
p6	9	1	1	
p7	9	4	1	1 genet has 6 ramets, and is siblings to the other genets
p8	9	3	1	1 genet has 7 ramets, and is sibling to the other genets

Table 2 Observed heterozygosity (H_O), expected heterozygosity (H_E), inbreeding coefficient (F_{IS}) for the sites of various *Pittosporum* species and overall values for *P.* sp. Coffs Harbour. Because of the high relatedness among the genets of P. sp. Coffs Harbour, population diversity could not be measured and instead we calculated the species' overall diversity.

	N	H_O	H_E	F_{IS}
Pittosporum sp. Coffs Harbour	9	0.281	0.254	-0.11
Pittosporum undulatum		0.215	0.222	0.03
Boambee Beach, Coffs Harbour	6	0.199	0.225	0.11
Moonpar, Nymboi Binderay NP	5	0.231	0.219	-0.05
Pittosporum revolutum		0.175	0.332	0.47
Reserve near Jordans Creek, Coffs Harbour	4	0.168	0.338	0.50
Treachery Beach, Seal Rocks	5	0.182	0.326	0.44
Pittosporum multiflorum		0.396	0.170	-1.33
Rolland Plains	4	0.405	0.164	-1.46
Way Way State forest	6	0.388	0.175	-1.21

Conservation genomics of *Pittosporum* sp. Coffs Harbour

Phase 2

prepared by the Research Centre for Ecosystem Resilience

(Royal Botanic Garden Sydney)

Background

A species-wide conservation genomic study of the newly discovered *Pittosporum* sp. Coffs Harbour was conducted to assess the genetic health, investigate the potential causes of its low reproductive output, and genomically select individuals to target for propagation in order to establish viable ex situ collections.

Results and Interpretations

We report results based on analyses of genomic data sourced from samples belonging to *Pittosporum* sp. Coffs Harbour. Sampling was undertaken across its known distribution at six sites/populations with more intensive sampling conducted at the site within the Coffs Harbour bypass development area across 23 patches/subsites (Table 1).

- A high-quality genome scan of 13,000 SNPs (single nucleotide polymorphisms) was successfully obtained and analysed for 354 individuals of *Pittosporum* sp. Coffs Harbour.
- Kinship analysis detected high relatedness among individuals from across the whole distribution (Fig. 1, Table 1): 62 unique genets were identified among the 354 individual ramets sampled. 17 of these unique genets were identified among 144 individuals sampled from the 10 patches scheduled to be cleared. Clonality was more extensive within than outside the development area, with multiple cases of ramets belonging to a single genet distributed among more than one patch.
- Diversity measures obtained across all populations detected reasonable levels of genetic diversity remaining across *P.* sp. Coffs Harbour (Table 2). All sites were differentiated, suggesting that at least one representative from each site is required to preserve overall species' evolutionary potential.
- Principal component analysis (Fig. 2) further validated the presence and distribution of genetic diversity, identifying some of genets recovered within the development area as particularly distinct from those located across the other sites outside the development area. This suggests that ex situ collections that maximises the species' evolutionary potential should include representative individuals from all sites including the impacted ones.
- Patches that produce fruit (patch 2,3,4) had similar levels of clonality than patches that did not produce fruit, but were geographically close to other genetically differentiated patches.
- Optimised scenarios representing selection of ex situ individuals to be included in representative collections that capture diversity within impacted sites and across the whole species are presented in Table 3. Non-optimisable

scenarios for salvage digs are also discussed in relation to those results (Fig. 3).

Figure 1. Study sites of *Pittosporum* sp. Coffs Harbour to assess the extent of its clonality. Each map indicates a site and each dot within a map indicates a sampled individual.

(a) shows all sites that are outside of the Coffs Harbour Bypass development area, with each differently coloured dot indicating a distinct genet, and dots of the same colour representing ramets of a single genet. Note that ramets of single genets were not shared across sites.

(b) shows all sampled patches within the Coffs Harbour Bypass Development area, labelled with a patch number code near each of the patches. The number of genets per patch are highlighted in the figure. The map shows that in the development site higher clonality was detected (despite more intensive sampling), and that sufficient representation of diversity can be obtained from a smaller number of individuals. More extensive clonality at the development site (38 genets from 281 samples vs. 24 genets from 73 samples for other sites) could be the result of historical disturbance events.

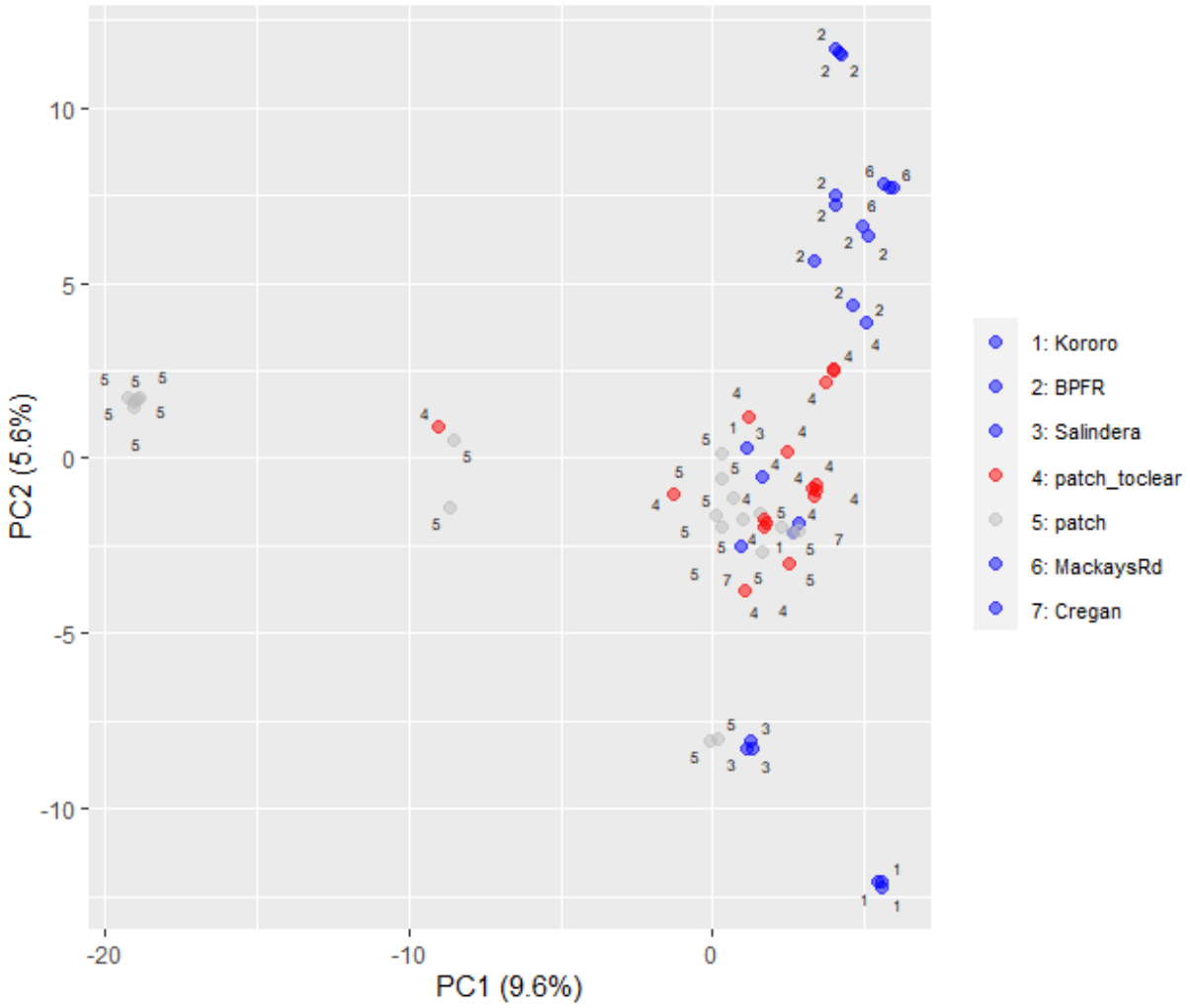

Figure 2. Principal Component Analysis (PCA) of the Single Nucleotide Polymorphism (SNP) data from the 62 unique genets of *Pittosporum* sp. Coffs Harbour.

The 62 genets were determined from the kinship analysis. Note that each dot represents a unique genet. Any genets belonging to "patch" is within the Coffs Harbour Bypass development area but not impacted, and any genets belonging to "patch_toclear" is within the area that will be impacted. Refer to Table 1 and Fig.1 for details on specific patches.

This PCA indicates genetic diversity is present in the species and that some of the diversity within the development area is measurably distinct from the other sampled sites.

Table 1. Sampling information and kinship results for *Pittosporum* sp. Coffs Harbour. Patches with an asterisk (*) are those to be removed as part of the Coffs Harbour Bypass development.

Sampling of this study further validates that the species is extensively clonal with only 62 genets identified out of 354 samples.

Site	Number of unique genets	Number of samples	Average distance between individuals (m)	Notes
Sites outside of the Coffs Harbour Bypass Development area				
BPFR	10	28	85.8	
Cregan	2	8	9.4	
Kororo	5	19	53.1	
Mackays Rd	3	8	6	
Salindera	4	10	77.1	
Site within Coffs Harbour Bypass Development area				
p1	1	9	0	
p2	6	22	7.1	patch 2,3 share a genet of multiple ramets. Number of genets identified differs from previous (9 genets) because previously, sampling of *P.* sp. CH was less representative of species' diversity, resulting in fewer SNPs detected and consequently less sensitive detection of relatedness among individuals.
p3	2	12	11.5	
p4	3	22	15.9	patch 4,13,14 share a genet of multiple ramets
p5	2	9	10.1	
p6	1	9	10.4	patch 6,7,8 share a genet of multiple ramets
p7	1	9	6.7	patch 6,7,8 share a genet of multiple ramets
p8		4	10.3	patch 6,7,8 share a genet of multiple ramets
p9	1	9	36.5	
p10	1	9	6.5	
p11	1	9	15.6	
p12*	2	12	10.9	
p13*		3	0	patch 4,13,14 share a genet of multiple ramets
p14*	1	11	8.3	patch 4,13,14 share a genet of multiple ramets
p15*	4	23	7.6	
p16*	2	22	7.5	
p17*	1	22	6.5	
p18*	3	22	5.7	
p19*	1	9	5.5	
p20*	1	8	0.6	
p21	1	9	7.9	
p22*	2	12	8.6	
p23	1	5	9.8	
Total	62	354		

Table 2. Measures of diversity for all sites of *P.* sp. Coffs Harbour: observed heterozygosity (H_O), expected heterozygosity (H_E), inbreeding coefficient (F_{IS}).

All populations within *P.* sp. Coffs Harbour display genetic variability despite high relatedness and clonality among individuals within each site. Positive F_{is} values at the development site suggests the likelihood of biparental inbreeding (as expected in circumstances of recent bottleneck and relatively limited gene flow).

Site	H_O	H_E	F_{IS}	Number of genets
Sites outside of the Coffs Harbour Bypass Development area				

BPFR	0.281	0.279	-0.012	10
Cregan	0.299	0.196	-0.495	2
Kororo	0.282	0.241	-0.13	5
Mackays Rd	0.283	0.152	-0.842	3
Salindera	0.297	0.214	-0.307	4
Site within Coffs Harbour Bypass Development area				
Combined patches that are **not scheduled** to be cleared	0.285	0.293	0.038	21
Combined patches that are **scheduled** to be cleared	0.284	0.298	0.044	17
	Ave: 0.287	**Ave: 0.239**		**Total: 62 genets**

Figure 3. The Table below is extracted from the Pittosporum Management Plan developed by transport for NSW and shows the current translocation strategy.

A range of optimised propagation planting scenarios are presented in Table 3 (following this figure). They include optimised solutions including an option considering the representative genets from impacted sites only (also used to trial propagation success), and a range of optimised solutions that include all living genets and are more evolutionary representative for overall conservation targets. The solutions are based on 600 propagated individuals (as per prior agreements) and once propagation success is evaluated, optimal plantings including 125 individuals (as per Plan below) each can be quickly finalised.

Recipient Site Name	*Fontainea* sp. Coffs Harbour		*Pittosporum* sp. Coffs Harbour	
	Salvage Translocation	Propagation Planting	Salvage Translocation	Propagation Planting
Jordans Creek Exclusion Zone	1 mature individual	83 propagated plants	4 discrete patches	-
APO 38	-	83 propagated plants	3 discrete patches	125 propagated plants
APO 75 & 78	-	-	3 discrete patches	-
Kororo Nature Reserve	-	84 propagated plants	-	125 propagated plants

Salvage plantings cannot be optimised as numbers of ramets to be moved (as well as survival rates) are unknown. Furthermore, replicating existing conditions is unlikely to fully re-invigorate the fitness of patches and fully re-establish reproductive success that appears to having been lost from most of the current patches due to high clonality and inbreeding levels (as suggested by the data presented above). Nevertheless, the information presented in Figure 2 and Tables 2,3 can support planning. Figure 2 shows that there is little genetic divergence among the patches to be removed so that mixing can focus on minimising kinship rather than on maximising divergence.

Tables 2, 3 identify where the single genets are located within each of the patches (as well as where single genets are replicated among patches). That will help identify what the patches contain and how to keep track of numbers of genets included within each salvage dig. The solutions on Table 3 can then provide additional information on how to mix the salvage digs. Although section (b) is designed for a species-wide translocation strategy, it identifies solutions for increasing numbers of samples based on increasing relatedness (i.e. the solution for 20 genets suggests 4 genets from the impacted area that can be mixed, the solution for 30 identifies another 4, and so on). Following this genet-based strategy should satisfy the needs of the salvage translocation plan (as listed in the Management Plan) since it is now possible to identify what genets are present within each dig.

Table 3. List of individuals and respective numbers of cuttings needed to generate propagation populations of optimal genetic diversity (a) for the impacted sites only and (b) across the whole species. Selected patches with an asterisk (*) are those to be removed by the development.

Numbers are based on an ex situ collection of 600 cuttings representing (a) a sampling strategy to obtain maximum diversity from the impacted sites. (b) Alternative strategies are also provided, to generate propagation populations representative of species diversity across all sites (within and outside the impacted area) to increase the species' chance of survival in the longer term.

The table lists all unique genets identified in this study. To help with identification, for each genets with multiple ramets, unique genetic sample numbers assigned by ReCER (NSW number) and the individual number assigned by Andrew Benwell (Individual) are listed.

When sampling, cuttings should be taken from a different ramet/stem where possible, and so more details about other ramets (for impacted genets) are provided below to assist with the sampling. More details about ramets for all populations can be provided if requested. Once the number of plants that have been successfully propagated is known, ReCER will provide further advice on the exact planting strategy (and possibly more if the opportunity arises).

NSW sample	Site	Individual	(a) To capture diversity within impacted sites	(b) To capture maximum species diversity				
			N cuttings to obtain from 17 genets	N cuttings to obtain from 10 genets	N cuttings to obtain from 20 genets	N cuttings to obtain from 30 genets	N cuttings to obtain from 40 genets	N cuttings to obtain from 50 genets
NSW1154164	BPFR	indiv no. 250		60	30	20	15	12
NSW1154160	BPFR	indiv no. 256		60	30	20	15	12
NSW1154161	BPFR	indiv no. 257		0	0	0	0	12
NSW1154154	BPFR	indiv no. 260		0	30	20	15	12
NSW1154150	BPFR	indiv no. 266		0	0	20	15	12
NSW1154305	BPFR	indiv no. 277		0	30	20	15	12
NSW1154303	BPFR	indiv no. 275		0	0	0	0	0
NSW1154309	BPFR	indiv no. 271		0	0	0	15	12
NSW1154298	BPFR	indiv no. 280		60	30	20	15	12
NSW1154306	BPFR	indiv no. 278		0	0	20	15	12

NSW sample	Site	Individual	(a) To capture diversity within impacted sites	(b) To capture maximum species diversity				
			N cuttings to obtain from 17 genets	N cuttings to obtain from 10 genets	N cuttings to obtain from 20 genets	N cuttings to obtain from 30 genets	N cuttings to obtain from 40 genets	N cuttings to obtain from 50 genets
NSW1154225	Cregan	indiv no. 191		0	0	0	0	12
NSW1154220	Cregan	indiv no. 196		0	30	20	15	12
NSW1087627	Kororo	indiv no. 75		0	0	0	0	12
NSW1087622	Kororo	indiv no. 77		0	0	20	15	12
NSW1087602	Kororo	indiv no. 78		60	30	20	15	12
NSW1154174	Kororo	indiv no. 240		0	30	20	15	12
NSW1154170	Kororo	indiv no. 246		0	0	20	15	12
NSW1150492	MackaysRd	indiv no. 5		0	0	0	0	12
NSW1150491	MackaysRd	indiv no. 4		60	30	20	15	12
NSW1150495	MackaysRd	indiv no. 8		0	0	20	15	12
NSW1154213	Salindera	indiv no. 199		0	0	0	15	12
NSW1154208	Salindera	indiv no. 203		0	0	0	15	12
NSW1154209	Salindera	indiv no. 205		0	0	0	0	0
NSW1154216	Salindera	indiv no. 202		0	30	20	15	12
NSW1087675	p1	indiv no. 23		60	30	20	15	12
NSW1087630	p2	indiv no. 46		0	0	0	0	0
NSW1087615	p2	indiv no. 48		0	0	0	0	0
NSW1087645	p2	indiv no. 42		0	0	0	0	0
NSW1087582	p2	indiv no. 49		0	0	0	15	12
NSW1087635	p2	indiv no. 44		0	0	20	15	12
NSW1087605	p2	indiv no. 41		60	30	20	15	12

NSW sample	Site	Individual	(a) To capture diversity within impacted sites	(b) To capture maximum species diversity				
			N cuttings to obtain from 17 genets	N cuttings to obtain from 10 genets	N cuttings to obtain from 20 genets	N cuttings to obtain from 30 genets	N cuttings to obtain from 40 genets	N cuttings to obtain from 50 genets
NSW1152869	p3	indiv no. 23		0	0	0	0	0
NSW1152872	p3	indiv no. 24		0	0	0	0	0
NSW1152896	p4	indiv no. 49		0	0	0	0	12
NSW1087633	p4	indiv no. 32		60	30	20	15	12
NSW1087678	p4	indiv no. 37		0	0	20	15	12
NSW1154081	p5	indiv no. 128		0	30	20	15	12
NSW1154084	p5	indiv no. 131		0	0	0	0	0
NSW1154133	p6	indiv no. 140		60	30	20	15	12
NSW1154137	p7	indiv no. 144		0	0	0	15	12
NSW1154113	p9	indiv no. 164		0	0	0	0	12
NSW1154094	p10	indiv no. 168		0	0	0	0	0
NSW1154102	p11	indiv no. 186		0	0	20	15	12
NSW1154062	p12*	indiv no. 110	30 (indiv no. 102-110)	0	0	0	15	12
NSW1154064	p12*	indiv no. 112	30 (indiv no. 111-113)	0	30	20	15	12
	p13*		60 (indiv no. 99-101)					
NSW1152945	p14*	indiv no. 98	60 (indiv no. 87-98)	0	0	0	0	12
NSW1087577	p15*	indiv no. 61	15 (indiv no. 61-69)	0	0	0	0	12
NSW1087624	p15*	indiv no. 70	15	0	0	20	15	12
NSW1087668	p15*	indiv no. 64	15	60	30	20	15	12
NSW1152923	p15*	indiv no. 76	15 (indiv no. 75-86)	0	0	0	0	0
NSW1087650	p16*	indiv no. 51	30 (indiv no. 51-66)	0	0	0	0	0

NSW sample	Site	Individual	(a) To capture diversity within impacted sites	(b) To capture maximum species diversity				
			N cuttings to obtain from 17 genets	N cuttings to obtain from 10 genets	N cuttings to obtain from 20 genets	N cuttings to obtain from 30 genets	N cuttings to obtain from 40 genets	N cuttings to obtain from 50 genets
NSW1087651	p16*	indiv no. 57	30	0	30	20	15	12
NSW1087557	p17*	indiv no. 1	60	0	30	20	15	12
NSW1087483	p18*	indiv no. 12	20	0	0	0	0	0
NSW1087527	p18*	indiv no. 11	20	0	0	20	15	12
NSW1087537	p18*	indiv no. 17	20 (indiv no. 1-10)	0	30	0	15	12
NSW1154205	p19*	indiv no. 211	60 (indiv no. 7-15)	0	0	0	15	12
NSW1152915	p20*	indiv no. 68	60 (indiv no 67-74)	0	0	20	15	12
NSW1152885	p21	indiv no. 38		0	0	0	0	12
NSW1154066	p22*	indiv no. 114	30	0	0	0	0	12
NSW1154067	p22*	indiv no. 115	30 (indiv no. 115-125)	0	0	0	15	12
NSW1154112	p23	indiv no. 176		0	0	0	15	12