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Small research and development activity

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prepared by	David Bush
co-authors/ contributors/ collaborators	David Bush, Lex Thomson, Linda Broadhurst, Sonu Dutt, Ponijesi Bulai, Tevita Faka'osi, Maloni Havea, Sunia Napa'a, Leody Vainikolo
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1 Acknowledgments

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2 Executive summary

Native sandalwood (*Santalum yasi*) is an important cultural and economic forest product throughout its natural range in Fiji and Tonga where it is known as yasi dina and ahi, respectively. However, years of overharvesting have seriously depleted this resource, and may have significantly reduced the genetic diversity, especially in regions where cutting has been most heavy. Ongoing demand for high quality sandalwood and sandalwood oil creates an opportunity to develop a sustainable, planted resource in Fiji and Tonga, but basic questions about the species' genetic diversity and population structure must first be answered. An additional issue, and opportunity, is the widespread presence of introduced Indian sandalwood (*S. album*) throughout Fiji and Tonga: this species readily hybridises with *S. yasi* to produce trees that can be productive and vigorous on some site types. On one hand, the presence of the *S. album* and hybrids presents a threat to the diversity and integrity of the *S. yasi* population (being a source of so-called "genetic pollution"), while on the other the hybrids may present commercial opportunities.

The aim of this small research activity was to characterise and quantify the genetic diversity of native sandalwood (*Santalum yasi*) in Fiji and Tonga for use in future tree improvement and germplasm conservation programs. This was achieved by surveying planted and wild populations of natural and introduced sandalwood and applying morphological and molecular genetic markers to assess the levels of spontaneous hybridisation among the sandalwoods.

The research found that morphological markers including leaf size and shape, fruit characteristics and general habit of the tree can be effective for differentiating pure *S. yasi* from interspecific hybrids. It also found that the use of molecular markers provided greater certainty in differentiating pure *S. yasi* from *S. album* and their interspecific hybrid. The marker panel tested can be used to identify pure *S. yasi* with a high degree of certainty. There was agreement between in-field assessment of taxon using morphological traits and the molecular markers for the majority of trees. However, in some cases it would appear that incorrect determinations were made in the field where morphological traits such as fruit were absent. The ability to use molecular markers is likely to become increasingly vital where hybrids have back-crossed to pure species, producing offspring with only subtle morphological differences between the pure species and hybrid.

This project has determined that pure *S. yasi* has significant genetic diversity, with population differentiation throughout the islands of Fiji and Tonga. However, significant levels of inbreeding were detected, and wild populations are now scarce, highly fragmented and may possibly have lost the capacity to be self-sustaining. The assembly of genetically diverse seed production areas and gene conservation stands is strongly recommended in both Fiji and Tonga. It would then be possible to produce more vigorous planting stock that will improve commercial returns and play a role in *ex situ* or *circa situm* conservation of the species. It is recommended that these activities be carried out as part of an industry development plan for *S. yasi*.

Indications from this study are that, while widespread hybridisation does not seem to have occurred, a small proportion of hybrids were detectable. The widespread planting of hybrids is likely to increase the risks associated with pollen contamination and threatens the genetic integrity of *S. yasi*. Apart from the ecological consequences of this, widespread hybridisation may harm the potential to develop and market *S. yasi* as a unique and differentiated Pacific Islands sandalwood product (as distinct from Indian sandalwood which is now being planted in Australia and elsewhere at a large scale). This presents an additional motivation for taking active measures to conserve *S. yasi* in its genetically pure form.

3 Introduction

The sandalwoods (*Santalum* spp.) are a genus of hemiparasitic trees that, collectively, have a natural range from southern Australia extending north to Indonesia, India and Pakistan and east into the Pacific Ocean ranging from Hawai'i to the Juan Fernández islands in the south east. Many of the 15 species have commercially valuable heartwood that can be destructively harvested for essential oil extraction. Of the *Santalum* species, *S. album* (Indian sandalwood) is generally considered to be the most commercially valuable. It possesses a high yield of high quality oil that can be used in perfumery and incense wood applications. Other species with high quality, oil-yielding wood that are commercially exploited include *S. austrocaledonicum*, *S. spicatum* and *S. yasi*. Sandalwood is commonly used in India, Taiwan and Hong Kong, with smaller markets in Europe, Japan and North America (Page et al. 2012a). Commercial exploitation has now been underway for two centuries, with the result that *Santalum* is considered to be one of the most heavily exploited groups of plants across its range (Brennan and Merlin 1991). The phylogenetic relationships within the *Santalum* genus were resolved by Harbaugh and Baldwin (2007) who delineated relationships within 15 species of the genus and postulated an Australian ancestor, with species radiating out into the Pacific. According to this phylogeny, *S. album* is most closely related to *S. yasi*, a species that is native to Fiji. The Tongan population is considered by some authors to have been introduced from Fiji by humans in pre-European times (Brennan and Merlin 1991; Harbaugh and Baldwin 2007). A small population is also known to exist in Niue: this is also likely to have been introduced by humans possibly in ancient or Polynesian trade (Thomson 2006). *S. yasi* is an important cultural and economic plant in Fiji, where it is known as 'yasi dina' and Tonga where it is called 'ahi'. However harvesting over the last two centuries has severely depleted wild populations. A recent study and survey carried out in Fiji and Tonga by Huish et al. (2015) has revealed that large mature trees are absent in the wild, and that most stands show evidence of regenerative stress as evidenced by size class distributions.

Despite the widespread fragmentation and destruction of wild populations, the planting of *Santalum* spp. including *S. yasi*, *S. album* and to a lesser extent *S. austrocaledonicum* has been encouraged in Fiji and Tonga, and the practice of smallholder planting is rapidly increasing in popularity. While this does auger well for the conservation of *S. yasi* diversity, the increasingly widespread presence of the introduced species provides a potential threat through hybridisation and introgression of exogenous genes to the wild populations. That *S. album* and *S. yasi* produce spontaneous hybrids is well established, and Huish et al. (2015) noted that hybrid populations have now naturalised in Fiji and Tonga. Page et al. (2012b) have also found that there are no breeding barriers between *S. album* and *S. austrocaledonicum*, which implies that the possibility of spontaneous hybrids between *S. yasi* and *S. austrocaledonicum* is quite likely.

Securing the future of *Santalum yasi* throughout its natural range is important both for the sake of biodiversity conservation and also because it is a plant of considerable cultural value and commercial potential. To underpin a strategy to conserve and develop the species, basic information on its genetic structure and diversity is required, as well as assessment of the impact of hybridisation with introduced species. We report the results of genetic analysis undertaken on a broad sample of *S. yasi*, *S. album* and interspecific hybrid trees throughout Fiji and Tonga.

4 Research objectives

The objectives of this project were as follows:

1. To assess the overall diversity and population structure of *S. yasi* throughout its natural range in Fiji and Tonga.
    - Quantifying the overall diversity of *S. yasi* is a critical first step to managing its conservation and developing plans for genetic improvement and commercial development of the species.
2. To assess the level of introgression of *S. album* genes in wild populations.
    - It is well known that *S. yasi* hybridises with *S. album*, and with the widespread planting of both *S. album* and hybrids, “genetic pollution” of the pure *S. yasi* population (including wild and planted stands) might ensue. The long-term consequence might be the loss of *S. yasi* as a distinct species.
3. To ascertain whether or not morphological and/or molecular genetic markers can be used to discriminate between the pure species and their hybrid.
    - It is important that a means of discriminating between *Santalum* taxa, including the pure species and hybrids, is developed. Morphological markers such as leaf, fruit and other characteristics can be used, and have the advantages of being readily applied in the field and being cost-free. However it is unknown how effective comparative morphology is for detecting hybrids, especially as some hybrids can be almost indistinguishable from one or other of the parent species, particularly when traits such as fruit are absent at the time of assessment. Our objective was to compare in-field taxon determinations with molecular marker-based ones to assess the relative degree of reliability of each method.

5 Sampling strategy and acquisition

Samples were acquired throughout the natural range of *S. yasi* in Fiji and Tonga. Locations of known natural plantations and planted stands were supplied by project collaborators from Ministry of Agriculture, Food, Fisheries and Forests (MAFFF) in Tonga and Ministry of Fisheries and Forests (MAFF) in Fiji. In many cases samples were collected from within and around villages. Individual villages in some cases had plantings of local and imported sandalwood including pure *S. yasi*, *S. album* and hybrids. In some cases seedlings had been brought in from other locations or supplied by government forestry extension officers. A few *S. austrocaledonicum* trees were also sampled.

Localities of collections are given in Figures 1a (Fiji) and 1b (Tonga). Locality code information, such as the composition of taxa sampled, is given in Table 1.

In addition to these collections the MAFF *Santalum* gene bank and seed production plantings at Vunimaqo (Viti Levu, code VQM on Figure 1a) were more intensively sampled. The gene bank was established by the AusAID-SPRIG (South Pacific Regional Initiative on Forest Genetic Resources) project in 2002 and contained 11 families, 10 of which were pure *S. yasi* from Lakeba in the Lau island group and one *S. album* x *yasi* hybrid family. The seed production planting contained five bulked seedlots of *S. yasi* thought to be from Bua, Vanua Levu, five *S. album* and two *S. album* x *yasi* hybrids.

Data for each sample were recorded on a project data sheet (Appendix 1). The datasheet cross-references photographs of each tree's general situation, bark and reproductive structures (flowers, fruit and seed where available). Notes were also made about each sampled tree's host plant(s). Ten fully expanded adult leaves were collected from each tree. These were photographed on a sheet of white A4 paper so that scaled measurements could be made later using computer-based image analysis software. The position of each tree was recorded using an Etrex 30 hand-held GPS (global positioning system) unit (Garmin, Kansas, USA). The precise tree location data are held by MAFFF Tonga and MAFF Fiji and are not included in this report, due to the risk of illegal harvesting.

The ten leaves were then fully desiccated using silica for DNA extraction and long-term storage. Leaves were exported to Australia for analysis, with a duplicate set of leaves collected in Fiji remaining there.

Species determination

Due to the fairly widespread introduction of *S. album* throughout Fiji and Tonga, and natural and human-mediated dispersal of *S. album* x *yasi* hybrids, the taxonomic status of sampled trees was not immediately clear in all cases. In-field taxonomic determination was therefore made on the basis of morphological features (see later section on morphological determinations) in combination with expert knowledge of the origins of certain plantings (for example authors LT, PB, TF, MH, SN, LV have been closely involved with the promotion and distribution of economic sandalwood plantings through their forestry extension roles in their respective countries). Many of the sandalwood plantings at Vunimaqo, Fiji were implemented during the AusAID-SPRIG project, and thus the origins of each tree had been documented. Where, in a few cases, the morphological features led to somewhat uncertain taxonomic determination, the individual was still placed in a taxon, but a note was made on its data sheet. This needed to be done for around 10% of trees.

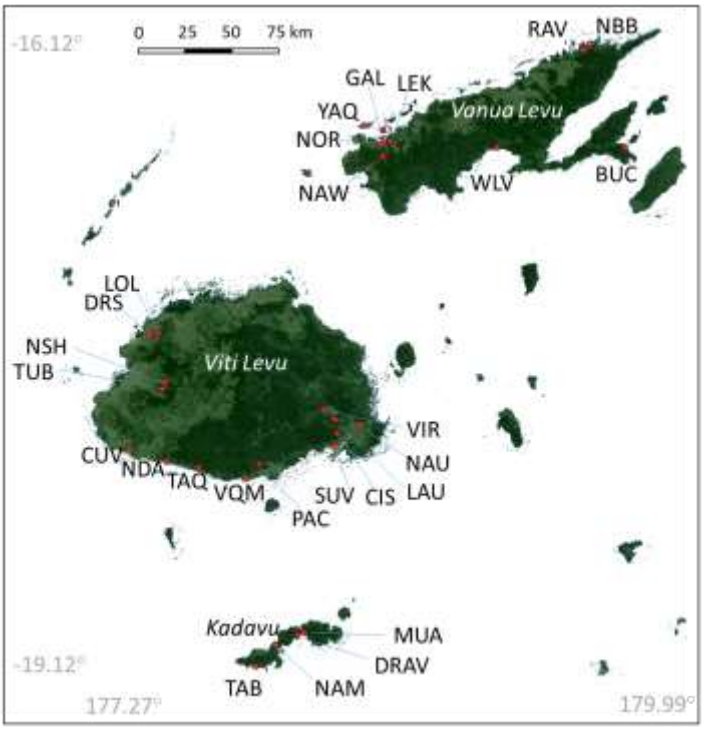

Figure 1a locations of sample collections on the islands of Galoa, Kadavu, Vanua Levu, Viti Levu and Yaqaga in Fiji

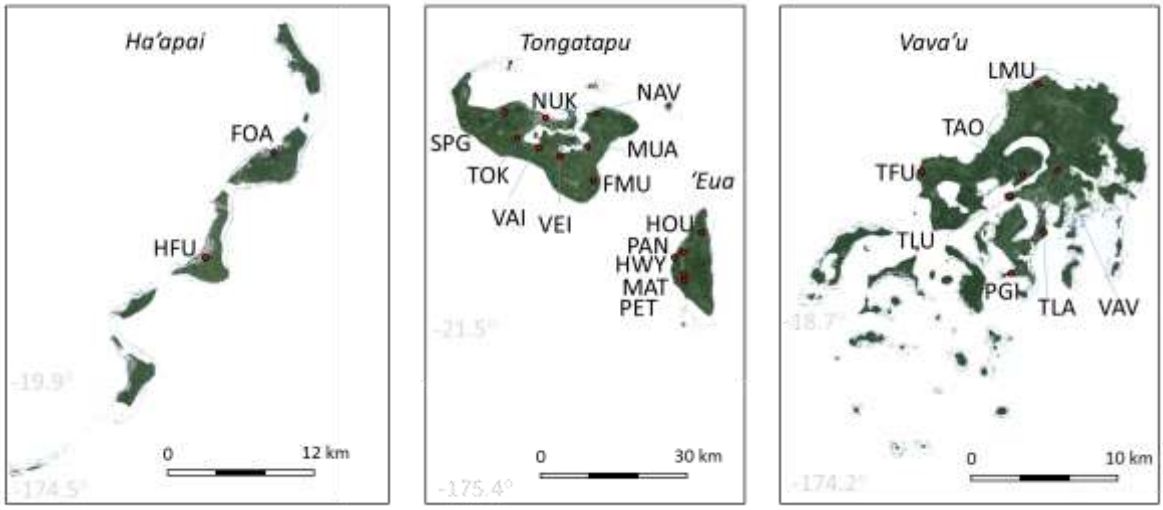

Figure 1b Locations of sample collections from the island groups of 'Eua, Ha'appai, Tongatapu and Vava'u, Tonga

Table 1 Summary information for sample locations by taxon (***S. a*** *album*; ***S. c*** *austrocaledonicum*; **S. h** *album* by *yasi* hybrid; ***S. y*** *yasi*) in Fiji and Tonga

Island	Code (Fig.1)	Subpopulation	No of samples by taxon				Forest type
			S. a	*S. c*	*S.* h	*S. y*	
Fiji							
Kadavu	DRAV	Dravuwalu Village			3	5	Planted
Kadavu	MUA	Muanisolo Village				8	Planted, Wild
Kadavu	NAM	Namuana Village				7	Planted
Kadavu	TAB	Tabuya Village			1	5	Planted
Viti Levu	CIS	Colo I Suva	4		8	4	Planted
Viti Levu	CUV	Cuvu, Sigatoka				4	Planted
Viti Levu	DRS	Drasa, Lautoka	5				Planted, natural regeneration (1)
Viti Levu	LAU	Laulau, Naitasivi		1	24	2	Planted
Viti Levu	LOL	Lololo, Lautoka			3	4	Planted
Viti Levu	NAU	Lakena and Naila, Nausori	1		10		Planted
Viti Levu	NDA	Namada Village	1		1		Planted
Viti Levu	NSH	Nausori Highland Village			1	5	Planted
Viti Levu	PAC	Pacific Harbour				2	Natural regeneration
Viti Levu	SUV	Suva: Wailoku, Civic Centre, Raiawaqa, Newtown, Nadera	1	1	4	4	Planted
Viti Levu	TAQ	Tagaqa			5		Planted
Viti Levu	TUB	Tubenasolo Village				7	Planted, Wild
Viti Levu	VIR	Viria			5	5	Planted
Viti Levu	VQM	Vunimaqo gene bank with material from Lau and Bua	7		2	18	Planted
Vanua Levu	BUC	Nawi Village, Savusavu				10	Planted
Vanua Levu	LEK	Votua Village, Lekutu				9	Planted (8), natural regeneration (1)
Vanua Levu	NAW	Nawailevu, Bua	3			5	Planted
Vanua Levu	NBB	Lakeba Village			1	9	Planted
Vanua Levu	NOR	Noro Village				5	Planted
Vanua Levu	RAV	Ravuka Village	1	1	1	2	Planted
Vanua Levu	WLV	Wailevu			1	4	Planted
Galoa	GAL	Galoa Island, Bua				8	Planted
Yaqaga	YAQ	Yaqaga Island, Bua				9	Planted (5), wild (4)
Tonga							
‘Eua	HOU	Houma				14	Wild
‘Eua	HTUA	Ha' Atu'a	2	2			Planted
‘Eua	HWY	Tutuvai, near Hideaway resort				3	Natural regeneration
‘Eua	PET	Mata'Aho	1			4	Planted/ Natural regeneration
‘Eua	PAN	Pangai				3	Natural regeneration
‘Eua	PET	Petani/Ha' Atu'A			2	7	Natural regeneration

Continued over…

Island	Code (Fig.1)	Subpopulation	No of samples by taxon				Forest type
			S. a	*S. c*	*S.* h	*S. y*	
Ha'apai	FOA	Lotofoa		2		4	Planted
Ha'apai	FOA	Lotofoa swamp				2	wild?
Ha'apai	FOA	Niu a Kalo				1	Wild?
Ha'apai	HFU	Salote Pilolevu airport				1	Wild
Ha'apai	HFU	Houmatafua, Lifuka	1		3	6	Planted
Tongatapu	TOK	Tokomalolo forestry station	1			2	Planted
Tongatapu	NUK	Church of Tonga Nuku'alofa	1				Planted
Tongatapu	MUA	Mua Village, Talasiu	1			1	Planted, natural regeneration
Tongatapu	NAV	Navutoka				3	Wild
Tongatapu	FMU	Fatumu				1	Planted
Tongatapu	VAI	Vaini	1				Planted
Tongatapu	VEI	Veitongo village	1				Planted
Tongatapu	SPG	SPRIG planting	2			1	Planted
Vava'u	LMU	Leimatu'a				2	Planted
Vava'u	PGI	Pangai, Pangaimotu				3	Natural regeneration
Vava'u	TAO	Taoa				2	Planted?
Vava'u	TFU	Toafa, Longomapu	3			2	Planted trial
Vava'u	TLA	Toula				2	Wild
Vava'u	VAV	Vavau MAFF station/ Mangia Village			1	4	Planted
Vava'u	TLU	Mo'unga Talau				1	Wild
Total			37	7	76	210	

6 Analysis of morphological characters

Leaf morphology, fruit, seed and tree habit characters are important for differentiating between *Santalum* species, and were used together for the in-field determination of taxon for each tree sampled in this project.

6.1 Fruit and seed characters

Though fruit and seed characters were found to be useful for differentiating among taxa, they were not available for all trees, and insufficient data were available for integration with the quantitative analysis of leaf data. However, a description of the observed differences between the taxa follows: *Santalum yasi* and *album* have broadly similar fruit, both are globose-ellipsoid, single-seeded, fleshy drupes crowned with a calyx scar (Thomson 2006). The position and size of the scar and the colour and shape of the seeds are differentiating traits. *S. yasi* has a small or very small scar enclosing a stout, cone-shaped point and a dark-coloured, pointed seed that is elliptical in profile, whereas *S. album* has a large scar, usually truncating the top of the fruit enclosing the flat or slightly depressed disc that ends in a small point, usually with a pale-coloured, almost spherical seed, sometimes also with a small point. Hybrid fruit are usually intermediate in these characters, but can sometimes be almost indistinguishable from one or other of the pure species. Images of typical fruit and seeds are shown in Figure 2.

Figure 2 Typical fruit and seed of *S. album* **A** fruit (ripe), **B** seed; *S. yasi* **C** fruit (ripe) **D** seed; *S. album* x *yasi* hybrid **E** fruit (ripening) **F** seed

6.2 Leaf characters

Generally, *S. yasi* has narrower leaves that are more lanceolate than are *S. album* leaves, while hybrid leaves are intermediate: differences in leaf width are most evident in seedlings and juvenile plants. The few *S. austrocaledonicum* leaves sampled in this project were generally very narrow and had a purplish colour: however some mature plants on 'Eua had very broad leaves. Though leaf colour was recorded for each tree sampled (ranging from yellowish-green to dark green in the *S. album*, *S. yasi* and hybrids), it was observed that this trait is strongly affected by the environment, particularly exposure to light and the overall relationship with the host plant(s), which influences access to nutrients and water. One of our objectives was to quantify leaf parameters for each taxon and determine whether or not they can be used to help quantitatively determine taxon.

A sample of ten, fully expanded and representative leaves was taken from each tree. These leaves were photographed against a sheet of white A4 paper, the edges of which were later used to calibrate computer-based measurements of the leaf (ADI Suite, University of California and Museum of Science, Boston, USA). Parameters measured included the length (L), width (W) and the wide point (WP), i.e. the distance from the leaf base along the leaf central axis to the point where the leaf is at its widest (Figure 3). Derived parameters include L/W and WP/L x 100. The average of each parameter was taken for each tree. Overall significance of differences among the traits and standard errors of differences among trait means were calculated using the REML (restricted maximum likelihood) procedure in Genstat 16 (VSN International, UK).

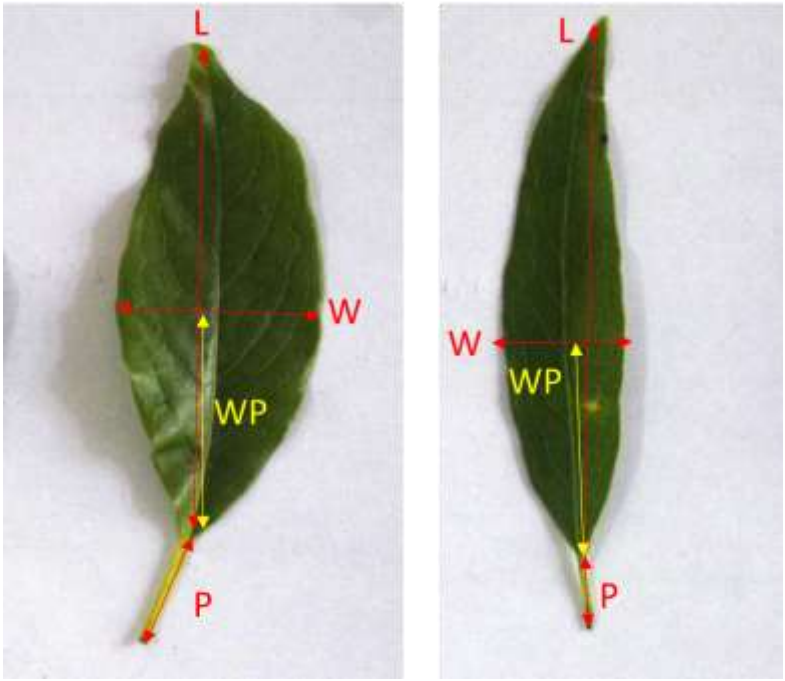

Figure 3 *S. album* (left) and *S. yasi* (right) leaves showing measured leaf parameters *L* (length from base to tip), *W* (width at widest point), *WP* distance from base to point of intersection of *W* and *L* and *P* petiole length.

Box plots describing the distribution of the measured and derived parameters are shown in Figure 4, with trait means, standard deviations and differences among means shown in Table 2. There were significant differences ($p<0.001$) among all traits. While differences in leaf length among taxa are not very marked, with significant overlap among taxa, *S. album* and *S. austrocaledonicum* are significantly wider and narrower, respectively, than either *S. yasi* or the *S. album* x *yasi* hybrid. Most *S. yasi* trees have a higher length to width ratio than either *S. album* or their hybrid, whereas *S. album* and the hybrid generally have longer petioles than *S. yasi* and the small sample of *S. austrocaledonicum*.

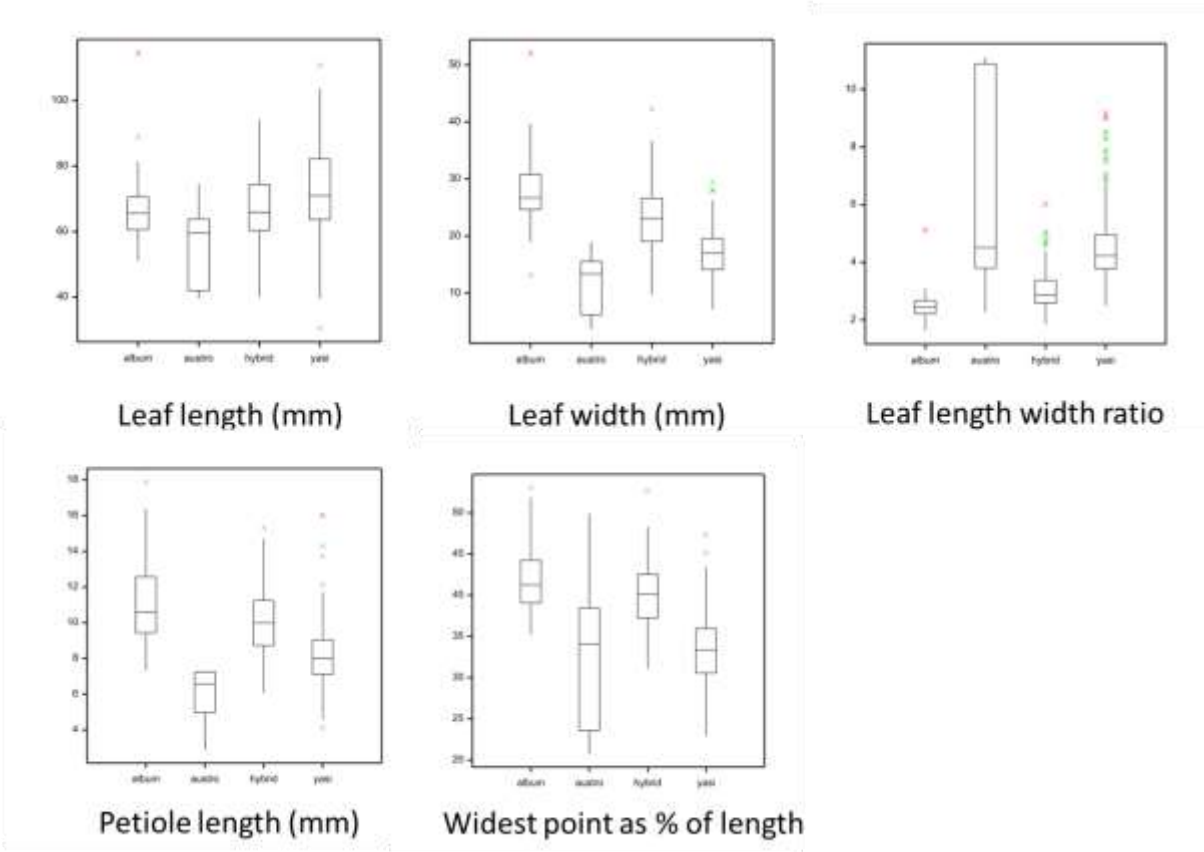

Figure 4 Box plots showing the distribution of data for leaf morphology traits. Boxes show the median and interquartile range while whiskers and outliers show the full range of the data.

Table 2 Mean, standard deviation and significant differences among treatments (calculated as twice the standard error of difference of REML means).

Trait	*S. album*	*S. yasi*	*S. album* x *yasi*	*S. austrocaledonicum*
Length (mm)	67.15^{bc}±11.74	72.4^{a}±13.39	67.64^{b}±11.55	55.3^{c}±14.32
Width (mm)	28.06^{a}±6.733	16.92^{c}±4.09	23.16^{b}±5.96	11.47^{d}±6.14
Length/width	2.49^{d}±0.558	4.60^{b}±1.274	3.09^{c}±0.79	6.60^{a}±4.08
Wide point intersect (% of length)	42.12^{a}±4.268	33.34^{c}±4.226	40.08^{b}±4.04	32.76^{c}±11.31
Petiole length (mm)	11.12^{a}±2.452	8.084^{c}±1.618	10.02^{b}±2.02	5.92^{d}±1.82

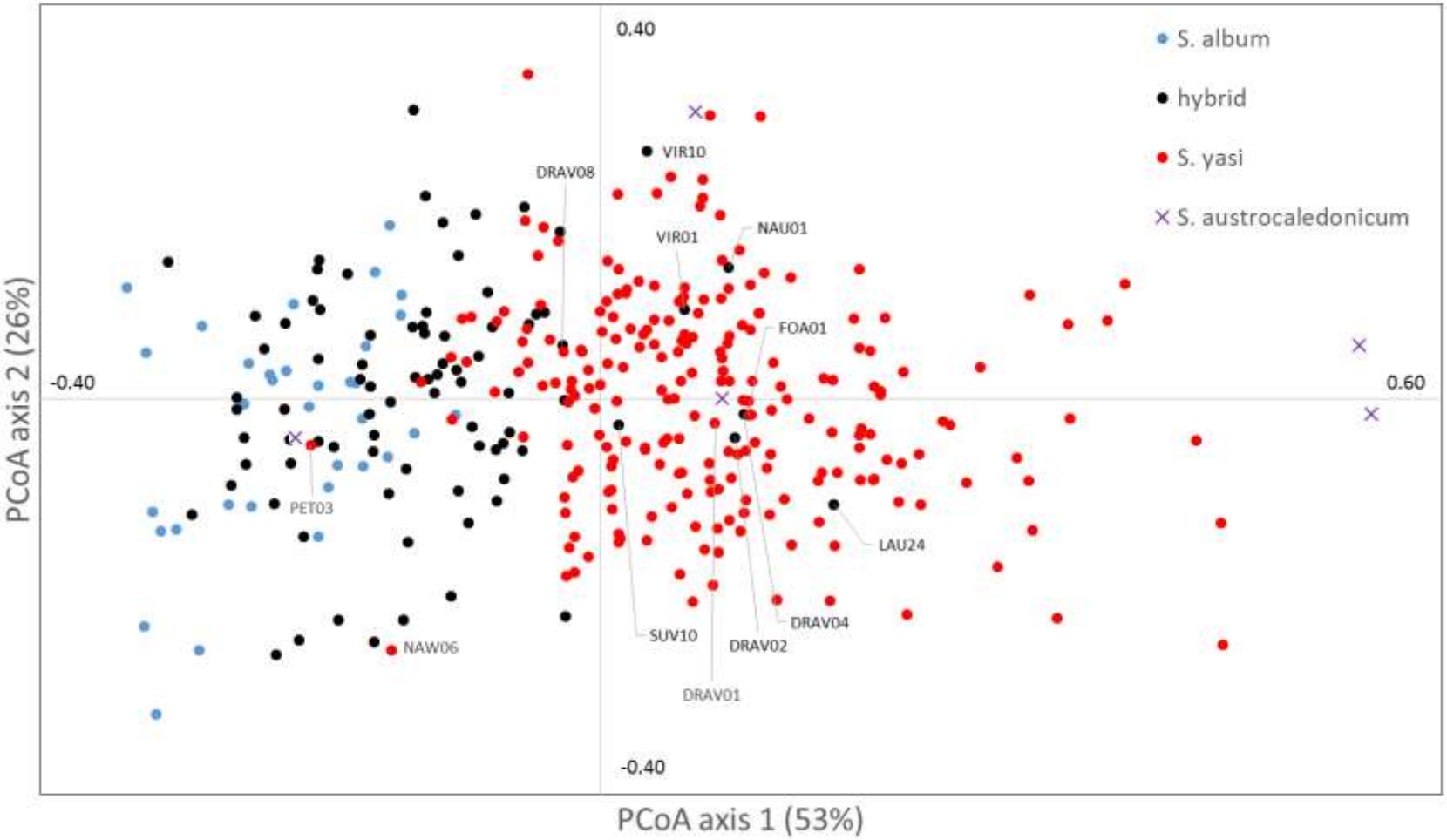

Figure 5 First and second axes of eigenvectors resulting from principle coordinates analysis of Euclidean distance matrix of leaf measure parameters for individual trees of *Santalum* taxa in Fiji and Tonga.

To examine the combined effect of the various leaf traits, the dataset was subjected to a multivariate principle coordinates analysis of a Euclidean distance matrix based on the traits listed in Table 3. The explanatory axes were then regressed on each trait to determine the underlying explanatory factors. This analysis was implemented in Genstat 16. The first two principal coordinate axes accounted for 79% of the observed variation and the resulting plot of the first and second axes is shown as Figure 5. This plot shows a clear grade between *S. album* and *S. yasi* on the first axis, with hybrids tending to occupy an intermediate region that heavily overlaps with *S. album*. Two of the *S. austrocaledonicum* points are located to the far right of the first axis[1], two amid the *S. yasi* cluster and one in the *S. album* and hybrid region of the plot.

Table 3 percent variance explained by regressions of underlying data parameters on the first two PCoA axes

	Percent variance explained by regression	
Trait	First PCoA axis	Second PCoA axis
Length	5	86
Width	58	26
L/W	79	-
Widepoint %	73	5
Petiole	46	14

A few points are notable in that they represent trees with apparently divergent morphology, for example PET3 and NAW6 were recorded in-field as *S. yasi* but appear to have leaf morphology more similar to *S. album* or their hybrid. Similarly DRAV2, DRAV4, FOA1, VIR1, VIR10 and NAU01 were determined in-field to be hybrids but have *S. yasi*-like morphological features. It should be remembered that the in-field determinations were influenced by the fruit morphology and/or the overall habit of the tree.

From Table 3 it is evident that the first axis (which explained 53% of observed variance in the data) is strongly associated with the length/width ratio and widepoint, while the second axis (which explained a further 26% of observed variance) was strongly correlated with leaf length. All measured traits appear to have had some explanatory value in differentiating between the taxa.

That there is good agreement between the in-field determination of taxon and the leaf morphological data is not surprising, as leaf morphology, together with fruit and seed morphology (where available) were major determinants of classification for each tree.

[1] *These two plants were younger specimens with seedling/juvenile leaves*

7 Molecular marker analysis

7.1 DNA extraction and amplification

Genomic DNA was extracted from silica gel-desiccated leaf tissue samples using the Invisorb® DNA Plant HTS 96 Kit (Stractec Biomedical AG, Germany) and 5 mg of tissue per sample. Twelve microsatellite (single sequence repeat, SSR) primer pairs were selected from 20 previously-published candidates with demonstrated functionality throughout the *Santalum* genus (Table 4). DNA samples were amplified in 5µL reactions comprising: 1 X PCR buffer (Invitrogen), 5 µM of each dNTP (Sigma), 0.25 µM of labelled M13 primer sequence (tagged with FAM, NED, PET or VIC Thermo Fisher Scientific), 0.05 µM 5'-tagged forward primer and 0.25 µM reverse primer, 5U/µL Platinum *Taq*(Invitrogen), 3 mM $MgCl_2$ (Invitrogen, USA)], 1 M Betaine (Sigma-Aldrich, USA)] and 10–30 ηg template DNA with a Mastercycler (Eppendorf, Germany). Amplicons were visualised on a 3130XL sequencer (Applied Biosystems, USA) with a LIZ 600bp internal standard and scored with GeneMapper Version 4.0.

7.2 Selection of SSR markers

Previously published SSR markers (Table 4) were screened with the aim of selecting at least eight polymorphic markers that were functional and that would produce reliable peaks across the three target taxa. The list of candidate markers included those already proven to work on *S. yasi*, those that had been screened by other authors but that did not work on *S. yasi* and markers that have been functional on other *Santalum* species but which were untested on *S. yasi*.

Table 4 List of SSR markers developed for various *Santalum* spp. screened for potential use with *S. album*, *S. yasi* and their hybrid.

Marker name	Developed for	Works with	Alleles	References
mSaCIRF04	au	au, y	10 (au), 2(y)	(Bottin et al. 2005; Huish 2009)
mSaCIRF10	au	au, y	20 (au), 6(y)	(Bottin et al. 2005; Huish 2009)
mSaCIRG10	au	au, y	33 (au), 2(y)	(Bottin et al. 2005; Huish 2009)
mSaCIRH09	au	au, y	14 (au), 3(y)	(Bottin et al. 2005; Huish 2009)
mSaCIRH10	au	au, la, le, y	16 (au), 10 (la), 6 (le), 3(y)	(Bottin et al. 2005; Huish 2009; Jones et al. 2010)
mSiCIR44	i	i, y	3(y)	(Huish 2009; Lhuillier et al. 2006)
mSiCIR148	i	i, y	2(y)	(Huish 2009; Lhuillier et al. 2006)
mSiCIR153	i	i, y	2(y)	(Huish 2009; Lhuillier et al. 2006)
Markers that have previously been tested but not worked on *S. yasi* and/or *album*				
mSaCIRE09	au	au, la, le	21 (au), 16 (la), 8 (le)	(Bottin et al. 2005; Huish 2009; Jones et al. 2009)
mSaCIRG01	au	au	3 (au)	(Bottin et al. 2005; Huish 2009)
mSiCIR42	i	i	8(i)	(Huish 2009; Lhuillier et al. 2006)
Markers not previously tested on *S. yasi* and/or *album*				
Lanc03	la, le	la, le	9(la)	Jones et al. (2010)
Lanc07	la, le	la, le	10(la)	Jones et al. (2010)
Lanc08	la, le	la, le	4(la)	Jones et al. (2010)
Lanc09	la, le	la, le	18(la)	Jones et al. (2010)
Lanc10	la, le	la, le	29(la)	Jones et al. (2010)
mSiCIR33	i	f, h, i, la, le	14 (i), 17(f,h)	(Harbaugh et al. 2010 ; Jones et al. 2010 ; Lhuillier et al. 2006)
mSiCIR39	i	i	7 (i)	Lhuillier (2006)
mSiCIR139	i	f, h, i	3 (i), 3(f,h)	(Harbaugh et al. 2010 ; Lhuillier et al. 2006)
mSiCIR153	i	f, h,i	10 (i), 6(f,h)	(Harbaugh et al. 2010 ; Lhuillier et al. 2006)
mSiCIR185	i	i	6 (i)	Lhuillier (2006)

a *S. album*, **au** *S. austrocaledonicum*; **f** S. *freycinetianum;* ***h*** *S. haleakalae,* **i** *S. insulare*; **la** *S. lanceolatum*; **le** S. *leptocladum*; **y** *S. yasi*

7.3 Analysis of SSR marker data

7.3.1 Suitable SSR markers

Preliminary screening of candidate SSRs yielded 11 reliable loci that were polymorphic in *S. album*, *S. yasi* and their putative hybrid. These markers yielded between 4 and 14 alleles across the whole study population (Table 5) and all proved to also be polymorphic for *S. austrocaledonicum*. The markers came from each of the three categories given in Table 4, including a marker that had previously been screened but found to be ineffective for *S. yasi (*mSaCIRE09*)*. Several alleles were found to be private to either *S. album* or *S. yasi* (i.e. they occurred only in one or the other of the pure species, as well as in some of the putative hybrids). Another locus (mSiCIR185) produced results that were considered marginal in that an unacceptable proportion of genotypes (<90%) failed to amplify or were difficult to score. It is possible that this locus might give reliable performance with protocol refinement and optimisation. Markers mSaCIRH09 and mSaCIRH10 may also work if optimised as they have functioned in a previously published study of *S. yasi* genetic diversity (Huish 2009).

Table 5 Number of alleles and number of alleles private to either *S. album* or *S. yasi* for each of the 11 selected SSR loci used in the study. The private alleles were also present in some *S. album* x *yasi* hybrid individuals. Values in parentheses are post-adjustments to each population for probable incorrect in-field assignment of taxon to some trees (described in section 7.3.2).

Locus	Alleles	Private to species	
		S. album	*S. yasi*
mSaCIRG10	4	1	1
mSaCIRF10	14	5	4 (5)
mSaCIRF04	8	4	-
mSiCIR44	6	-	2
mSiCIR148	7	1	2 (3)
mSiCIR153	9	-	2 (3)
mSaCIRE09	9	3	1
Lanc03	5	1	1
Lanc07	5	2	1
Lanc08	6	1	1
Lanc09	9	1	2 (3)

Some loci were found to be highly effective for differentiating hybrids. Examples include allele 184 at the mSaCIRE09 locus which was present in 53% of *S. album* samples, 20% of hybrids and 0% of *S. yasi*. Allele 148 of the mSiCIR148 locus was present in 0% of *S. album*, 19% of hybrids and 63% of *S. yasi*.

7.3.2 Population differentiation among *S. album*, *S. yasi* and their hybrid and *S. austrocaledonicum*

Principle coordinates analysis (PCoA) of genetic distances

Principal coordinates analysis (PCoA) of matrices formed from standardized genetic distances between (i) populations and (ii) individual trees were performed with GenAlex version 6.501 (Peakhill and Smouse 2006).The initial analysis was performed with only one representative tree per seedlot from the Vunimaqo seed orchard to avoid possible over-representation of these families. In the case of the population-level analysis, the first axis accounted for 90% of observed variation while the second axis accounted for a further 8%. The resulting plot of the first two axes shows clear separation of the taxa, each in separate plot quadrants (Figure 6).

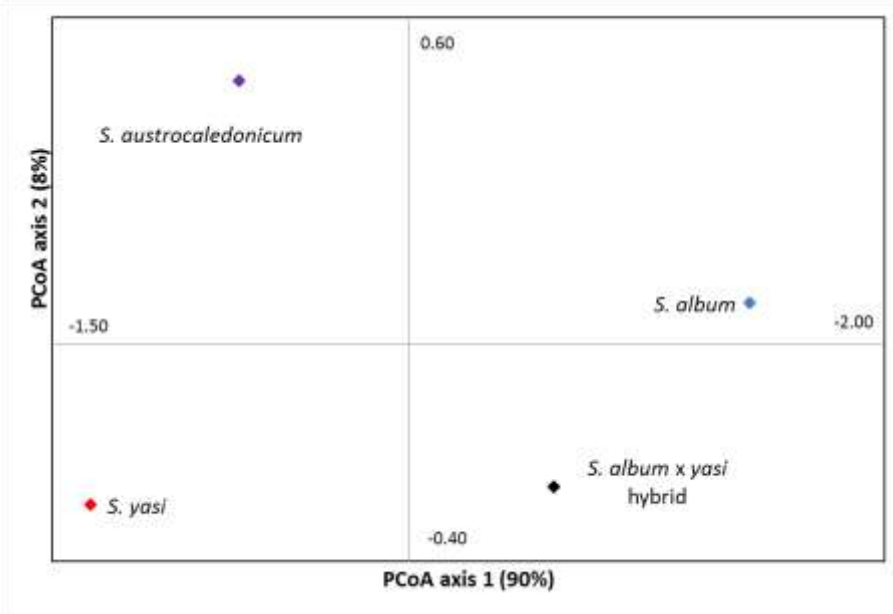

Figure 6 First and second axes of eigenvectors resulting from principle coordinates analysis of population genetic distance matrix for *Santalum* taxa in Fiji and Tonga.

The individual-tree diagram (Figure 7) shows clustering of the majority of *S. yasi* individuals to the left of the origin of the first PCoA axis (i.e. left of where the second axis intersects the first), with only one individual of *S. album* (TFU3) having a negative value on this axis. The five *S. austrocaledonicum* individuals also clustered to the left of origin on the first PCoA axis.

Individual-tree PCoA analysis and identification of trees with potentially incorrect in-field taxon assignment

A total of ten individuals that had been field-identified as *S. yasi* clustered to the right of the origin on the first PCoA axis (i.e. they had positive eigenvectors). Morphological data for these individuals were examined more closely to identify explanatory factors for this apparently incongruous result. Tree 0 from VQM family 4 from the Vunimaqo gene bank had been identified from its records as *S. yasi* from Lau, along with several other of the VQM entries. It was noted on the field sheets of HTUA4 (Ha'atu'a, 'Eua), PET3 (Petani, 'Eua) and VIR4 (Viria, Viti Levu) that the individuals were possibly hybrid, having ambiguous and/or some absent (fruiting) morphological characters. The four NAW individuals (Nawailevu, Vanua Levu) were growing in village gardens which contained very extensive *Santalum* plantings (probably a hundred individuals) from which 8 individuals were sampled. Of these, one was identified as *S. album* (probably correctly), and NAW5 was flagged as being a possible hybrid. The others had mostly *S. yasi*-like features, and our molecular analysis shows that three of these are in fact more likely to be pure *S. yasi*.

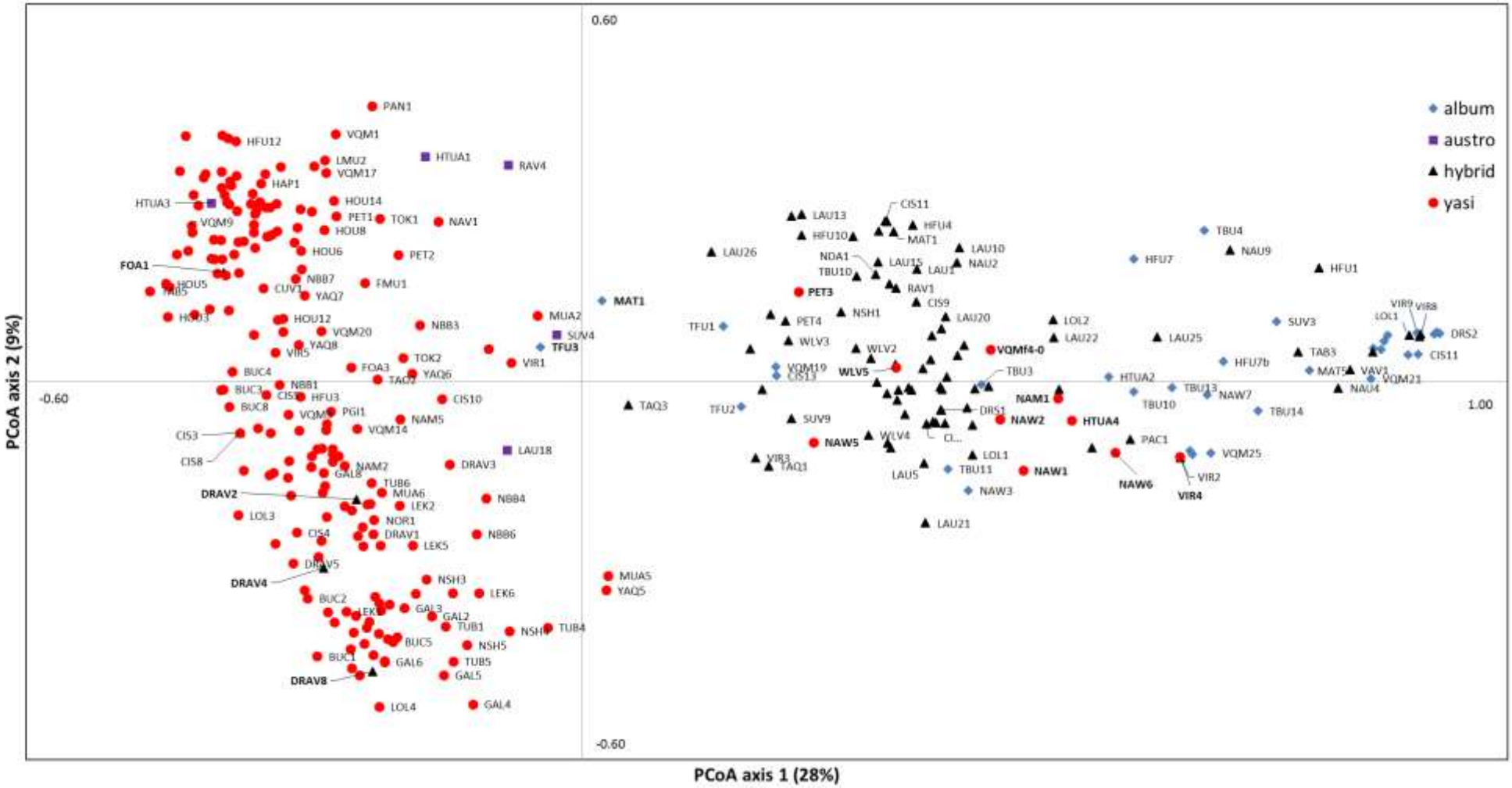

Figure 7 First and second axes of eigenvectors resulting from individual tree principle coordinates analysis of genetic distance matrix for *Santalum* taxa in Fiji and Tonga. The taxon of trees labelled in bold typeface have potentially been misidentified based on their position within the clusters evident in this figure.

Similarly, five field-identified *S. album* x *yasi* hybrids cluster to the left of the PCoA diagram together with pure *S. yasi* individuals. Three of these are from Dravuwalu Village, Kadavu. Eight trees were sampled from this village, five of which were assessed as pure *S. yasi,* in addition to the three putative hybrids identified in Figure 2. Re-examination of photographs and datasheets from these individuals does indicate that this population possessed some ambiguous morphological features (and one tree did not have reproductive structures present), making positive taxonomic differentiation between pure *S. yasi* and hybrids difficult in this case. However the leaf morphology of DRAV2 and DRAV4 was also *S. yasi*-like (Figure 3) as it was for FOA1, indicating that these are probably *S. yasi*. This situation also applies to CIS3, a planted tree from the Colo-I-Suva area which had relatively broad leaves and fruit consistent with an *S. album* x *yasi* hybrid.

Towards the positive end (right) of the PCoA 1 axis, the delineation between *S. album* and *S. album* x *yasi* hybrids is less well defined. Though several trees identified as *S. album* in-field cluster to the far right of the plot, others extend throughout the plot region where most hybrids are clustered, with one member of the TFU (Toafa, Vava'u) sample placed just to the left of the PCoA 1 axis intercept. The three TFU individuals (TFU1-3) in this plot region are almost certainly *S. album*, with very broad leaves (especially TFU3) and characteristic *S. album*-like fruit. In the case of VIR8 and 9 (Viria, Viti Levu), it was noted on the field sheets that these are possibly hybrids with more *S. album*-like morphology. LOL7 (Lololo, Kadavu) might well have been mis-identified and is actually *S. album*.

Analysis of molecular variance (AMOVA)

Analysis of molecular variance (AMOVA) indicated a significant difference among populations (p<0.001) with F_{ST} of 0.26, a moderately-high value as expected among different species within a genus. The range of F_{ST} values, pairwise between taxa, is given in Table 6. From this it is evident that *S. album* is relatively-less-well differentiated from the *S. album* x *S yasi* hybrid than is *S. yasi*. It is perhaps surprising that *S. austrocaledonicum* appears to be closer to *S. yasi* than does *S. album,* given that *S. yasi* and *S. album* were placed in the same clade by Harbaugh et al. (2010) while *S. austrocaledonicum* is more distant. However, it should be remembered that there were only six *S. austrocaledonicum* samples in the sample population, some of which may themselves have been hybrids with either *S. yasi* or *S. album*.

Table 6 Pairwise F_{ST} estimates among taxa from AMOVA. All estimates are significant (p=0.001).

	S. album	*S. austrocaledonicum*	hybrid
S. austrocaledonicum	0.241		
hybrid	0.058	0.142	
S. yasi	0.378	0.104	0.223

Removal of 16 of the above-mentioned points (listed in Appendix 2) with questionable taxon assignments from the dataset resulted in a minor increase of the overall F_{ST} estimate to 0.28 and pairwise values ranging between 70% and 90% of those given in Table 6.

7.3.3 Allele frequency-dependent measures of diversity and inbreeding across taxa

Estimates of allele-frequency-dependent parameters with trees listed in Appendix 2 removed from the dataset are presented in Table 7a and calculated from the raw data in Table 7b. The marker panel yielded an effective number of markers for all taxa as gauged

by N_a and N_e and a comparatively high ^{S}H. Genetic diversity for the pure species was moderate (H_e = 0.42 for *S. yasi*) though the possibility that this is being somewhat inflated by misidentified hybrids still exists. The large difference between observed and expected heterozygosity together with elevated *F* values indicate significant levels of inbreeding in these samples of the pure species. This analysis has not, however, accounted for subpopulation structure which might be expected given the distribution of the taxa, particularly *S. yasi*, over numerous islands. As might be anticipated from a population of interspecific hybrid individuals, expected and observed heterozygosity were markedly higher at around 0.6 and inbreeding values (*F*) were close to neutral.

Table 7 Population (taxon-level) allele frequency-dependent genetic parameters including number of individuals per population averaged across loci (N), average number of alleles per locus (*N*a), effective alleles per locus (*N*e), Shannon information index (^{S}H), observed and expected heterozygosity (*H*) and Wright's inbreeding index (*F*), each with standard errors.

7a. adjusted dataset with potentially misidentified trees removed

Taxon	N	*N*a	*N*e	^{S}H	H_o	H_e	*F*
S. album	31.18 (0.57)	5.00 (0.56)	2.17 (0.25)	0.94 (0.12)	0.28 (0.07)	0.47 (0.06)	0.47 (0.10)
S. yasi	191.36 (1.87)	5.09 (0.69)	2.08 (0.30)	0.75 (0.15)	0.24 (0.07)	0.42 (0.08)	0.39 (0.10)
S. album x *yasi*	72.27 (1.56)	4.55 (0.67)	2.74 (0.26)	1.06 (0.11)	0.69 (0.07)	0.59 (0.05)	-0.12 (0.12)
S. austrocal.	4.55 (0.21)	3.18 (0.35)	2.52 (0.32)	0.96 (0.11)	0.30 (0.08)	0.55 (0.05)	0.44 (0.13)

7b. Original data

Taxon	N	*N*a	*N*e	^{S}H	H_o	H_e	*F*
S. album	32.00 (0.52)	5.55 (0.67)	2.27 (0.26)	1.00 (0.12)	0.29 (0.07)	0.50 (0.06)	0.49 (0.09)
S. yasi	207.91 (2.18)	5.36 (0.69)	2.19 (0.31)	0.83 (0.14)	0.26 (0.07)	0.45 (0.07)	0.40 (0.09)
S. album x *yasi*	79.00 (1.62)	4.64 (0.66)	2.75 (0.26)	1.07 (0.11)	0.64 (0.07)	0.60 (0.04)	-0.03 (0.11)
S. austrocal.	4.73 (0.19)	3.73 (0.3)	2.77 (0.30)	1.09 (0.1)	0.40 (0.07)	0.59 (0.05)	0.30 (0.11)

7.3.4 A closer look at individuals planted at Vunimaqo gene bank

The Vunimaqo gene bank and seed stand are a valuable repository of *S. yasi* genetic material, especially as these contain several seedlots sourced from the Lau island group as well as from the Bua area of Vanua Levu. The Lau group is not otherwise represented in this study. As it lies relatively distant from Viti Levu and is the closest part of Fiji to Tonga, it may potentially contain a subpopulation of interest for conservation, testing and genetic improvement. However, the analysis carried out in 7.3.2 indicated that the mother of at least one family (family 4) may have been misidentified, or that some or all of its progeny may be the result of interspecific hybridisation with *S. album*. It was therefore seen as a worthy objective to more closely examine the data from Vunimaqo using the collection of between 5 and 11 leaves sampled per family.

The method used to examine the Vunimaqo data was a PCoA analysis similar to that described in section 7.3.2. The additional Vunimaqo data (92 trees) was added to the existing data and genetic distances were calculated using Genalex. PCoA was then conducted on the standardised genetic distance matrix. The plot of the first and second axes is given in Figure 8 and a summary of where each family clusters is given in Table 8.

Figure 8 shows clustering of six *S. album* trees to the far right of the first axis and a short distance from the origin on the second axis with one individual (VQM19) lying further to the left among a cluster of hybrids. Trial records show that VQM23 and VQM24 are hybrids of local origin, and these are clustered together with several non-VQM hybrids. Only single trees of these seed sources were sampled. Records for family 11 also show

that it is a local hybrid from Vunimaqo, and ten members of the family are close to VQM23 and VQM24, while one individual (f11-6) is situated in the bottom portion of the lower left quadrant in a space occupied by *S. yasi*. It is possible that this tree was either mislabelled or incorrectly sampled.

Examination of the seven trees in family 4 (*S. yasi* from Lakeba, Lau) indicates that three members of this family are likely to be hybrid, while the remainder, and therefore the originally selected mother, are likely to be pure *S. yasi*. A few additional individual trees (f6-8, f7-2 and possibly f10-2) from other families are also indicated as being hybrid, though this is by no means widespread in the Vunimaqo gene bank sample.

Table 8 Summary of PCoA analysis for Vunimaqo gene bank accessions including a tally of which PCoA quadrant (Figure 3) the family seedlots fell within (*UL* upper left, *UR* upper right, *LL* lower left, *LR* lower right)

Family	Trees	Taxon and origin	PCoA cluster quadrant	Comment
1	8	*S. yasi*, Lau	6 LL, 2 UL	
2	11	*S. yasi*, Lau	10 UL, 1 LL	Quite tightly clustered
3	8	*S. yasi*, Lau	7 LL, 1 UL	Quite tightly clustered
4	7	*S. yasi*, Lau	3 UR, 4 LL	3 in UR likely to be hybrid
5	7	*S. yasi*, Lau	1 LL, 6 UL	Quite tightly clustered
6	10	*S. yasi*, Lau	8 UL, 1 LL, 1 UR	1 hybrid individual likely
7	5	*S. yasi*, Lau	3 LL, 1UL, 1UR	f7-2 most likely a hybrid
8	5	*S. yasi*, Lau	All UL	Quite tightly clustered
9	5	*S. yasi*, Lau	3UL, 1LL	
10	4	*S. yasi*, Lau	1 UL, 1 L on PCoA1 axis, 1 LL, 1 LR	One individual may be hybrid
11	8	Hybrid	4 LR, 3UR, 1LL	f11-6 may be pure yasi
VQM14-15,17,18,20	1 each	*S. yasi*, Bua, Vanua Levu	VQM12,13,17, 20 UL; VQM 14,18 LL	Split across upper and lower left quadrants
VQM16,19,21 25-28	1 each	*S. album* (unknown)	UR and LR	Mainly to far right of plot except VQM19
VQM23-24	1 each	*S. album* x *yasi* hybrid, Vunimaqo	LR	

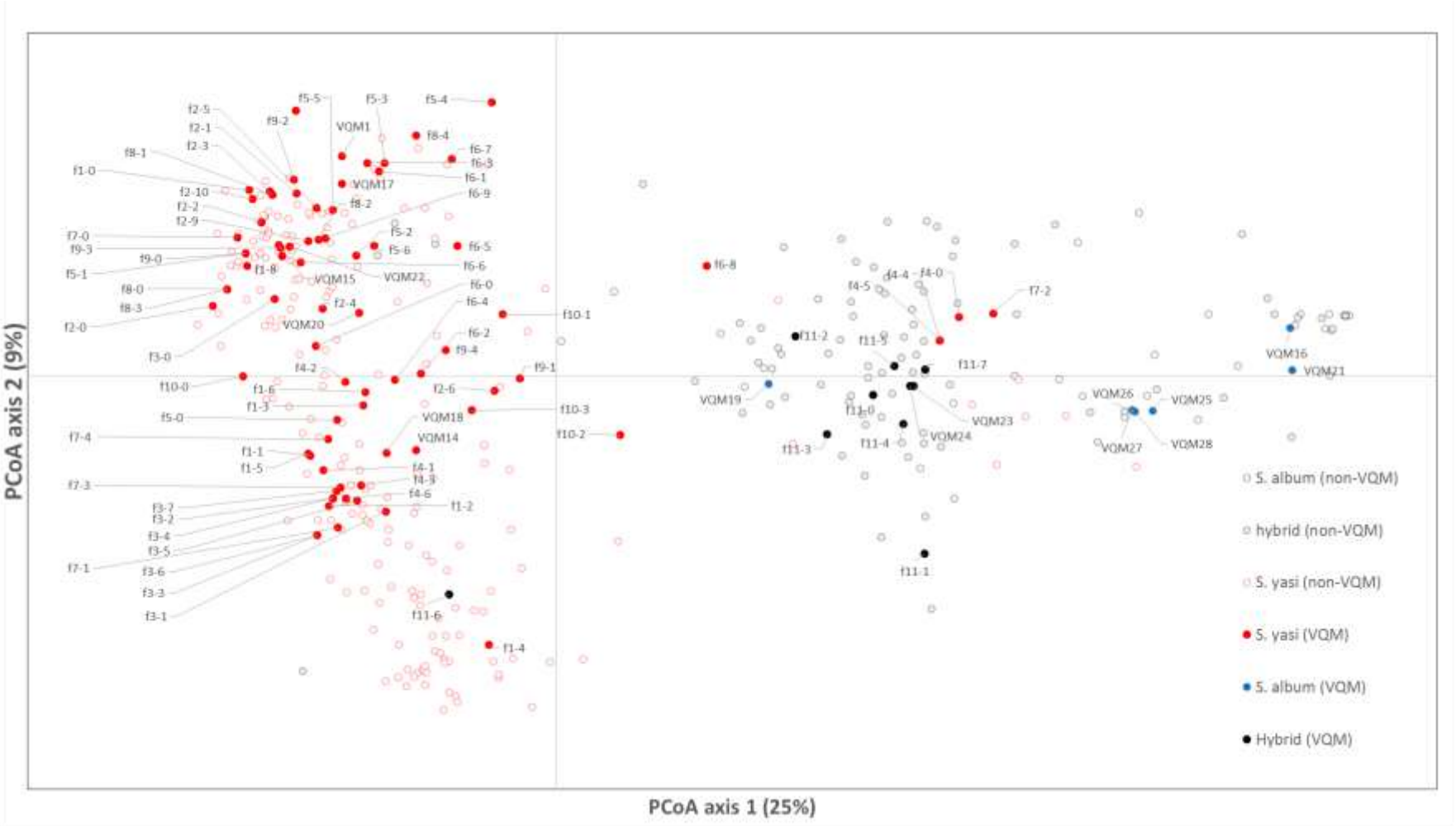

Figure 8 First and second axes of eigenvectors resulting from individual tree principle coordinates analysis of genetic distance matrix for *Santalum* taxa in Fiji and Tonga including all trees sampled from the Vunimaqo gene bank (VQM), Viti Levu.

Overall the Vunimaqo gene bank would appear to be a valuable repository of *Santalum* germplasm. The analysis of individual trees from the families has shown that some low levels of hybridisation occurred at the source of the seed collections (i.e. in Lakeba, Lau), though these appear to be readily detectable using the SSR marker panel.

7.3.5 Analysis of genetic diversity and structure within *S. yasi*

A major objective of the study was to determine the genetic diversity within the *S. yasi* population which is indigenous to Fiji and Tonga. This information is required to actively manage the conservation and breeding populations of the species.

The data used to make the determination was made as free as possible from *S. album* x *yasi* hybrid contamination: the effect of inclusion of hybrids would be to inflate the estimates of genetic diversity parameters. The data therefore included all of the *S. yasi* material discussed in section 7.3.2, but excluded the material identified as potentially hybrid in Appendix 2. The *S. yasi* material from Vunimaqo gene bank was also included, as this contained collections from the Lau group not otherwise represented in the sample population. The few individuals from Vunimaqo identified as likely to be hybrid in section 7.3.4 were also excluded. Principal coordinates analysis (PCoA) was carried out as described in previous sections.

Population differentiation among S. yasi *populations*

The results of this PCoA analysis are shown in Figure 9 (for individual trees) and Figure 10 (for subpopulations). Differentiation of subpopulations from Fiji and Tonga is evident with clustering of Tongan material to the right of both figures. Considering the information in Figure 9, no material sampled directly from Vanua Levu or Viti Levu is present in the lower right quadrant: the single Vanua Levu point (VQM15) situated there is a tree from the Vunimaqo seed stand recorded as originating from Bua, as are the two right-most Vanua Levu points (VQM17, VQM22) in the upper right quadrant. Though there appear to be clusters of Tongan trees in both the upper and lower right quadrants, there appears to be no geographic-based explanation for these.

The material of Lau origin generally overlaps with both the Tongan and Fiji material, and is in congruence with the geographic position of the Lau island group between Fiji and Tonga. Referring to Figure 10, tight clustering of Tongan material along the positive side of the first PCoA axis with minimal deviation from zero on the second axis is evident, while Vanua Levu, Viti Levu and Kadavu subpopulations are located to the far left of the first PCoA axis and there is a significant divergence between Kadavu, Viti Levu and Vanua Levu on the second axis. The Lauan plants appears to have closer affinity to the Tongan plants than to plants from the Fijian subpopulations.

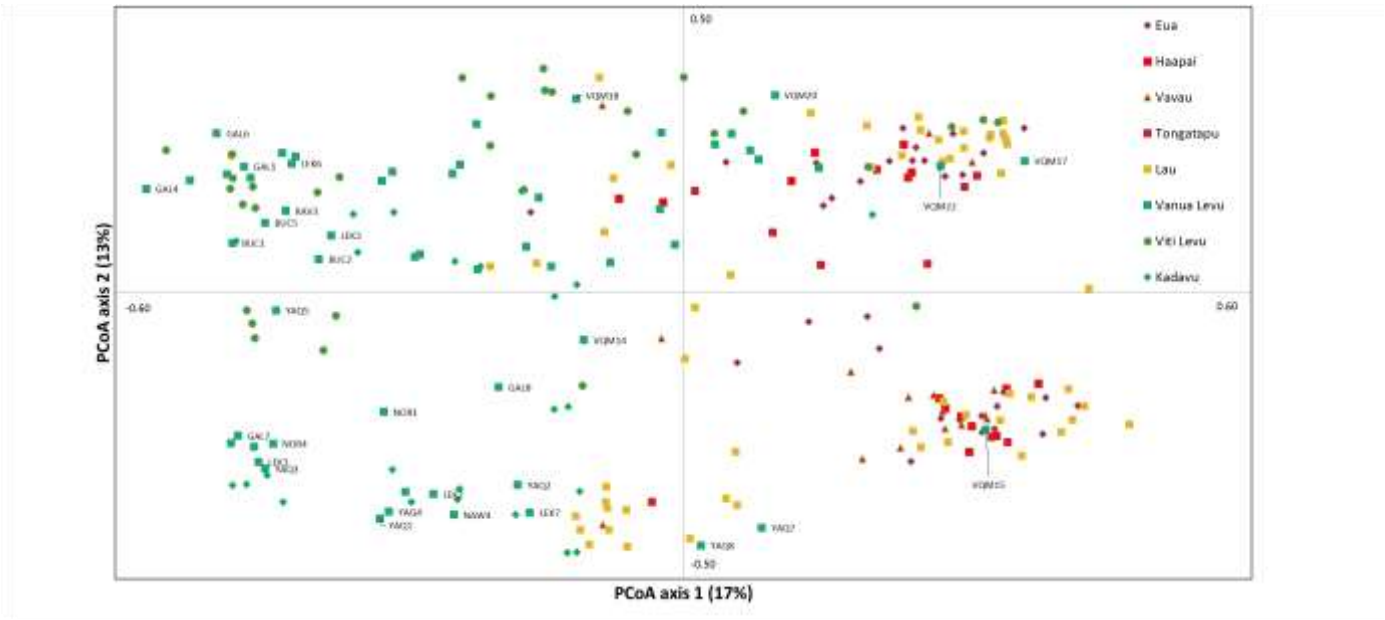

Figure 9 First and second axes of eigenvectors resulting from principle coordinates analysis of genetic distance matrix for individual *S. yasi* trees sampled in Fiji and Tonga.

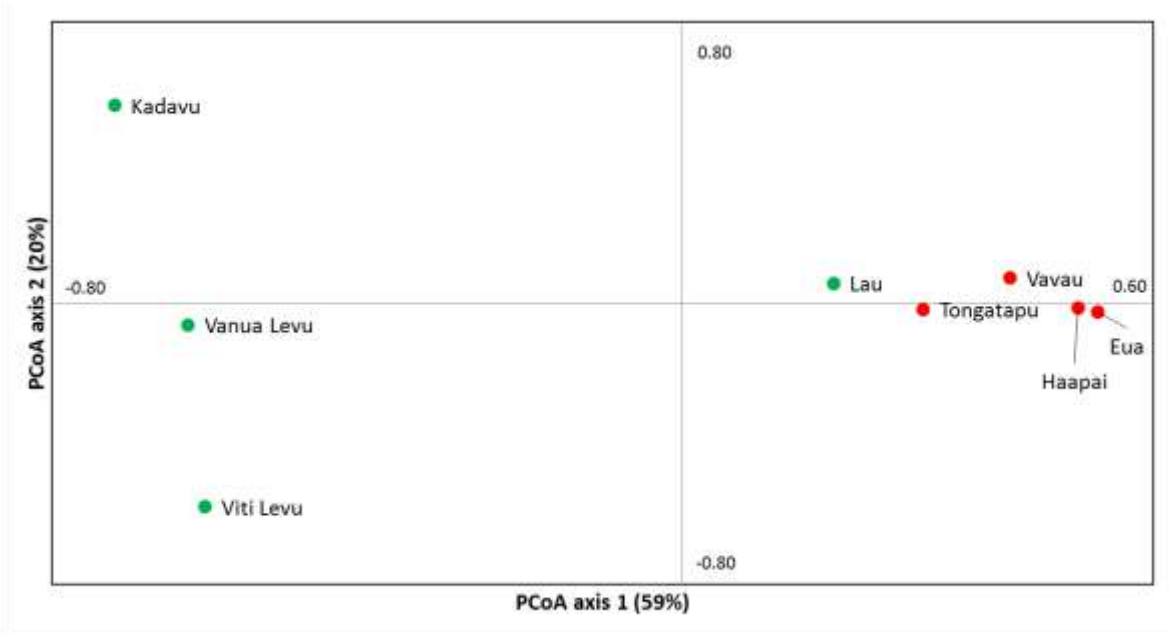

Figure 10 First and second axes of eigenvectors resulting from principle coordinates analysis of genetic distance matrix for populations (defined by island groups) of *S. yasi* sampled in Fiji and Tonga.

AMOVA of the *S. yasi* data showed significant regional and subpopulation structure within the population (p<0.001) and partitioning of F_{ST} 8% between countries and 11% among subpopulations. Table 9 gives estimates of F_{ST} pairwise between subpopulations. Differences between 'Eua, Ha'apai and Vava'u were minor and mostly non-significant. The Lauan plants (which were sampled from the Vunimaqo gene bank) appear to have genetic affinities to those of both Tonga and Vanua Levu, being more strongly differentiated from both Kadavu and Viti Levu. Maximum subpopulation differentiation was between 'Eua and Kadavu with a substantial estimate of F_{ST} = 0.25.

Table 9 matrix of F_{ST} pairwise values between subpopulations (heat mapped). Entries marked with an asterisk are not significantly different at $p<0.05$

Population	'Eua	Ha'apai	Vava'u	Tongatapu	Vanua Levu	Viti Levu	Lau
Ha'apai	0.01*						
Vava'u	0.07	0.02*					
Tongatapu	0.06	0.04*	0.06				
Vanua Levu	0.18	0.16	0.17	0.15			
Viti Levu	0.21	0.19	0.18	0.15	0.07		
Lau	0.09	0.04	0.03	0.03*	0.13	0.15	
Kadavu	0.25	0.23	0.21	0.19	0.08	0.17	0.16

Clearly the material held at the Vunimaqo gene bank from Lau is valuable and appears not to be represented in other plantings sampled from either Vanua Levu or Viti Levu.

Genetic diversity parameters

Genetic diversity parameters for the *S. yasi* material were calculated for each population. This included the material sampled from Vunimaqo with its recorded subpopulation identity retained despite the possibility that a small number of the trees recorded as being from Vanua Levu may be from Lau. Results are given in Table 10.

Table 10 Allele frequency-dependent genetic parameters for *S. yasi* subpopulations and across subpopulations including number of individuals per population averaged across loci (N), average number of alleles per locus (*Na*), effective alleles per locus (*Ne*), Shannon information index (^{S}H), observed and expected heterozygosity (*H*) and Wrights inbreeding index (*F*), each with standard errors.

Subpopulation	N	*Na*	*Ne*	^{S}H	H_o	H_e	*F*
'Eua	30.09 (0.44)	2.82 (0.52)	1.9 (0.30)	0.61 (0.16)	0.27 (0.08)	0.34 (0.09)	0.23 (0.10)
Ha'apai	15.82 (0.12)	2.36 (0.39)	1.88 (0.25)	0.59 (0.14)	0.28 (0.09)	0.36 (0.08)	0.26 (0.14)
Vava'u	15.64 (0.20)	3.00 (0.47)	1.81 (0.23)	0.64 (0.13)	0.31 (0.09)	0.36 (0.07)	0.27 (0.15)
Tongatapu	8.36 (0.31)	2.64 (0.41)	1.96 (0.26)	0.67 (0.14)	0.26 (0.10)	0.39 (0.08)	0.37 (0.16)
Vanua Levu	65.82 (0.82)	4.00 (0.66)	2.08 (0.30)	0.74 (0.14)	0.26 (0.07)	0.40 (0.08)	0.33 (0.10)
Viti Levu	30.09 (0.37)	2.73 (0.49)	1.86 (0.29)	0.60 (0.15)	0.19 (0.06)	0.35 (0.08)	0.44 (0.13)
Lau	51.18 (0.63)	3.36 (0.61)	1.80 (0.20)	0.65 (0.12)	0.23(0.07)	0.38 (0.07)	0.44 (0.11)
Kadavu	21.64 (0.96)	2.18 (0.26)	1.50 (0.15)	0.43 (0.11)	0.19 (0.06)	0.27 (0.07)	0.26 (0.14)
Across subpopulations	29.83 (1.97)	2.88 (0.18)	1.84 (0.09)	0.61 (0.05)	0.25 (0.03)	0.36 (0.03)	0.33 (0.04)

Generally, genetic diversity was moderate with H_e=0.36 across the eight subpopulations. However, as foreshadowed by the taxon-level analysis, there was an overall deficit of heterozygotes which was also evident in each subpopulation. This is accompanied by elevated *F* values, suggesting that inbreeding may be a problem in many of the sampled subpopulations. It is not known whether inbreeding has particularly serious effects on *Santalum* species, though it commonly leads to lack of vigour and poor survival in many tree species. This has important implications for population turnover and long term population persistence. Another possibility is that population sub-structuring is present in some or all of the island subpopulations which, if present, might be another explanation for the apparent deficit of heterozygosity. Founder effects caused by human mediated dispersal, as postulated by Harbaugh and Baldwin (2007), may also be a causative factor. This is difficult to account for among the samples from Viti Levu and Vanua Levu,

because of the large number of planted stands of unknown genetic origin, though the substantial sizes of these islands makes the phenomenon more likely. However in Tonga, the islands are relatively small and the sampling carried out there was relatively intense. Major underlying population structure in Tonga is less likely to be an explanation for the observed heterozygosity deficit: it is more likely as a result of fragmentation and depletion of the few remaining subpopulations leading to selfing and other inbreeding (mating between related trees).

7.3.6 General discussion of molecular marker study

The molecular marker study was effective in characterising genetic differences at the taxon level between *S. yasi*, *S. album* and their interspecific hybrid as well as *S. austrocaledonicum*. An important finding is that, overall, genetic diversity is moderate, and if carefully managed will be able to provide the basis for effective conservation and domestication programs.

A challenge with this study has been that very few of the small number of remaining *S. yasi* populations are known to be wild (natural occurrences) and uncontaminated by interspecific pollen, making the establishment of a 'baseline' set of marker information for the species difficult. A further challenge is that many of the sampled trees are from planted stands, many of which anecdotally are known to contain trees that have been imported from other islands, often situated together with hybrids and *S. album*. With the exception of the accessions from Vunimaqo, for which written records are available, in carrying out the population-level analyses we have generally assumed that trees sampled in a given location are likely to have originated nearby.

Though overall diversity appears to be moderately high, an apparent lack of observed heterozygosity relative to Hardy-Weinberg expectation in *S. yasi* is of concern. While this may be partially due to population sub-structuring, it is most likely that this deficit is due to inbreeding, the consequences of which are likely to be negative for plant health, vigour and population persistence. It should also be noted that the inbreeding coefficient for *S. album* was very high (0.47), indicating that many of the trees sampled may be the product of mating between related trees. The observation that the hybrid is relatively vigorous may in fact be due to its having been compared in trials and plantings with inbred pure *S. yasi*. If this is the case, then a significant improvement in health and vigour of new plantings of *S. yasi* could be expected if seed was to be produced in a well-designed seed orchard.

Despite the challenges of highly heterogeneous planted and depleted wild populations, the study has demonstrated subpopulation differentiation within *S. yasi* among the islands, with some marked differences between Tonga and the Fijian islands of Vanua Levu, Viti Levu and Kadavu. The Lau group were not directly sampled by this study, though Lau material sampled from the Vunimaqo gene bank appeared to have affinities with both the Fijian and Tongan populations, indicating a geographic grade. It would be a useful extension of this study to acquire additional samples directly from the Lau island group to confirm this pattern.

8 Conclusions and recommendations

8.1 Conclusions

The present study has examined the genetic parameters of *Santalum* species in Fiji and Tonga in some detail, with the objective of underpinning future conservation, domestication and industry development programs. The study has examined the suitability and application of both morphological and molecular (DNA) markers for differentiating between the indigenous *S. yasi* and introduced and fairly widely-planted *S. album*, their interspecific hybrid and also the occasionally-planted *S. austrocaledonicum*. We conclude that morphological markers including leaf size and shape, fruit characteristics and general habit of the tree can be effective for differentiating pure *S. yasi* from interspecific hybrids. However, this can be done with greater certainty using molecular markers. This is likely to become increasingly vital in cases where hybrids have back-crossed to pure species, a situation that is likely to give rise to only subtle morphological differences between the pure species and hybrid.

Though the genetic diversity of *S. yasi* in Fiji and Tonga appears to be sufficient to form an adequate breeding base population, it should be noted that few wild stands now remain. Our results indicate that significant levels of inbreeding of the wild and planted *S. yasi* may be prevalent, and if this is the case it is likely that growth and performance of the trees will be suboptimal. However, an encouraging trend is the rising popularity of planting sandalwood on farms and in gardens in rural, village and even urban situations. This presents opportunities for both economic benefit, possibly (but not assuredly) alleviating pressure on dwindling wild stands and also circa situm conservation (see discussion of principles in Dawson et al. (2013)). For the conservation objective to be effective, however, the planting of pure *S. yasi* of appropriate local provenance would need to be encouraged. It is not yet clear whether this would be economically desirable for many growers who may wish to plant the *S. album* x *yasi* hybrid which has a capacity for rapid growth on some sites.

If the spread of more *S. album* and hybrid material is seen as inevitable, then active conservation measures will need to be taken to conserve the genetic diversity of *S. yasi*. The Vunimaqo gene bank serves as a good model for establishment. Desirable features include its high levels of security and maintenance and ready accessibility to MAFF staff. The development of additional, carefully pedigreed gene banks and seed stands in both Fiji and Tonga is to be strongly encouraged.

The finding that significant differentiation among populations within and between Fiji and Tonga and the various island groups of Fiji has important potential implications for domestication and breeding. Systematic testing of the performance of both pure *S. yasi* and hybrids in terms of growth and heartwood productivity, as well as site adaptability of the full range of material need to be undertaken at the earliest opportunity throughout Fiji and Tonga. The opportunity for maintenance of genetic diversity and management of inbreeding of *S. yasi* throughout successive cycles of genetic improvement as well as intraspecific crosses are important opportunities to be exploited. A systematic exchange of material between Fiji and Tonga may be a desirable undertaking, broadening the genetic base at the outset, for the development of commercial breeding populations of *S. yasi*.

8.2 Recommendations

The further development of the sandalwood industry in Fiji and Tonga will depend on a transition from harvesting wild material to a sustainable planting program. This transition will be dependent on the development of high quality planting stock that carries sufficient genetic diversity for ongoing selection and breeding without the risk of inbreeding.

We recommend the development of a conservation, domestication and breeding plan for sandalwood in Fiji, Tonga and Niue that should be written in concert with a strategy to further develop the entire sandalwood value chain. The following elements will need to be addressed:

- A widespread collection of seed and grafting material of confirmed *S. yasi* material should be undertaken throughout Fiji and Tonga.
- The small endemic population on Niue ought to be sampled and genetically assessed, as well as remote populations such as Ono-I-Lau in Fiji and the Niuas in Tonga which was not possible in the current study (due to available resources).
- Screening of seedling material collected in the above activities should be undertaken to ensure that it is pure *S. yasi*: the molecular marker panel described in this report will be highly effective for this.
- A series of gene conservation and seed production plantings should be established on well-protected sites, similar to the existing MAFF Vunimaqo gene bank and seed stand in Fiji. It is important that these plantings be at more than one site to provide redundancy.
- An exchange of genetic material of *S. yasi* between Fiji and Tonga (and perhaps Niue) be actively considered. There may be benefits to sharing material, such as in the development of wide intraspecific crosses, which this report has shown substantial genetic differentiation between the island groups. The benefits would be both commercial and also for gene conservation (as availability of wide-crossed and diverse *S. yasi* germplasm would lead to wider planting of the pure species).
- Seed produced from the above-mentioned plantings need to be made widely available. Extension programs within MAFF Fiji and MAFFF Tonga are already working in this area. These appear to be effective but need to be strengthened and expanded, especially to remote islands which have very limited other opportunities for generating cash income. There are also considerable opportunities for greater involvement of women and young farmers, including through plantings in home gardens.
- Careful consideration of the management of *S. album* in Fiji and Tonga needs to be taken. The benefits of development of fast-growing interspecific hybrids between *S. album* and *S. yasi* need to be weighed against the opportunity to develop *S. yasi* as a recognised “clean, green, unique Pacific” market segment, in anticipation of large volumes of *S. album* becoming available from industrial-scale plantings in Australia. These objectives need not be mutually exclusive, but would need to be carefully managed.

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10 Appendixes

10.1 Appendix 1: Sample field record sheet

Santalum DNA collection, Tonga 2015

Tree number	Species		Date
Location			
GPS Point		Lat	Long
Photo (tree)			
Height	Width	DBH	
Host plants			
Photo (leaf)	Fruit/flowers/seed		Forest type
Leaf width			
leaf length	Bark and Habit		
Petiole length			
Leaf colour			
Notes			

10.2 Appendix 2: list of trees removed from analyses

The following trees were removed from the taxon-level analysis because of the likelihood that their taxon was incorrectly identified in the field based on PCoA clustering:

(i) Trees assessed in-field as hybrid but probably *S. yasi*

DRAV2,4,8, FOA1

(ii) Trees assessed in-field or from records as *S. yasi* but probably hybrid

NAM1, NAW1,2,5,6; PET3, VIR4, VQM11, WLV5

(iii) Trees assessed in-field as hybrid but probably *S. album*

VIR8,9; LOL7

The following trees from Vunimaqo gene bank have records indicating that they are *S. yasi*, but analyses have indicated that they are likely hybrids

VQM family 4-4 and 4-5, VQM family 6-8, VQM family 7-2

VQM family 11-6 is part of a hybrid seedlot but it is likely to be *S. yasi*.