ORIGINAL PAPER

Diversification history and hybridization of *Dacrydium* (Podocarpaceae) in Remote Oceania

Gunnar Keppel[A,B], *Peter Prentis*[C], *Ed Biffin*[C], *Paul Hodgskiss*[D], *Susana Tuisese*[E], *Marika V. Tuiwawa*[F] *and Andrew J. Lowe*[C,G]

[A] Ecology Center, School of Integrative Biology, University of Queensland, St. Lucia, 4072 QLD, Brisbane, Australia

[B] Current address: Curtin Institute for Climate and Biodiversity, Department of Environment and Agriculture, Curtin University, GPO Box U1987, Perth, WA 6845, Australia. E-mail: G.Keppel@curtin.edu.au

[C] Australian Centre for Evolutionary Biology and Biodiversity, School of Earth and Environmental Sciences, University of Adelaide, 5005 SA, Adelaide, Australia

[D] USDA Forest Service, Pacific Southwest Research Station, Institute of Forest Genetics, Davis, CA, USA

[E] Tropik Wood Industries Ltd., Lautoka, Fiji

[F] South Pacific Regional Herbarium, University of the South Pacific, PO Box 1168, Suva, Fiji

[G] State Herbarium of South Australia, Science Resource Centre, Department for Environment and Natural Resources, Hackney Road, Adelaide, SA 5005, Australia

Running Header: Diversification of *Dacrydium* in Remote Oceania

Abstract. We examine evolutionary relationships, hybridization and genetic diversity in species of *Dacrydium* (Podocarpaceae) in Remote Oceania, where it is restricted to New Caledonia and Fiji. We use cpDNA sequence (*trnL-trnF*) data to construct a phylogeny and estimate taxon divergence using a relaxed molecular clock approach. The phylogeny is verified using allozymes, which are also used to investigate genetic diversity of all species and the hybridization dynamics of two endangered species, *D*. *guillauminii* and *D*. *nidulum*. Our results suggest that *Dacrydium* species in Remote Oceania form a monophyletic group that arose and diversified within the last 20 my through long-distance dispersal and a range of speciation mechanisms. While we detect no hybridization between the Fijian species *D*. *nausoriense* and *D*. *nidulum*, we confirm hybridization between *D*. *guillauminii* and *D*. *araucarioides* in New Caledonia and determine introgression to be assymetric from the widespread *D*. *araucarioides* into the rare, restricted-range species *D*. *guillauminii*. In addition, *D*. *guillauminii* has lower genetic diversity than the other species of *Dacrydium* studied, which had genetic diversity similar to other gymnosperms. Our results provide evidence for the recent and complex diversification of *Dacrydium* in Remote Oceania. In addition, low genetic diversity of and introgression from *D*. *araucarioides*, are of grave concern for the conservation of *D*. *guillauminii*.

INTRODUCTION

Molecular studies have shown that several lineages of gymnosperms (e.g., Araucariaceae - Setoguchi *et al*. 1998; *Pinus* - Willyard *et al*. 2007) and many flowering plants (Rieseberg and Willis 2007) are the products of recent radiations. Studying divergence will increase our understanding of the evolutionary processes underlying species diversification and the generation of biological diversity. It may also allow us to better understand the implications of recent evolutionary history on rare species and design better conservation strategies for these species.

A number of processes can lead to speciation, including changes in gene flow dynamics and directional selection (Butlin *et al.* 2008). Several geographic barriers may reduce gene flow and thereby facilitate speciation (Coyne 1992; Pavlacky *et al.* 2009). Islands are often separated by large stretches of ocean, which restrict gene flow between species on different islands (e.g. Gillespie 2002; Keppel *et al.* 2002). Alternatively, divergent natural selection associated with ecological differences between habitats may drive phenotypic divergence and speciation under varying levels of gene flow (Rieseberg *et al*. 2002; Fitzpatrick *et al*. 2008b). In addition, sympatric speciation (speciation in the same location with no physical barriers to gene flow; Nosil 2008) has been documented (Barluenga *et al.* 2006; Savolainen *et al.* 2006). Allopatric and sympatric speciation are extremes that occur over a continuum of geographical scales, environmental conditions and levels of gene flow, which may vary during different stages of the speciation process (Butlin *et al.* 2008; Fitzpatrick *et al.* 2008a).

Hybridization has also played a major role in plant evolution and speciation (Mallet 2007; Rieseberg and Willis 2007) and is common in organisms that have recently evolved but come into secondary contact (Gow *et al.* 2006; Seehausen 2006). However, the outcome of hybridization, however, is not easily predictable. For example, it can produce novel gene combinations or introgress new adaptive variation that can enhance the evolutionary potential of populations (Whitney *et al.* 2006; Prentis *et al.*

2008). Conversely, hybridization can also drive local species extinction (Wolf *et al.* 2001; Prentis *et al.* 2007), collapsing one or both parental species into a single hybrid swarm (Seehausen *et al.* 2008).

The South Pacific harbours several biodiversity hot spots, with high levels of restricted-range and endemic species (Myers *et al.* 2000). A large proportion of this biodiversity is of recent evolutionary origin (Grandcolas *et al.* 2008; Keppel *et al.* 2009). Evolutionary radiations have made major contributions to Pacific diversity and were facilitated through geographical isolation between different islands within archipelagos and divergent natural selection associated with ecological differences between environments, presumably driving phenotypic divergence and speciation (Baldwin and Sanderson 1998; Filardi and Moyle 2005). Considering that in many cases reproductive barriers may be ecological rather than genetic, it is not surprising that hybridization often occurs in the Pacific flora (Howarth and Baum 2005; Pillon *et al.* 2009).

We focus on the genus *Dacrydium* Sol. ex Lamb. to examine the poorly understood evolutionary dynamics of plants in Remote Oceania. *Dacrydium* is a member of the Southern Hemisphere conifer family Podocarpaceae and part of the Dacrydioid clade, which also includes *Dacrycarpus* de Laub. and *Falcatifolium* de Laub. (Conran *et al.* 2000; Sinclair *et al.* 2002). It extends from SE Asia eastward into the Southwest Pacific and from southern China to New Zealand (Quinn 1982). Six of the 21 extant *Dacrydium* species (29%) are found in Remote Oceania (Fig. 1), areas north, east or south of the Solomon Islands (Matisoo-Smith and Robins 2004), making this region a centre of diversity for the genus. Five of the species are endemic to three islands: Grande Terre (New Caledonia), Viti Levu and Vanua Levu (both Fiji), meaning that more than a fifth of all *Dacrydium* species are restricted to some 32,000 km^2 of land in the Pacific Ocean.

New Caledonia’s main island (Grande Terre), a Gondwanan fragment that was mostly or entirely submerged 40 mya (Grandcolas *et al.* 2008), has four endemic species of *Dacrydium* occurring in

ecologically distinct habitats (de Laubenfels 1972; Jaffré 1995). *Dacrydium guillauminii* is one of the world's rarest conifers, restricted to nine small gallery forest populations in a single river basin (Fig. 1). It is classified as critically endangered (CR B1+2c, C1; Conifer Specialist Group 2000), because populations are experiencing a continuing and projected decline in area and quality of habitat and are under threat from increasing tourism, potential fires and pollution as a result of upstream mining (Jaffré *et al.* 1998; Oldfield *et al.* 1998). The other three species are classified as "lower risk" (LR), although *D. lycopodioides* is considered "conservation dependent" (LR/cd), and occur mainly on ultramafic

former is restricted to moister habitats in the south and the latter occurs in drier habitats throughout the island. *Dacrydium lycopodioides* is found in very moist, high elevation (above 900 m) forests. Recent evidence indicates that *D. guillauminii* is hybridizing with *D. araucarioides* to form *D*. × *suprinii* Nimsch. The hybrid has foliage characteristics and seed size intermediate between the two parent species and shares the same habitat as *D. guillauminii*, growing in or near water (Knopf *et al.* 2007).

The Fiji archipelago is of mostly volcanic origin, began to form some 40 mya (Yan and Kroenke 1993) and has two *Dacrydium* species that differ in adult leaf and pollen cone size (Smith 1979). *Dacrydium nausoriense* is classified as endangered (EN A1cd, B1+2ce, C1; Conifer Specialist Group 1998) and restricted to two locations in Fiji (Fig. 1), one on each of the two major islands Viti Levu (Nausori Highlands; 2 adjacent populations) and Vanua Levu (Sarava; 1 population). The latter population is small and was only recently discovered (mid-1990s). None of the populations has formal protection and are threatened by anthropogenic subsistence activities and poor regeneration (Oldfield *et al.* 1998). The other species, *D. nidulum*, is widespread (Malesia to Fiji, Fig. 1) and not considered to be of conservation concern. Ash (1986) suggested that *D. nausoriense* has a well-defined ecological niche, mostly occupying re-growth sites in relatively dry locations, and should be considered an ecotype of *D.*

nidulum adapted to drier climatic conditions. However, *D. nidulum* also occurs in drier climates, dominating many forests on the leeward sides of Fiji's largest islands (Keppel *et al.* 2006) and thus appears to have a broad ecological niche, likely including that of *D. nausoriense*. The distributions of the two species are not known to overlap and no intermediate forms are known (Ash 1986).

In this paper we use phylogenetic (sequences) and population genetic (allozymes) methods to investigate the diversification history and hybridization dynamics of *Dacrydium* in Remote Oceania. We use the resulting data to discuss: 1) the evolutionary history and age of diversification amongst extant species (including a discussion of the likelihood of different processes of speciation), 2) the distinctiveness of currently recognised species, 3) the incidence, frequency and direction of hybridization among sample species pairs, 4) the genetic diversity of various species, and 5) the implications of our data for conservation.

Materials and methods

Molecular data

DNA extraction and sequencing

We generated plastid *trnL-trnF* spacer and intron for four species of *Dacrydium*, 3 species of *Dacrycarpus* and *Falcatifolium falciforme* de novo using primers 'C' and 'F' of Taberlet *et al.* (1991) and added published sequences for 5 species of *Dacrydium*, 2 species of *Dacrycarpus* and *Falcatifolium* from Genebank (Table 1). These were used to infer phylogenetic relationships amongst Pacific *Dacrydium*, using *Dacrycarpus* and *Falcatifolium* as outgroups. DNA was extracted from silica-dried leaf material using a DNeasy Plant Minikit (QIAGEN; following manufacturers' protocols). PCR and sequencing reactions used standard conditions (Sinclair *et al.* 2002), and sequencing of both forward and

reverse strands was performed on an AB 3730*xl* sequencer using fluorescent dye-labelled terminators (BigDye v.3.1, Perkin Elmer).

Phylogenetic and Dating Analyses

Sequence alignment was performed using Clustal W (Thompson *et al.* 1994) and adjusted manually. Phylogenetic analyses were carried out using Bayesian relaxed molecular clock (BRC) implementation BEAST v. 1.4.7 of Drummond and Rambaut (2007), which applies a molecular clock to the data and simultaneously estimates topology, incorporating uncertainty in branch length estimates and relationships amongst sequences. *Dacrydium, Dacrycarpus* and *Falcatifolium* have a macrofossil record extending back to the Eocene (Hill and Brodribb 1999; Hill and Christophel 2001). However, it is unclear whether the oldest of these fossils belong within extant crown groups and under these circumstances, the most objective method for fossil placement is to constrain the stem group node (Renner 2004). As relationships amongst *Dacrydium, Dacrycarpus* and *Falcatifolium* are not well-resolved (Conran *et al.* 2000; Sinclair *et al.* 2002), the age of the root was constrained using a translated log-normal prior probability distribution with a mean of 50 million years (my) and a zero off-set of 35 my. A log-normal calibration prior appears to reasonably reflect potential bias in the fossil record (e.g., Sanders and Lee 2007) and, for instance, does not exclude the possibility that the root is substantially older than the oldest known fossil. In addition, Pole (1992; 1997) reports vegetative material of *Dacrycarpus* from the Miocene of New Zealand which he considers to be closely related to the extant species *Dacrycarpus dacrydioides* (endemic to New Zealand). This material was used to constrain the *Dacrycarpus* crown group node using a normal prior probability distribution with a mean of 20 my.

Analyses in BEAST were performed using the above temporal priors, and an uncorrelated log-normal distribution of branch rates and a GTR+I+Γ model of sequence evolution were assumed *a priori*. Five

separate analyses were run over 5 million generations (sampling topology and parameter values every 1000th generation) and after excluding an appropriate burn-in fraction, tree and parameter log files were combined and summarised using Tracer v. 1.4 (Rambaut and Drummond 2007) and TreeAnnotator v.1.4.7 (Drummond and Rambaut 2007) respectively.

The maximum likelihood (ML) topology for the *trnL-trnF* data was estimated in PAUP* 4.0 b.10a (Swofford 2002) under a GTR+I+Γ model of sequence evolution using the successive approximations approach of Swofford *et al*. (1996). The estimated model parameter values were fixed and 100 non-parametic bootstrap replicates were used to estimate the topological uncertainty arising from stochastic error in the data.

Starch gel electrophoresis

Leaf samples were collected from four populations of *D. nidulum,* three populations of *D. nausoriense*, two populations of *D. araucardioides*, two populations of *D. guillauminii* and one each of *D. balansae* and *D. lycopodioides* (Table 2, Fig. 1) for starch gel electrophoresis. In addition, four hybrids (*D.* × *suprinii*) were encountered in the field and sampled. Preparation of samples and starch gel electrophoresis followed methods described by Conkle *et al*. (1982) and Keppel *et al*. (2002). Twelve (six polymorphic) presumptive loci of seven enzyme systems were resolved consistently (Table 3).

POPGENE, version 1.21 (Yeh *et al.* 1997) was used to calculate the allele frequencies, the mean number of alleles per locus (*A*), the effective number of alleles per locus (*AE*; Kimura & Crow, 1964), percentage of loci polymorphic (*P*), observed mean heterozygosity (H_o) and the heterozygosity expected (H_e) in Hardy-Weinberg equilibrium (Nei 1973). Gene flow was calculated from the F_{ST} estimates using $Nm = (1/ F_{ST}-1)/4$ (Wright 1951). TFPGA, version 1.3 (Miller 1998) was used to create an UPGMA

dendogram base on pairwise genetic distances (*D*; Nei 1978) between the populations investigated. Bootstrap support was calculated using 1,000 permutations.

Hybridization between species pairs

To test for recent hybridization between *Dacrydium araucarioides* and *D. guillauminii*; and between *D. nidulum* and *D. nausoriense* we used Newhybrids 1.1 (Anderson and Thompson 2002), a Bayesian genetic clustering program, that tests for the presence of individuals displaying hybrid multilocus genotypes by sorting individuals into six genetic classes (pure populations P0 and P1, F1 and F2 hybrids, two backcross classes BC-P0 and BC-P1) and calculating the posterior probabilities of an individual belonging to each class. To distinguish between different classes this program requires diagnostic alleles. As none of the allozyme loci were fixed between the species pairs, it was not possible to distinguish between different hybrid classes. Consequently, posterior probability values were summed across hybrid classes for each individual and this value was used to estimate if a genotype was of pure or hybrid origin (Vähä and Primmer 2006). We used uniform priors to estimate posterior probabilities from the average of five runs of 2 million iterations following a burn-in of 100,000 iterations. The four hybrids (*D*. × *suprinii*) are used in the *D. araucarioides* and *D. guillauminii* data set to confirm the hybrid status of these individuals. Principal co-ordinates analysis (PCOA) was used to examine clustering of individual *D. araucarioides*, *D. guillauminii* and *D*. × *suprinii* genotypes from all sampled sites using GENALEX (Peakall and Smouse 2006).

Results

Phylogenetic relationships of Dacrydium *in Remote Oceania*

The plastid relationships of *Dacrydium* in Remote Oceania*,* estimated under ML and the BRC implementation BEAST, are shown in Figure 2 and 3, respectively. Topologies are generally congruent irrespective of the optimisation criteria used, and nodes that received a high BS percentage (i.e. >75%) under ML also had a high posterior probability (PP; i.e. >0.95) of being correct (given the data and the model of sequence evolution). *Dacrydium, Dacrycarpus* and *Falcatifolium* each form strongly supported clades, although the relationships amongst these are poorly resolved. Within *Dacrydium,* there is weak support for a sister group relationship of *D. cupressinum* and the remaining included representatives, strong support for a relationship between *D. elatum* and *D. pectinatum,* and moderate (ML BS = 64%) to strong (PP = 1.0) support for the monophyly of *Dacrydium* in Remote Oceania. Amongst taxa in Remote Oceania, *D. araucarioides* and *D. balansae* form a well-supported clade, as do *D. nidulum* and *D. nausoriense.* The former pair are distinguished from each other by a one base insertion/deletion (indel) in the *trnL* intron (position 230 in the aligned sequences), while a 2 base indel (positions 232 and 233 in the *trnL* intron) distinguishes *D. nausoriense* from *D. nidulum*. There is at best weak support for the monophyly of the New Caledonian *Dacrydium* (Figs. 2 and 3).

Figure 3 shows the BRC estimation of lineage diverergence times. Given the poor resolution amongst genera, the age of the root (median 52.6 my, 95% highest posterior density (HPD) 43–68 my) estimates the timing of the evolution of *Dacrydium.* The *Dacrydium* crown group arose an estimated 22 my (95% HPD 11–38 my) before present. For the Remote Oceania clade, the timing of lineage diversification is estimated at 10 my (95% HPD 4–20 my) before present and within this group, the pairs *D. nausoriense/D. nidulum* and *D. araucarioides/D. balansae* arose within the last 10 my (Fig. 3).

Allozyme data generally concurs with the topologies obtained from plastid DNA sequences (Fig. 4), as relationships within *Dacrydium* are poorly resolved and the close relationships between the species pairs *D. araucarioides*/*D. balansae* and *D. nidulum*/*D. nausoriense* receive moderate to strong support. Although *D. guillauminii* hybridizes with *D. araucarioides*, the species are genetically distinct and appear to be discrete species. All species pairs, except *D. nidulum* and *D. nausoriense*, have an average genetic distance of 0.075 or higher (Fig. 2). Calculated gene flow between the two Fijian species is relatively high (Nm = 2.43) but populations cluster by taxa (Fig. 4). Most of the differentiation between the species results from the ACO 1 locus (Nm = 1.10 for this locus), with allele 1 being the most common in populations of *D. nidulum* and allele 2 being most common in *D. nausoriense* (Fig. 5).

Hybridization between species pairs

Allozyme data support the hybrid nature of the four *D.* × *suprinii* samples in PCOA and hybrid-class assignment analysis using Newhybrids 1.1. Individual genotypes clustered largely according to species based on PCOA (Fig. 6a), where the first two axes account for 82% of the total variation, with the species-differentiating axis 1 explaining > 69% of the total variation. *Dacrydium* × *suprinii* individuals were intermediately distributed between the two species, as were some *D. guillauminii* genotypes.

Newhybrids 1.1 did not detect any hybrids between *D. nidulum* and *D. nausoriense*, but 6.25 % of the genotypes were identified as hybrids between *D. araucarioides* and *D. guillauminii*. Based on posterior probabilities of > 0.9 all *D. nidulum* and *D. nausoriense* individuals were strongly allocated to pure populations. Hybridization between *D. araucarioides* and *D. guillauminii* was asymmetric, as no hybrid genotypes could be attributed to *D. araucarioides* individuals, but 12.5 % of *D. guillauminii* individuals exhibited hybrid genotypes (Fig. 6b). All except one *D. araucarioides* individual could be attributed to pure populations (posterior probabilities > 0.9), but the unassigned individual still had a high probability

(> 0.7) of being pure *D. araucarioides*. All non-hybrid *D. guillauminii* individuals were assigned to a pure population with a high probability (> 0.9). The four *D.* × *suprinii* individuals were all found to be of hybrid origin with a strong posterior probability (> 0.75).

Genetic diversity

The number of effective alleles (*NE*) ranged from 1.16 to 1.28 (Table 4), with the exception of *D. guillauminii* (*NE* = 1.08), which also had the lowest observed heterozygosity (H_o = 0.038). *D. araucarioides* also had a relatively low observed heterozygosity (H_o = 0.075), which ranged between 0.117 and 0.182 for the other species analysed here. *D. guillauminii*, therefore had low genetic diversity, a phenomenon that was more pronounced in the lake population than in the river population (*NE* = 1.05 vs. 1.16; H_o = 0.006 vs. 0.096). The other rare species, *D. nausoriense* exhibited levels of genetic diversity similar to the other species studied (*NE* = 1.28; H_o = 0.138).

Discussion

Diversification history of Dacrydium *in Remote Oceania*

The present distribution of *Dacrydium* in Remote Oceania is the result of a complex interplay of speciation, dispersal and extinction. *D. cupressinum* is sister to the other sampled *Dacrydium*, which appears to support the importance of New Zealand as a source area of biota in Remote Oceania (Wright *et al.* 2000; Knapp *et al.* 2007; but see Biffin *et al*. in press). However, interpretation of a colonization sequence is extremely difficult, as many relationships are poorly supported by the molecular data and taxon sampling was not exhaustive. The latter also precludes the assessment of phylogenetic relationships within the genus as a whole. In addition, *Dacrydium* occurred in Australia into the

Pleistocene, but is presently absent (Hill and Christophel 2001). These extinction events likely impact the interpretation of our *trnL-trnF* topologies and can lead to false conclusions (Hill 2001; Crisp and Cook 2005).

Considering that the Remote Oceania lineage evolved only about 20 mya, long-distance dispersal events likely played a pivotal role in facilitating the present distribution of *Dacrydium*, as has been shown in many other groups in the Pacific (Sanmartín *et al.* 2007; Keppel *et al.* 2009; Baldwin and Wagner 2010). This includes several gymnosperm lineages (e.g., Setoguchi *et al.* 1998; Keppel *et al.* 2008) that arose prior to the fragmentation of the southern super-continent Gondwana. Recent dispersal between Fiji and New Caledonia is also supported by the estimated divergence dates in this study, which place the split between the Fijian and New Caledonian species at approximately 10 mya (median; 95% HPD 20–5 mya).

It is increasingly being recognized that the high diversity and endemism of the New Caledonian (Lowry II 1998) flora has complex origins. Presence of a great variety of soil types (de Kok 2002), complex topography (Murienne *et al.* 2008), climatic changes (Pintaud *et al.* 2001; Murienne *et al.* 2008) and hybridization (Pillon *et al.* 2009) have all been implicated in various groups. The distinct ecological niches (gallery forest, moist climate ultramafics, dry climate ultramafics and high elevation ultramafics) and high genetic differentiation between the New Caledonian *Dacrydium* species suggest allopatric ecological divergence to have been an important force during speciation. Hybridization between *D. guillauminii* and *D. araucarioides*, which are not sister taxa, provides additional support for ecological divergence in allopatry because it suggests weak reproductive isolation.

Although *D. nausoriense* and *D. nidulum* are seemingly sister species, the processes leading to speciation in Fiji are less clear. Recent divergence, wind pollination (gene flow can occur over long distances) and close proximity of populations (within 20 km), suggest that reproductive isolation may

have evolved in the presence of gene flow (Bolnick and Fitzpatrick 2007). In addition, the species occupy similar ecological niches and do not appear to hybridize, despite low genetic differentiation, fulfilling the expectations of strong reproductive isolation arising in sympatric conditions (Seehausen and van Alphen 1999; Rundle *et al.* 2000). However, the Fiji archipelago is composed of numerous islands and has a complex geological and tectonic history (Yan and Kroenke 1993), leading to numerous plausible speciation scenarios.

Hybridization between species pairs

Hybridization between *D. araucarioides* and *D. guillauminii* occurs in the wild, which is also the case in some other plant species in secondary contact (Rieseberg and Wendel 1993). However our results indicate that hybridization between this species pair is asymmetric; from the common *D. araucarioides* into the critically endangered *D. guillauminii*. Asymmetric introgression has been reported for a range of hybrid zones (Rieseberg and Wendel 1993). Our results are probably an underestimate of the actual level of introgression into *D. guillauminii*, as the four *D.* × *suprinii* individuals were all found within or very near to *D. guillauminii* habitat. There appears to be no hybridization between *D. nidulum* and *D. nausoriense*, and the two species are genetically divergent. However, more extensive sampling should be conducted to confirm the absence of hybrids at earlier life-history stages.

Distinctiveness of recognised species

Although species of *Dacrydium* in Remote Oceania are closely related, they are distinct taxonomic units. *Dacrydium guillauminii* and *D. araucarioides* hybridize but are not sister species and are strongly differentiated (F_{ST} = 0.575). Despite the close relationship between *D. nidulum* and *D. nausoriense* (low genetic distances, high gene flow (Nm = 2.43)), the two species are clearly genetically divergent and

should be treated as distinct taxonomic units and not ecotypes (cf. Ash 1986). Differentiation in allele frequencies at the ACO 1 locus suggests that the high indirect measure of gene flow between the two species may be an artefact of recent divergence. At which taxonomic level *D. nausoriense* should be recognized, however, remains an unanswered question that requires more detailed genetic and ecological data.

Genetic diversity

Dacrydium lycopodioides, which had a small sample size, and the rare *D. guillauminii* had considerably lower genetic diversity than the other species studied. Excluding these two species, estimates of genetic diversity are similar to average values reported more broadly for gymnosperms (cf. Hamrick and Godt 1989). This suggests that most conifers found on the islands of New Caledonia and Fiji.are likely to have genetic diversities similar to mainland conifer species.

Conservation of Rare Species

Low genetic diversity and asymmetric hybridization potentially pose serious threats to the survival of *D. guillauminii*, as rare plant species of recent evolutionary origin are most vulnerable to the effects of introgressive hybridization (Levin *et al.* 1996; Seehausen 2006). Hybridization is of even greater concern, if reproductive barriers between species are weak and the species exist as small isolated populations (Levin *et al.* 1996), as is the case for *D. guillauminii*. In fact, introgressive hybridization has been implicated in the extinction and decline of some rare plant species (Wolf *et al.* 2001), including *Argyranthemum coronopifolium* (Willd.) Humphries and *Cercocarpus traskiae* Eastw. (Brochmann 1984; Rieseberg and Gerber 1995). Consequently, detailed studies are needed to determine the precise nature and impact of hybridization on the genomic composition and morphology of *D. guillauminii*.

In addition, *Dacrydium guillauminii* has much lower genetic diversity (A = 1.55, H_e = 0.059) than its congeners, a phenomenon regularly observed in rare species (Spielman *et al.* 2004). Lower genetic diversity appears to compromise evolutionary potential and reproductive fitness, resulting in a higher extinction risk (Reed and Frankham 2003; Spielman *et al.* 2004). The low heterozygosity implies an excess of homozygotes (F_{IS} = 0.165) and could be the result of inbreeding, bottlenecks and/or asexual reproduction in the remnant population, with the former potentially raising further conservation concerns (Keller and Waller 2002).

In comparison to *D. guillauminii*, the high genetic diversity in most *D. nausoriense* populations bodes well for the survival of this endangered Fijian species. In fact, genetic diversity in most populations is similar to or higher than in its widespread cogeners, indicating that *D. nausoriense* should have sufficient genetic diversity to respond to changes in environmental conditions. The small Savara population, which has low genetic variation, should be given highest conservation priority. In addition, *D. nausoriense* does not appear to hybridize with the other Fijian species, *D. nidulum*, although populations often occur within about 30 km of each other.

Acknowledgments

We would like to thank M. Pole, L. Cook, H.P. Possingham, D. Ortiz-Barrientos for kindly reviewing this paper. M.F. Doyle organised the collection of the population samples and J.G. Conran supplied several of the samples for sequencing. B. Suprin assisted in the collection of New Caledonian samples. We are also grateful to the University of Queensland (UQ) for providing an International Postgraduate Research Scholarship (IPRS) and International Living Allowance Scholarship (ILAS) to GK, Australia-Pacific Science Foundation (APSF) for providing funding for part of this project through grants to AJL

and GK, the Ecology Centre at the School of Integrative Biology of the University of Queensland for supporting GK, and the Australian Research Committee (ARC – DP 0665859) for support to AJL. PJP wishes to acknowledge Ortiz-Barrientos lab at UQ for providing resources during his research visit.

References

Anderson EC, Thompson EA (2002) A model-based method for identifying species hybrids using multilocus genetic data. *Genetics* **160**, 1217–1229.

Ash J (1986) Growth rings, age and taxonomy of *Dacrydium* (Podocarpaceae) in Fiji. *Australian Journal of Botany* **34**, 197–205.

Baldwin BG, Sanderson MJ (1998) Age and rate of diversification of the Hawaiian silversword alliance (Compositae). *Proceedings of the National Academy of Sciences, USA* **95**, 9402–9406.

Baldwin BG, Wagner WL (2010) Hawaiian angiosperm radiations of North American origin. *Annals of Botany* **105**, 849–879.

Barluenga M, Stölting KN, Salzburger W, Muschick M, Meyer A (2006) Sympatric speciation in Nicaraguan crater lake cichlid fish. *Nature* **439**, 719–723.

Biffin E, Hill RS, Lowe AJ (2010) Did kauri (*Agathis*: Araucariaceae) really survive the Oligocene drowning of New Zealand? *Systematic Biology* **59**, 594–602.

Bolnick DI, Fitzpatrick BM (2007) Sympatric speciation: models and empirical evidence. *Annual Review of Ecology, Evolution, and Systematics* **38**, 459–487.

Brochmann C (1984) Hybridization and distribution of *Argyranthemum coronopifolium* (Asteraceae - Anthemideae) in the Canary Islands. *Nordic Journal of Botany* **4**, 729–736.

Butlin RK, Galindo J, Grahame JW (2008) Sympatric, parapatric or allopatric: the most important way to classify speciation? *Philosophical Transactions of the Royal Society, London, Series B* **363**, 2997–3007.

Conifer Specialist Group (1998) Dacrydium nausoriense. In '2007 IUCN red list of threatened species. Version 2010.2.' Available at http://www.iucnredlist.org [accessed on 08 July 2010].

Conifer Specialist Group (2000) Dacrydium guillauminii. In '2007 IUCN red list of threatened species. Version 2010.2.' Available at http://www.iucnredlist.org [accessed on 08 July 2010].

Conkle MT, Hodgskiss PD, Nunnally LB, Hunter SC (1982) 'Starch gel electrophoresis of conifer seeds: a laboratory manual.' (Pacific Southwest Forest and Range Experiment Station: Berkeley, CA)

Conran JG, Wood GM, Martin PG, Dowd JM, Quinn CJ, Gadek PA, Price RA (2000) Generic relationships within and between the gymnosperm families Podocarpaceae and Phyllocladaceae based on an analysis of the chloroplast gene *rbcL*. *Australian Journal of Botany* **48**, 715–724.

Coyne JA (1992) Genetics and speciation. *Nature* **355**, 511–515.

Crisp MD, Cook LG (2005) Do early branching lineages signify ancestral traits? *Trends in Ecology and Evolution* **20**, 122–126.

de Kok R (2002) Are plant adaptations to growing on serpentine soils rare or common? A few case studies from New Caledonia. *Adansonia* **24**, 229–238.

de Laubenfels DJ (1972) *Gymnospermes*. Muséum National d'Histoire Naturelle, Paris, France.

Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **7**, 214.

Earle CJ (2008) 'The gymnosperm database'. Available at http://www.conifers.org/ [accessed on 10 April 2010].

Filardi CE, Moyle RG (2005) Single origin of a pan-Pacific bird group and upstream colonization of Australasia. *Nature* **438**, 216–219.

Fitzpatrick BM, Fordyce JA, Gavrilets S (2008a) What, if anything, is sympatric speciation? *Journal of Evolutionary Biology* **21**, 1452–1459.

Fitzpatrick BM, Placyk JS, Niemiller ML, Casper GS, Burghardt GM (2008b) Distinctiveness in the face of gene flow: hybridization between specialist and generalist gartersnakes. *Molecular Ecology* **17**, 4107–4117.

Gillespie RG (2002) Biogeography of spiders on remote oceanic islands of the Pacific: archipelagos as stepping stones? *Journal of Biogeography* **29**, 655–662.

Gow JL, Peichel CL, Taylor EB (2006) Contrasting hybridization rates between sympatric three-spined sticklebacks highlight the fragility of reproductive barriers between evolutionary young species. *Molecular Ecology* **15**, 739–752.

Grandcolas P, Murienne J, Robillard T, Desutter-Grandcolas L, Jourdan H, Guilbert E, Deharveng L (2008) New Caledonia: a very old Darwinian island? *Philosophical Transactions of the Royal Society, B* **363**, 3309–3317.

Hamrick JL, Godt MJW (1989) Allozyme diversity in plant species. In 'Plant population genetics, breeding and genetic resources' (Eds AHD Brown, MT Clegg, AL Kahler, BS Weir) pp. 43–63. (Sinauer Associates: Sunderland, MA)

Hill RS (2001) Biogeography, evolution and palaeoecology of *Nothofagus* (Nothofagaceae): the contribution of the fossil record. *Australian Journal of Botany* **21**, 321–332.

Hill RS, Brodribb TJ (1999) Southern conifers in time and space. *Australian Journal of Botany* **47**, 639–696.

Hill RS, Christophel DC (2001) Two new species of *Dacrydium* (Podocarpaceae) based on vegetative fossils from Middle Eocene sediments at Nelly Creek, South Australia. *Australian Systematic Botany* **14**, 193–205.

Howarth DG, Baum DA (2005) Genealogical evidence of homoploid hybrid speciation in an adaptive radiation of *Scaevola* (Goodeniaceae) in the Hawaiian Islands. *Evolution* **59**, 948–961.

Jaffré T (1995) Distribution and ecology of conifer in New Caledonia. In '*Ecology of Southern Conifers*. (Eds NJ Enright, RS Hill) pp. 171–196. [Melbourne University Press: Melbourne].

Jaffré T, Bouchet P, Veillon J-M (1998) Threatened plants of New Caledonia: is the system of protected areas adequate? *Biodiversity and Conservation* **7**, 109–135.

Keller LF, Waller DM (2002) Inbreeding effects in wild populations. *Trends in Ecology and Evolution* **17**, 230–241.

Keppel G, Hodgskiss PD, Plunkett GM (2008) Cycads in the insular Southwest Pacific: dispersal or vicariance? *Journal of Biogeography* **35**, 1004–1015.

Keppel G, Lee S-W, Hodgskiss PD (2002) Evidence for long isolation among populations of a Pacific cycad: genetic diversity and differentiation in *Cycas seemannii* A.Br. (Cycadaceae). *The Journal of Heredity* **93**, 133–139.

Keppel G., Lowe A. J. & Possingham H. P. (2009) Changing perspectives on the biogeography of the tropical South Pacific: influences of dispersal, vicariance and extinction. *Journal of Biogeography* **36**, 1035–1054.

Keppel G, Rounds IA, Thomas NT (2006) The flora, vegetation, and conservation value of mesic forest at Dogotuki, Vanua Levu, Fiji Islands. *New Zealand Journal of Botany* **44**, 273–292.

Kimura M, Crow JF (1964) The number of alleles that can be maintained in a finite population. *Genetics* **49**, 725–738.

Knapp M, Mudaliar R, Havell D, Wagstaff SJ, Lockhart PJ (2007) The drowning of New Zealand and the problem of *Agathis*. *Systematic Biology* **56**, 862–870.

Knopf P, Nimsch H, Stützel T (2007) *Dacrydium* × *suprinii*, sp. nova - a natural hybrid of *Dacrydium araucarioides* × *D. guillauminii*. *Feddes Repertorium* **118**, 51–59.

Levin DA, Francisco-Ortega J, Jansen RK (1996) Hybridization and the extinction of rare plant species. *Conservation Biology* **10**, 10–16.

Lowry II PP (1998) Diversity, endemism, and extinction in the flora of New Caledonia: a review. In 'Rare, Threatened, and Endangered Floras of Asia and the Pacific Rim' (Eds C-I Peng, PP Lowry II) pp. 181–206. (Academia Sinica: Taipei)

Mallet J (2007) Hybrid speciation. *Nature* **446**, 279–283.

Matisoo-Smith E, Robins JH (2004) Origins and dispersal of Pacific peoples: evidence from mtDNA phylogenies of the Pacific rat. *Proceedings of the National Academy of Sciences, USA* **101**, 9167–9172.

Miller M (1998) 'Tools for Population Genetic Analyses (TFPGA), version 1.3. A Windows program for the analysis of allozyme and molecular population genetic data'. Available at: http://herb.bio.nau.edu/~miller/tfpga.htm [accessed on 24 April 2009].

Murienne J, Pellens R, Budinoff RB, Wheeler WC, Grandcolas P (2008) Phylogenetic analysis of the endemic New Caledonian cockroach *Lauraesilpha*. Testing competing hypotheses of diversification. *Cladistics* **24**, 802–812.

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. *Nature* **403**, 853–858.

Nei M (1973) Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences, USA* **70**, 3321–3323.

Nei M (1978) Estimation of average heterozygosity and genetic distance from small number of individuals. *Genetics* **89**, 583–590.

Nosil P (2008) Speciation with gene flow could be common. *Molecular Ecology* **17**, 2103–2106.

Oldfield S, Lusty C, Mackinven A (1998) 'The world list of threatened trees'. (World Conservation Press: Cambridge, UK)

Pavlacky DC, Goldizen AW, Prentis PJ, Nicholls JA (2009) A landscape genetics approach for quantifying the relative influence of historic and contemporary habitat heterogeneity on the genetic connectivity of a rainforest bird. *Molecular Ecology* **18**, 2945–2960.

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* **6**, 288–295.

Pillon Y, Munzinger J, Amir H, Hopkins HCF, Chase MW (2009) Reticulate evolution on a mosaic of soils: diversification of the New Caledonian endemic genus *Codia* (Cunoniaceae). *Molecular Ecology* **18**, 2263–2275.

Pintaud JC, Jaffre T, Puig H (2001) Chorology of New Caledonian palms and possible evidence of Pleistocene rain forest refugia. *Comptes Rendus de L'Academie des Sciences Serie III-Sciences de la Vie* **324**, 453–463.

Pole M (1992) Early Miocene flora of the Manuhenkia Group, New Zealand. 2. Conifers. *Journal of the Royal Society of New Zealand* **22**, 287–302.

Pole M (1997) Miocene conifers from the Manuherikia Group, New Zealand. *Journal of the Royal Society of New Zealand* **27**, 353–370.

Prentis PJ, White EM, Radford IJ, Lowe AJ, Clarke AR (2007) Can hybridization cause local extinction: a case for demographic swamping of the Australian native *Senecio pinntifolius* by the invasive *Senecio madagascariensis*? *New Phytologist* **176**, 902–912.

Prentis PJ, Wilson JRU, Dormontt EE, Richardson DM, Lowe AJ (2008) Adaptive evolution in invasive species. *Trends in Ecology and Evolution* **13**, 288–294.

Quinn CJ (1982) Taxonomy of *Dacrydium* Sol. ex Lamb. emend. de Laub. (Podocarpaceae). *Australian Journal of Botany* **30**, 311–320.

Rambaut A, Drummond AJ (2007) 'Tracer v1.4.' Available at http://beast.bio.ed.ac.uk [accessed on 24 April 2009].

Reed DH, Frankham R (2003) Correlation between fitness and genetic diversity. *Conservation Biology* **17**, 230–237.

Renner SS (2004) Relaxed molecular clocks for dating historical plant dispersal events. *Trends in Plant Science* **10**, 550–558.

Rieseberg LH, Gerber D (1995) Hybridization in the Catalina Island mountain mahogany (*Cercocarpus traskiae*): RAPD evidence. *Conservation Biology* **9**, 199–203.

Rieseberg LH, Wendel JH (1993) Introgression and its consequences in plants. In 'Hybrid zones and the evolutionary process.' (Ed RG Harrison) pp. 70–109. (Oxford University Press: New York, NY)

Rieseberg LH, Widmer A, Arntz M, Burke JM (2002) Directional selection is the primary cause of phenotypic diversification. *Proceedings of the National Academy of Natural Sciences, USA* **99**, 12242–12245.

Rieseberg LH, Willis JH (2007) Plant Speciation. *Science* **317**, 910–914.

Rundle HD, Nagel L, Bougham JW, Schluter D (2000) Natural selection and parallel speciation in sympatric sticklebacks. *Science* **287**, 306–308.

Sanders KL, Lee MSY (2007) Evaluating molecular clock calibrations using Bayesian analysis with soft and hard bounds. *Biology Letters* **3**, 275–279.

Sanmartín I, Wanntorp L, Winkworth RC (2007) West wind drift revisited: testing for directional dispersal in the Southern Hemisphere using event-based tree fitting. *Journal of Biogeography* **34**, 398–416.

Savolainen V, Anstett M-C, Lexer C, Hutton I, Clarkson JJ, Norup MV, Powell MP, Springate D, Salamin N, Baker WJ (2006) Sympatric speciation in palms on an oceanic island. *Nature* **441**, 210–203.

Seehausen O (2006) Conservation: loosing biodiversity by reverse speciation. *Current Biology* **16**, R334–R337.

Seehausen O, Takimoto G, Roy DN, Jokela J (2008) Speciation reversal and biodiversity dynamics with hybridization in changing environments. *Molecular Ecology* **17**, 30–44.

Seehausen O, van Alphen JJM (1999) Can sympatric speciation by disruptive sexual selection explain rapid evolution of cichlid diversity in Lake Victoria? *Ecology Letters* **2**, 262–271.

Setoguchi H, Osawa TA, Pintaud J-C, Jaffré T, Veillon J-M (1998) Phylogenetic relationships within Araucariaceae based on *rbcL* gene sequences. *American Journal of Botany* **85**, 1507–1516.

Sinclair WT, Mill RR, Gardner MF, Woltz P, Jaffré T, Preston J, Hollingsworth PM, Ponge A, Möller M (2002) Evolutionary relationships of the New Caledonian heterotrophic conifer, *Parasitaxus usta* (Podocarpaceae), inferred from chloroplast *trn*L-F intron/spacer and nuclear rDNA ITS2 sequences. *Plant Systematics and Evolution* **233**, 79–104.

Smith AC (1979) 'Flora Vitiensis nova: a new flora of Fiji (spermatophytes only).' (Pacific Tropical Botanical Garden: Lawai, Hawai'i)

Spielman D, Brook BW, Frankham R (2004) Most species are not driven to extinction before genetic factors impact them. *Proceedings of the National Academy of Natural Sciences, USA* **101**, 15261–15264.

Swofford DL (2002) 'PAUP*: Phylogenetic analysis using parsimony (*and other methods): version 4.' (Sinauer Associates: Sunderland, MA).

Swofford DL, Olsen GJ, Waddell PJ, Hillis DM (1996) Phylogenetic inference. In 'Molecular Systematics. 2nd edition.' (Eds DM. Hillis, C Moritz, B Mable) pp. 407–514. (Sinauer Associates, Sunderland, MA)

Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* **17**, 1105–1109.

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position -specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**, 4673–4680.

Vähä JP, Primmer CR (2006) Efficiency of model-based Bayesian methods for detecting hybrid individuals under different hybridization scenarios and with different numbers of loci. *Molecular Ecology* **15**, 63–72.

Whitney KD, Randell RA, Rieseberg LH (2006) Adaptive introgression of herbivore resistance traits in the weedy sunflower *Helianthus annus*. *American Naturalist* **167**, 794–807.

Willyard A, Syring J, Gernandt DS, Liston A, Cronn R (2007) Fossil calibration of molecular divergence infers moderate mutation rate and recent radiations for *Pinus*. *Molecular Biology and Evolution* **24**, 90–101.

Wolf DE, Takebayashi N, Rieseberg LH (2001) Predicting the risk of extinction through hybridization. *Conservation Biology* **15**, 1039–1053.

Wright S (1951) The genetical structure of populations. *Annals of Eugenics* **15**, 323–354.

Wright SD, Yong CG, Dawson JW, Whittaker DJ, Gardner RC (2000) Riding the ice age El Niño? Pacific biogeography and evolution of *Metrosideros* subg. *Metrosideros* (Myrtaceae) inferred from nuclear ribosomal DNA. *Proceedings of the National Academy of Sciences, USA* **97**, 4118–4123.

Yan CY, Kroenke LW (1993) A plate tectonic reconstruction of the Southwest Pacific, 0-100 Ma. *Proceedings of the Ocean Drilling Program, Scientific Results* **130**, 697–709.

Yeh FC, Yang R-C, Boyle TJB, Ye Z-H, Mao JX (1997) 'POPGENE, the user friendly shareware for population genetic analysis.' (University of Alberta: Alberta)

Table 1. Genbank numbers and vouchers (in brackets).

Taxon	Genbank accession (voucher)
Dacrycarpus compactus (Wassch.) de Laub.	AY083139.1/AY083095.1
Dacrycarpus dacrydioides (A.Rich.) de Laub.	(RBGSyd living collections/cult)
Dacrycarpus imbricatus (Blume) de Laub.	AY083140.1/AY083096.1
Dacrycarpus imbricatus var. *patulus* de Laub.	AY013727.1
Dacrycarpus veillardii (Parl.)	(M.Doyle s.n. New Caledonia)
Dacrydium araucarioides Brongn. & Gris	AY083138.1/AY083094.1
Dacrydium balansae Brongn. & Gris	AY083137.1/AY083093.1
Dacrydium cupressinum Sol. ex Lamb.	AY083136.1/AY083092.1
Dacrydium elatum (Roxb.) Wall. ex Hook.	AJ441090.1
Dacrydium guillauminii J.Buchholz	(M.Doyle s.n. New Caledonia)
Dacrydium lycopodioides Brongn. & Gris	(M.Doyle s.n. New Caledonia)
Dacrydium nausoriense de Laub.	(SBG A883952/cult.)
Dacrydium nidulum de Laub.	(GK304/SUVA)
Dacrydium pectinatum de Laub.	AY534797.1
Falcatifolium falciforme (Parl.) de Laub.	(J. Conran s.n./cult.)
Falcatifolium gruezoi de Laub.	AY083144.1/ AY083100.1
Falcatifolium taxoides (Brongn. & Gris.) de Laub.	AY083143.1/ AY083099.1

Table 2. Locations of *Dacrydium* study populations in Remote Oceania.

Species	**Population Name**	**Location**	**Altitude (m)**	**Longitude/ Latitude**
D. nidulum	Korobaba	Mt. Korobaba & Mt. Kobalevu, Naitasiri Province, Viti Levu	360	18°04’40 S 178°22’57 E
D. nidulum	Waisoi	Waisoi, Namosi Province, Viti Levu	400	18°00’00 S 178°08’25 E
D. nidulum	Kasi	Mt. Kasi, Cakaudrove Province, Vanua Levu	310	16°46’20 S 179°01’40 E
D. nidulum	Namosi	Near Namosi Village, Namosi Province, Viti Levu	530	18°00’45 S 178°07’42 E
D. nausoriense	Nausori 1	Population 1, Nausori Highlands,	540	17°47’14 S 177°50’11 E
D. nausoriense	Nausori 2	Population 2, Nausori Highlands	540	17°46’41 S 177°49’10 E
D. nausoriense	Sarava	Sarava	270	16°29’43 S

				179°27’51 E
D. araucarioides	Plain	Includes the lower lying populations on Plaines des Lacs and near Lac de Yaté	170-250	22°16’13 S 166°54’20 E
D. araucarioides	Hills	Includes the populations in hills west of Lac de Yaté	300-400	22°07’23 S 166°38’38 E
D. balansae		Around Mt. Dzumac	500-1,100	21°59’21 S 166°31’54 E
D. guillauminii	Grand	Around Grand Lac	235	22°15’40 S 166°54’31 E
D. guillauminii	Huít	Around Lac en Huít and Chute de la Madeleine	350	22°16’04 S 166°53’40 E
D. lycopodioides		Around Mt. Dzumac	700	22°01’37 S 166°29’27 E
D. × *suprinii*		Around Grand Lac and Chute de la Madeleine	230-260	22°13’45 S 166°51’17 E

Table 3. Polymorphic enzyme systems consistently resolved in this study. Nomenclature and abbreviations follow Murphy *et al.* 1996, based on IUBNC. E.C. No. = Enzyme Commission number.

Enzyme	**Locus Abbreviation**	**E.C. No.**	**Buffer***
Aspartate aminotransferase	AAT-1	2.6.1.1	B
Aconitate hydratase	ACO-1	4.2.1.3	D
Glucose-6-phosphate dehydrogenase	G6PDH-1, -2	1.1.1.49	B
Glucose-phosphate isomerase	GPI-1	5.3.1.9	A
Isocitrate dehydrogenase	IDH-1	1.1.1.42	D
Malate dehydrogenase	MDH-2	1.1.1.37	D

* = A, B, D refer to buffers A (tris citrate/ lithium borate), B (citrate/ sodium borate) and a pH 8 version of buffer D (morpholine citrate) of Conkle *et al.* 1982.

Table 4. Genetic diversity parameters in *Dacrydium* study populations of Remote Oceania (standard deviation in parentheses). *x* = mean sample size per locus, *A* = mean number of alleles per locus, *NE* = number of effective alleles per locus, *P* = percentage of loci polymorphic, H_o = mean observed heterozygosity, H_e = expected heterozygosity (Nei 1973). Total values for each species (including all populations sampled) are in bold.

Population	***X***	***A***	***NE***	***P***	H_o	H_e
D. nidulum (Korobaba)	67	1.64 (1.03)	1.14 (0.25)	36.4	0.083 (0.138)	0.092 (0.157)
D. nidulum (Waisoi)	36	1.55 (0.69)	1.12 (0.29)	45.5	0.071 (0.132)	0.074 (0.146)
D. nidulum (Kasi)	36	1.36 (0.81)	1.13 (0.30)	18.2	0.091 (0.204)	0.075 (0.168)
D. nidulum (Namosi)	71	1.82 (1.17)	1.30 (0.47)	45.5	0.191 (0.284)	0.160 (0.230)
D. nidulum	**212**	**2.18 (1.33)**	**1.18 (0.29)**	**54.6**	**0.117 (0.163)**	**0.117 (0.168)**
D. nausoriense (Nausori 1)	66	1.82 (1.08)	1.64 (1.03)	45.5	0.154 (0.253)	0.140 (0.229)
D. nausoriense (Nausori 2)	48	1.64 (1.03)	1.32 (0.64)	36.4	0.121 (0.206)	0.137 (0.240)
D. nausoriense (Sarava)	14	1.18 (0.41)	1.11 (0.27)	18.2	0.091 (0.224)	0.064 (0.150)

D. nausoriense	**128**	**1.82 (1.08)**	**1.28 (0.49)**	**45.5**	**0.138 (0.230)**	**0.137 (0.228)**
D. araucarioides (Plain)	52	1.82 (1.17)	1.16 (0.29)	36.4	0.093 (0.152)	0.099 (0.163)
D. araucarioides (Hills)	46	1.55 (0.93)	1.18 (0.33)	36.4	0.056 (0.096)	0.104 (0.179)
D. araucarioides	**97**	**1.91 (1.38)**	**1.16 (0.27)**	**36.4**	**0.075 (0.125)**	**0.106 (0.166)**
D. balansae	**18**	**1.55 (0.82)**	**1.35 (0.59)**	**36.4**	**0.182 (0.282)**	**0.164 (0.249)**
D. guillauminii (Grand)	36	1.55 (0.93)	1.16 (0.47)	36.4	0.096 (0.262)	0.071 (0.181)
D. guillauminii (Huít)	64	1.36 (0.51)	1.05 (0.14)	36.4	0.006 (0.013)	0.040 (0.093)
D. guillauminii	**99**	**1.55 (0.93)**	**1.08 (0.16)**	**36.4**	**0.038 (0.099)**	**0.059 (0.111)**
D. lycopodioides	**12**	**1.82 (0.41)**	**1.16 (0.37)**	**18.2**	**0.152 (0.345)**	**0.086 (0.191)**
TOTAL	**566**	**2.73 (1.74)**	**1.35 (0.44)**	**54.6**	**0.103 (0.118)**	**0.200 (0.217)**

Fig. 1. Distribution of *Dacrydium*. Insert shows the populations of *D. araucarioides* (black-shaded circles), *D. guillauminii* (non-shaded circles and corresponding to entire range of the species) and their hybrid *D*. × *suprinii* (grey-shaded circles). * = also found in the Moluccas, west of New Guinea.

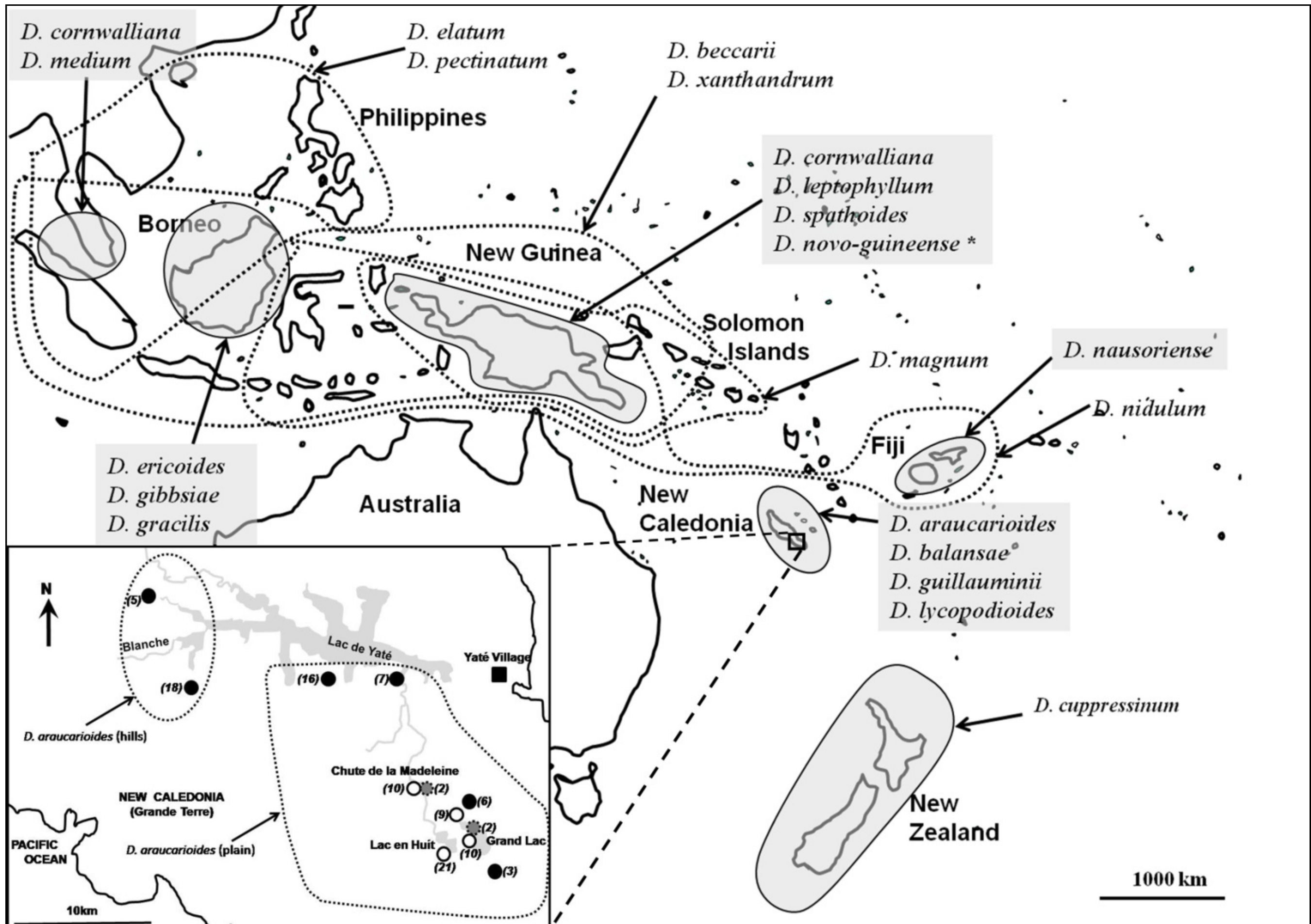

Fig. 2. Maximum likelihood topology for *Dacrydium* in Remote Oceania and outgroups inferred from *trnL-trnF* intron and spacer sequences using a GTR+I+Γ model of sequence evolution. Numbers below the branches are bootstrap proportions based upon 100 non-parametric bootstrap pseudoreplicates. Branches are proportional to the number of changes.

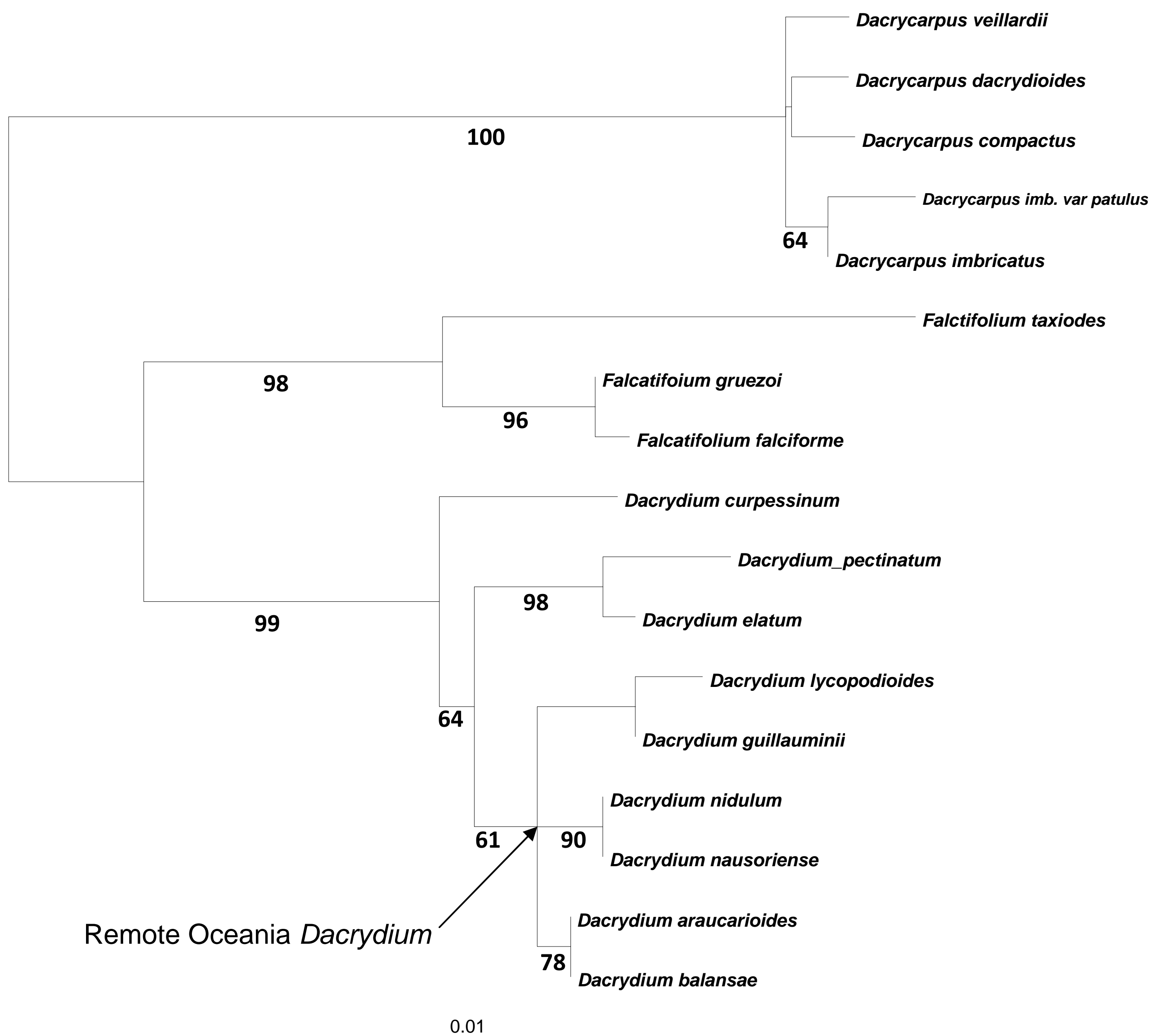

Fig. 3. Maximum clade credibility topology for Remote Oceania *Dacrydium* and outgroups inferred using Bayesian relaxed clock methods. Potserior probability values, estimated from 25000 sampled topologies are indicated above the branch. Grey bars indicate the 95% highest posterior density divergence time estimates for the corresponding node. Branch lengths are proportional to time (millions of years).

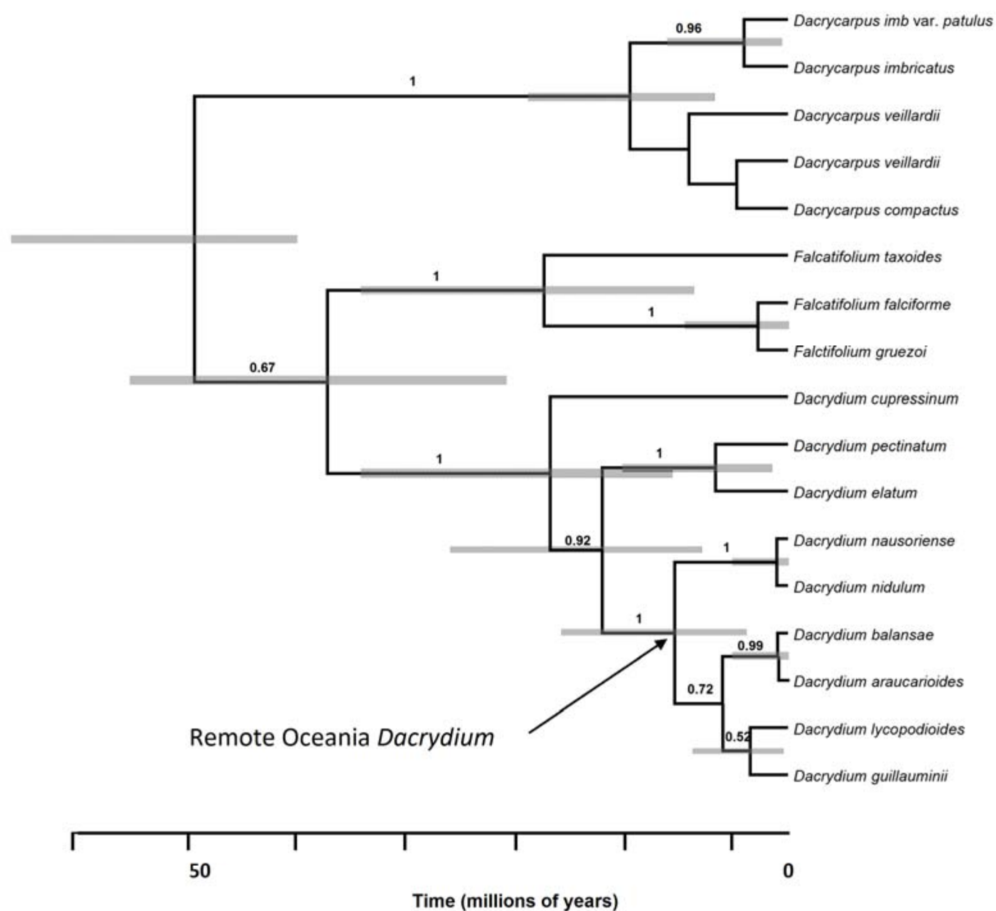

Fig. 4. UPGMA tree showing relationships among *Dacrydium* populations in Remote Oceania based on Nei's (1978) genetic distances of allozyme data. Percentage values indicate bootstrap values for the different nodes.

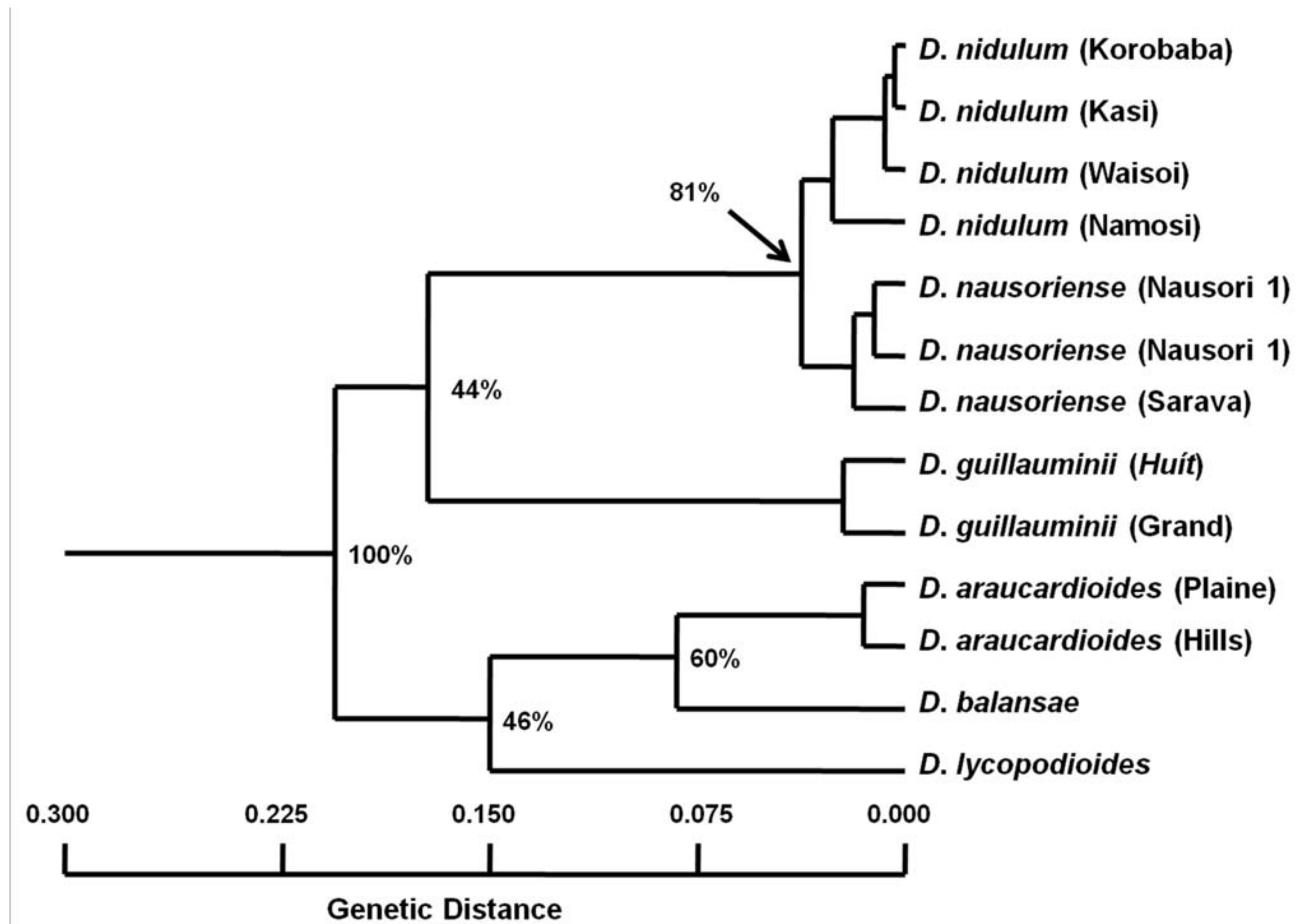

Fig. 5. Map of Fiji showing the location of *Dacrydium* study populations and pie charts showing the percentage distribution of alleles for the ACO-1 locus. The pie charts in boxes are of *Dacrydium nausoriense*, the unboxed ones of *Dacrydium nidulum*. Black areas of pie charts correspond to the percentage of allele 1, striped areas to the percentage of allele 2 and the non-shaded areas the combined percentage for alleles 3 and 4.

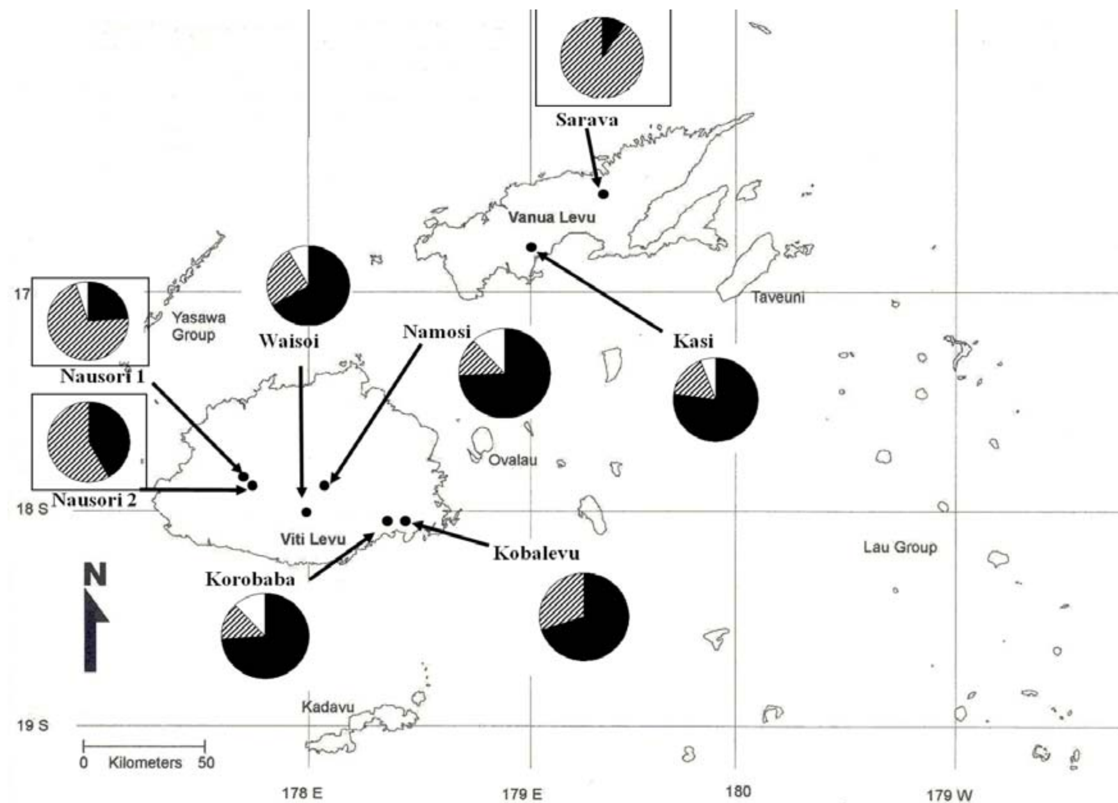

Fig. 6. Plots showing the direction of hybridization between *Dacrydium araucarioides* (n = 49) and *D. guillauminii* (n = 50), and their relationship to the their supposed hybrid *D. x suprinii* (x = 4). a) principal co-ordinates analysis depicting clustering of *D. araucarioides*, *D. guillauminii* and *D. x suprinii* individuals collected and analysed with allozymes, b) percentage of pure and hybrid individuals identified as *D. araucarioides* (*Da*), *D. guillauminii* (*Dg*) or *D. x suprinii* plants in the field.