Seasonal rainfall regime modulates genetic variation in the moss *Pseudocrossidium crinitum*

Nicholas Wilding

October 2009

Honours degree

Supervisor: Professor T.A.J. Hedderson

Acknowledgements

I would like to thank Alastair Potts for his help and enthusiasm in collecting *Pseudocrossidium crinitum* in the Eastern Cape, his collections have proven invaluable for this study. Jeff Duckett and Howard Matcham provided the material from Lesotho and Chile, demonstrating the importance for bryologists to travel the world and make collections!

Thanks to Tracey Nowell and Krystal Tolley for their willingness to tackle my issues with SAMOVA. You guys saved me hours of pulling my hair out! Many other people helped with running of analyses, thanks for your help (Terry, Yanis and Vera). Last but not least I would like to thank Terry Hedderson for his support and enthusiasm in this very cool project. This project made me realise how exciting and challenging science can be.

Abstract

Patterns of genetic variation, resulting from Pliocene-Pleistocene climate shifts have been largely documented for species from Europe. However, little is known from Africa and especially South Africa, where climate shifts have often been invoked to explain the amazingly high diversity of the Cape Floristic Region. An analysis of cpDNA and nDNA sequence variation for 65 populations of the moss *Pseudocrossidium crinitum* across South Africa revealed the presence of a phylogeographic break corresponding to the split between the winter-rainfall zone (WRZ) and the all-year-(ARZ) and summer-rainfall zones (SRZ). Coalescent estimates of the time since these populations split (1.3 - 3.4 Mya) are highly consistent with the onset of winter-rainfall in the south-western Cape. Estimates of gene flow indicate much higher levels of gene flow into the WRZ, fitting the expected direction of gene flow based on wind patterns and differences in phenology. Haplotype diversity was observed to be highest in the WRZ, suggesting a number of genetic structuring factors in play within the WRZ. Additional analysis of populations from Chile and Lesotho suggest recent dispersal from Chile and possibly high levels of trans-continental gene flow between these populations. The study provides a first look at the genetic consequences of paleo-climate shifts on a moss species in South Africa. These results, in combination with other similar studies, may help to piece together the factors and processes responsible for the high diversity in the CFR today.

Table of Contents

Table of Figures

List of Tables

Introduction

The onset and intensification of the Benguela uprising over the past 2-4 Mya has wrought profound changes on the climate of the western part of South Africa (Dupont *et al.* 2005). Prior to this, rainfall in South Africa was largely restricted to summer months, but the shift placed the south-western part of the country into a winter rainfall zone (WRZ)(see Figure 2)(Chase and Thomas 2007).

These Pliocene developments are important because of their apparently large impact on the evolution of a range of organisms which is evident in vegetation records (Vogel *et al.* 1978). The interplay of contrasting climate systems between the winter and summer rainfall zones has produced a truly unique flora, and is frequently invoked as a significant factor in the evolution of the astonishingly diverse and endemic-rich Cape Floristic region (see Linder and Mann 1998; Klak *et al.* 2004; Verboom *et al.* 2003; Davis and Scholtz 2004; Hedderson and Zander 2007a). The flora is a globally significant model system for many forms of scientific study, especially of speciation (Caujape-Castells *et al.* 2004; Klak *et al.* 2004; Rowe 2005; Hedderson and Zander 2007a).

In addition to its apparent impact on speciation rates, the onset of winter rainfall is also a potential structuring factor within species that span the seasonality boundary (Cowling *et al.* 1999). Gene flow within such species could be significantly affected if reproduction and timing of rainfall are highly correlated (e.g. bryophytes). Bergh *et al.* (2007) were able to show how rainfall dynamics, originating in the Holocene, have resulted in genetic disjunction between northern and southern populations of *Elytropappus rhinocerotis* in the WRZ.

A large amount of phylogeographic literature exists documenting the effects of paleo climatic shifts on the genetic structure of species and populations (Bucci *et al.* 2007; Hedderson and Zander 2007b; Hedderson and Nowell 2006; Nunez-Avila and Armesto 2006; Trewick *et al.* 2002; Mitton *et al.* 2000; Tremblay and Schoen 1999). To date the majority of this literature is associated with elucidating the genetic consequences of glacial periods on selected species from Europe and America. However comparatively little work has been done in Africa, and almost nothing is known about how climate shifts have impacted on species phylogeography.

Although less well studied, the bryophyte flora of the CFR is also species rich, and recent work has revealed the presence of many phylogenetically isolated endemic genera

(Hedderson and Zander 2007a; Hedderson and Zander 2008a; Hedderson and Zander 2008b). Not surprisingly, an increasing number of new bryophyte species are also being described for the CFR and surrounding areas (see Hedderson and Zander 2007b). Estimates place the age of many of these species around the end of the Pliocene (2.6 Mya) (Hedderson and Zander 2007b). The combination of enhanced aridity and prevalent high winds that characterized some periods of the Pleiocene–Pleistocene (Dupont et al., 2006; Chase & Thomas, 2007) may have rendered these particularly favourable for dispersal of many bryophytes, ultimately resulting in genetic drift and speciation of isolated populations.

According to a recent review by Shaw (2009), very little is known about speciation in bryophytes. This is surprising in light of traits such as fast generation times and the ability to disperse long distances, all of which appear to make bryophytes suitable candidates for studies of speciation. However, if one wishes to study the origin of species, one must first address this question: What is a species? This is still one of the most controversial and perplexing questions in systematic biology, and to date no unanimously accepted concept exists. One problematic area in developing such a concept is where to place intermediate cases or populations that are undergoing speciation (Dobzhansky 1937), and may not exhibit any morphological differentiation and/or reproductive/geographic isolation.

Interestingly, many bryophyte species are characterized by having cosmopolitan distributions with little morphological differentiation between individuals from different continents (Fernandez *et al.* 2006). However, phylogeographic studies, with the aid of DNA sequence variation, have often revealed genetic divergence among populations from different regions and provided evidence of cryptic species in both mosses and liverworts (e.g. Shaw 2000; Szweykowski and Krzakowa 1979; Dewey 1989; Shaw and Rooks 1994; Shaw and Schneider 1995). In many cases, cryptic species exhibit broadly overlapping geographical ranges, although many may be locally adapted ecotypes (Shaw 2001).

Pseudocrossidium crinitum (Schultz) Zander (Bryopsida, Pottiaceae), formerly *Barbula crinita* Schultz, is distributed among the major southern continental land masses extending into south-west North America (Figure 1). It is particularly common in South Africa, with numerous populations recorded from South America, Australia, New Zealand and North America. In habit, the species is primarily terricolous but it can be observed growing on a range of substrates (on humus, in crevices and on trees) with varying levels of disturbance. The plants occur in two forms, one displaying a golden-yellow hairpoint/awn and the other

white (see Appendix 1); in all other aspects the two forms appear to be completely indistinguishable. The white form is only known from South Africa (Zander 1993) and appears to be weedy within and around Cape Town, often growing on sidewalks, while the yellow form is more abundant throughout the rest of the country. It is noteworthy that the two forms are often observed growing in discrete patches adjacent to each other. Growth of gametophyte cushions is suspected to be primarily by means of fragments which give rise to new gametophytes, while spores probably act as the most effective dispersal agent in the local environment.

As with all other bryophytes there is an alternation of generation in which the gametophyte (n) is the dominant stage. The sporophyte (2n) is relatively short lived and is parasitic on the gametophyte. Sexual reproduction relies on the presence of water for male sperms to fertilize female egg cells. This means that the production of sporophytes is tightly linked to periods of rainfall.

The objectives of this study are to: (i) establish the phylogeographic structure of *Pseudocrossidium crinitum* in South Africa, giving explanations for the distribution of the two forms in terms of known phylogeographic breaks and/or past climatic conditions and processes (ii) test the hypothesis that seasonality of rainfall is a genetic structuring force for bryophytes (iii) determine whether there are grounds (molecular or morphological) for the two forms to be recognized as separate species.

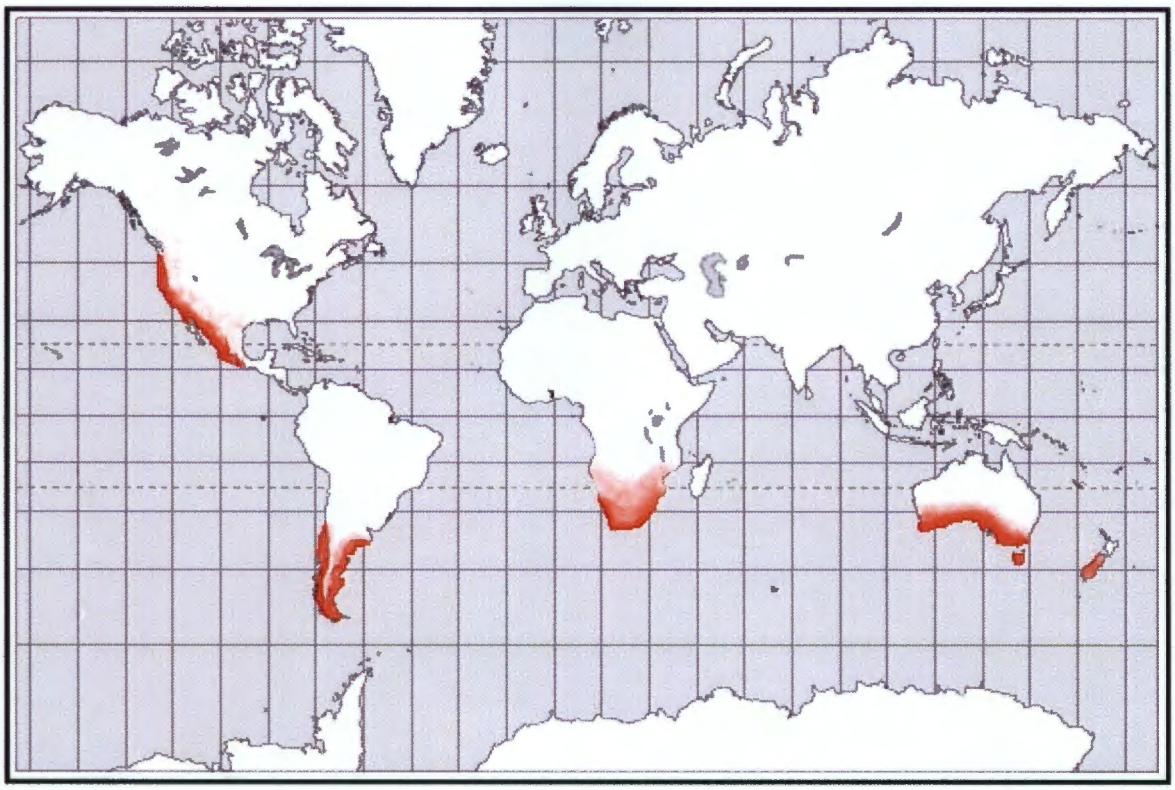


Figure 1 : Distribution of *Pseudocrossidium crinitum* around the world. Frequency of occurrences is shaded in red.

Figure 2 : Rainfall seasonality in South Africa. The region shaded blue receives mostly winter rain (WRZ) while the orange receives the majority of its rain during summer months (SRZ). The area shaded green is the all-year-round rainfall zone (ARZ). Map adapted from Chase and Thomas (2007).

Material and Methods

Sample size per population?

Sampling

Sixty-five populations of *P. crintium* were sampled covering much of the species range in the Eastern, Northern and Western Cape provinces of South Africa (Figure 3). Where it was possible, white- and yellow-awned populations growing adjacent to each other were both sampled. Additionally, two yellow-awned populations from Lesotho and four from Chile were included in the study.

per pop?

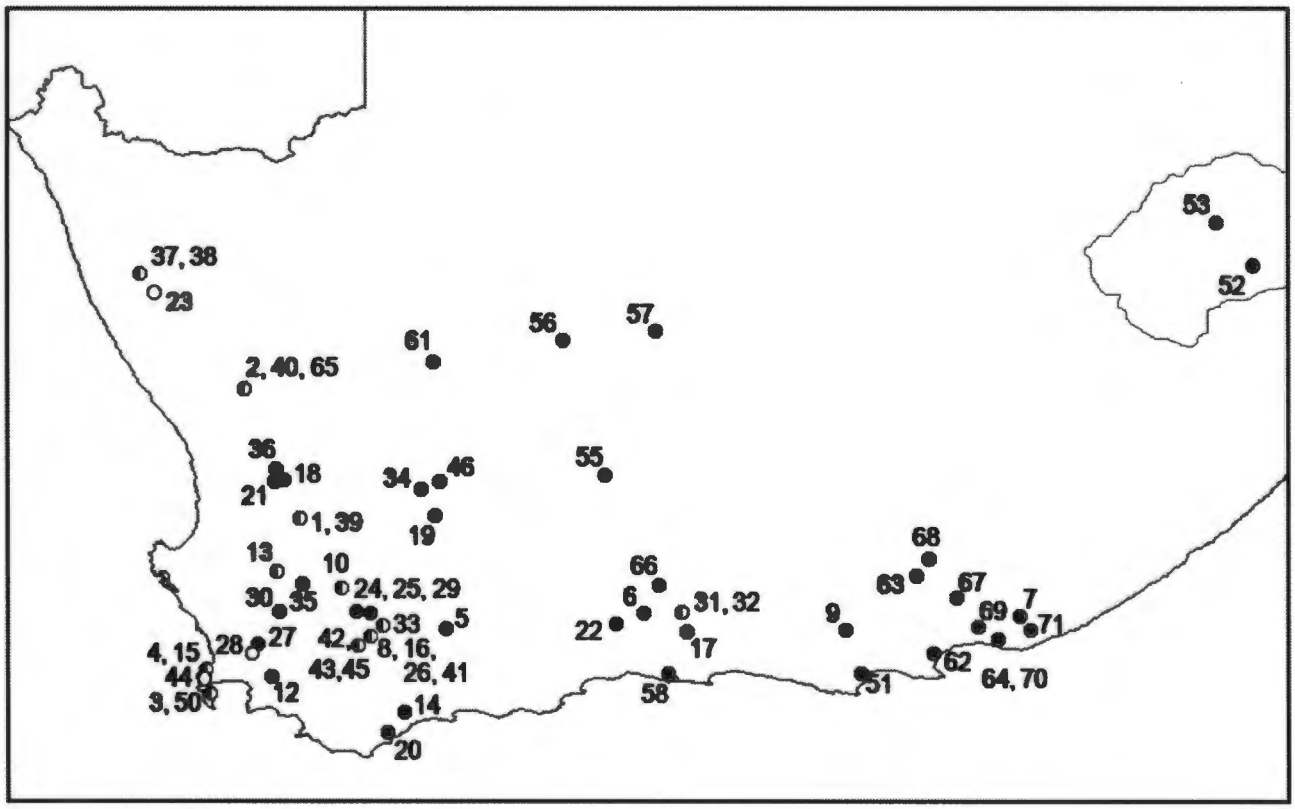


Figure 3 : Map of South Africa and Lesotho, indicating sampling localities of *Pseudocrossidium crinitum*. Numbers correspond to populations listed in Appendix 2. Black circles are populations with yellow hairpoints. White circles are populations with white hairpoints and half-black-half-white circles are localities with both forms present.

DNA extraction and PCR amplification

Total DNA was isolated from dried plant material using the CTAB method outlined in Doyle and Doyle (1987). Quantities of reagents were scaled down for ca. 20 mg of plant material, and slight modifications made as follows. Samples were ground in a pestle and mortar with 700 µl of extraction buffer. Following incubation, 600 µl of chloroform–isoamyl alcohol (24:1 v/v) was added, mixed by inversion for 5 min and spun. The aqueous phase was transferred to a clean micro-centrifuge tube. An equal volume of ice-cold isopropanol was added, and DNA was precipitated at -20°C overnight. After centrifugation, the DNA pellet recovered was washed with 75% ethanol and spun. Ethanol was discarded and DNA was left to air-dry prior to re-suspension in 50µl of nanopure water. Primers 18KRC (Hedderson and Nowell 2006) and ITS2 (Baldwin 1992) were used to amplify *ITS1*. The *trnL-F* region was amplified with primers tabC and tabF (Taberlet *et al.* 1991).

PCRs were performed using 0.75 units of KapaTaq DNA polymerase (Kapa Biosystems) in 30 µl volumes also containing 1x NH_4 buffer and 5 mM $MgCl_2$, 0.1 mM of each dNTP, 0.5 mM of each primer and 3µl of unquantified DNA template.

Thermo-cycling was carried out on a Applied Biosystems Gene Amp PCR system 2700, set to the following thermal conditions: initial denaturation at 97°C for 2 min, followed by 25 cycles of 97°C for 1 min, 50°C for 1 min, 72°C for 2 min, with a final polymerization step of 72°C for 7 min. Cycle sequencing was carried out at the University of Stellenbosch sequencing facility.

Sequence Data Analysis

Sequences were assembled with SeqMan (Lasergene System Software, DNA* Inc.). Assembled sequences were aligned manually using MegAlign (Lasergene System Software). The nuclear DNA region was analysed for possible recombinants, as these can confound the reconstruction and interpretation of phylogenetic relationships (Schierup and Hein 2000). To detect recombination in the *ITS1* region we used IMgc (Woerner *et al.* 2007), which infers recombination from violations of the four-gamete rule. The 4-gamete rule is based on the observation that two nearby mutations can only lead to three out of four possible haplotypes. The fourth haplotype can only be created by a recombination event, which becomes more likely with larger distance between the two SNPs (Hudson and Kaplan 1985).

meaning?

Haplotype networks were constructed using statistical parsimony (Templeton *et al.* 1992) implemented in the computer program TCS (Clement *et al.* 2000). The TCS-analysis is based upon a matrix of absolute pairwise distances, for which the probability of parsimony (as defined in Templeton *et al.* 1992) up to a threshold probability of 0.95 is calculated. The probability just before this 95% cut-off is then the maximum number of mutational connections between pairs of sequences justified by the 'parsimony' criterion (Clement *et al.* 2002).

Spatial analysis of molecular variance (SAMOVA) was used to define geographically homogeneous populations using the SAMOVA v1.0 software (Dupanloup *et al.* 2002). The method makes use of a simulated annealing approach that aims at maximizing the proportion of total genetic variance due to differences between groups of populations. As a by-product the program also enables the identification of genetic barriers between groups of populations (Dupanloup *et al.* 2002). The South African populations were divided into 13 geographic clusters, varying between 2 and 11 populations in size.

Basic population demographic parameters including timing of separation of populations (t), directional gene flow (m_1 and m_2) and effective population sizes of daughter (N_1 and N_2) and ancestral populations (N_A) were estimated using the IMa (Isolation with migration) software (Hey and Nielsen 2007). The program utilizes a Bayesian approach based on coalescence models to infer the demographic parameter θ of the ancestral (θ_A) and two daughter (θ_1 and θ_2) populations ($\theta=N_e\mu$, where N_e is the effective population size and μ is the neutral mutation rate), the time of separation of populations t (where $t = t\mu$, in number of generations), and the scaled migration rate m in each direction ($m = m/\mu$).

Bryophytes present a unique case in which the primary means of dispersal (i.e. by spores or asexually (using gemmae or by fragmentation)) contain a haploid copies of both the nuclear and chloroplast genomes (ploidy level=n). Further, dispersal distances of bryophyte sperm, which contain only the nuclear genome, have been shown to be negligible (i.e. a few centimetres) and not influencing spatial genetic structure (Shaw 2001). This means that the effective population size and dispersal history of the two genomes is comparable. Due to this similarity, combination of the cpDNA and nDNA data sets was possible, allowing for both loci to be used in a single IMa analysis.

Why didn't you use a parsimony approach?

Initial analyses were run using very wide prior distributions for model parameters (i.e. $q1$ = 10, $m1 = m2 = 10$, $t = 10$) based on the recommendations from the IMa manual. This ensured maximum sampling of the prior distribution. The use of wide initial prior distributions in this Bayesian approach allowed us to express our paucity of prior beliefs on parameter values. Additional runs of 10^7 steps were then performed with more appropriate parameter values to identify trends in parameter estimation and to ensure that Markov chains were mixing adequately for all parameters. Primary runs all consisted of a burn-in period of 10^5 steps followed by an additional run of 10^7 steps with 20 Markov chains and geometric heating (g1 = 0.8 g2 = 0.9). Markov chains were assumed to be mixing properly when parameter update rates of greater than 2% were obtained and multiple independent runs of 10^7 steps yielded similar results. Additionally, ESS (effective sample size) estimates of > 50 were required for all model parameters before a final run was accepted (Hey *et al.* 2004).

Generation times for bryophytes are largely unknown. This is not surprising given the myriad of factors which could affect estimates based on expected rates of DNA substitution. In order to calculate generation time in bryophytes, confounding factors like the indefinite persistence of old gametophytes, retention of somatic mutations in populations arising from gametophyte fragments and the frequency of asexual and sexual reproduction need to be taken into account. Unfortunately many of these factors are intrinsically hard, if not impossible to estimate. Additional metabolic rates have been shown to affect generation time (Martin and Palumbi 1993) and most importantly an estimate of minimum time between parent and daughter generations could be very useful. Clearly an in-depth study of generation time in bryophytes is long overdue. An estimated generation time of one year was used to calculate basic demographic parameters from scaled model parameters. This is based on the fact that apparently young shoots of *P. crinitum* are frequently observed with sporophytes.

"like" = "similar to but not including"

meaning? unclear

A divergence rate of 0.8 – 2% per million years was used for the *ITS* region (Bakker *et al.* 1995) and a rate of 0.024 – 0.116%per million years for the *trnL-F* region (Zhang and Hewitt 2003). Divergence rates were weighted by the amount of information contributed by each marker. Except where mentioned, parameter estimates were converted to more easily interpretable units using the weighted-geometric mean divergence rate for both loci. Upper (1.3% divergence per million years) and lower (0.5% divergence per million years) limits of divergence rates were used to calculate a range for the maximum likelihood estimate of t.

why did you use this?

Phylogenetic analysis was carried out using a Bayesian Markov chain Monte Carlo (MCMC) method as implemented in the program MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001). Bayesian analyses of sequence data was performed with parameters being estimated during two independent runs with four Markov chains under the GTR G + I model (nst = 6). The Monte Carlo Markov chain (MCMC) length was 1000 000 generations, and every 100th generation was sampled to minimize autocorrelation. The burnin length (2000 generations) was determined by examination of MCMC generation versus lnL plots. Runs were considered to have converged when the standard deviation of split frequencies was less than 0.01. The consensus topology was constructed using the *sumt* option in MrBayes, trees were edited in FigTree v1.2 (Rambaut 2007).

slang

?

meaning?

Results

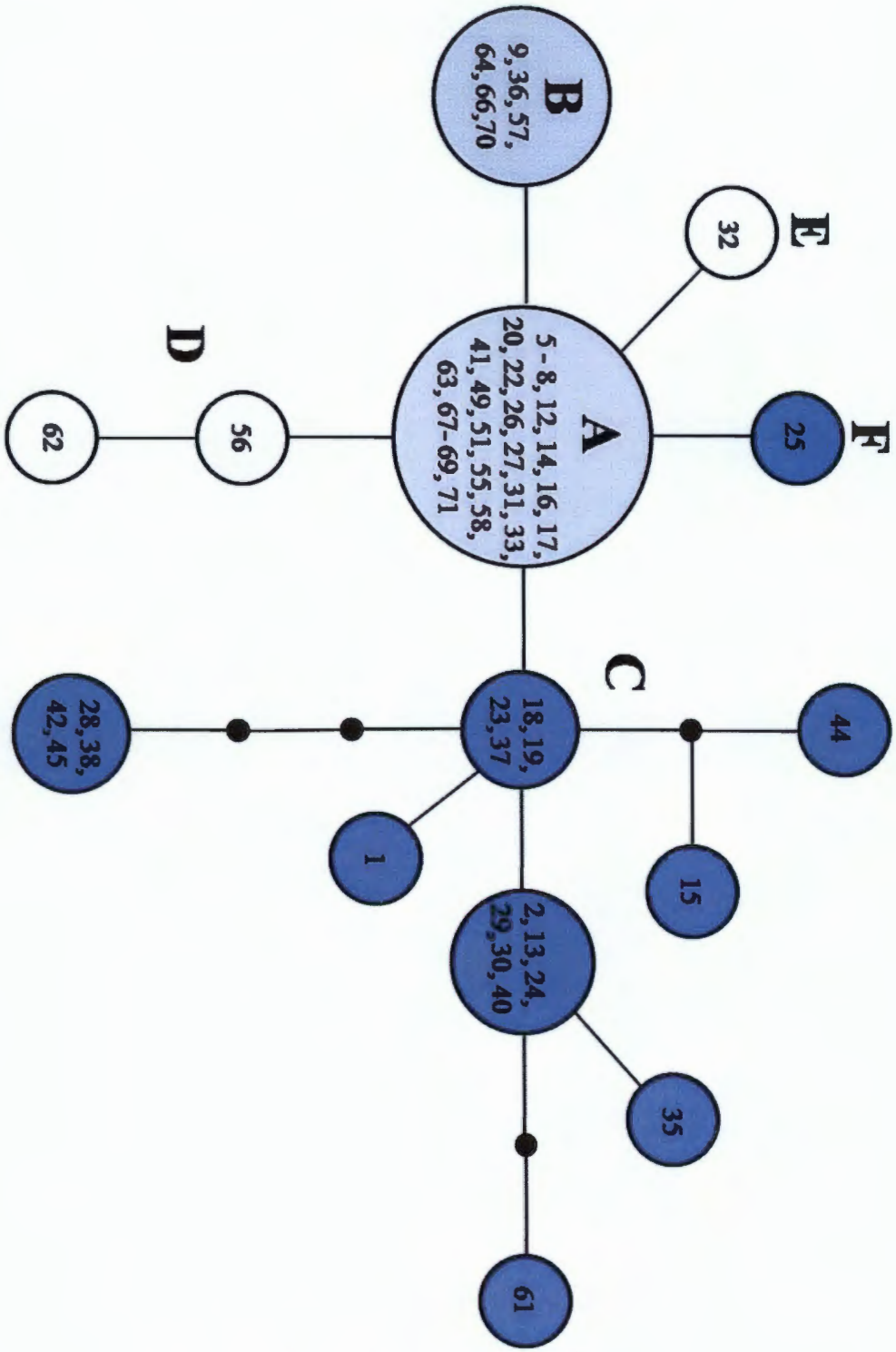
Sequence Data and Recombination

The *trnL-F* region varied from 348 to 349 bp. The sequences comprise 8 haplotypes, among which there are 17 variable characters comprising 16 nucleotide substitutions and 1 insertion/deletion (indel) event. In addition to *ITS1*, the DNA region sampled also included 241 bp from the 3' end of the *18S* rRNA gene: this was discarded for all subsequent analyses. The *ITS* region exhibited moderate length variation, ranging from 248 to 257 bp.

The IMgc analysis identified seven recombinants among the *ITS* sequences, as well as 425bp of recombinant code from the 18S and 5.8S regions. The 7 recombinants, representing 2 white-awned populations and 5 yellow, were all from the WRZ. Subsequent analysis was based on the remaining sequences, which constitute 14 haplotypes. Among these haplotypes there are 18 variable characters comprising 15 nucleotide substitutions and 3 insertion/deletion (indel) events.

Haplotype networks

The unrooted statistical parsimony network for the *ITS1* sequences is shown in (Figure 4). The largest and most widespread haplotype (denoted A in Figure 4) comprises 24 sequences from 24 populations and is confined almost entirely (21/24) to the all-year (ARZ) and summer (SRZ) rainfall zones of South Africa. The frequency of this haplotype increases with proximity to the summer rainfall zone. Under neutrality, this is also identified as the likely ancestral node for the network as it is the most frequent and widespread of all the haplotypes sampled. Interestingly the single Chilean population (49) is also included in this widespread haplotype. The second most frequent haplotype (denoted B in Figure 4) comprises 6 sequences from 6 populations. This haplotype is also comparatively widespread and almost entirely restricted to the ARZ and SRZ (1 population) with the exception of a single population (36) in the Cederberg. The group comprising 8 haplotypes (denoted C in Figure 4), derived from the putative ancestral haplotype (Haplotype A), are all exclusively found in the winter rainfall zone (WRZ) of South Africa. Two haplotypes (denoted D in Figure 4), both dependently derived from haplotype A, are found positioned within the SRZ (56) and ARZ (62). Similarly, haplotypes E and F which are both independently derived by one step from



Chilean & Lesotho haplotypes?

Figure 4 : Haplotype network for the *ITS1* sequences of *Pseudocrossidium crinitum*. The size of the circle representing each haplotype is proportional to its frequency and numbers indicate the populations in which each haplotype is found. Black circles indicate 'missing' haplotypes, whilst haplotypes connected by single lines differ by a single mutational difference. The degree of blue shading, indicates the proportion of populations found in the WRZ, 0 shading = none; darkest shading = all.

the putative ancestral haplotype are found either within the ARZ (haplotype E) or only just within the limits of the WRZ *sensu stricto* (haplotype F).

The unrooted statistical parsimony network for the *trnL-F* sequences is shown in Figure 5. The largest and most widespread haplotype (denoted A in Figure 5) comprises 45 sequences from 44 populations. This haplotype is distributed extensively throughout most of the sampled range of *P. crinitum,* except in the Klein Karoo (ARZ) where the second largest haplotype occurs (denoted B in Figure 5). Haplotype B comprises 10 sequences from 10 populations, reaching its highest frequency in the Klein Karoo where it is also the most abundant haplotype (5 out of 7 populations). The frequency of B decreases with proximity to the WRZ. Three haplotypes (C, D and E), independently derived from haplotype A by one step, one step and two steps respectively are all restricted to the WRZ. A single Chilean haplotype representing one of the four populations sampled (denoted F in Figure 5) is derived by two steps from B whilst the remaining three Chilean populations form part of a highly differentiated group of haplotypes (G in Figure 5), requiring 7 mutational steps to connect to the remainder of the network. This group contains 2 haplotypes, one of which is shared by the three populations from Chile and one from Lesotho, while the other belongs to a single South African WRZ population. Interestingly this WRZ haplotype from the Koue Bokkeveld is more closely related to populations from Chile and Lesotho than to any of the other South African populations. not indicated

Phylogenetic trees

Trees were constructed using a representative of each haplotype and rooted with *Pseudocrossidium porphyreoneurum* (Müll. Hal.) Zander. Low support on nodes is probably due to the small number of variable characters within the data. Trees contain polytomies because multiple haplotypes are derived from a single ancestor. The separation of haplotype groups by rainfall zone is clear in the *ITS* tree (Figure 6). The ARZ haplotypes (green branches) form a relatively well supported (0.86) group comprising 5 haplotypes from the ARZ, two of which are shared by WRZ populations (light blue branches), and a single haplotype from the WRZ (dark blue branches) (E in Figure 4), which represents a single population very close to the ARZ. WRZ haplotypes form a paraphyletic group. The red star indicates the haplotype shared by the Chilean population. The same separation by rainfall zone is not as evident in the *trnL-F* tree (Figure 7). The single ARZ-SRZ haplotype is sister to

outgroup should be mentioned in Methods

meaning?

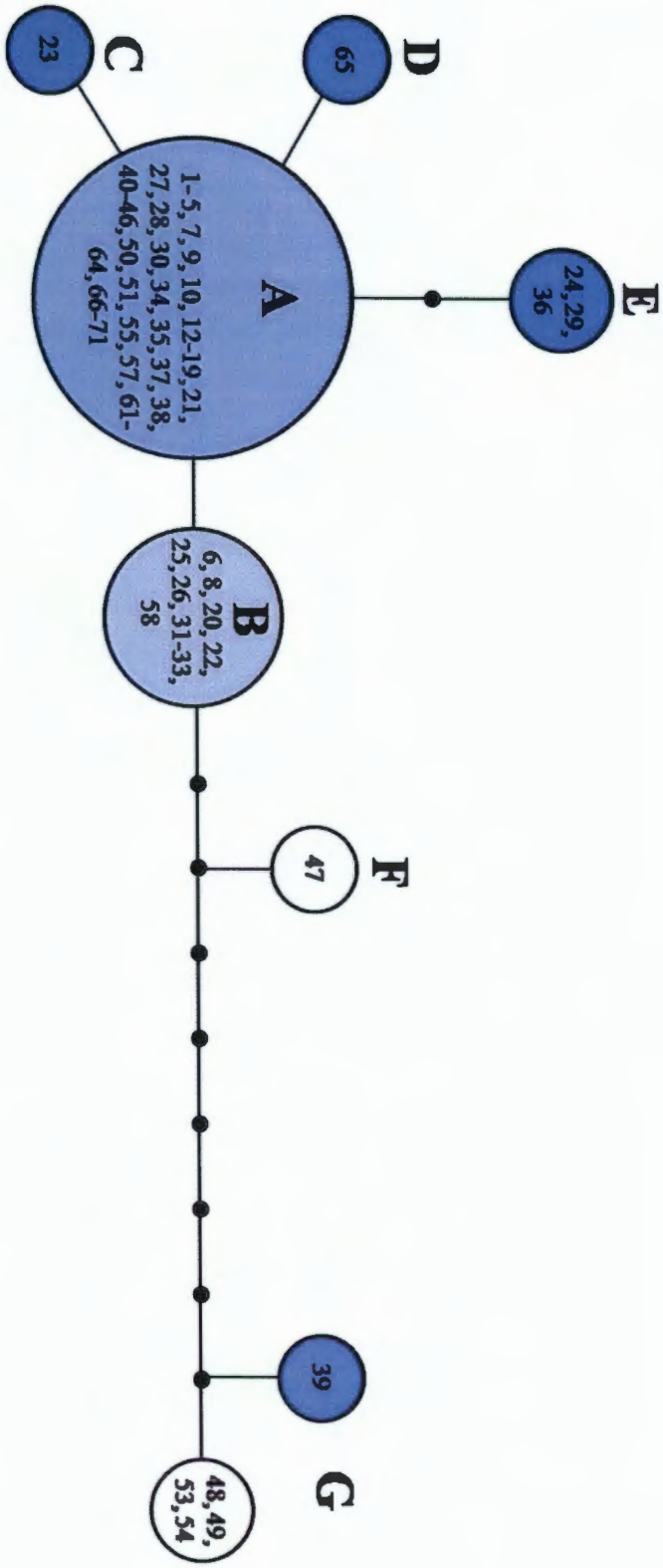


Figure 5 : Haplotype network for the *trnL-F* sequences of *Pseudocrossidium crinitum*. The size of the circle representing each haplotype is proportional to its frequency and numbers indicate the populations in which each haplotype is found. Black circles indicate 'missing' haplotypes, whilst haplotypes connected by single lines differ by a single mutational difference. The degree of blue shading, indicates the proportion of populations found in the WRZ, 0 shading = none; darkest shading = all.

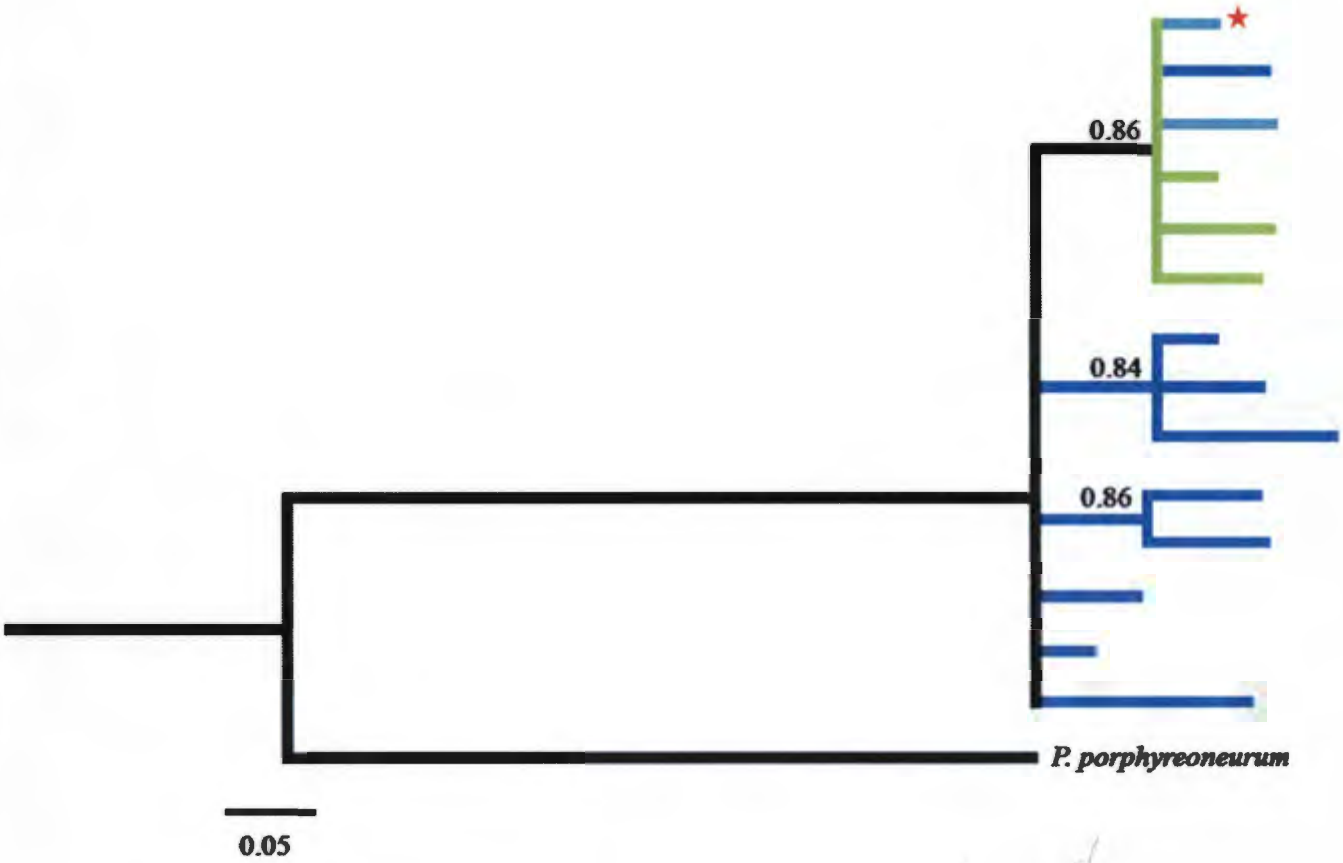


Figure 6 : Phylogenetic tree of *Pseudocrossidium crinitum ITS* haplotypes. The 50% majority-rule consensus tree from the Bayesian analysis of *P. crinitum ITS* haplotypes. Numbers above branches correspond to posterior probabilities estimated using the Bayesian approach. Colours reflect the geographic location of the haplotypes: green branches are haplotypes found in the ARZ and SRZ, blue branches are haplotypes from the WRZ and light blue branches are haplotypes found in both the WRZ and ARZ-SRZ. The red star indicates the haplotype shared with the Chilean population.

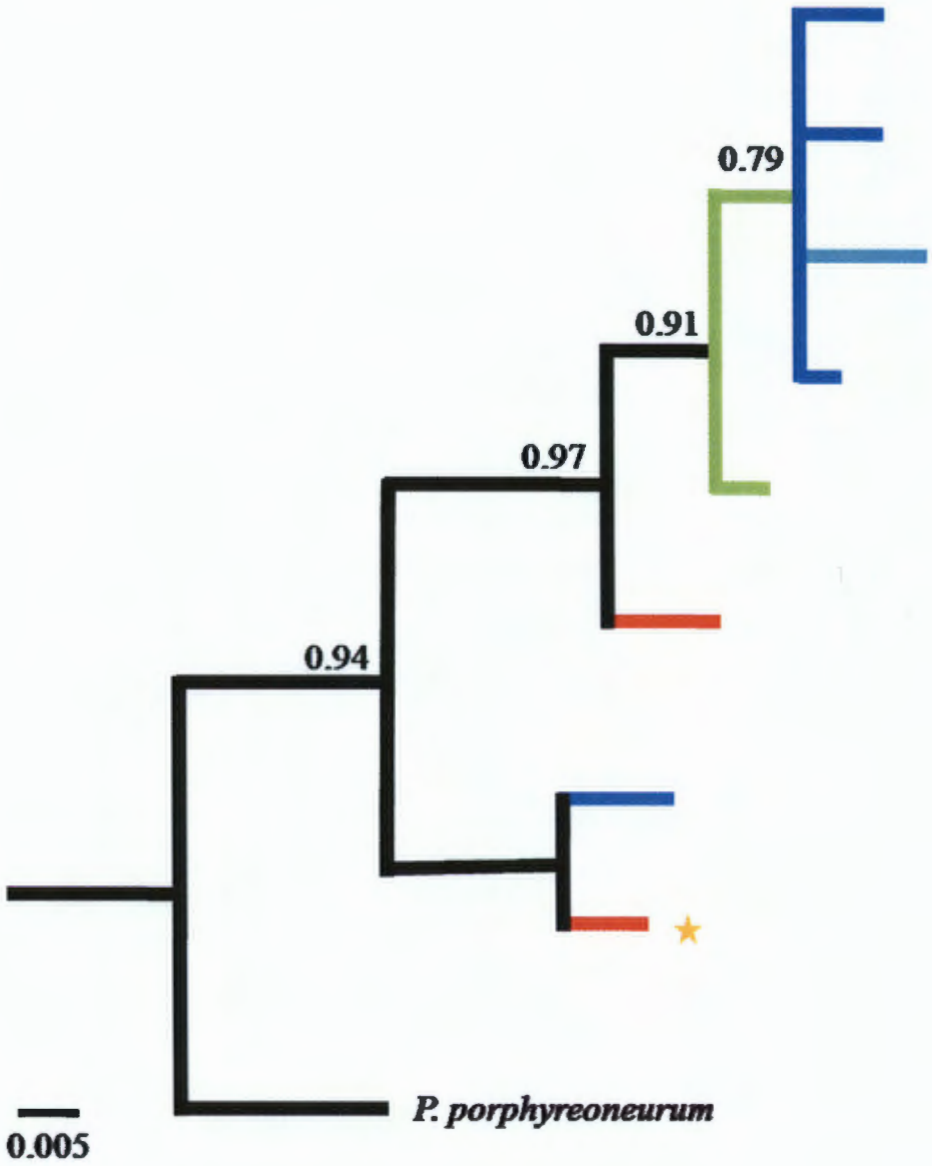


Figure 7 : Phylogenetic tree of *Pseudocrossidium crinitum trnL-F* haplotypes. The 50% majority-rule consensus tree from the Bayesian analysis of *P. crinitum trnL-F* haplotypes. Numbers above branches correspond to posterior probabilities estimated using the Bayesian approach. Colours reflect the geographic location of the haplotypes: red branches are Chilean haplotypes, green branches are haplotypes found in the ARZ and SRZ, blue branches are haplotypes from the WRZ and light blue branches are haplotypes found in both the WRZ and ARZ-SRZ. The yellow star indicates the haplotype shared by populations from Chile and Lesotho.

the group comprising 3 WRZ haplotypes and one haplotype shared between the WRZ and ARZ. The remaining WRZ haplotype is sister to the two Chilean haplotypes, of which one of these is shared with the populations from Lesotho (denoted by a yellow star in Figure 7).

where is the overall phylogeny?

Spatial Analysis of Molecular Variance

The SAMOVA analysis indicated that the best spatial genetic structure was obtained when populations were separated into two groups, F_{ct}=0.71 and 0.52 for *ITS* and *trnL-F* haplotype data respectively. However, groupings were uninformative and always comprised the most highly differentiated population group being separated from the remaining 12. It was mentioned in the previous section that SAMOVA functions by defining groups of populations that are geographically homogeneous and maximally differentiated from each other. The small ranges occupied by WRZ haplotypes resulted in low within group variation, because groups usually only contained a single haplotype, and very high differentiation between groups. This reduced the program's ability to distinguish significant population structure in the data.

Isolation with Migration **(IMa)**

The ESS values from the final run ranged from 65 to greater than 19,000. Although some parameters (*t*) had low ESS scores, examination of parameter trend plots suggested that Markov chains were sufficiently exploring parameter space, and posterior estimates produced during the preliminary and final runs had very similar values. The analysis examined the split between WRZ and ARZ (including the two populations from the SRZ) populations. The scaled and basic population parameters are given in Table 1. Figures 8a and b show the marginal posterior probability distributions of the estimates of N_e (in millions) for the WRZ and ARZ-SRZ (a) populations and the ancestral population (b). The results for both populations (N_1 and N_2) indicate negative growth in effective population since time of splitting because N_e for both populations (Table 1) is smaller than the ancestral N_e (N_A). The highest posterior probability for the estimated ancestral N_e is however very low and additional data ~~is~~ are needed to provide more certainty around this estimate and any subsequent inferences. The N_e in the geographically more widespread ARZ-SRZ population is significantly smaller than N_e in the more restricted WRZ population.

meaning?

The coalescent estimates of time since population divergence (Figure 8c) reveal a sharp peak in the marginal likelihood which translates to the period ranging from the end of the Pliocene (3.4Mya), assuming a slow molecular clock (0.5%), to a divergence estimate of 1.3Mya, assuming a fast molecular clock of 1.3% divergence per million years. This estimate also falls within the range of the upper and lower HPD interval for t (Table 1).

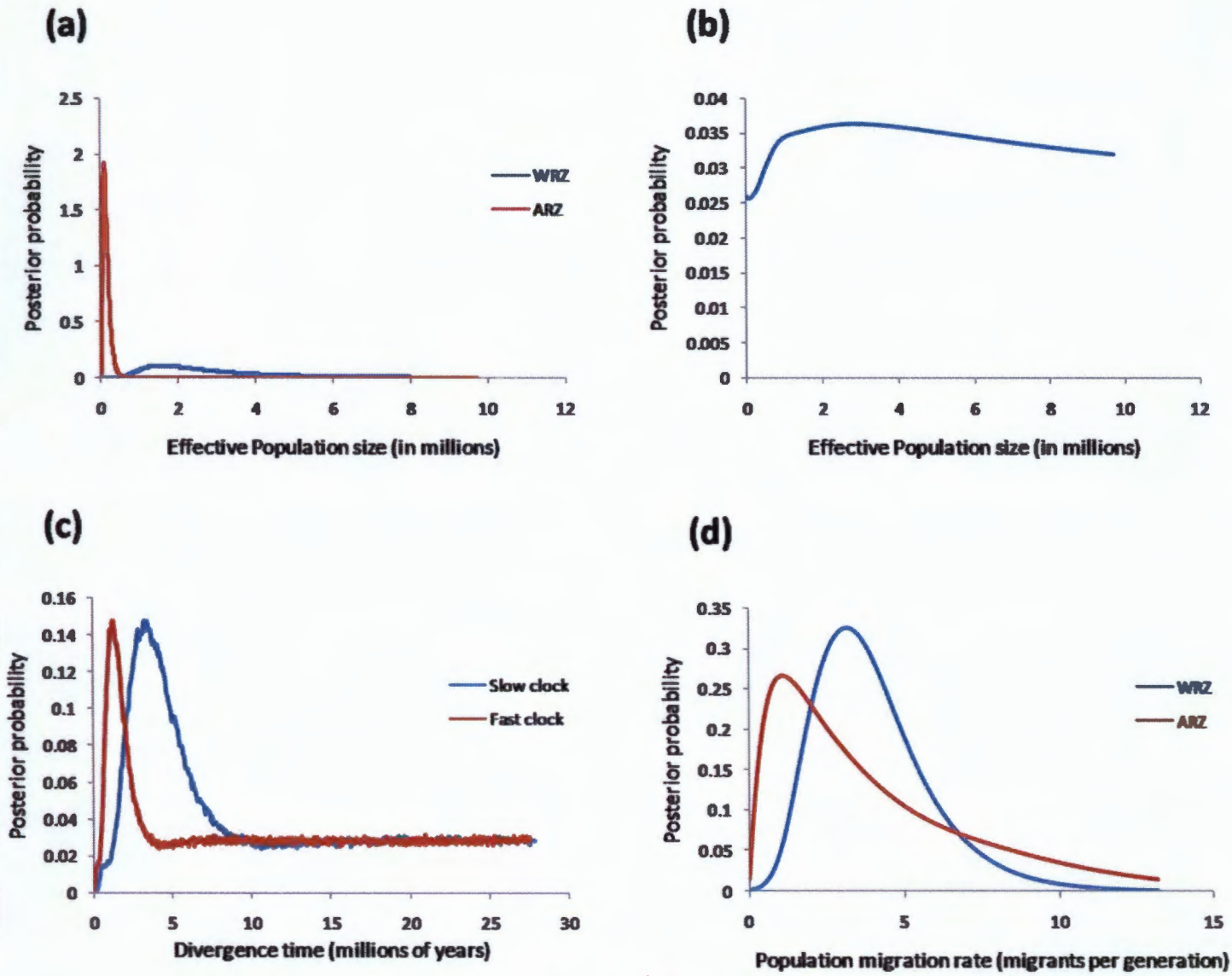
Estimates of migration (Figure 8d) suggest a considerable amount of gene flow from the ARZ-SRZ into the WRZ (1.8 migrants per generation) compared to gene flow in the opposite direction which is only 0.64 migrants per generation.

Table 1 : Maximum-Likelihood Estimates[a] (MLE) and the 90% Highest Posterior Density (HPD) Intervals[b] of Demographic Parameters Estimated by IMa for the WRZ (θ_1, m_1, N_1, m_1), ARZ (θ_2, m_2, N_2, m_2) and ancestral populations (θ_A, N_A).

	θ_1	θ_2	θ_A	m_1	m_2	t	N_1	N_2	N_A	m_1	m_2	t (Mya)
MLE	4.744	0.338	8.651	2.395	0.843	1.497	1.567	0.112	2.857	1.813	0.638	1.978
Lower 90% HPD	1.835	0.132	2.1	0.865	0.122	0.767	0.606	0.044	0.694	0.654	0.092	1.013
Upper 90% HPD	19.43	0.925	28.066	5.165	4.182	4.997	6.417	0.305	9.269	3.909	3.165	6.601

[a] MLE estimates for the basic parameters (N_1, N_2, N_A, m_1 and m_2) are the locations of the peaks in the curves shown in Figure 8, the MLE estimate of t was calculated using the weighted mean mutation rate for the two loci. The scaled parameters (θ_1, θ_2, θ_A, m_1, m_2 and t) are the direct outputs from the IMa analysis. and what do they mean?

[b] The 90% HPD intervals are the shortest spans, along the x axes of Figure 8, that contain 90% of the area of those histograms. For basic parameters not scaled by the mutation rate including N_1, N_2, N_A, m_1,m_2 and t (see Material and Methods), these HPD intervals are not directly available. However, estimates of the 90% HPD intervals were made as the products of those for the corresponding scaled parameters, upper and lower HPD intervals were calculated using the geometric mean mutation rate for the two loci.



in simple English?

Figure 8 : Marginal posterior probability distributions for demographic estimates of population size, population migration rates and time since population divergence from the IMa analysis. (a) Estimates of N_e for the WRZ and ARZ, (b) Estimate of ancestral N_e, (c) Time since divergence between WRZ and ARZ populations and (d) Estimates of the population migration rate in WRZ and ARZ populations.

DISCUSSION

Many Results not explained here
Much speculation

Sequence variation and haplotype diversity

P. crinitum contains a moderate amount of genetic variation within the DNA regions sampled (average of 11 haplotypes in 66 sequences) and is comparable to variation in many angiosperm species (e.g. Tremblay and Schoen 1999). This is contrary to earlier hypotheses that bryophytes are genetically impoverished in comparison to other plant groups (Shaw 2002; Hedderson and Nowell 2006) and provides further evidence to refute this.

Recombination

Strong evidence for the importance of sexual reproduction and consequently the production of spores in *P. crinitum* was evident in the *ca* 11% of ITS sequences which contained traces of recombination, particularly when it is remembered that only a proportion of the progeny is likely to show recombination at a particular locus (Hedderson and Nowell 2006). The exact importance of spores in the dispersal of bryophytes has been debated with little resolve (see review in Miles and Longton 1990), due to the technical difficulties in determining the fate of spores in the field (Hedderson and Nowell 2006).

In comparison to the very coarse methods used in this study to estimate recombination, fine scale estimates of recombination rate may provide the necessary means to answer this question. A new full-likelihood method developed by Wang and Rannala (2008) is able to determine fine scale recombination rates using a Bayesian MCMC method. The method uses marginal individual SNP (single nucleotide polymorphism) genealogies related through an ancestral recombination graph to effectively model genealogies underlying a sampling of chromosomes. The new method is far more accurate in comparison to previous statistical methods which are limited to approximated likelihood approaches. Obtaining fine scale estimates of recombination rates will greatly increase our knowledge of the extent of sexual reproduction in bryophytes. Additionally, this knowledge could also be used to provide the ground work for a better understanding of generation time in bryophytes.

at last!
references?
why?
why?

Spatial genetic structure of populations

Despite the proportionately small area sampled relative to the full distribution of *P. crinitum* in South Africa, the large number of populations sampled allowed for the examination of fine scale patterns of spatial genetic structure between winter and all-year-round rainfall zones. The variation within *P. crinitum* is not strongly structured according to the geographical distribution of its haplotypes. However, populations from the WRZ present an entirely unique set of haplotypes compared to those found in the ARZ and SRZ. This separation is also made clear in the phylogenetic trees. The lack of a strong geographic structure is probably due to the high dispersal ability of *P. crinitum*, a trait which has been shown to be characteristic of many bryophyte species. A study by Bergh *et al.* (2007) on the species *E. rhinocerotis* in South Africa, attributed a similar lack of spatial genetic structure to the high dispersal ability of the species.

later you say the opposite!

this is not a bryophyte relevance?

Unfortunately plant phylogeographic studies for the region are lacking and to date nothing is known about how past climate shifts have impacted on the population genetic structure of bryophyte species.

White and Yellow awned populations Results?

The presence and distribution of the two forms is still an interesting question which has yet to be answered. This study revealed that the two forms are not genetically distinct. Rather, white and yellow awned populations share a lot of the same genetic variation, indicating the definite presence of gene flow between the two forms. Currently, the distribution of genetic variation within white awned populations does not suggest any geographic structuring and the presence of a few populations in the ARZ precludes any hypotheses concerning an adaptation to different climates. It may be possible that the white and yellow pigment is controlled by a single allele which was accidentally switched on or off, allowing this apparently harmless mutation to spread throughout the populations. The lack of genetic variation between forms is compatable with this hypothesis and could be tested through a series of crossing experiments. Genetic differentiation would only be expected if the two forms evolved in isolation. Morphometric analyses of the two forms (N Wilding and T Hedderson unpublished data) are unable to find characters unique to either form.

neutral?

ARZ and SRZ

One hypothesis for the low levels of genetic variation in the ARZ may be that the history of *P. crinitum* in this area is relatively recent and that too little time has passed for genetic variation to accumulate to the levels present in the WRZ. Frequencies of the putative ancestral haplotype reach their highest in the ARZ which could provide further evidence for a recent expansion into the region. However, the converse could be true and large numbers of the ancestral haplotype could also point to a very old occupation of the region. Alternatively the effects of aridity may explain why it has much lower levels of genetic variation than the areas in the west. Oscillating climatic conditions with no directional change characterized the ARZ over the last 8-10 Mya (Cowling *et al.* 2009). This could have made it impossible for different ecotypes to establish and adapt to environmental conditions. It is also possible that the continuous variability and aseasonality of rainfall favoured dispersal by fragmentation over sexual reproduction and production of spores, a hypothesis which is consistent with the lack of recombination in nDNA sequences from the region. This would also result in lower levels of diversity.

Strange claim! low genetic variability = evidence for palaeo-climatic shifts!

Similar patterns of low genetic variability were found in the study by Bergh *et al.* (2007), suggesting that paleo-climate shifts may be responsible for the pattern we see today. Evidence from the fossil record indicate that temperate afromontane forests were greatly restricted during the last glacial period (Butzer and Helgren 1972). However, increases in moisture during phases of the Holocene and the resulting expansion of forests (Martin 1968; Partridge *et al.* 1999) may have displaced *P. crinitum* from much of the ARZ. Aridification during the late Holocene and subsequent anthropological influence caused contraction of these forests and may have resulted in more recent recolonization and lower genetic variation.

Another genetic feature shared with the study of Bergh *et al.* (2007) is the presence of a group of populations with unique genetic variation centred around the Klein Karoo area of the South Coast. Due to a lack of sampling in the area Bergh *et al.* (2007) could not speculate at the reason for the extreme genetic difference they observed. Sampling in the current study is comprehensive for the area and the genetic differentiation observed may point towards the presence of an ecotype of *P. crinitum,* adapted to higher levels of rainfall that were present during the Mid-Miocene (Linder and Hardy 2004). Peaks of greater than 950mm mean annual precipitation occur at two places in the Western Cape; one in the coastal region of the Klein Karoo and, the second spanning the area between Stellenbosch, Hermanus and Worcester, just inside of the WRZ (Environmentek, CSIR). The same cpDNA haplotype

previously you said they found low genetic variability!

where are these Results?

just inside!

not "just inside"

? reference

which is found in the Klein Karoo is also found in the second high rainfall area. At this time, the existence of different ecotypes and potential refuges is only speculation given the patterns in the data. This hypothesis would need to be tested with the use of morphological and physiological experiments which aim at establishing differences in life-form and water use efficiency. The presence of high rainfall refuges does however present an interesting hypothesis that could be tested by examining the genetic structure in other species occurring over the same range.

WRZ

Haplotype diversity in the WRZ is comparatively higher (*ca* 50%) than the ARZ and SRZ, and the most widespread WRZ haplotypes are distributed along a North-South axis.

The high genetic diversity present in the WRZ is assumed to reflect the impact of past climatic shifts on the genetic structure of *P. crinitum*. A scenario involving adaptation to a new environment might assume that if *P. crinitum* occupied the same range before the onset of winter rain, then a prior adaptation to summer rainfall and better light conditions may have resulted in a population bottleneck during the build-up to full winter rainfall conditions. During this period, significant reductions in range due to fewer habitable niches, may have resulted in a patchy distribution of only those fit enough to survive in the new environment. This scenario could explain the appearance of many unique haplotypes, as selection weeded out unfit individuals and genetic drift acted on populations. Simple tests of light use efficiency on plants from the different rainfall zones could provide a simple way to test the validity of this scenario.

Other scenarios involving range fragmentation during periods of increased Holocene aridity could be attributed to the creation of new diversity through drift. Taking into account model suggestions that increased aridity began in the North and spread South, significant levels of genetic disjunction would be expected between northern and southern populations coupled with a decrease in diversity moving northwards, patterns not evident in the data (van Zinderen-Bakker 1978). Further, given the biology of the species and its ability to grow in very arid environments (see Figure 1), increased aridification may not have presented a problem for northern populations. The most feasible hypothesis for the North-South distribution of haplotypes is dispersal along this axis, a theory which is consistent with the prevailing wind directions for the south-western part of the country (i.e. N.W. and S.E.).

Gene flow between WRZ and ARZ-SRZ Not in Results

The split between WRZ and ARZ-SRZ haplotypes is probably the most obvious feature of the data and by far the most significant. It is clear from this example that the Pliocene climate have not only impacted on the genetic structure of species in Europe and America but also in Africa. This is one of the few studies which has tried to show how paleo-climatic shifts have impacted on spatial genetic structure of a species and possibly the first to demonstrate how the presence of different rainfall regimes can preclude gene flow between parts of an otherwise homogeneous and contiguous area.

Maximum likelihood estimates place the time of population divergence (i.e. WRZ and ARZ) between 1.98 Mya, this date is in agreement with estimates for the shift from summer to winter rainfall during the late Pliocene (DeMenocal 2004). Growth and the production of sporophytes in bryophytes is strongly linked to rainfall. It would seem obvious then that a change in the timing of rainfall for a set of populations should significantly alter aspects of their phenology. It is unclear at present whether production of sporophytes is common in populations from the ARZ and at what time of the year sporophytes mature. However, it is suspected that isolation, resulting from poor sperm dispersal distances and a differential timing of sporophyte production and thus dispersal of spores, has played a large role in limiting gene flow between the two areas.

Of relevance here is the recent demonstration by Munoz *et al.* (2004) that patterns of wind intensity and direction better explain floristic similarity in a range of plant groups among sub-Antarctic islands than does geographic proximity. Seasonal patterns of air circulation indicate that dispersal during summer months should be facilitated by wind from the south-east. This would send propagules from the ARZ into the WRZ, assuming that mature sporophytes are present and that rainfall in the ARZ doesn't wash spores from the atmosphere. During winter months, at which time mature sporophytes should be present in SRZ populations, dispersal is facilitated by wind from the south-west (Chase and Thomas 2007). This means that spores would be dispersed in a north-easterly direction, along the coast of Kwa-Zulu Natal. In essence dispersal out of the WRZ is almost impossible as a result of the prevailing wind direction and the timing of sporophyte maturation and dispersal into the WRZ is thwarted by the inconsistency of ARZ rainfall and its affect on timing of sporophyte production.

Estimates of migration rate support this theory and are in proportion with expected levels of gene flow in each direction. A migration rate of one migrant per generation in each direction

is expected to provide sufficient gene flow to result in a homogenizing effect between two populations. Therefore, migration into the WRZ is predicted to provide sufficient gene flow for this process to take place. Since gene flow is only taking place in one direction, this might allow process like genetic drift to act on the WRZ populations, ultimately resulting in divergence between ARZ and WRZ populations.

lots of unwarranted speculation

Relationship between populations from Chile, Lesotho and South Africa

The genetic differentiation of populations from Chile is clear from the cpDNA data and its position in the tree, suggesting that the two populations are quite genetically distinct. Recombination and high mutation rates in the nDNA have probably resulted in this signal being slowly lost. nDNA is expected to show more recent genetic history, this may effectively overwrite older information contained within the genome (T. Hedderson pers. comm.). Due to the ~~small number of~~ single nDNA sample from Chile (n=1) we can only make a preliminary guess at the more recent history of gene flow between the two continents. Gene flow between Africa and South America has been well documented and a number of species have been shown to have similar haplotypes on the two continents (T. Hedderson pers. comm). The fact that the Chilean population (n=1?) forms part of the most widespread nDNA haplotype might point to recent gene flow between the continents. Of particular interest here is the direct evidence of gene flow between Chile and a population from the Koue Bokkeveld. Its position in the cpDNA tree points to a recent dispersal event from Chile. The population from the Koue Bokkeveld is more closely related to Chilean and Lesotho haplotypes than it is to the remaining South African populations and the converse is true for one of the Chilean haplotypes. Could this be dispersal in the other direction? If so, then this provides a number of interesting questions that need to be answered regarding gene flow and the mechanisms which facilitate it. Answers to such questions could have many implications for biogeographic studies. Many more samples are needed in order to estimate gene flow and it would be interesting to compare this gene flow to that between the two rainfall zones.

The populations from Lesotho form part of the same cpDNA haplotype as 3 of the Chilean populations, indicating recent gene flow between these two disjunct populations or dispersal in a single direction.

Implications for bryophyte speciation and evolution of the regions flora

The patterns of genetic variation exhibited by *P. crinitum* may provide invaluable insight into the processes and mechanisms which ensue during the speciation of bryophytes and quite possibly other plant groups too. Although evidence does not support complete and rapid divergence between populations, it is clear that significant population differentiation is occurring. It is intriguing that patterns of genetic diversity exhibited in *P. crinitum* largely reflect those of the Cape flora at the species level (Linder 2005) and may reflect a common set of structuring factors acting at varying scales. Thus, it is possible that the same factors responsible for high species diversity in the CFR might act at a finer scale and produce similar inter-specific variation in taxa with different life histories. Without additional studies it is not known whether this same pattern will be evident in other species that span the seasonality boundary and to what degree they will be affected (i.e. high gene flow or complete divergence).

Conclusion

Not mentioned in Discussion

The lack of distinguishing molecular variation, between the white and yellow forms of *P. crinitum,* point to the presence of a neutral mutation and not one that confers any selective advantage among populations. Future investigation using genetic and physiological techniques may provide further understanding of the white form and its highly restricted distribution.

? really ?

Distribution of genetic variation in *P. crinitum* clearly exposes rainfall seasonality as a significant genetic structuring factor. A lack of sampling precluded much speculation regarding diversity in the SRZ and hence the degree to which rainfall seasonality has affected genetic variation in WRZ populations. In order to elucidate the full extent to which rainfall seasonality has affected genetic variation, future work should include comprehensive sampling of winter-, summer- and all-year-rainfall zones, providing increased support for coalescent estimates of population divergence and gene flow.

Additional work similar to this may provide much needed evidence and support for hypotheses regarding the evolution of the Cape flora and other endemic groups. Studies which are interested in the history of populations and species are relying increasingly more on coalescent methods. Soon, with the advances in sequencing technology these methods could be applied more effectively and one day provide alternative means of calibrating phylogenies, providing significantly higher resolution for phylogeographic studies and studies of speciation.

References

Bakker, F.T., Olsen, J.J., & Stam, W.T. 1995. Evolution of rDNA ITS sequences in the *Cladophora albida/sericea* clade (Chlorophyta). *Journal of Molecular Evolution* 40: 640–651.

Baldwin, G.B. 1992. Phylogenetic utility of the Internal Transcribed Spacers of nuclear ribosomal DNA in plants: an example from the Compositae. *Molecular Phylogenetics and Evolution* 1: 3–16.

Bergh, N.G., Hedderson, T.A., Linder, H.P. Bond, W.J. 2007. Palaeoclimate-induced range shifts may explain current patterns of spatial genetic variation in renosterbos (*Elytropappus rhinocerotis*, Asteraceae). *Taxon* 56(2):393-408

Butzer, K.W. & Helgren, D.M. 1972. Late cenozoic evolution of the Cape coast between Knysna and Cape St. Francis, South Africa. *Quaternary Research* 2(1): 143-169

Bucci, G., González-Martínez, S.C., Le Provost, G., Plomion, C., Ribeiro, M.M., Sebastiani, F., Alía, R., Vendramin, G.G. 2007. Range-wide phylogeography and gene zones in *Pinus pinaster*. revealed by chloroplast microsatellite markers. *Molecular Ecology* 16(10): 2137-2153.

Caujapé-Castells, J., Jansen, R.K., Pedrola-Monfort, J., & Membrives, N. 2002. Space-time diversification of *Androcymbium* Willd. (Colchicaceae) in western South Africa. *Plant Systematics and Evolution* 232(1): 73-88.

Chase, B.M., & Thomas, D.S. 2007. Multiphase late Quaternary aeolian sediment accumulation in western South Africa: Timing and relationship to palaeoclimatic changes inferred from the marine record. *Quaternary International* 166(1): 29-41.

Clement, M., Posada, D., & Crandall, K.A. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* 9(10): 1657-1659.

Cowling, R.M., Esler, K.J., & Rundel, P.W. 1999. Namaqualand, South Africa: An Overview of a Unique Winter-Rainfall Desert Ecosystem. *Plant Ecology* 142(1/2): 3-21.

Davis, A.L.V., & Scholtz, C.H. 2004. Local and regional species ranges of a dung beetle assemblage from the semi-arid Karoo/Kalahari margins, South Africa. *Journal of Arid Environments* 57(1): 61-85.

DeMenocal, P.B. 2004. African climate change and faunal evolution during the Pliocene-Pleistocene. *Earth and Planetary Science Letters* 220(1-2): 3-24.

Dewey, R.M. 1989. Genetic variation in the liverwort *Riccia dictyospora* (Ricciaceae, Hepaticae). *Systematic Botany* (14): 155-167.

Dobzhansky, T. 1937. Genetics and the Origin of Species. Columbia Univ. Press, New York. 1972. Species of Drosophila. *Science* 177: 664- 669.

Doyle, J.J., & Doyle, J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin. 19*(1): 11–15.

Dupanloup, I., Schneider, S., & Excoffier, L. 2002. A simulated annealing approach to define the genetic structure of populations. *Molecular Ecology* 11(12): 2571-2581.

Dupont, L.M., Donner, B., Vidal, L., Pérez, E.M., & Wefer, G. 2005. Linking desert evolution and coastal upwelling: Pliocene climate change in Namibia. *Geology* 33(6): 461-464.

Envirnmentek, CSIR: http://www.ngo.grida.no/soesa/nsoer/indicatr/m_rain.htm: accessed 1/10/2009

Fernandez, C.C., Shevock, J.R., Glazer, A.N., & Thompson, J.N. 2006. Cryptic species within the cosmopolitan desiccation-tolerant moss *Grimmia laevigata. Proceedings of the National Academy of Sciences of the United States of America* 103(3): 637-642.

Hedderson, T.A., & Nowell, T.A. 2006. Phylogeography of *Homalothecium sericeum* (Hedw.) Br. Eur.; toward a reconstruction of glacial survival and postglacial migration. *Journal of Bryology* 28(4): 283-292.

Hedderson, T.A., & Zander, R.H. 2008a. *Algaria nataliei* (Pottiaceae), a new moss genus and species from the Western Cape Province of South Africa. *Journal of Bryology* 30(3): 192-195.

Hedderson, T.A., & Zander, R.H. 2008b. *Vrolijkheidia circumscissa* (Pottiaceae), a new moss genus and species from the Succulent Karoo of South Africa. *Journal of Bryology* 30(2): 143-146.

Hedderson, T.A., & Zander, R.H. 2007a. *Ludorugbya springbokorum* (Pottiaceae) a new moss genus and species from the Western Cape Province of South Africa. *Journal of Bryology* 29(4): 222-227.

Hedderson, T.A., & Zander, R.H. 2007b. *Triquetrella mxinwana*, a new moss species from South Africa, with a phylogenetic and biogeographic hypothesis for the genus. *Journal of Bryology* 29(3): 151-160.

Hey, J., & Nielsen, R. 2007. Integration within the Felsenstein equation for improved Markov chain Monte Carlo methods in population genetics. *Proceedings of the National Academy of Sciences of the United States of America* 104(8): 2785-2790.

Hey, J., Yong-Jin Won, J., Sivasundar, A., Nielsen, R., & Markert, J.A. 2004. Using nuclear haplotypes with microsatellites to study gene flow between recently separated Cichlid species. *Molecular Ecology* 13(4): 909-919.

Hudson, R.R., & Kaplan, N.L. 1985. Statistical properties of the number of recombination events in the history of a sample of DNA sequences. *Genetics* 111(1): 147-164.

Huelsenbeck, J.P., & Ronquist, F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17(8): 754-755.

Klak, C., Reeves, G., & Hedderson, T. 2004. Unmatched tempo of evolution in Southern African semi-desert ice plants. *Nature* 427(6969): 63-65.

Linder, H.P. 2005. Evolution of diversity: the Cape flora. *Trends in Plant Science* 10(11): 536-541.

Linder, H.P., & Hardy, C.R. 2004. Evolution of the Species-Rich Cape Flora. *Philosophical Transactions: Biological Sciences* 359(1450): 1623-1632.

Linder, H.P. & Mann, D.M. 1998. The phylogeny and biogeography of *Thamnochortus* (Restionaceae). *Botanical Journal of the Linnean Society*. 128: 319-357

Martin, A.R.H. 1968. Pollen analysis of Groenvlei lake sediments, Knysna (South Africa).*Review of Palaeobotany and Palynology* 7(1): 107-144

Martin, A.P., & Palumbi, S.R. 1993. Body Size, Metabolic Rate, Generation Time, and the Molecular Clock. *Proceedings of the National Academy of Sciences of the United States of America* 90(9): 4087-4091.

Miles, C.J., & Longton R.E. 1990. The role of spores in reproduction in mosses. *Botanical Journal of the Linnean Society* 104(1): 149–173.

Mitton, J.B., Kreiser, B.R., & Latta, R.G. 2000. Glacial refugia of limber pine (*Pinus flexilis* James) inferred from the population structure of mitochondrial DNA. *Molecular Ecology* 9(1): 91-97.

Munoz, J., Felicisimo, A., M., Cabezas, F., Burgaz, A.R., Martinez, I. 2004. Wind as a long-distance dispersal vehicle in the Southern Hemisphere. *Science* 304(1): 1114–1147.

Núñez-Avila, M., & Armesto, J.J. 2006. Relict islands of the temperate rainforest tree *Aextoxicon punctatum* (Aextoxicaceae) in semiarid Chile: genetic diversity and biogeographic history. *Australian Journal of Botany* (54):733–743

Partridge, T.C., Scott, L., & Hamilton, J.E. 1999. Synthetic reconstructions of southern African environments during the Last Glacial Maximum (21-18 kyr) and the Holocene Altithermal (8-6 kyr). *Quaternary International* 57-58: 207-214.

Rambaut, A. 2007. FigTree, a graphical viewer of phylogenetic trees. Latest version 1.2.2, March 2009. Available from http://tree.bio.ed.ac.uk/software/figtree/

Rowe, G., 2005. Speciation and phylogeography: Coalescent-based models applied to the Cape plant genus *Pauridia*. Unpublished MSc Dissertation. University of Cape Town.

Schierup, M.H., & Hein, J. 2000. Recombination and the Molecular Clock. *Molecular Biology and Evolution* 17(10): 1578-1579.

Shaw, A.J., & Rooks, P.E. 1994. Systematics of *Mielichhoferia* (Bryaceae: Musci) I. Morphological and Genetic Analyses of *M. elongata* and *M. mielichhoferiana*. *The Bryologist* 97(1): 1-12.

Shaw, A.J., & Schneider, R.E. 1995. Genetic Biogeography of the Rare "Copper Moss," *Mielichhoferia elongata* (Bryaceae). *American Journal of Botany* 82(1): 8-17.

Shaw, A.J. 2000. Molecular phylogeography and cryptic speciation in the mosses, *Mielichhoferia elongate* and *M. mielichhoferiana* (Bryaceae). *Molecular Ecology* (9): 595–608

Shaw, A.J. 2001. Biogeographic patterns and cryptic speciation in bryophytes. *Journal of Biogeography* (28): 253-261

Shaw, AJ. 2009. Bryophyte species and speciation, in Bryophyte Biology: Second Edition Eds B. Goffinet & J. Shaw, Cambridge University Press 445-485

Szweykowski, J., & Krzakowa, M. 1979. Variation of four enzyme systems in Polish populations of *Conocephalum conicum* (L.) Dum. (Hepaticae, Marchantiales). *Bulletin of the L'Académie Polonicae, Sér. Sciences Biologiques.* (27): 37-41.

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(5): 1105-1109.

Tremblay, N.O., & Schoen, D.J. 1999. Molecular phylogeography of *Dryas integrifolia*: glacial refugia and postglacial recolonization. *Molecular Ecology* 8(7): 1187.

Trewick, S.A. et al. 2002. Polyploidy, phylogeography and Pleistocene refugia of the rockfern Asplenium ceterach: evidence from chloroplast DNA. *Molecular Ecology* 11(10): 2003-2012.

VanZinderenBakker, E.M. 1976. The evolution of Late Quarternary paleoclimates of Southern Africa. Pp.160-202. In: VanZinderenBakker, E.M., Sr. (ed.), *Palaeoecology of Africa*: 1972-1974, vol. 9. Balkema, Rotterdam.

Verboom, G.A., Linder, H.P., & Stock, W.D. 2003. Phylogenetics of the Grass Genus Ehrharta: Evidence for Radiation in the Summer-Arid Zone of the South African Cape. *Evolution* 57(5): 1008-1021.

Vogel, J.C., Fuls, A., and Ellis, R.P., 1978. The geographical distribution of Kranz grasses in South Africa: *South African Journal of Science*, (74): 209–215.

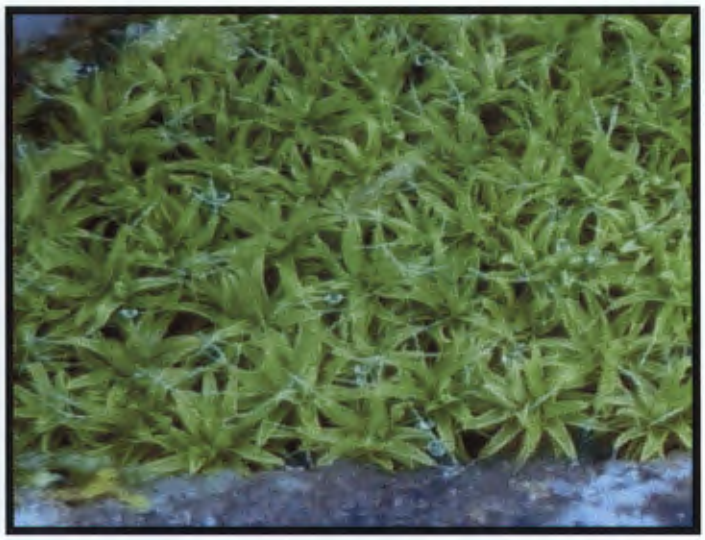
Wang, Y., & Rannala, B. 2008. Bayesian inference of fine-scale recombination rates using population genomic data. *Philosophical Transactions of the Royal Society B: Biological Sciences* 363(1512): 3921-3930.

Woerner, A.E., M.P. Cox & M.F. Hammer., 2007. Recombination-Filtered Genomic Datasets by Information Maximization. *Bioinformatics* 23(1): 1851-1853.

Zander, R.H. 1993. Genera of the Pottiaceae: mosses of harsh environments. *Bulletin of the Buffalo Society of Natural Sciences* 32(1): 1–378

Zhang, D., & Hewitt, G.M. 2003. Nuclear DNA analyses in genetic studies of populations: practice, problems and prospects. *Molecular Ecology* 12(3): 563-584.

Appendix 1



Picture 1: The white hairpoint form of *P. crinitum*. Picture taken on a dewy morning. Water droplets are visible on the hairpoints which act as condensation points for any moisture in the air.

Picture 2: The yellow hairpoint form of *P. crinitum*. Picture of dry plants, leaves are twisted and pressed against the stem. Sporophytes are also present.

Appendix 2

Sample	Collection info.
1	Hedderson 13772. Western Cape. Koue Bokkeveld Mntns. Yellow.
2	Hedderson 14331. Nieuwoudtville area. Oorlogues kloof NR. White.
3	Hedderson 12804a. Cape Point. White
4	Hedderson 13483. Cape peninsula. Table Mntn. White.
5	Hedderson 14918. Riversdale district. Tradouws pass. Yellow.
6	Hedderson 15149. Dysseldorp. Oudemuragie farm. Yellow.
7	Hedderson 14903. Grahamstown. Signal Hill. Yellow.
8	Hedderson 15529. Robertson area. Bergendal farm. White.
9	Hedderson 15122. Addo elephant park. Spekboom lookout area. Yellow.
10	Hedderson 13841. Ceres district. Btwn ceres and Touwsrivier. Yellow.
11	Hedderson 13069. Cederberg. Algeria. White.
12	Hedderson 13139. Hottentots Holland Mntns. Sir lowrys pass. Yellow.
13	Hedderson 14289. Groot Winterhoek NR. White.
14	Wilding 81108. De Hoop. Yellow.
15	Hedderson 16428. Hout bay. Epiphytic. Yellow.
16	Hedderson 15539. Robertson area.Klaas Voogds West. Yellow.
17	Hedderson 16452. Uniondal. De rust. Yellow.
18	Hedderson 15946. Clanwilliam-Wuppetaal. Yellow.
19	Hedderson 16030. Sutherland-ceres. N-cape pass. Yellow.
20	Hedderson 16009. Arniston on thatch. Yellow.
21	Hedderson 15953. Wolfdrif-clanwilliam. Yellow.
22	Hedderson 16453. Oudtshoorn area. White and yellow?
23	Hedderson 16565. Ncape Rd to kamieskroon. White.
24	Wilding 211208b. Monatgu.Simonskloof. Yellow.
25	Wilding 211208a. Monatgu.Simonskloof. White.
26	Wilding 201208a. Robertson area. Vrojlikheid NR. White.
27	Wilding 231208b. Barrydale.Kylaken farm. Yellow.
28	Wilding 231208. Barrydale. Kylaken farm. White.
29	Wilding 201208c. Montagu. Yellow.
30	Wilding 171208. Baineskloof.Yellow.
31	Hedderson 16456. De rust. Uniondale. White.
32	Hedderson 16454(1). De rust. Uniondale. White.
33	Hedderson 16112. Robertson-Dysseldorp. Yellow.
34	Hedderson 16134. Ceres-Sutherland. Yellow.
35	Hedderson 15225. Yellow.
36	Hedderson 15941. Clanwilliam-Wuppetaal. Yellow.
37	Hedderson 16563. Kamieskroon. White.
38	Hedderson 16554. Kamieskroon. Yellow.
39	Hedderson 13784. Kouebokkeveld Mntns. White.
40	Hedderson 14331. Nieuwoudtville area. Oorlogues kloof NR. White.
41	Hedderson 15540. Robertson area. Yellow.
42	Hedderson 14061. McGregor area. Kleinberg. Yellow.
43	Hedderson 13921. McGregor area. Kranz NR. White.
44	Hedderson 13159. Silvermine. White.
45	Hedderson 13922. McGregor area. Yellow.
46	Hedderson 16037. Sutherland Rd to obs. Yellow.
47	Matcham 164. Chile. Yellow.
48	Duckett 424. Chile. Yellow.
49	Matcham 129. Chile. Yellow.

50	Matcham 5381. Cape point. White.
51	Matcham 5390. Jeffreys Bay. Yellow.
52	Matcham 5041a. Lesotho. Yellow.
53	Matcham 1126a. Lesotho.Yellow.
54	Matcham 143. Chile. Yellow.
55	Wilding 30-3-09 Karoo NP. Yellow.
56	Wilding 31-3-09 Loxton. Yellow.
57	Wilding 1-4-09 Victoria West. Yellow.
58	Wilding 6-5-09 Sedgefield. Yellow.
59	G. Williamson 2675a Buchuberg. Yellow.
60	E.A. Schelpe 7996. Springbok. Yellow.
61	AJP Calvinia. Eastern Cape. Yellow.
62	AJP 195. Eastern Cape. Yellow.
63	AJP 237. Eastern Cape. Yellow.
64	AJP 213. Eastern Cape. Yellow.
65	AJP Vanrhynsdorp. Yellow.
66	AJP 222. Eastern Cape. Yellow.
67	AJP 203. Eastern Cape. Yellow.
68	AJP 197. Eastern Cape. Yellow
69	AJP 206. Eastern Cape. Yellow.
70	AJP 210. Eastern Cape. Yellow.
71	AJP 217. Eastern Cape. Yellow.
x	H. Toelken 5557.*Pseudocrossidium porphyreoneuru.* Farm sturmfield. Gobabis district