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Sexual reproduction in populations of *Austropuccinia psidii*

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Abstract *Austropuccinia psidii* is a rust fungus that has expanded its known geographic distribution and host range on Myrtaceae. Invasions by rust fungi are often caused by asexual urediniospores that give rise to populations with low genotypic diversity. Recently it was shown that basidiospores, the gametic spores of *A. psidii*, were able to infect species of Myrtaceae under controlled conditions. The present study tested the hypothesis that sexual reproduction occurs through infection of Myrtaceae by basidiospores of *A. psidii* in recently invasive populations from New Zealand and South Africa. We provided three lines of evidence to test this hypothesis: i) presence of a sexual stage, ii) high genotypic diversity within an invasive population, and iii) no genetic linkage between microsatellite markers in multilocus genotypes. Our results provide evidence that invasions of *A. psidii* are caused by both urediniospores that spread clonal genotypes, and teliospores that produce recombinant basidiospores, which infect Myrtaceae. We reject the hypothesis that field infections of *A. psidii* are only caused by asexual urediniospores, and support that sexual reproduction occurs in invasive populations and may accelerate adaptation to environmental change.

Keywords Biological invasions · Clonal fungi · Life cycle · Mixed mating system · Myrtle rust · Pucciniales

Alistair R. McTaggart and Esna du Plessis contributed equally to this work.

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Introduction

The rust fungus *Austropuccinia psidii* (syn. *Puccinia psidii*, Sphaerophragmiaceae, Pucciniales) causes disease on species of Myrtaceae. Winter (1884) described *A. psidii* on a species of *Psidium* from Brazil, and it was soon found on other native genera in South America, such as *Eugenia* and *Plinia* (Rangel 1916). It was a new encounter pathogen on introduced species of *Eucalyptus* in Brazil (Joffily 1944), which foreshadowed its

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potential to cause disease on a wider host range of the Myrtaceae. *Austropuccinia psidii* spread from its hypothesized native range in Central and South America (Coutinho et al. 1998) and is currently present in at least 27 countries (Carnegie and Pegg 2018).

The life cycle stages of *A. psidii* include urediniospores, a dikaryotic clonal spore stage, and diploid teliospores that produce basidiospores through meiosis. Mature basidiospores contain two nuclei, but it is unknown whether these are hetero- or homokaryotic (Morin et al. 2014; McTaggart et al. 2018). Two different life cycles of *A. psidii* have been hypothesized. Coutinho et al. (1998) suggested completion of its life cycle on a single host without production of spermogonia and aecia. Ruiz et al. (1989) postulated that spermogonia and aecia were formed on an alternate, but unknown host, similar to other heteroecious rust fungi.

The role of basidiospores has been investigated to resolve the life cycle of *A. psidii*. Figueiredo et al. (1984) and Figueiredo (2001) inoculated *Syzygium jambos* and a species of *Eucalyptus* with basidiospores, which infected and produced uredinia on both hosts. However, Morin et al. (2014) observed that basidiospores did not penetrate the cuticle of inoculated *Agonis flexuosa*, and despite production of uredinia from inoculations with basidiospores, there were no signs of recombination measured by genotypic diversity. Morin et al. (2014) suggested urediniospores had contaminated inocula in their own, and earlier studies. The lack of evidence for sexual recombination in populations of *A. psidii* sampled from cultivated hosts and invasion events led to the conclusion that it reproduces clonally (Graça et al. 2013; Machado et al. 2015). The observed genotypic diversity in populations of *A. psidii* was treated as the product of mutation rather than sexual reproduction (Graça et al. 2013; Machado et al. 2015).

McTaggart et al. (2018) queried the assumption that *A. psidii* only reproduces asexually and provided evidence for the alternative hypothesis that genotypic diversity is a product of recombination and infection of plants by basidiospores. They filtered urediniospores from their inocula and used microsatellite markers to confirm pustules that developed after inoculations with basidiospores on *S. jambos* were the product of sexual reproduction. These experiments showed *A. psidii* completes the asexual and sexual parts of its life cycle on a single host (autoecious) and that meiotic recombination produces basidiospores that are able to infect species of Myrtaceae under controlled conditions (McTaggart et al. 2018).

Fungal populations acquire genetic diversity in different ways (Milgroom 1996). A population that reproduces sexually may produce sexual structures, have increased genotypic diversity and many possible recombinant genotypes, and different regions of the genome may have different histories of descent (Milgroom 1996). Alleles are randomly distributed among individuals after sexual reproduction, which can be measured in diploid and dikaryotic organisms using the inbreeding coefficient (De Meeûs et al. 2006). Clonal populations will have low genotypic diversity, show linkage disequilibrium between markers, and be mostly either homozygous or heterozygous at individual loci (De Meeûs et al. 2006; Grunwald et al. 2017; Milgroom 1996). Clonal populations from a single invasion event may also have low allelic diversity when a single genotype infects, colonizes and spreads (Gladieux et al. 2015; McDonald and Linde 2002).

The recent incursions of *A. psidii* in South Africa (Roux et al. 2013) and New Zealand (Ho et al. 2019) provided us with an opportunity to test the hypothesis that sexual reproduction of this rust occurs in the field as part of, or immediately following, an invasion event. Roux et al. (2016) reported one genotype was present in South Africa and noted that telia, the precursors to sexual reproduction, were abundant. In a separate invasion event of *A. psidii* in New Zealand, several genotypes were identified (du Plessis et al. 2019) and telia were present (Ho et al. 2019). We specifically sought to test if: (i) basidiospores of *A. psidii* play a role in the epidemiology of invasive populations, and (ii) recombination gives rise to a random association of genetic markers in the invading population. Insight into the reproductive biology of invasive species such as rusts, and understanding their disease cycle and reproductive biology, including the generation of genetic diversity through recombination, are important in efforts to either eradicate, contain or manage these invasions.

Materials and methods

Sampling and DNA extraction

We used an ad hoc sampling strategy for specimens collected in South Africa as part of an ongoing study to monitor the distribution of *A. psidii*. We sampled the

first five leaf specimens of *A. psidii* from the disease incursion in New Zealand, sent to South Africa under import permit P0079592, as described in the study of du Plessis et al. (2019). The presence or absence of telia was recorded by visual inspection for all specimens from New Zealand and South Africa. All specimens used in the present study are listed in Table 1.

Two to four discrete pustules were sampled from single leaves of each specimen to detect genotypic diversity expected from infections caused by basidiospores. If four or more of the five microsatellite loci amplified, the pustule was included in the analyses of genotypic diversity and recombination. These analyses included 35 (out of 62) pustules of *A. psidii* from South Africa and nine (out of 22) pustules from New Zealand (Table 1).

DNA was extracted from single pustules using either the Gentra Puregene Yeast/Bacteria Kit (Qiagen, Germany) or the Ultraclean® Microbial DNA Isolation Kit (MoBio Laboratories, USA). The protocols supplied by the manufacturer were followed, with the exception that DNA was eluted in a final volume of 15 μL.

Selection, amplification and analysis of microsatellite loci

We tested whether sexual reproduction occurred in populations of *A. psidii* from South Africa and New Zealand with five microsatellite markers (PpSSR012, PpSSR014, PpSSR018, PpSSR102, PpSSR161) originally developed by Zhong et al. (2008), and modified by Graça et al. (2013). The markers were labeled with FAM™, NED™, PET® or VIC® fluorescent dye. These markers had two alleles each in the invasive population of *A. psidii* in South Africa (Roux et al. 2016), and had two alleles each in the population from New Zealand, with the exception of PpSSR102 that had one allele (du Plessis et al. 2019).

PCR mixtures included 1× PCR FastStart™ Taq Buffer with $MgCl_2$ (Sigma-Aldrich, USA), 200 μM dNTPs, 0.1 μM fluorescent primers, 1 unit FastStart™ Taq DNA polymerase and DNA template in 12.5 μL reaction volumes. PCR products were amplified with the following conditions: one cycle at 95 °C for 10 min, followed by 10 cycles at 95 °C for 30s, 52–56 °C (depending on the primer pair) for 45 s, 72 °C for 60 s, 30 cycles on an auto-delta step at 95 °C for 30 s, 52–56 °C (depending on the primer pair) for 45 s plus 5 s added after each cycle, and 72 °C for 60 s, one cycle at 60 °C for 30 min. Primers PpSSR 012, PpSSR 014, PpSSR 018, PpSSR 161 were amplified at 56 °C, and PpSSR 102 at 52 °C. An ABI® Applied Biosystems Veriti 96 Well Thermal Cycler (ThermoFisher Scientific, USA) was used for PCRs.

PCR fragments were analysed on an ABI Applied Biosystems PRISM® 3500xl Autosequencer (ThermoFisher Scientific, USA) at the Sequencing Facility of the Faculty of Natural and Agricultural Sciences, University of Pretoria. Samples were run with GeneScan™ 500 LIZ® Size Standard (ThermoFisher Scientific, USA) and scored with GeneMapper® Software 5 (Thermo Fischer Scientific, USA).

Alleles for isolates that could not be amplified were treated as a result of limited DNA available per extraction rather than PCR bias or null alleles, as discussed by McTaggart et al. (2018). The allele profiles for single-pustule isolates are shown in Supplementary Fig. 1 and the scored alleles are listed in Table 1.

Genotypic diversity, gene diversity and tests for sexual reproduction

We tested whether basidiospores played a role in the life cycle through analyses of genotypic diversity. If the population had high genotypic diversity there was evidence that recombinant spores (basidiospores) caused infection, rather than clonal urediniospores. Genotypic diversity of the populations was assessed using the genotypic evenness and Simpson Index, calculated from 1000 permutations of the poppr function in *poppr* (Kamvar et al. 2014; R Core Team 2014). All commands and data are provided in Supplementary File 2.

We used the level of gene diversity (heterozygosity) to determine whether sexual reproduction occurred in the invading population or if there were multiple sequential invasions, as a higher level of gene diversity may indicate multiple introductions. We used GenoDive (Meirmans and Van Tienderen 2004) to determine overall diversity for populations by the frequency of alleles across all five loci.

We used the Index of Association (I_A) to test whether there was evidence of sexual reproduction within populations from South Africa and New Zealand, calculated with the ia function in *poppr*. This tested a null hypothesis of random mating and showed whether the observed multi-locus genotypes were best explained by clonal propagation (if there was linkage between markers) or recombination (if loci were not linked).

Table 1 Collection information and allele sizes of *Austropuccinia psidii* at five microsatellite loci used in this study. NA is used if the microsatellite locus was not amplified

Herbarium number (PREM[a])	Origin	Host	Telia	Allele sizes				
				PpSSR 12	PpSSR 14	PpSSR 18	PpSSR 102	PpSSR 161
62,050	LIM[b], RSA[c]	*Eugenia natalitia*	Yes	237, 237	205, 205	165, 174	140, 140	288, 288
62,051	LIM, RSA	*E. natalitia*	Yes	NA	205, 205	165, 174	140, 142	270, 288
62,052	WC[d], RSA	*Myrtus communis*	No	233, 237	205, 213	165, 174	140, 142	270, 288
62,053	KZN[e], RSA	*E. erythrophylla*	Yes	NA	213, 213	174, 174	140, 142	288, 288
62,054	KZN, RSA	*E. verdoorniae*	Yes	233, 233	213, 213	NA	140, 140	270, 288
62,055	KZN, RSA	*E. umtamvunensis*	Yes	237, 237	205, 205	165, 174	142, 142	288, 288
62,056	KZN, RSA	*E. natalitia*	Yes	237, 237	205, 213	165, 165	140, 142	288, 288
62,057	KZN, RSA	*E. natalitia*	Yes	233, 237	205, 213	NA	140, 140	270, 288
62,058	KZN, RSA	*E. natalitia*	No	233, 237	205, 213	165, 174	140, 142	270, 288
62,059	KZN, RSA	*E. simii*	No	233, 237	205, 213	165, 174	142, 142	NA
62,060	KZN, RSA	*E. simii*	No	233, 237	205, 213	165, 174	NA	270, 288
62,061	KZN, RSA	*E. erythrophylla*	Yes	237, 237	205, 213	165, 165	140, 142	270, 270
62,062	KZN, RSA	*E. verdoorniae*	Yes	233, 237	205, 213	165, 174	140, 142	270, 288
62,063	KZN, RSA	*E. umtamvunensis*	Yes	237, 237	205, 205	174, 174	NA	270, 270
62,064	KZN, RSA	*E. erythrophylla*	No	237, 237	205, 213	165, 165	140, 140	270, 288
62,065	KZN, RSA	*E. verdoorniae*	Yes	233, 237	205, 205	172, 172	142, 142	270, 288
62,066	KZN, RSA	*E. umtamvunensis*	No	NA	213, 213	174, 174	140, 140	270, 270
62,067	KZN, RSA	*E. umtamvunensis*	No	237, 237	NA	174, 174	142, 142	288, 288
62,068	MPU[f], RSA	*Melaleuca viminalis*	Yes	233, 233	213, 213	165, 174	140, 142	288, 288
62,069	KZN, RSA	*E. capensis*	Yes	233, 237	213, 213	165, 174	142, 142	270, 270
62,070	LIM, RSA	*E. natalitia*	No	233, 233	205, 213	165, 165	140, 142	288, 288
62,071	KZN, RSA	*My. communis*	No	233, 237	205, 213	165, 174	140, 142	270, 288
62,072	MPU, RSA	*Mel. viminalis*	Yes	233, 237	213, 213	174, 174	140, 142	288, 288
62,073	KZN, RSA	*E. capensis*	Yes	233, 233	205, 205	165, 174	142, 142	270, 288
62,074	EC[g], RSA	*My. communis*	No	NA	205, 213	165, 174	140, 142	270, 288
62,075	KZN, RSA	*E. capensis*	No	237, 237	205, 213	165, 174	140, 142	270, 288
62,076	KZN, RSA	*E. capensis*	No	233, 237	213, 213	165, 174	142, 142	270, 288
62,077	KZN, RSA	*E. capensis*	No	233, 233	213, 213	174, 174	140, 140	NA
62,078	KZN, RSA	*E. capensis*	No	233, 237	205, 213	NA	140, 142	270, 288
62,079	KZN, RSA	*E. verdoorniae*	No	233, 233	205, 213	165, 172	140, 142	NA
62,080	KZN, RSA	*E. verdoorniae*	No	233, 237	205, 213	165, 174	140, 142	270, 288
62,081	KZN, RSA	*E. erythrophylla*	No	233, 233	205, 213	165, 174	140, 142	270, 288
62,082	MPU, RSA	*Mel. viminalis*	No	233, 237	205, 213	NA	140, 142	270, 270
62,083	KZN, RSA	*E. capensis*	No	233, 237	205, 213	174, 174	140, 142	270, 288
62,084	KZN, RSA	*E. capensis*	Yes	233, 237	205, 213	165, 174	140, 142	NA
62,096–1	RI[h], NZ[i]	*Metrosideros kermadecensis*	Yes	230, 236	207, 211	172, 172	140, 140	276, 276
62,096–2	RI, NZ	*Met. kermadecensis*	Yes	230, 236	211, 211	170, 172	NA	290, 290
62,099–1	NI[j], NZ	*Met. excelsa*	Yes	230, 236	211, 211	170, 172	NA	276, 290
62,099–2	NI, NZ	*Met. excelsa*	Yes	230, 236	207, 207	170, 170	140, 140	NA
62,099–3	NI, NZ	*Met. excelsa*	Yes	230, 236	207, 207	170, 172	140, 140	276, 290
62,099–4	NI, NZ	*Met. excelsa*	Yes	236, 236	207, 211	170, 172	140, 140	NA

Table 1 (continued)

Herbarium number (PREM[a])	Origin	Host	Telia	Allele sizes				
				PpSSR 12	PpSSR 14	PpSSR 18	PpSSR 102	PpSSR 161
62,102	NI, NZ	*Lophomyrtus bullata*	Yes	236, 236	207, 211	170, 170	140, 140	NA
62,103	NI, NZ	*Lophomyrtus bullata*	Yes	236, 236	207, 211	170, 172	NA	276, 290
62,104	NI, NZ	*Lophomyrtus bullata*	Yes	230, 230	207, 211	170, 172	140, 140	NA

[a] Herbarium of the South African National Fungus Collection
[b] Limpopo
[c] Republic of South Africa
[d] Western Cape
[e] KwaZulu-Natal
[f] Mpumalanga
[g] Eastern Cape
[h] Raoul Island
[i] New Zealand
[j] North Island

The I_A was tested on clone-corrected and non-clone corrected data. We tested whether there was a random distribution of alleles among individuals using the inbreeding coefficient calculated in GenoDive.

We visualized recombination events using a Neighbour-Net in SplitsTree4 v4.14.8 (Huson and Bryant 2005). Allelic data were converted to a distance matrix with *PopGenReport* (Adamack and Gruber 2014) using the genetic distance measure of Kosman and Leonard (2005), appropriate for organisms with mixed mating methods (Kosman and Leonard 2007). Reticulations within the Neighbour-Net were treated as putative recombination events in populations (Huson and Bryant 2005).

Results

Presence of sexual stage

Telia were present on 16 out of the 35 (~46%) specimens of *A. psidii* from South Africa used in this study (Table 1), which supported the observation by Roux et al. (2016). Telia were observed on all five specimens from New Zealand. Telia were brown and compact, whereas uredinia were bright yellow and powdery (Fig. 1).

Genotypic diversity, gene diversity and tests for sexual reproduction

Genotypic diversity was high in both populations. The genotypic evenness of populations from New Zealand and South Africa showed a single (clonal) genotype was not dominant (Table 2). The Simpson Indices showed a high probability that two randomly selected samples from each population had a different genotype (Table 2).

Fig. 1 Telia (T) and uredinia (U) of *Austropuccinia psidii* on *Eugenia natalitia* in Dlinza Forest Reserve, KwaZulu Natal, South Africa (PREM 62056). Telia (meiotic stage) and uredinia (mitotic stage) were distinguished by their colour, where uredinia are yellow and telia are brown

Table 2 Genotypic diversity in populations of *Austropuccinia psidii* from New Zealand and South Africa. Genotypic evenness and the Simpson index show high genotypic diversity in the two populations of *A. psidii*

Population	Number of pustules	Number of MLGs[a]	Expected number of MLGs	Standard error	Genotypic evenness	Simpson index
New Zealand	9	8	9	0	1	0.89
South Africa	35	31	31	0	0.79	0.96

[a] Multilocus genotype

Gene diversity showed both populations had one or two alleles for each locus. The exception was marker PpSSR018, which had three alleles, one of which was unique in two pustules of the South African population (Table 3). There were different alleles in the populations from New Zealand and South Africa, which shows they are not connected.

The clone-corrected I_A values for the New Zealand and South African populations were not significant for linkage disequilibrium ($P < 0.05$), which suggests random mating and provided evidence for sexual reproduction in the two populations (Table 4). The inbreeding coefficient of populations from New Zealand and South Africa was close to zero, which showed alleles were randomly distributed among the population, indicative of sexual reproduction (Table 4). The Neighbour-Net (Fig. 2) was reticulated within the populations, a sign of potential recombination events in the two examined populations.

Discussion

Our results support the hypothesis that invasive populations of *A. psidii* in New Zealand and South Africa reproduce sexually. This is based on three lines of evidence: (i) telia, the sexual stage, were present in the field, (ii) there was a high level of genotypic diversity within these invasive populations, and (iii) there were many recombinant multilocus genotypes with unlinked loci; all of which are putative signs of sexual reproduction (Milgroom 1996; De Meeûs et al. 2006). Teliospores, the precursors of sexual recombination in rust fungi, were identified in 46% ($n = 35$) of the South African samples and in 100% ($n = 5$) of the New Zealand samples. There was high genotypic diversity, despite low allelic diversity, in invasive populations from New Zealand and South Africa, as measured by the genotypic evenness and Simpson index. There was also no linkage between individual microsatellite markers, there was a random distribution of alleles, and the Neighbour-Net was reticulate, all of which are expected in recombinant populations.

There are three competing hypotheses to explain high genotypic diversity in an invasive population with low allelic diversity, namely sexual or parasexual reproduction, or parallel evolution. Graça et al. (2013) treated *A. psidii* as clonal and hypothesized genotypic diversity was a result of parallel evolution. Wu et al. (2019) showed a species of rust reproduced by mitotic recombination (parasexual reproduction) when unable to complete its sexual life cycle. We cannot reject parasexual reproduction and parallel evolution as hypotheses based on our current data. However, McTaggart et al. (2018) resolved the sexual life cycle of *A. psidii* under controlled conditions; basidiospores infected Myrtaceae and dikaryotic, recombinant

Table 3 Gene diversity in populations of *Austropuccinia psidii* from New Zealand and South Africa

Population	Pustules	Frequency of alleles at each microsatellite locus										Gene diversity	Inbreeding coefficient (*P* value)
		PpSSR 012		PpSSR 014		PpSSR 018		PpSSR 102		PpSSR 161			
New Zealand	9	A	0.39	A	0.5	A	0.56	A	1	A	0.5	0.45	−0.069 (0.97)
		B	0.61	B	0.5	B	0.44			B	0.5		
South Africa	35	C	0.45	C	0.47	C	0.42	A	0.45	C	0.47	0.58	−0.138 (0.55)
		D	0.55	D	0.53	D	0.53	B	0.55	D	0.53		
						B	0.05						

Table 4 Test for linkage disequilibrium in populations of *Austropuccinia psidii* from New Zealand and South Africa. The clone corrected tests supported that loci were in linkage equilibrium (P I_A, P $\overline{r_d} \geq 0.01$)

Population	Number of pustules	Number of MLGs	Non-clone corrected				Clone corrected			
			I_A[a]	P I_A	$\overline{r_d}$	P $\overline{r_d}$	I_A	P I_A	$\overline{r_d}$	P $\overline{r_d}$
New Zealand	9	8	−0.50	0.998	−0.170	0.991	−0.50	0.998	−0.170	0.993
South Africa	35	31	0.22	0.001	0.055	0.001	0.066	0.053	0.017	0.053

[a] I_A = Index of Association

uredinia and telia were produced. With knowledge that *A. psidii* reproduces sexually, the most logical explanation from our findings is that basidiospores of *A. psidii* infect species of Myrtaceae to complete its sexual life cycle under field conditions.

An alternative hypothesis to explain high genotypic diversity is that observed multilocus genotypes were part of genetically different and genotypically diverse invasive populations. This alternative hypothesis is unlikely because there was only one locus that had a third allele, and agrees with findings that incursions in New Zealand and South Africa were caused by single strains (du Plessis et al. 2019; Roux et al. 2016). The third allele in the locus from South Africa was attributed to mutation based on its low frequency and that stepwise mutations are a characteristic of microsatellite loci (Valdes et al. 1993).

Our results, analyses and conclusions are in contrast to studies that found low genotypic diversity and clonal spread of *A. psidii* in Australia (Machado et al. 2015; Sandhu et al. 2016), Brazil (Graça et al. 2013), Colombia (Granados et al. 2017), Hawaii (Stewart et al. 2017), New Caledonia (Soewarto et al. 2017) and South Africa (Roux et al. 2016). Those studies sampled plants grown in monoculture, such as *Eucalyptus* and guava, sampled at different geographic scales, or were from recently invasive populations in which a founder effect may have led to low sampled genotypic diversity (Drenth et al. 2019; Gladieux et al. 2015; Taylor et al. 2015). Future studies need to investigate whether these populations are truly clonal or whether there is evidence of recombination, as shown in New Zealand and South Africa.

Our results demonstrate that *A. psidii* has a mixed mating system. Urediniospores are formed in abundance, spread quickly and maintain desirable genotypes by clonal reproduction. Teliospores and basidiospores play a role in dispersal of the pathogen and infection of

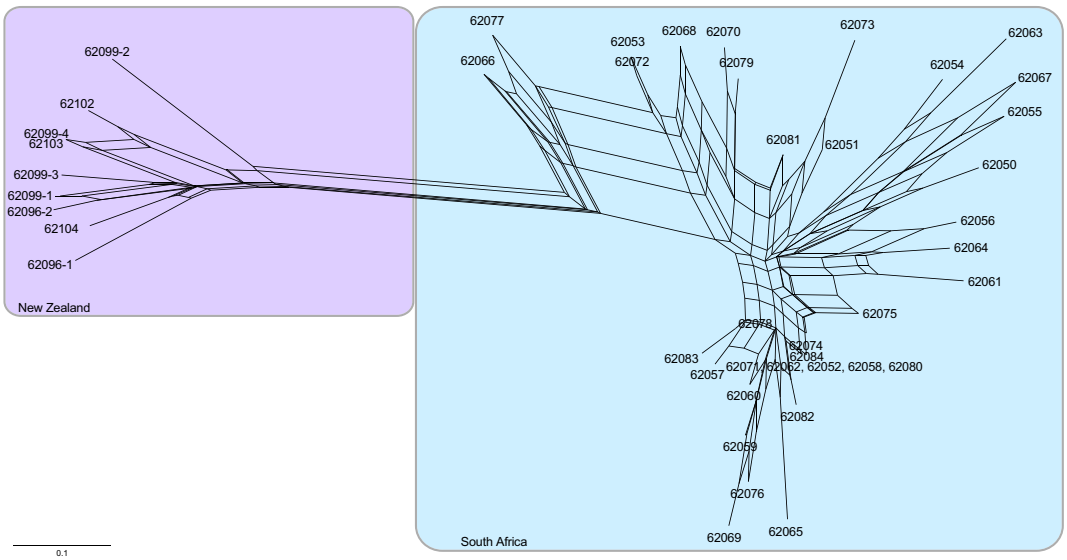

Fig. 2 Neighbour-Net made in SplitsTree based on Kosman genetic distance between multilocus genotypes of *A. psidii* from New Zealand and South Africa. Reticulations indicate possible recombinant relationships between the multilocus genotypes

Myrtaceae in addition to the long-term benefits of sexual recombination. Gladieux et al. (2015) proposed that mixed mating systems, with asexual and sexual reproduction, benefit fungal invasions. Clonal reproduction allows propagation without losing advantageous gene combinations (McDonald and Linde 2002), and recombination through sexual reproduction allows populations to increase diversity after a bottleneck (Barrett 2015).

Different genotypes of *A. psidii* could potentially recombine if their basidiospores infected the same host, based on the life cycle hypothesized by McTaggart et al. (2018). Recombination within or between genotypes enables pathogens to increase their speed of adaptation (reviewed by Nieuwenhuis and James 2016) and overcome resistance of host plants in fewer generations (McDonald and Linde 2002). Another species of rust, *Hemileia vastatrix*, the cause of coffee rust, undergoes cryptic sexual reproduction, and recombinant genotypes with new combinations of alleles overcame resistance in coffee plants (Carvalho et al. 2011). New combinations of pathogenicity and virulence may occur through sexual recombination, which typically reduces the lifespan of selective breeding for resistance.

Our findings show that invasive populations of *A. psidii* in New Zealand and South Africa reproduce sexually, which may increase its ability to more rapidly adapt to changes in the environment. *Austropuccinia psidii* is capable of quickly building inocula through clonal reproduction in the form of urediniospores, and of overcoming population bottlenecks and host resistance through recombination and potential outcrossing between genotypes.

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Compliance with ethical standards The authors declare that there are no potential conflicts of interest and that the study did not involve human participants or animals.

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