Research Note

The pandemic strain of *Austropuccinia psidii* causes myrtle rust in New Zealand and Singapore

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Abstract: The myrtle rust pathogen, *Austropuccinia psidii*, was recently detected in New Zealand and Singapore. We used microsatellite markers to identify the strain of *A. psidii* that caused these incursions. Our results show that the pandemic strain of the pathogen caused outbreaks in both New Zealand and Singapore.

Keywords: invasion; myrtle rust; population; Pucciniales

Austropuccinia psidii causes rust on species of Myrtaceae. It is an invasive pathogen that has expanded its distribution and host range, and threatens naïve ecosystems rich in Myrtaceae (Carnegie et al., 2015; Pegg et al., 2017). For example, *A. psidii* has caused stem blight and tree die-back, resulting in localized extinction of vulnerable native species in Australia (Pegg et al. 2017).

Two different strains of *A. psidii* occur outside of its putative native range in Central and South America. The pandemic strain, as described by Ross-Davis et al. (2014) and Stewart et al. (2017), is reported from Asia, Australia, Colombia and the Pacific region (Graça, 2011; Granados et al., 2017; Machado et al., 2015; McTaggart et al., 2016; Stewart et al., 2017). A second invasive strain was reported from South Africa (Roux et al., 2016).

Austropuccinia psidii was discovered on a single host from Raoul Island in 2017 (Ministry of Primary Industries Manatū Ahu Matua, 2017). The rust was soon detected in the North Island

of New Zealand, where it became widespread, infecting over twenty hosts. It has also recently been found from northern parts of the South Island (Ministry of Primary Industries Manatū Ahu Matua, 2018). du Plessis et al. (2017) reported *A. psidii* on *Rhodomyrtus tomentosa* from Singapore. The present study aimed to identify the strain of *A. psidii* invasive in New Zealand and Singapore. We hypothesized it was the pandemic strain based on its spread in Australia, Indonesia and New Caledonia (Machado et al., 2015; McTaggart et al., 2016; Soewarto et al., 2017).

Three hosts of *A. psidii* were sampled in New Zealand for this study, namely *Lophomyrtus bullata*, *Metrosideros excelsa* and *M. kermadecensis*, and one host, *Rhodomyrtus tomentosa*, was sampled in Singapore. Specimens were sent to South Africa under import permit P0079592 and lodged in the herbarium of the South African National Fungus Collection (PREM). We used five microsatellite markers developed by (Zhong et al., 2008) to screen 16 single pustules of *A. psidii* on seven specimens from New Zealand, and two single pustules on two specimens from Singapore (Table 1). DNA was extracted from uredinial pustules using the Ultraclean® Microbial DNA Isolation Kit (MoBio Laboratories, California, USA) as specified by the manufacturer, with the exception that DNA was eluted in a final volume of 15 µL. The microsatellite loci were amplified and scored according to the protocols of Granados et al. (2017).

The amplified microsatellites were run on an ABI® Applied Biosystems PRISM® 3500xl Autosequencer (Life Technologies) at the Sequencing Facility of the Faculty of Natural and Agricultural Sciences (NAS), University of Pretoria and included a control sample from the study of Granados et al. (2017). We used a Principal Component Analysis (PCA) in the R package *adegenet* (Jombart, 2008; R Core Team, 2014) to cluster multilocus genotypes from New Zealand and Singapore with populations from Granados et al. (2017). Pustules were included in the analyses if they amplified at three or more microsatellite loci. The multilocus genotypes of *A. psidii* from New Zealand and Singapore clustered with a genotype of the pandemic strain found in Australia, Colombia and Indonesia (Figure 1). The results confirmed our hypothesis that the pandemic strain invaded New Zealand and Singapore, and this is the first study to identify the strain of *A. psidii* in these countries.

Table 1 Specimen details and microsatellite alleles scored for each pustule obtained in the present study and used in the principal component analysis

Collection number	PREM number	Host	Origin	Allele size				
				PpSSR012	PpSSR014	PpSSR018	PpSSR102	PpSSR161
NZ02–1	62096	*Metrosideros kermadecensis*	New Zealand, Raoul Island	230, 236	207, 211	172, 172	140, 140	276, 276
NZ03–2	62096			230, 236	211, 211	170, 172	NA	290, 290
NZ04–1	62097	*Metrosideros excelsa*	New Zealand, Raoul Island	NA	207, 207	NA	140, 140	276, 276
NZ09	62099	*Metrosideros excelsa*	New Zealand, North Island	230, 236	211, 211	170, 172	NA	276, 290
NZ10	62099			230, 236	207, 207	170, 170	140, 140	NA
NZ11	62099			236, 236	207, 211	170, 172	NA	NA
NZ12	62099			230, 236	207, 207	170, 172	140, 140	276, 290
NZ13	62099			236, 236	207, 211	170, 172	140, 140	NA
NZ14	62100	*Metrosideros excelsa*	New Zealand, North Island	230, 236	207, 211	170, 172	NA	NA
NZ16	62102	*Lophomyrtus bullata*	New Zealand, North Island	236, 236	207, 211	170, 172	NA	NA
NZ17	62102			230, 236	207, 211	170, 170	NA	NA
NZ18	62102			236, 236	207, 211	170, 170	140, 140	NA
NZ19–2	62103	*Lophomyrtus bullata*	New Zealand, North Island	236, 236	207, 211	170, 172	NA	276, 290
NZ19–3	62103			230, 236	207, 211	170, 172	NA	NA
NZ19–4	62104	*Lophomyrtus bullata*	New Zealand, North Island	230, 236	207, 211	NA	140, 140	NA
NZ19–5	62104			230, 230	207, 211	170, 172	140, 140	NA
SIN208	61592	*Rhodomyrtus tomentosa*	Singapore	230, 230	207, 211	170, 172	140, 140	NA
SIN209	61593	*Rhodomyrtus tomentosa*	Singapore	236, 236	207, 211	170, 172	140, 140	276, 276

NA Data were not amplified at that marker, *PREM* South African National Fungus Collection

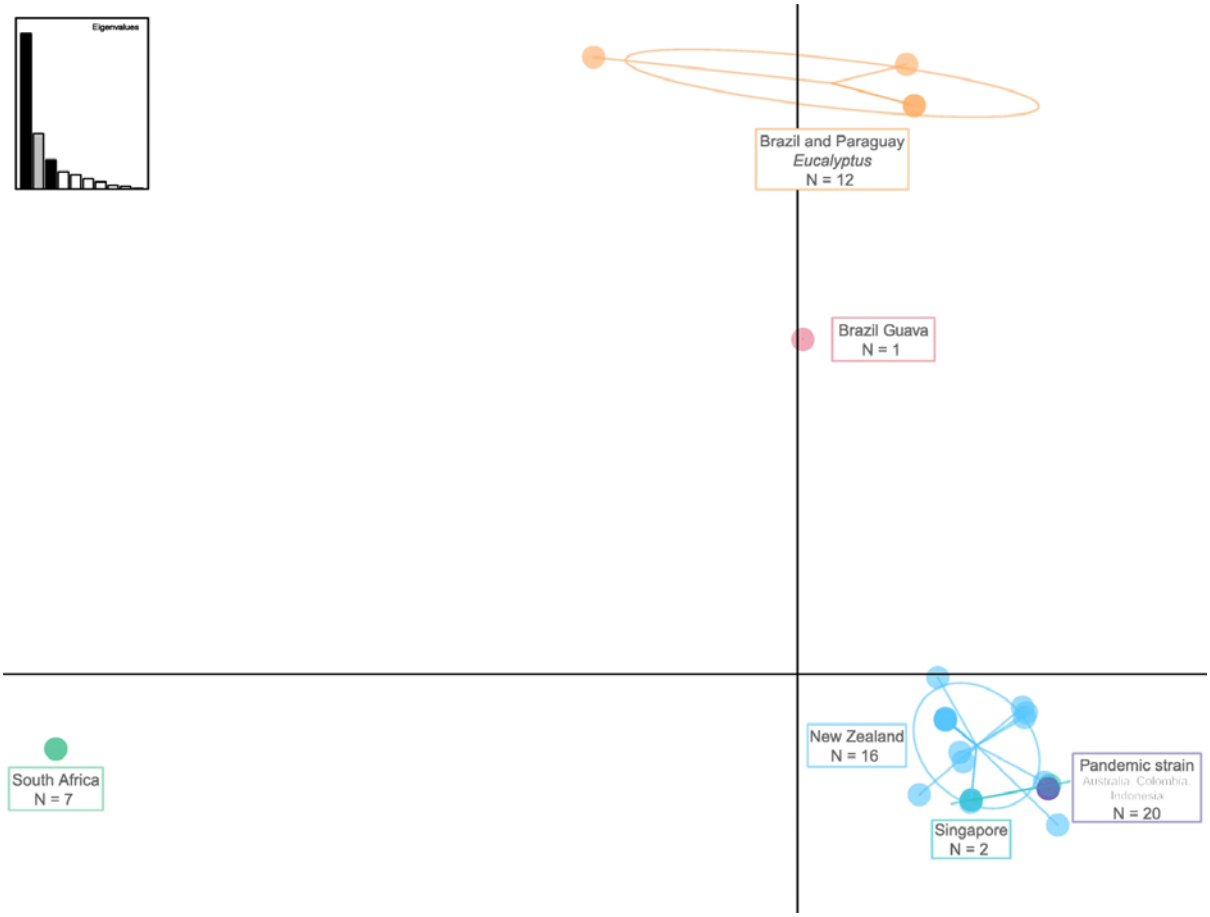

Figure 1. Principal component analysis for pustules of *Austropuccinia psidii* from New Zealand and Singapore included with the dataset of Granados et al. (2017). Points represent multilocus genotypes (MLGs) of five microsatellite loci; N indicates the number of isolates used per population. The populations in New Zealand and Singapore clustered with the pandemic strain, represented by one MLG from 20 pustules collected in Australia, Colombia and Indonesia.

There were ten multilocus genotypes in the population of *A. psidii* from New Zealand, however the cause of this genotypic diversity was not tested in the present study. Graça et al. (2013) explained that parallel mutations caused genotypic diversity in populations of *A. psidii* from Brazil. Another explanation was possibly provided by McTaggart et al. (2018), who showed that *A. psidii* can reproduce sexually. Sexual reproduction by *A. psidii* is yet to be tested in a natural environment.

Acknowledgements

This work is based on research partially supported by the Tree Protection Co-operative Programme (TPCP) and the National Research Foundation of South Africa (Grant specific unique reference numbers UID 78566 and UID 83924) and the DST-NRF Centre of

Excellence in Tree Health Biotechnology (CTHB). The grant holders acknowledge that opinions, findings and conclusions or recommendations expressed in any publication generated by NRF supported research are that of the authors and that the NRF accepts no liability whatsoever in this regard. ARM acknowledges the University of Queensland Development Fellowships (UQFEL1718905) and support from the Department of the Environment and Energy under the Australian Biological Resources Study (grant number RG18-43). WHH and BJRA acknowledge the support from the Ministry for Primary Industries (MPI) Plant Health and Environment Laboratory and the MPI Myrtle Rust Response Team.

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