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ORIGINAL PAPER

Microsatellite analysis indicates that *Puccinia psidii* in Australia is mutating but not recombining

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Abstract *Puccinia psidii* is considered a biosecurity threat in Australia because of its broad host range that includes many species of Myrtaceae which dominate Australian ecosystems. Since it was first reported in this country, in April 2010, there has been little information about the population structure of the pathogen. In this study, six microsatellite loci were analysed to determine the genetic relationship among rust specimens from different hosts and locations in Australia, New Caledonia, Hawaii and China. The Chinese and New Caledonian specimens share a multi-locus genotype with the majority of the Australian specimens. The results also indicated a close relationship between Australian and Hawaiian samples. At present, the *P. psidii* population in Australia is genetically uniform with no evidence of sexual recombination. Five of the 104 collections varied by one allele at single loci, indicating that mutations are common but persistence of the mutants in the population may be less common.

Keywords Myrtaceae · Myrtle rust · Plant disease · Genotyping

Introduction

Australia is the centre of origin of eucalypt and melaleuca biodiversity (Ladiges et al. 2003; Crisp and Cook 2013) and has over 1,646 species of Myrtaceae (Australian National Botanic Gardens and Centre for Australian National Biodiversity Research 2012) Australian Flora Statistic, https://www.anbg.gov.au/aust-veg/australian-flora-statistics.html). *Eucalyptus*, one of the largest genera within Myrtaceae and including several industrially important species, is susceptible to two types of rust fungi, *Phakopsora myrtacearum* and *Puccina psidii* Winter. *Phakopsora myrtacearum* was recently reported infecting *Eucalyptus* species in three countries from Africa (Maier et al. 2015). *P. psidii*, known colloquially as guava rust, eucalyptus rust or myrtle rust, is considered a biosecurity threat to many Myrtaceae species worldwide, especially Australia. Plants belonging to the Myrtaceae are dominant in this country in ecosystems ranging from tall forests to swamps and wetlands (Australian Government Department of Agriculture 2015). *Puccinia psidii* was first reported in Australia in April 2010, on *Agonis flexuosa* on the Central Coast of New South Wales (Carnegie et al. 2010) and spread rapidly along the east coast where it was detected in Queensland in December 2010 and 1 year later in Victoria (Pegg et al. 2013). It was not detected in Tasmania until February 2015 and in the Northern Territory (Tiwi Islands) until May 2015, and is still (May 2015) not recorded from South Australia or Western Australia.

Up to 2015, the pathogen has been reported on about 56 genera and 244 species of host in the Myrtaceae distributed in different continents: from South and North America (Carnegie and Lidbetter 2012), Asia (Kawanishi et al. 2009; Zhuang and Wei 2011), Oceania (Carnegie et al. 2010; Giblin 2013) and Africa (Roux et al. 2013). In Brazil, the pathogen is considered endemic (Tommerup et al. 2003) and is not usually severe on native hosts with the exception of occasional epidemics in guava orchards (de Goes et al. 2004; Ribeiro and Pommer 2004), but it can be a problem in eucalypt plantations, an important industrial crop for the country (Alfenas et al.

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2009). After introduction into new areas with Myrtaceae species that have not previously been exposed to this pathogen, *P. psidii* can rapidly expand its host range. This has been observed in Jamaica, Florida, Hawaii and Australia (MacLachlan 1938; Rayachhetry et al. 2001; Loope 2010; Pegg et al. 2013).

The ultimate impact of the pathogen on Australian biodiversity is yet to be determined, but considering its rapid dissemination, wide host range and the severe damage reported to some species such as *Rhodamnia rubescens* (Benth.) Miq., *Rhodomyrtus psidioides* (G.Don) Benth., *Syzygium anisatum* (Vickery) Craven & Biffen and *Melaleuca quinquenervia* (Cav.) S.T.Blake (Carnegie and Cooper 2011), *P. psidii* is a threat not only for the vegetation but also animal species which may depend on native plant species (Tommerup et al. 2003; Glen et al. 2007). Besides biodiversity, the pathogen can have a commercial impact on the forest and timber industry, lemon myrtle plantations (Plant Health Australia 2007) and commercial nurseries (Plant Health Australia 2010).

Since the pathogen was reported in 2010, studies have been conducted to identify vulnerable areas (Booth and Jovanovic 2012; Elith et al. 2013) and the host range of susceptible and resistant plant species (Carnegie and Lidbetter 2012). However, there is no information about the genetic variation of the pathogen population, how the rust was introduced into the country and how it spreads. Recent studies using microsatellite markers have determined the population structure and the host specificity of *P. psidii* in different areas such as Hawaii and Brazil (Zhong et al. 2011; Graça et al. 2013). In Hawaii, *P. psidii* collections are genetically uniform, indicating that the population consists of a single clonal lineage originating from one introduction (Zhong et al. 2011). In Brazil, host species provide strong selection pressure on *P. psidii* populations, regardless of geographic location (Graça et al. 2013). Principal coordinate analysis also indicated a high degree of genetic differentiation among collections from nine Brazilian states on different host species, revealing five major groups, the first formed by specimens from *Eucalyptus* spp. and *Syzygium jambos*, the second included collections from *Psidium guajava* and *Psidium guineense*, and three weakly separated groups formed by specimens collected from *Syzygium cumini*, *Myrciaria cauliflora* and *Eugenia uniflora* (Graça et al. 2013). The existence of host-specific genotypes may indicate the occurrence of cryptic species within the *P. psidii* complex or potential evolution to the level of "formae speciales". Genetic analysis of pathogen populations is required to understand the mechanisms generating genetic variation, host-pathogen co-evolution, and in the management of resistance (Keiper et al. 2003). An initial study based on a small number of collections of *P. psidii* soon after its introduction demonstrated the presence of a single multi-locus genotype in Australia (Glen and Mohammed 2011), consistent with the Hawaiian population, but did not include specimens from other countries where the pathogen was also recently introduced, such as China and New Caledonia. In this study microsatellite loci were analysed to determine the genetic relationship among rust specimens from different hosts and locations from Australia and recent incursions in other countries.

Material and methods

Sampling

A total of 104 single uredinial pustules of *P. psidii* were collected on 55 Myrtaceae species in Australia, New Caledonia (Fig. 1) China and Hawaii (Table 1). The samples were collected in mainland Australia in 2010 and 2013 and from Tasmania in 2015. The survey points were geo-referenced and most collections were deposited in the Queensland plant pathology herbarium (BRIP). A portion of each specimen was preserved in ethanol and retained for DNA extraction. Single pustules were excised and placed separately into 1.5-mL microcentrifuge tubes and stored at −80 °C prior to DNA extraction. Samples from New Caledonia, consisting of urediniospores collected from multiple *Syzygium jambos* plants in New Caledonia, were preserved in 70 % ethanol and imported into Australian under import permit IP13103123. DNA from the Chinese and Hawaiian collections was extracted and imported under IP13007011 and IP07020087.

DNA extraction

Genomic DNA was extracted directly from a single *P. psidii* pustule (fungus+host tissue) using one metal bead placed in a 1.5-ml microcentrifuge tube followed by two rounds of maceration using a TissueLyser II (Qiagen) for 2 min at the frequency 30 Hz. A total of 250 μl extraction buffer (Raeder and Broda 1985) was added and the tubes incubated at 65 °C for 1 h. Tubes were centrifuged at 14,000 rpm for 15 min and the supernatant removed. DNA was purified by binding to silica (Boyle and Lew 1995). Briefly, 600 μl of 100 % NaI and 10 μl silica were added to 200 μl of the supernatant and vortexed briefly. The mixture was incubated on ice for 15 min with occasional shaking. Tubes were centrifuged for 10 s at 14,000 rpm, the supernatant removed, and the pellet resuspended in 600 μl of wash buffer (100 mM NaCl, 10 mM Tris HCl pH 7.5, 1 mM EDTA in 50 % ethanol). Following centrifugation for 10 s at 14,000 rpm, the supernatant was removed, the pellet suspended in 600 μl 100 % ethanol and centrifuged as before. Finally, the supernatant was removed and the pellet dried for 20 min. DNA was eluted by adding 20 μl of TE buffer, vortexing briefly and incubating at 45 °C for 10 min. Supernatant containing DNA was removed following centrifugation for 2 min at 14,000 rpm and stored at –20 °C.

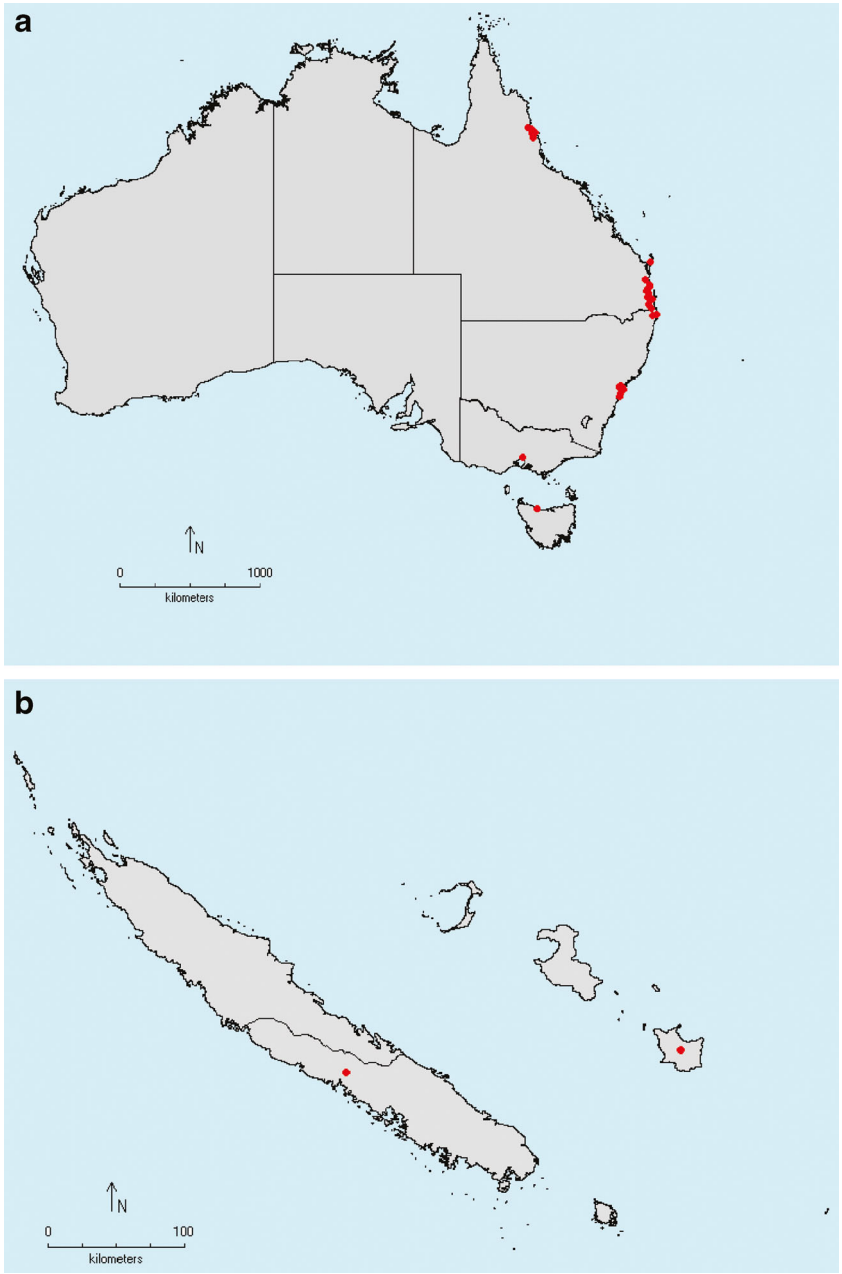

Fig. 1 Sampling locations of *Puccinia psidii* in Australia (**a**) and in New Caledonia (**b**)

Microsatellite genotyping

The samples were genotyped at 6 microsatellite loci (EF523503, EF523504, EF523507, EF523508, EF523510, EF523513) (Zhong et al. 2008; Graça et al. 2013). For each 10 μL PCR reaction we used 5 μL of 2× Master Mix (Type-It Microsatellite PCR kit, Qiagen), 0.1 μL (20 μM) of forward (labelled with either D2, D3 or D4 Well-RED fluorescent dye, Sigma-Aldrich) and reverse primers, 0.2 mg/mL of Bovine Serum Albumin (BSA, Fisher BioReagents™), 3.6 μL of nuclease-free water and 1 μL genomic DNA. PCR amplifications were performed using a thermal cycler (model 2720, Applied Biosystems) and the following program: 95 °C for 3 min, then 34 cycles of 94 °C for 15 s, 45 to 50 °C (depending on the locus) for 15 s, 72 °C for 45 s, ending with a hold at 60 °C for 30 min, then 14 °C. Fragment analysis was conducted on a CEQ™ 8000 Genetic Analysis System (Beckman Coulter), using 1 μL of PCR product mixed with 38.5 μL Sample Loading Solution (Beckman Coulter) and 0.5 μL size marker (DNA Size Standard Kit – 400, Beckman Coulter).

Results

Very little genetic variability was found among the 104 specimens of *P. psidii*; a single multilocus genotype was observed

Table 1 Host and geographic origin of *Puccinia psidii* collections

Herbarium or specimen code[a,b]	Host	Location[c]	Latitude	Longitude
MR1	*Syzygium jambos*	NSW, Australia	−33,868	151,207
MR2	*Syzygium jambos*	Qld, Australia	−27,919	153,051
MR3	*Syzygium australe*	Qld, Australia	−27,475	152,973
MR4	*Rhodomyrtus canescens*	Qld, Australia	−27,475	152,973
MR5	*Rhodomyrtus pervagata*	Qld, Australia	−27,475	152,973
MR6	*Syzygium nervosum*	Qld, Australia	−27,475	152,973
MR7	*Syzygium macilwraithianum*	Qld, Australia	−27,475	152,973
BRIP59494a	*Rhodamnia blairiana*	Qld, Australia	−27,475	152,973
BRIP59495a	*Chamelaucium uncinatum*	Qld, Australia	−27,475	152,973
BRIP59496a	*Backhousia oligantha*	Qld, Australia	−27,475	152,973
BRIP59497a	*Austromyrtus dulcis*	NSW, Australia	−28,605	153,572
BRIP59499a	*Melaleuca quinquenervia*	NSW, Australia	−28,605	153,572
BRIP59500a	*Rhodomyrtus psidioides*	NSW, Australia	−28,605	153,572
BRIP59502a	*Agonis flexuosa*	NSW, Australia	−28,672	153,279
BRIP59503a	*Syzygium jambos*	Qld, Australia	−27,485	152,992
BRIP59504a	*Melaleuca fluviatilis*	Qld, Australia	−27,404	153,073
BRIP59505a	*Melaleuca quinquenervia*	Qld, Australia	−26,333	152,82
BRIP59506a	*Rhodamnia rubescens*	Qld, Australia	−26,333	152,82
BRIP59507a	*Melaleuca quinquenervia*	Qld, Australia	−26,941	152,974
BRIP59508a	*Leptospermum trinervium*	Qld, Australia	−26,941	152,974
BRIP59509a	*Melaleuca quinquenervia*	Qld, Australia	−26,941	152,974
BRIP59510a	*Melaleuca quinquenervia*	Qld, Australia	−26,941	152,974
BRIP59511a	*Rhodamnia sessiliflora*	Qld, Australia	−27,494	152,944
BRIP59512a	*Eugenia reinwardtiana*	Qld, Australia	−16,912	145,767
BRIP59513a	*Melaleuca quinquenervia*	Qld, Australia	−17,222	145, 664
BRIP59514a	*Eugenia reinwardtiana*	Qld, Australia	−17,222	145, 664
BRIP59515a	*Syzygium* sp.	Qld, Australia	−17,222	145, 664
BRIP59516a	*Melaleuca* sp.	Qld, Australia	−17,222	145, 664
BRIP59517a	*Gossia* sp.	Qld, Australia	−16,816	145,686
BRIP59518a	*Eugenia reinwardtiana*	Qld, Australia	−16,816	145,686
BRIP59519a	*Melaleuca leucadendron*	Qld, Australia	−16,816	145,686
BRIP59520a	*Austromyrtus dulcis*	Qld, Australia	−16,816	145,686
BRIP59521a	*Gossia inophloia*	Qld, Australia	−16,816	145,686
BRIP59522a	Hybrid *Syzygium leuhmannii x S. wilsonii*	Qld, Australia	−16,816	145,686
BRIP59523a	*Leptospermum* sp.	Qld, Australia	−16,816	145,686
BRIP59524a	*Xanthostemon* sp.	Qld, Australia	−16,816	145,686
BRIP59525a	*Gossia myrsinocarpa*	Qld, Australia	−16,812	145,685
BRIP59526a	*Syzygium cormiflorum*	Qld, Australia	−16,812	145,685
BRIP59527a	*Rhodamnia sessiliflora*	Qld, Australia	−16,812	145,685
BRIP59528a	*Gossia* sp.	Qld, Australia	−16,812	145,685
BRIP59529a	*Rhodamnia spongiosa*	Qld, Australia	−16,812	145,685
BRIP59530a	*Leptospermum madidum subsp. sativum*	Qld, Australia	−16,82	145,642
BRIP59531a	*Tristaniopsis exiliflora*	Qld, Australia	−16,82	145,642
BRIP59532a	*Leptospermum petersonii*	Qld, Australia	−16,825	145,624
BRIP59533a	*Syzygium wilsonii subsp. wilsonii*	Qld, Australia	−16,825	145,624
BRIP59534a	*Melaleuca viminalis*	Qld, Australia	−16,825	145,624
BRIP59535a	*Gossia* sp.	Qld, Australia	−16,825	145,624
BRIP59536a	*Eugenia reinwardtiana*	Qld, Australia	−16,825	145,624
BRIP59537a	*Rhodamnia sessiliflora*	Qld, Australia	−17,037	145,613

Table 1 (continued)

Herbarium or specimen code[a,b]	Host	Location[c]	Latitude	Longitude
BRIP59538a	*Rhodamnia* sp.	Qld, Australia	−17,037	145,613
BRIP59539a	*Gossia myrsinocarpa*	Qld, Australia	−17,037	145,613
BRIP59540a	*Syzygium sayeri*	Qld, Australia	−17,037	145,613
BRIP59541a	*Melaleuca viridiflora*	Qld, Australia	−16,98	145,552
BRIP59542a	*Melaleuca* sp.	Qld, Australia	−16,98	145,552
BRIP59543a	*Syzygium jambos*	Qld, Australia	−16,876	145,757
BRIP59544a	*Syzygium kuranda*	Qld, Australia	−16,831	145,667
BRIP59545a	*Gossia myrsinocarpa*	Qld, Australia	−16,804	145,636
BRIP59546a	*Tristaniopsis exiliflora*	Qld, Australia	−16,804	145,636
BRIP59547a	*Rhodamnia spongiosa*	Qld, Australia	−16,804	145,636
BRIP59548a	*Decaspermum humile*	Qld, Australia	−16,804	145,636
BRIP59549a	*Rhodomyrtus pervagata*	Qld, Australia	−16,796	145,622
BRIP59550a	*Eucalyptus tereticornis*	Qld, Australia	−16,699	145,529
BRIP59551a	*Melaleuca nervosa*	Qld, Australia	−16,699	145,529
BRIP59552a	*Backhousia hughesii*	Qld, Australia	−16,681	145,519
BRIP59553a	*Melaleuca nervosa*	Qld, Australia	−16,658	145,477
BRIP59554a	*Rhodomyrtus effusa*	Qld, Australia	−16,582	145,321
BRIP59555a	*Rhodomyrtus pervagata*	Qld, Australia	−16,582	145,321
BRIP59556a	*Gossia lewisensis*	Qld, Australia	−16,594	145,284
BRIP59557a	*Rhodomyrtus canescens*	Qld, Australia	−16,588	145,275
DAR80674	*Agonis flexuosa*	NSW, Australia	−33,224	151,219
DAR80675	*Syncarpia glomuifera*	NSW, Australia	−33,224	151,219
DAR80678	*Agonis flexuosa*	NSW, Australia	−33,224	151,219
MR73	*Syzygium apodophyllum*	Qld, Australia	−16,898	145,747
MR74	*Myrtus communis*	Vic, Australia	−37,807	144,953
MR75	*Melaleuca quinquenervia*	Qld, Australia	−25,191	153,151
MR77	*Rhodamnia maideniana*	Qld, Australia	−28,209	153,270
MR78	*Syzygium jambos*	Qld, Australia	−28,209	153,270
M10-13851	*Melaleuca quinquinervia*	NSW, Australia	−33,389	151,469
M10-14107	*Melaleuca quinquinervia*	NSW, Australia	−33,271	151,422
M10-15047	*Rhodamnia rubescens*	NSW, Australia	−33,173	151,261
ON10/0304	*Tristania neriifolia*	NSW, Australia	np[d]	np
ON10/0307	*Tristania neriifolia*	NSW, Australia	np	np
O10-00033	*Metrosideros collina*	NSW, Australia	−33,320	151,179
O10-00113	*Tristania neriifolia*	NSW, Australia	−33,683	151,227
PPS001	*Austromyrtus inophloia*	Qld, Australia	−27,345	153,010
PPS002	*Austromyrtus inophloia*	Qld, Australia	−27,364	153,016
PPS003	*Austromyrtus inophloia*	Qld, Australia	−27,080	152,933
PPS004	*Syzygium australe* 'Golden Hedge'	Qld, Australia	−27,080	152,933
PPS009	*Austromyrtus inophloia*	Qld, Australia	−27,108	152,947
PPS010	*Austromyrtus inophloia*	Qld, Australia	−27,584	153,302
PPS013	*Austromyrtus inophloia*	Qld, Australia	−27,471	153,095
PPS021	*Austromyrtus inophloia*	Qld, Australia	−26,654	153,081
PPS022	*Austromyrtus inophloia*	Qld, Australia	−26,804	153,124
PPS023	*Austromyrtus inophloia*	Qld, Australia	−27,207	153,052
35-15	*Lophomyrtus* sp.	Tas, Australia	np	np
42-15	*Lophomyrtus* sp.	Tas, Australia	np	np
65-15	*Ugni molinae*	Tas, Australia	np	np
87-15	*Lophomyrtus* sp.	Tas, Australia	np	np

Table 1 (continued)

Herbarium or specimen code[a,b]	Host	Location[c]	Latitude	Longitude
NC3	*Syzygium jambos*	Farino, NC	np	np
NC4	*Syzygium jambos*	Farino, NC	np	np
NCC	*Syzygium jambos*	Farino, NC	np	np
NCE	*Eugenia gacognei*	Mare, NC	np	np
HMAS242567	*Syzygium jambos*	Hainan, China	np	np
HAW45011	*Syzygium jambos*	Oahu, USA	np	np

[a] BRIP=Queensland Plant Pathology Herbarium, DAR=Orange Agricultural Institute, HMAS=Herbarium Mycologicum Academiae Sinicae, HAW=Joseph F. Rock Herbarium

[b] Herbarium specimens with a code not beginning with BRP, DAR, HMAS or HAW were not retained

[c] *Qld* Queensland, *NC* New Caledonia, *NSW* New South Wales, *Tas* Tasmania, *USA* United States of America

[d] *np* not provided

in the majority of the Australian collections and samples from New Caledonia, Hawaii, and China (Table 2). However, in five collections from Australia (BRIP59525a, BRIP59529a, BRIP59543a, BRIP59545a and 65-15) an unusual allele was detected at four loci (Table 2). The first four of these collections were from Cairns and surrounds on three different hosts and the fifth from yet another host species in Tasmania (Table 2). Genotyping was repeated 2–3 times for these collections from the original DNA, providing the same result each time. Further DNA was extracted from additional pustules from the same collections, with variable genotyping results (Table 2). In three instances, further variation at the variable locus was detected; in the other two, the common MLG was detected.

Discussion

Low genetic variation was demonstrated in *P. psidii* collections from Australia, New Caledonia and China using six microsatellite loci previously shown to be polymorphic among different *P. psidii* populations (Zhong et al. 2008, 2011; Graça et al. 2013). The same heterozygous genotype found among the majority of collections in Australia indicates

Table 2 Allele sizes for microsatellite loci of collections from Australia, New Caledonia (NC) and China. Variant allele sizes are in *bold* text

Collection	Locus					
	503	504	507	508	510	513
MR1[a]	217, 219	155, 157	162, 171	173, 179	68, 78	215, 227
NC3[b]	217, 219	155, 157	162, 171	173, 179	68, 78	215, 227
HMAS242567	217, 219	155, 157	162, 171	173, 179	68, 78	215, 227
HAW45011	217,219	155, 157	162,171	173,179	68,78	215, 227
BRIP59525a (pustule 1)	**215**, **217**	155, 157	162, 171	173, 179	68, 78	215, 227
BRIP59525a (pustule 2)	**213**, **215**	155, 157	162, 171	173, 179	68, 78	215, 227
BRIP59525a (pustule 3)	217, 219	155, 157	162, 171	173, 179	68, 78	215, 227
BRIP59529a (pustule 1)	217, 219	155, 157	162, 171	173, 179	68, 78	**215**, **221**
BRIP59529a (pustule 2)	217, 219	155, 157	162, 171	173, 179	68, 78	**215**, **223**
BRIP59543a (pustule1)	217, 219	155, 157	162, 171	**175**, **177**	68, 78	215, 227
BRIP59543a (pustule2)	217, 219	155, 157	162, 171	**177**, **179**	68, 78	215, 227
BRIP59545a (pustule 1)	217, 219	155, 157	162, 171	173, 179	68, 78	**215**, **215**
BRIP59545a (pustule 2)	217, 219	155, 157	162, 171	173, 179	68, 78	215, 227
65-15 (pustule 1)	217, 219	155, 157	162, 171	173, 179	68, 78	215, 227
65-15 (pustule 2)	217, 219	155, 157	**162**, **173**	173, 179	68, 78	215, 227

Four collections from the Tiwi Islands, Northern Territory, had multilocus genotypes consistent with MR1 and the majority of collections from mainland Australia

[a] MR1 and the majority of collections (=99) from Australia have this same genotype

[b] All collections from New Caledonia have the same genotype

a lack of genetic recombination and no selection by host, consistent with a recent introduction of a single, clonally-reproducing rust genotype in Australia. Although teliospores were identified in 20 % of the samples in a survey in Queensland (Pegg et al. 2013), the lack of recombination and structure of Australian collections is consistent with the lack of recombination in the Hawaiian rust population (Zhong et al. 2011; Graça 2011), where the pathogen was reported 9 years ago (Uchida et al. 2006). The low variability in the Australian population is consistent with clonal reproduction, precluding analysis with GenAlex 6.4 (Peakall and Smouse 2006). The collections that showed an unusual allele size were from Cairns and surrounds from three different hosts as well as from a fourth host species in Tasmania and no correlation was found among host, allele size or loci, indicating that these mutations are random occurrences. Similar levels of mutation have been observed in clonal populations of *Puccinia triticina* in wheat cultivars, where a strong correlation between genotype and pathotype has been demonstrated (Goyeau et al. 2007).

Microsatellite markers have been used to infer the origin of the *P. psidii* incursion in Hawaii. A unique genotype found in four Hawaiian Islands (Maui, Oahu, Kauai, and Big Island) was also found in two collections from different hosts in California, indicating that California may have been the source of the *P. psidii* introduction into Hawaii, probably by the trade of Myrtaceae plant between both states (Graça 2011). The origin of the genotype in California is unknown.

The collections from New Caledonia and China and the majority of Australian collections have the same genotype as that present in Hawaii. Although *P. psidii* was reported first in Hawaii followed by China, Australia and most recently, New Caledonia, it is not possible to confirm the origin of the incursion in these countries, unlike in Japan where the rust was detected on *Metrosideros* plants imported from Hawaii (Kawanishi et al. 2009). The rust may have been distributed from California to all of the other countries or may have travelled from e.g., California to Hawaii, from Hawaii to China, from China to Australia, and finally from Australia to New Caledonia. The multilocus genotypes of the *P. psidii* population in South Africa is unknown.

In contrast with the rust populations in Australia and Hawaii, the genetic variability of *P. psidii* collections in Brazil is high. In a recent study based on analysis of 10 microsatellite loci in 148 *P. psidii* collections from seven host species (Graça et al. 2013), all loci were polymorphic and strong selection by host species regardless of geographic location was demonstrated. As no evidence of recent sexual recombination among the host populations on guava and eucalypts, it is likely that they have become differentiated by a series of mutations similar to those observed in the Australian population. As the mutations accumulate, the mutants that are better adapted to a particular host species would have a better chance of survival and eventually a strain that has a MLG quite different to the original would evolve. Despite the high genetic variability and broad distribution of this pathogen in Brazil, the genotype present in Australia, Hawaii and California has not been detected in Brazil (Graça 2011). It may be present at low levels in native vegetation in Brazil or may have arisen outside of Brazil.

The source and pathway of the incursion in Australia is unknown. Although the country has a continental size, wind combined with susceptible host and suitable climatic conditions provided a near-continuous corridor where the spores of the pathogen were spread along the east coast, also assisted by human movement of host plants (Carnegie and Lidbetter 2012). There is also evidence of aerial dispersal of two other rust species, *Melampsora larici-populina* Klebah and *Melampsora medus*a Thümenth, from the east coast of Australia to New Zealand across the Tasman Sea (Close et al. 1978). Whether this also occurred with *P. psidii* moving between Australia and New Caledonia is unknown. Besides airflows, the commercial trade of plants and movements of people and commodities are likely to be the other long-distance dispersal pathways for pathogen spores (Sheridan 1989; Williams et al. 2000).

In Brazil the populations of *P. psidii* collected on different host species are genetically distinct (Graça et al. 2013). This contrast with the population of the pathogen in Australia, where 5 years after the first report of the pathogen in this country a few mutants of the dominant genotype were observed. Artificial inoculations showed that at least 107 native host species in 30 genera are susceptible to this predominant genotype (Carnegie and Lidbetter 2012). While mutations in microsatellite loci are unlikely to affect host range, and the persistence of mutant genotypes in the population has not yet been demonstrated, this indicates the potential for genetic changes in genomic regions that may affect host adaptation and the possible emergence of new pathotypes. This has been demonstrated in Brazil where the genotype that is widespread on eucalyptus has mutated to create a new race that has overcome rust resistance (Graça et al. 2011). Thus, avoiding the introduction of new *P. psidii* genotypes into, and dispersal around the country in areas of high Myrtaceae biodiversity which have not previously been exposed to this rust, is highly desirable.

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