Genetic and Genotypic Diversity of *Puccinia psidii*—The Cause of Guava/Eucalypt/Myrtle Rust—And Preliminary Predictions of Global Areas at Risk

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INTRODUCTION

Puccinia psidii (Basidiomycota, Uredinales) is a biotrophic rust fungus that was first reported in Brazil from guava (*Psidium guajava*) in 1884 (Winter 1884) and later from nonnative eucalypt (*Eucalyptus capitelata* and *Corymbia citriodora*, syn. *Eucalyptus citriodora*; Joffily 1944). Considered to be of Neotropical origin, the rust has also been reported to infect diverse myrtaceous hosts elsewhere in South America, Central America, the Caribbean, Mexico, the United States (California, Florida, and Hawaii), Japan, Australia, China, and most recently South Africa and New Caledonia (Carnegie et al. 2010; Giblin 2013; Kawanishi et al. 2009; MacLachlan 1938; Marlatt and Kimbrough 1979; Mellano 2006; Pérez et al. 2011; Roux et al. 2013; Uchida et al. 2006; Zambino and

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Table 1—Geographic origin and host of confirmed *Puccinia psidii* genotypes.

Origin	Host	Cluster[a]
Australia	*Pilidiostigma glabrum*	Pandemic
	Backhousia oligantha	Pandemic
	Rhodomyrtus psidioides	Pandemic
Brazil	*Eucalyptus* spp.	Eucalypt
	Eugenia uniflora	Guava
	Myrciaria cauliflora	Guava
	Psidium guajava	Guava
	Psidium guineenese	Guava
	Syzygium cumini	Guava
	Syzygium jambos	Eucalypt
Costa Rica	*Callistemon lanceolatus*	Pandemic
	Syzygium jambos	Pandemic
Jamaica	*Pimenta dioica*	Allspice
	Syzygium jambos	Pandemic
Mexico	*Syzygium jambos*	Pandemic
Paraguay	*Eucalyptus grandis*	Eucalypt
Puerto Rico	*Syzygium jambos*	Pandemic
Uruguay	*Psidium guajava*	Guava
	Eucalyptus grandis	Eucalypt
	Eucalyptus globulus	Eucalypt
	Myrrhinium atropurpurea	Guava
United States	*Eugenia koolauensis*	Pandemic
	Melaleuca quinquenervia	Pandemic
	Metrosideros excelsa	Pandemic
	Metrosideros polymorpha	Pandemic
	Myrcianthes fragrans	Pandemic
	Myrtus communis	Pandemic
	Rhodomyrtus tomentosa	Pandemic
	Syzygium jambos	Pandemic

[a] (1) Allspice = collections from *Pimenta dioica* from Jamaica; (2) Pandemic = collections from diverse hosts from Australia, the Caribbean, Mexico, and the United States; (3) Eucalypt = collections from *Eucalyptus* spp. and *Syzygium jambos* from Brazil, Paraguay, and Uruguay; (4) Guava = collections from *Psidium guajava*, *Psidium guineense*, *Syzygium cumini*, *Myrciaria cauliflora*, *Eugenia uniflora*, and *Myrrhinium atropurpureum* var *octandrum* from Brazil and Uruguay.

Nolan 2011; Zhuang and Wei 2011). Given the rate at which the pathogen is spreading and its wide host range, the objectives of this study are to determine how much genetic diversity exists within populations across the species' putative native range as well as areas of recent introduction, evaluate possible pathways of spread, and predict the geographic area that is climatically suitable for the species to determine areas at risk of invasion.

METHODS

Six microsatellite loci were scored for *P. psidii* samples derived from diverse hosts and geographic locations (table 1). Samples were assigned to genetic clusters using a Bayesian genetic clustering algorithm implemented in STRUCTURE v2.3.4 (Pritchard et al. 2000). Posterior probabilities were estimated for $K = 1$ to $K = 12$ assuming an admixture model.

Table 2—Summary of genetic variation among clusters of *Puccinia psidii*.

	Allspice[a]	Pandemic	Eucalypt	Guava
Alleles per locus	2.50 (0.43)	3.50 (0.67)	2.33 (0.21)	4.83 (0.60)
Effective alleles per locus	2.26 (0.35)	2.91 (0.45)	1.97 (0.25)	3.40 (0.39)
Private alleles	4	9	1	11
Shannon's index	0.78 (0.18)	1.07 (0.16)	0.70 (0.12)	1.35 (0.12)
Number of MGs	5	9	6	6
Observed heterozygosity	0.77 (0.16)	1.00 (0.00)	0.69 (0.19)	0.61 (0.13)
Expected heterozygosity	0.49 (0.10)	0.62 (0.05)	0.44 (0.08)	0.69 (0.04)
Fixation index	-0.62 (0.16)	-0.67 (0.13)	-0.39 (0.31)	0.14 (0.16)

[a]Clusters of Allspice, Pandemic, Eucalypt, and Guava are described in Table 1.

STRUCTURE analyses were performed with clone-corrected data (unique multilocus genotypes, MGs). Fifty thousand burn-in generations were employed for each of 10 replicate runs of 1,000,000 generations of the Markov chain Monte Carlo (MCMC) sampler for each *K*. The optimal value of *K* was inferred using the method of Evanno et al. (2005). Population genetic analyses of all samples grouped by genetic cluster were performed using GenAlEx v6.501 (Peakall and Smouse 2006). To examine relationships among MGs, a minimum spanning network was estimated using the genetic distance measure of Bruvo et al. (2004). Bioclimatic modeling was performed using all documented rust occurrences and reduced sets of the eucalypt, pandemic, and guava biotypes coupled with 19 bioclimatic variables in MaxEnt (Maximum Entropy Species Distribution Modeling) v3.3.3K. Cross-validation was used to verify results among 10 replicate runs. Quantum GIS was used to create the final output in a cumulative format according to prediction probability.

RESULTS AND DISCUSSION

Among the *P. psidii* samples collected from Australia, Brazil, Costa Rica, Jamaica, Mexico, Paraguay, Puerto Rico, Uruguay, and the United States, 26 unique MGs were found. A Bayesian clustering analysis and a minimum spanning network revealed four major genetic clusters among the MGs: (1) Allspice = collections from *Pimenta dioica* from Jamaica; (2) Pandemic = collections from diverse hosts from Australia, the Caribbean, Mexico, and the United States; (3) Eucalypt = collections from Eucalyptus species and *Syzygium jambos* from Brazil, Paraguay, and Uruguay; and (4) Guava = collections from *Psidium guajava*, *Psidium guineense*, *Syzygium cumini*, *Myrciaria cauliflora*, *Eugenia uniflora*, and *Myrrhinium atropurpureum* var. *octandrum* from Brazil and Uruguay (table 1; figs. 1 and 2).

All loci were polymorphic, with 5 to 11 alleles detected per locus. Genetic diversity was highest within the guava cluster, followed by the pandemic, the allspice, and finally the eucalypt clusters (table 2). Similar to previous attempts to assess the geographic invasive potential of the species (Booth and Jovanovic 2012; Booth et al. 2000; Elith et al. 2013; Glen et al. 2007; Kriticos et al. 2013; Magarey et al. 2007), the model using all records of occurrence projects many areas throughout the world as having some degree of probability of suitable climate for *P. psidii* (fig. 3). Models using reduced sets of the eucalypt, pandemic, and guava biotypes show predictions of different distributions (figs. 3 and 4). Inoculation tests can also help to assess threats posed by different pathogen biotypes. For example, Silva et al. (2014) showed that the eucalypt biotype was highly virulent on Hawaiian ʼōhiʼa lehua (*Metrosideros polymorpha*) plants (fig. 5), and Zauza et al. (2010) showed that diverse myrtaceous species from Australia varied greatly in their resistance/susceptibility to one race of the eucalypt biotype.

When bioclimatic modeling is coupled with inoculation tests, it suggests that different biotypes pose different risks to various global regions, should they be introduced. This work builds on previous research that shows that diversity must be considered when assessing the invasive threat posed by this pathogen

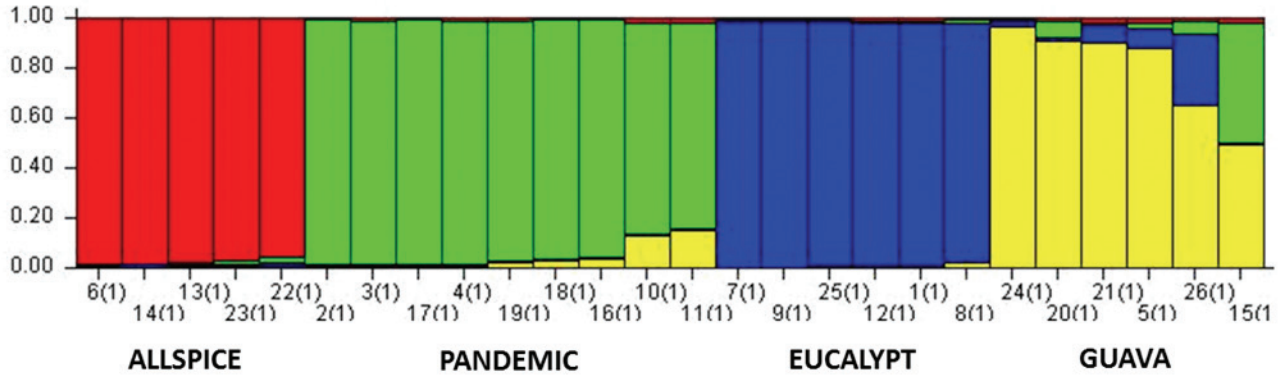

Figure 1—A Population structure inferred using a Bayesian clustering algorithm implemented in STRUCTURE at *K* = 4 (estimated ln probability = -418.9).

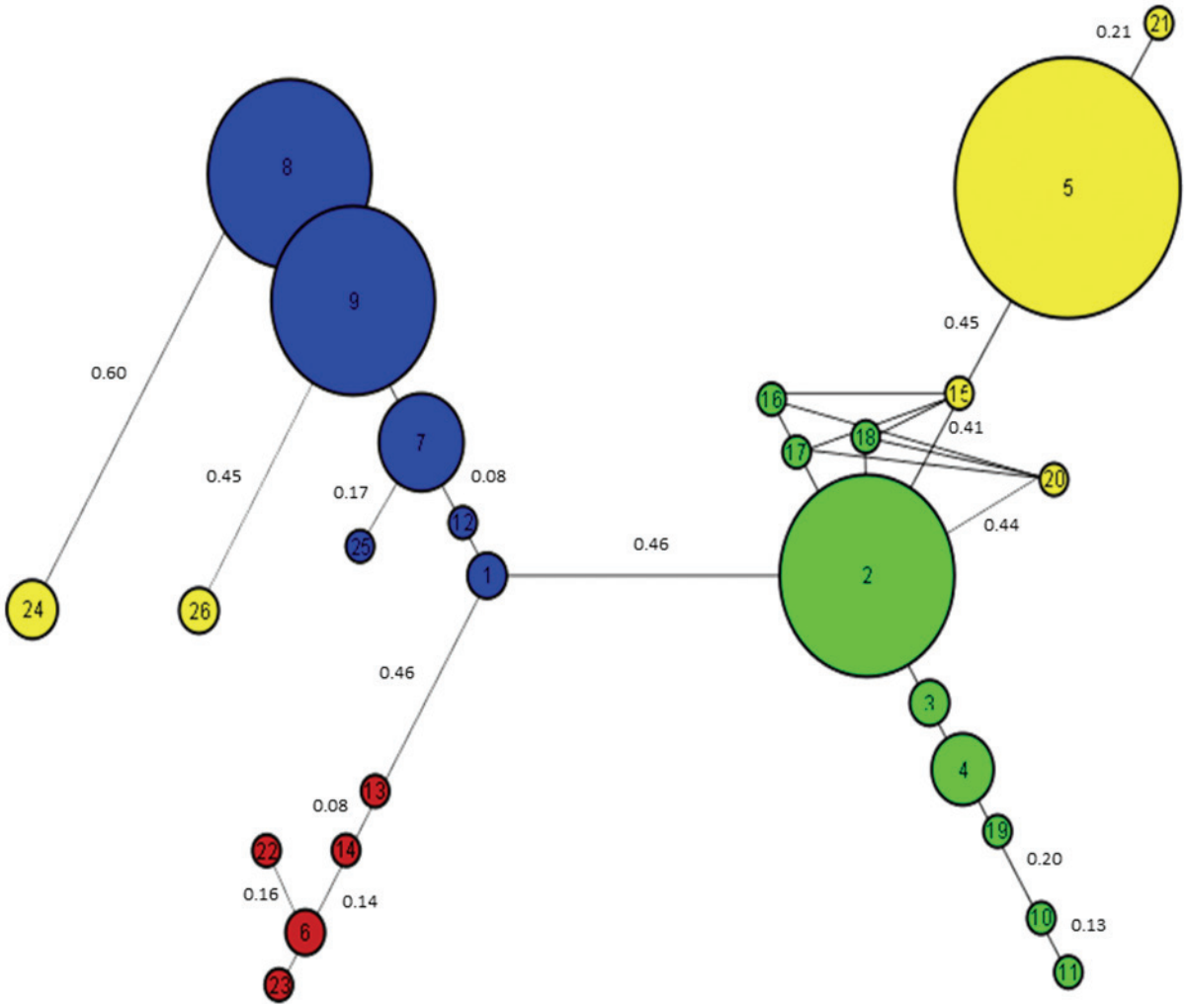

Figure 2—Minimum spanning network of multilocus genotypes represented by red for collections from *Pimenta dioica* (Jamaica), blue for collections from *Eucalyptus* spp. and *Syzygium jambos* (Brazil, Paraguay, and Uruguay), green from the pandemic cluster (which includes collections from diverse hosts from the Australia, the Caribbean, Mexico, and United States), and yellow from other hosts (collections from *Psidium guajava*, *Psidium guineense*, *Syzygium cumini*, *Myrciaria cauliflora*, *Eugenia uniflora*, and *Myrrhinium atropurpureum* var *octandrum*). Sizes of circles are proportional to MG frequency and connections are labeled with Bruvo genetic distances if different from 0.06, which corresponds to one mutational step at one locus. Loops in the network indicate multiple tied minimum spanning trees.

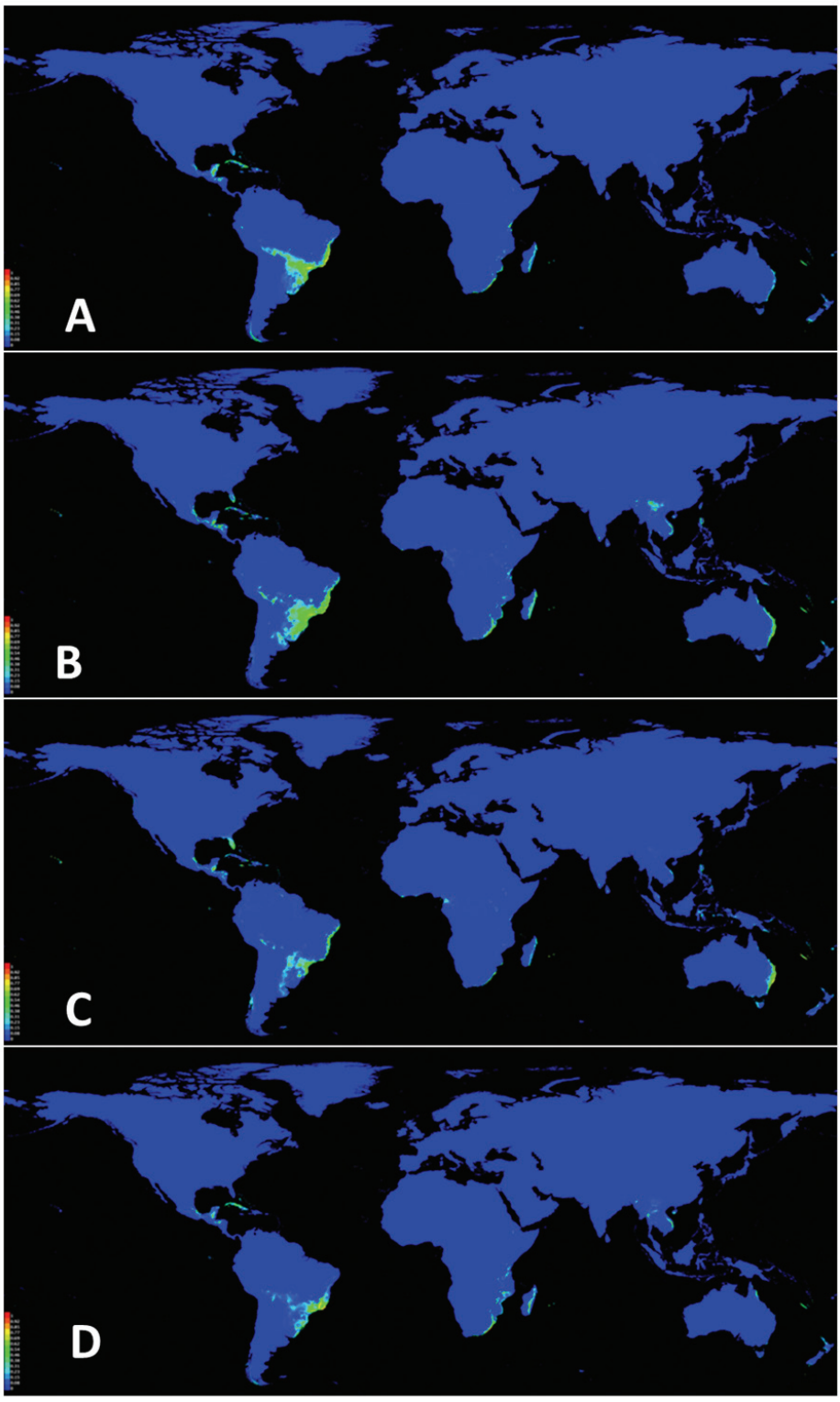

Figure 3—Maxent models of suitable climate space (potential distribution) for *Puccinia psidii* based on 19 global bioclimatic variables derived from the WorldClim (worldclim.org) database for A all biotypes (245 occurrence points); B eucalypt biotype (88 occurrence points); C pandemic biotype (89 occurrence points); and D guava biotype (65 occurrence points).

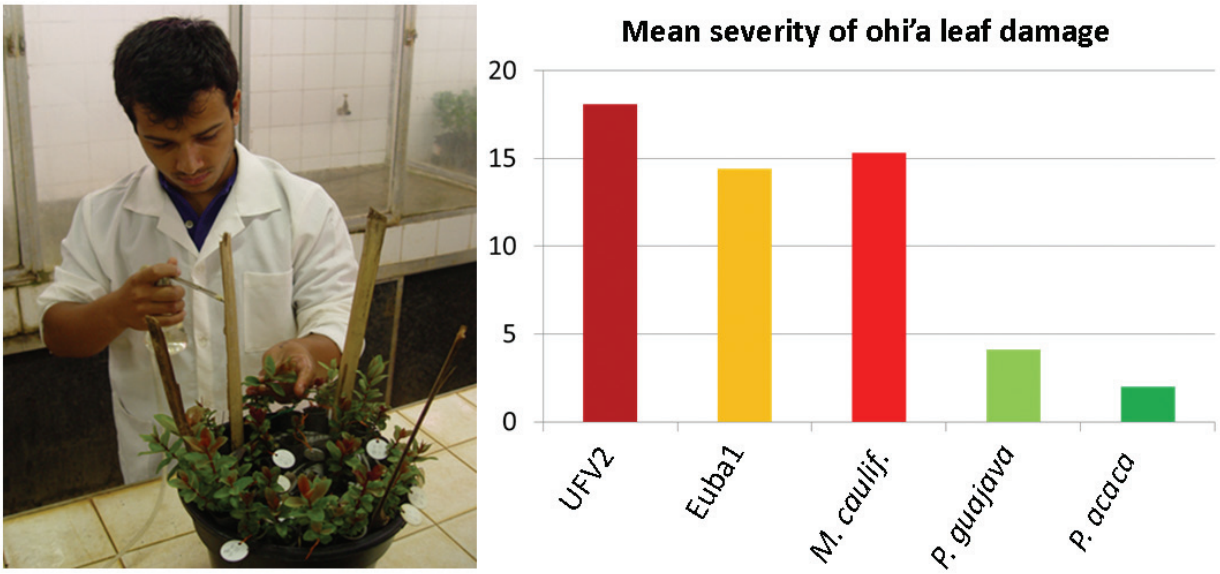

Figure 4—Assessment of Brazilian strain virulence to Hawaiian ʼŌhiʼa (*Metrosideros polymorpha*) (Silva et al. 2014). The Brazilian *Puccinia psidii* eucalypt biotype was highly virulent on Hawaiian ʼōhiʼa plants.

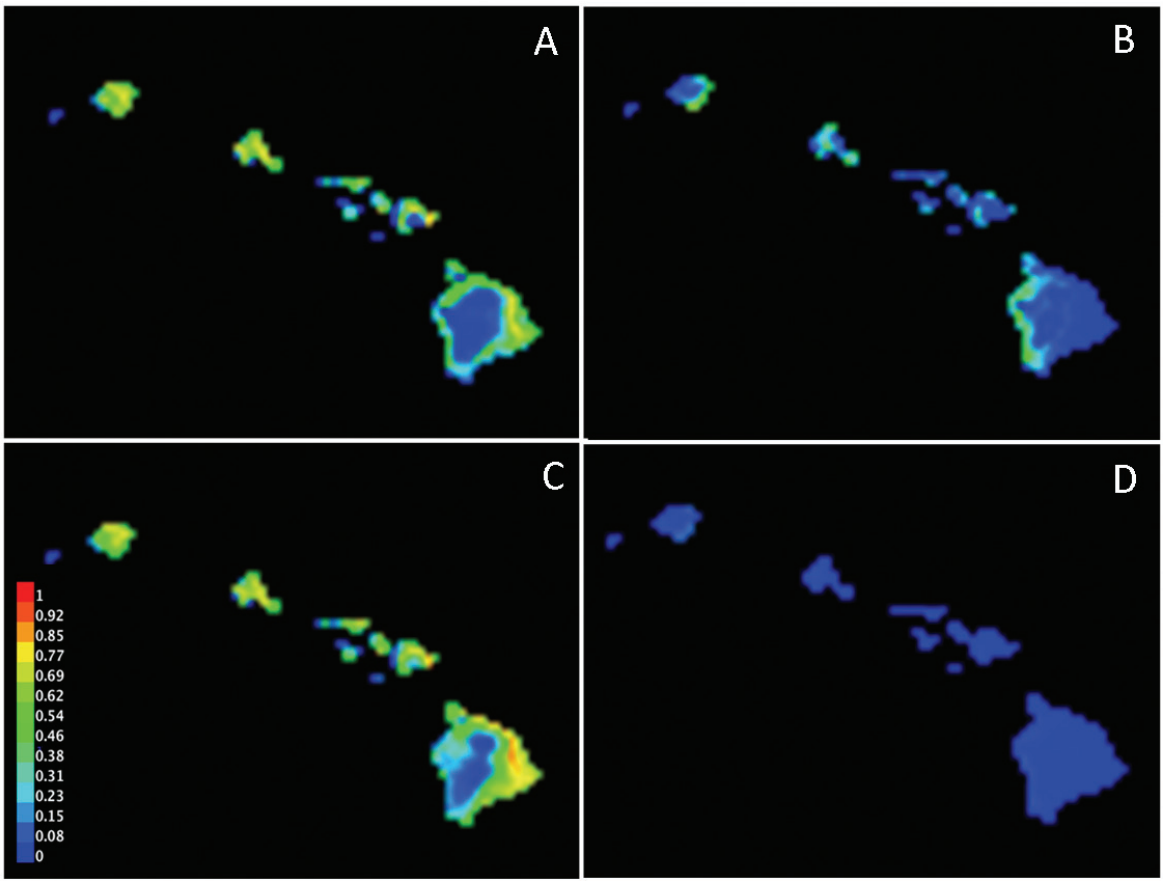

Figure 5—Maxent models of suitable climate space (potential distribution) for *Puccinia psidii* in Hawaii based on 19 global bioclimatic variables derived from the WorldClim (worldclim.org) database for A all biotypes; B eucalypt biotype; C pandemic biotype; and D guava biotype.

to myrtaceous hosts worldwide (Graça et al. 2013). Future studies that include *P. psidii* genotypes from additional geographic areas will allow a better prediction of global geographic areas that are climatically suitable for *P. psidii* and for subspecies-level genetic clusters, which will provide a more robust assessment for pathways of spread and areas at risk of invasion.

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REFERENCES

Booth, T.H.; Old, K.M.; Jovanovic, T. 2000. A preliminary assessment of high risk areas for *Puccinia psidii* (Eucalyptus rust) in the Neotropics and Australia. Agriculture Ecosystems and Environment. 82: 295–301.

Booth, T.H.; Jovanovic, T. 2012. Assessing vulnerable areas for *Puccinia psidii* (eucalyptus rust) in Australia. Australasian Plant Pathology. 41: 425–429.

Bruvo, R.; Michiels, N.K.; D'Souza, T.G.; [et al.]. 2004. A simple method for the calculation of microsatellite genotype distances irrespective of ploidy level. Molecular Ecology. 13: 2101–2106.

Carnegie, A.J.; Lidbetter, J.R.; Walker, J.; [et al.]. 2010. *Uredo rangelii*, a taxon in the guava rust complex, newly recorded on Myrtaceae in Australia. Australasian Plant Pathology. 39: 463–466.

Elith, J.; Simpson, J.; Hirsch, M.; [et al.]. 2013. Taxonomic uncertainty and decision making for biosecurity: Spatial models for myrtle/guava rust. Australasian Plant Pathology. 42: 43–51.

Evanno, G.; Regnaut, S.; Goudet, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. Molecular Ecology. 14: 2611–2620.

Giblin, F. 2013. Myrtle rust report: New Caledonia. Maroochydore, Queensland: University of the Sunshine Coast. 18 p.

Glen, M.; Alfenas, A.C.; Zauza, E.A.V.; [et al.]. 2007. *Puccinia psidii*: A threat to the Australian environment and economy—A review. Australasian Plant Pathology. 36: 1–16.

Graça, R.N.; Ross-Davis, A.L.; Klopfenstein, N.B.; [et al.]. 2013. Rust disease of eucalypts, caused by *Puccinia psidii*, did not originate via host jump from guava in Brazil. Molecular Ecology. 22: 6033–6047.

Joffily, J. 1944. Ferrugem do eucalipto. Bragantia. 4: 475–487.

Kawanishi, T.; Uemastu, S.; Kakishima, M.; [et al.]. 2009. First report of rust disease on ohia and the causal fungus, *Puccinia psidii*, in Japan. Journal of Genetic Plant Pathology. 75: 428–431.

Kriticos, D.J.; Morin, L.; Leriche, A.; [et al.]. 2013. Combining a climatic niche model of an invasive fungus with its host species distributions to identify risks to natural assets: *Puccinia psidii sensu lato* in Australia. PLoS ONE. 8(5): e64479. doi:10.1371/journal.pone.0064479.

MacLachlan, J.D. 1938. A rust of the pimento tree in Jamaica, BWI. Phytopathology. 28: 157–170.

Magarey R.D.; Fowler. G.A.; Borchert, D.M.; [et al.]. 2007. NAPPFAST: An internet system for the weather-based mapping of plant pathogens. Plant Disease. 91: 336–345.

Marlatt, R.B.; Kimbrough, J.W. 1979. *Puccinia psidii* on *Pimenta dioica* in south Florida. Plant Disease Reporter. 63: 510–512.

Mellano, V. 2006. Rust on myrtle found in San Diego County. Healthy Garden-Healthy Home, University of California Cooperative Extension Retail Nursery Newsletter. 1: 3.

Peakall, R.; Smouse, P.E. 2006. GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes. 6: 288–295.

Pérez, C.A.; Wingfield, M.J.; Altier, N.A.; [et al.]. 2011. *Puccinia psidii* infecting cultivated Eucalyptus and native myrtaceae in Uruguay. Mycological Progress. 10: 273–282.

Pritchard, J.K.; Stephens, P.; Donnelly, P. 2000. Inference of population structure using multilocus genotype data. Genetics. 155: 945–959.

Roux, J.; Greyling, I.; Coutinho, T.A.; [et al.]. 2013. The Myrtle rust pathogen, *Puccinia psidii*, discovered in Africa. IMA Fungus. 4: 155–159.

Silva, A.C.; Andrade, P.M.T.; Alfenas, A.C.; [et al.]. 2014. Virulence and impact of Brazilian strains of *Puccinia psidii* on Hawaiian 'ohi'a (*Metrosideros polymorpha*). Pacific Science. 68: 47–56.

Uchida, J.; Zhong, S.; Kilgore, E. 2006. First report of a rust disease on 'ōhi'a caused by *Puccinia psidii* in Hawaii. Plant Disease. 90: 524.

Winter, G. 1884. Repertorium. Rabenhorstii fungi europaei et extraeuropaei, Centuria XXXI et XXXII. Hedwigia. 23: 164–172.

Zambino P.J.; Nolan, P.A. 2011. First report of rust caused by *Puccinia psidii* on paperbark, *Melaleuca quinquenervi*a, in California. Plant Disease. 95: 1314–1315.

Zauza, E.A.V.; Alfenas, A.C.; Old, K.; [et al.]. 2010. Myrtaceae species resistance to rust caused by *Puccinia psidii*. Australasian Plant Pathology. 39: 406–411

Zhuang, J.-Y.; Wei, S.-X. 2011. Additional materials for the rust flora of Hainan Province, China. Mycosystema. 30: 853–860.