



Multilocus Genotypes Indicate Differentiation Among *Puccinia psidii* Populations from South America and Hawaii

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ABSTRACT

Puccinia psidii is the cause of rust disease of many host species in the Myrtaceae family, including guava (*Psidium* spp.), eucalypt (*Eucalyptus* spp.), rose apple (*Syzygium jambos*), and 'ohi'a (*Metrosideros polymorpha*). First reported in 1884 on guava in Brazil, the rust has since been detected in South America (Argentina, Brazil, Colombia, Paraguay, Uruguay, and Venezuela), Central America (Costa Rica and Panama), Caribbean (Cuba, Dominica, Dominican Republic, Jamaica, Puerto Rico, Trinidad and Tobago, and Virgin Islands), Mexico, USA (Florida, California, and Hawaii), Japan, and most recently it was potentially found in Australia. Of present concern is the recent introduction of the pathogen to Hawaii, where it infects an endemic tree species known as 'ohi'a, the dominant tree species in Hawaii's remaining native forests. The rust also poses serious threats to several Myrtaceae species including Eucalyptus, a genus native to Australia and planted extensively in numerous tropical and subtropical countries. Despite the potential threat toward many forest ecosystems worldwide and the expanding geographic range of this disease, little is known about the genetic structure of *P. psidii* populations, migratory routes and sources of introductions. To determine genetic structure

of pathogen populations in the putative center of origin, approximately 150 single-pustule isolates of *P. psidii* have been collected from diverse host species and locations in Brazil. These isolates were scored for variation at 10 microsatellite loci. Additional isolates have been collected from Hawaii, California, Paraguay, and Uruguay; and collaborators are currently being sought to obtain isolates from other regions. Collections from reputedly introduced populations will facilitate inferences about the spread of this rust pathogen throughout the world. Preliminary results for Brazil indicate that: 1) considerable genetic diversity is present; 2) species of hosts strongly influence population structure; and 3) distinct multilocus genotypes are uniquely associated with specific hosts across diverse geographic locations. In contrast, all 49 rust isolates collected from five different hosts in Hawaii (Oahu, Maui, Kauai, and Hawaii - "Big Island") share a unique, multilocus genotype, indicating a recent introduction of a single rust genotype. This information will help identify rust races that pose threats to global populations of Myrtaceae and help prevent their introduction into new regions. For example, none of the rust genotypes in Brazil are known to occur in Hawaii. At present, it seems prudent to focus on avoiding the introduction of any novel genotypes to new regions with populations of known hosts. Furthermore, potential recombination of pathogen genotypes could generate new virulent races with unpredictable consequences. Population genetics and molecular characterization of *P. psidii* isolates collected from around the world will provide critical information on pathways of spread and assessment of future risk.

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BACKGROUND

Puccinia psidii causes rust disease on many host species in the Myrtaceae family (Farr and Rossman 2010), including guava, Surinam cherry (*Eugenia uniflora*), and 'ohi'a (figure 1). First reported in 1884 on guava in south Brazil (Maclachlan 1938), the rust has since been detected in other regions of South America (Ferreira 1981; Lindquist 1982; Kern et al. 1928; Lindquist 1982; Chardon and Toro 1934),

Central America (Bernt 2004; Perdomo-Sanches and Piepenbring 2008), Caribbean (Spaulding 1961; Baker and Dale 1948; Kern et al. 1933; Dale 1955; Stevenson 1975), Mexico (Gallegos and Cummins, 1981), USA (Florida, Rayachhertry et al. 1997; California, Mellano 2006; and Hawaii; Uchida et al. 2006), and most recently in Japan (Kawanishi et al. 2009). Based on *P. psidii*'s widespread geographic distribution and its large host range, this rust pathogen is considered a serious threat to several hosts in the Myrtaceae family in numerous tropical and subtropical countries (figure 2) (Tommerup et al. 2003; Glen et al. 2007). Of special note is rust that was found infecting Myrtaceae species in Australia and reported as *Uredo rangellii*, based on the tomsure found in urediniospores surface (Carnegie et al. 2010). However, DNA sequence data did not differentiate the rust from

P. psidii. Studies are needed to elucidate the disease etiology. In Hawaii, *P. psidii* infects an endemic tree species known as 'ohi'a, the dominant tree species in Hawaii's native forests that provides habitat for much of the Hawaiian flora and fauna (Uchida et al. 2006; Loope 2010). This disease has also been highly damaging to the last remaining populations of endemic *Eugenia koolauensis*, a federally listed endangered species. Of present concern is the potential introduction of new pathogen genotypes into Hawaiian Islands (Loope 2010). However, despite the potential threats to many forest ecosystems worldwide and the expanding geographic range of this disease, little is known about the genetic structure of pathogen populations, migratory routes, and sources of introductions.

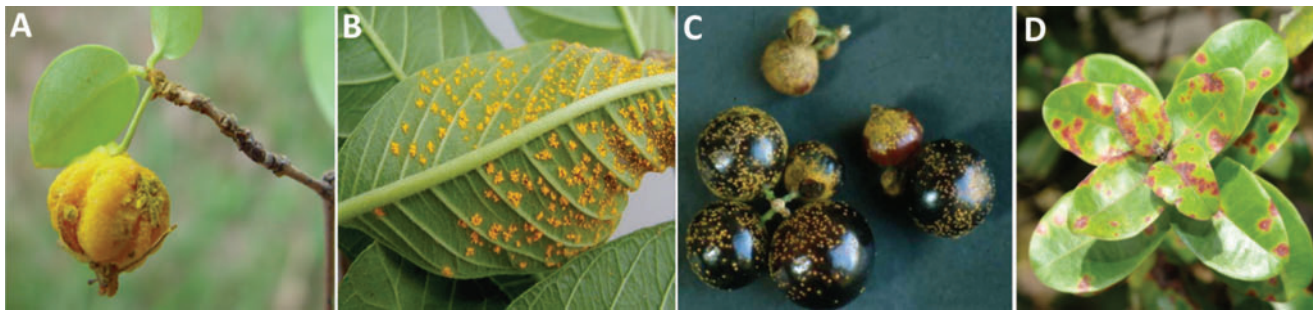


Figure 1—Some hosts of the rust caused by *Puccinia psidii*. A. *Eugenia uniflora* (Surinam cherry); B. *Psidium guajava* (guava); C. *Myrciaria cauliflora* (jaboticaba); D. *Metrosideros polymorpha* ('ohi'a) (From Alfenas et al., 2009).

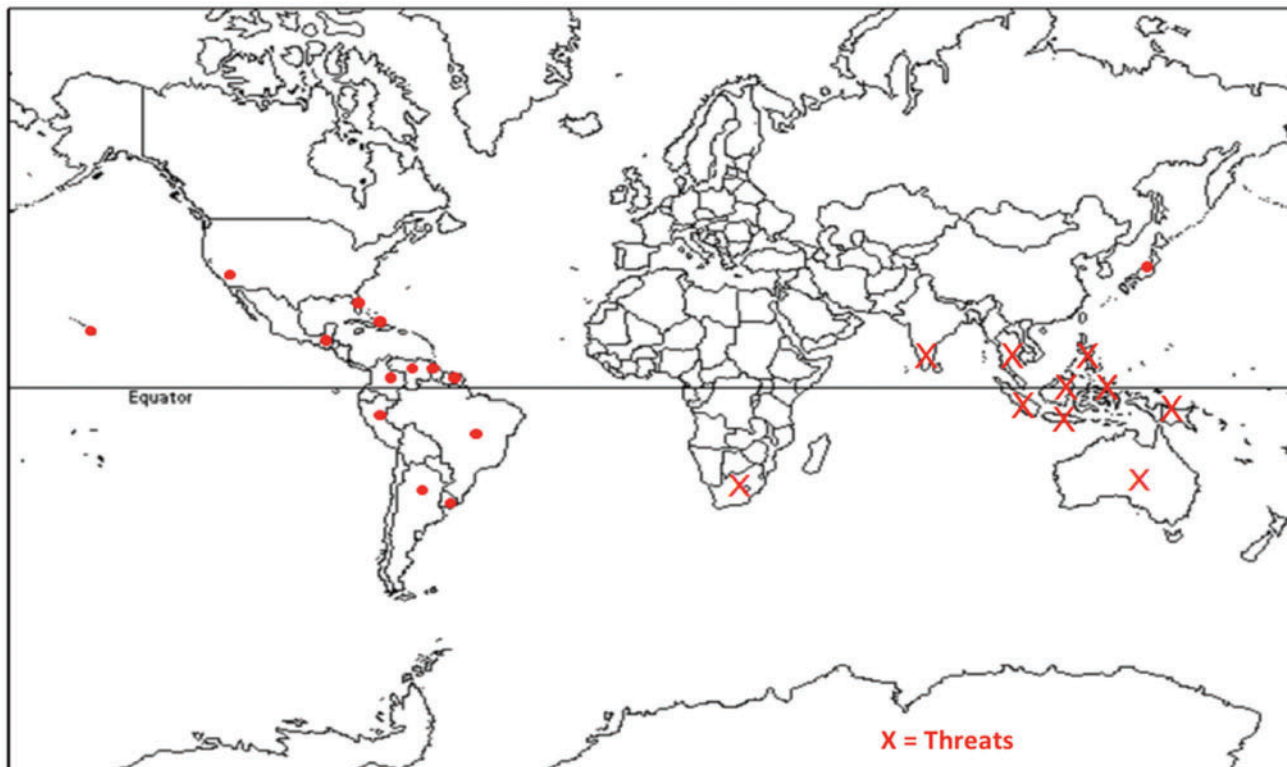


Figure 2—Current (red dot) and potential (red x) geographic distribution of *Puccinia psidii*.

OBJECTIVE

The main goal of this study is to provide baseline information to identify rust genotypes that pose threats to global populations of Myrtaceae. This information can be used to help prevent the introduction of critical rust genotypes into new regions and increase the efficacy in selecting rust-resistant host individuals.

METHODS

To determine the genetic structure of pathogen populations, approximately 150 single-uredinial isolates of *P. psidii* were collected from diverse host species and locations in Brazil, Uruguay, and Paraguay. DNA was extracted from each isolate and subsequently scored for variation at 10 microsatellite loci (Zhong et al. 2007). Additionally, 49 isolates were collected on *Metrosideros polymorpha*, *M. excelsa*, *Eugenia koolauensis*, *Syzygium jambos* and *Rhodomytrus tomentosa* from four Hawaiian Islands (Oahu, Maui, Kauai, and Hawaii - "Big Island") and, so far, scored for variation at 7 microsatellite loci (Zhong et al. 2007). Collaborators are currently being sought to sample isolates from other global regions, such as California, Florida, Puerto Rico, Central America, Colombia, Venezuela, and Japan. Collections from these diverse geographic locations will facilitate inferences about the spread of this rust pathogen throughout the world.

RESULTS

Preliminary results indicate that host species strongly influences *P. psidii* population structure in South America (figure 3). Distinct multilocus genotypes were uniquely associated with specific hosts across diverse geographic locations in South America. Higher genetic diversity of *P. psidii* was detected in Brazil compared to Hawaii, which is consistent with the hypothesis that Brazil is the evolutionary origin of this pathogen. However, additional samples from diverse global locations are needed to confirm this hypothesis. In contrast, all of the 49 Hawaiian isolates had the same multilocus genotype across the three islands despite being sampled from six different species of hosts (figure 3). The low genetic diversity in Hawaii is consistent with a recent introduction of a single rust genotype into Hawaii. Four alleles at three loci in the Hawaiian sample were not found in the South American sample (private alleles, sensu Slatkin 1985) indicating that the hosts sampled in South America were unlikely to have been the source of the introduction to Hawaii.

PRELIMINARY CONCLUSIONS

Structure of *P. psidii* populations in South America is strongly associated with species of hosts. Hosts may have selected particular *P. psidii* genotypes in South America followed by little or no gene flow among host-adapted pathogen genotypes.

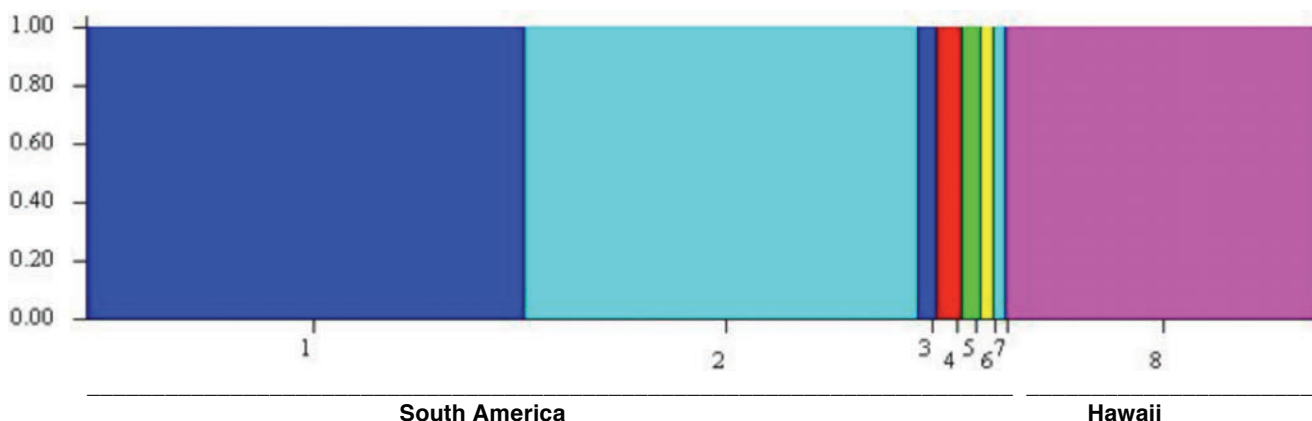


Figure 3—STRUCTURE analysis showing posterior probability (y-axis) of each isolate belonging to one of six genetic clusters (K = 6), shown in different colors. Each isolate is represented by a vertical colored bar on the x-axis, where bars of the same color represent isolates assigned to the same genetic cluster. Thus, the width of the colored bar represents the number of isolates assigned to the genetic cluster. Numbers under genetic clusters are as follows: 1 = *Eucalyptus* spp.; 2 = *Psidium guajava*; 3 = *Syzygium jambos*; 4= *Syzygium cumini*; 5 = *Myciaria cauliflora*; 6 = *Eugenia uniflora*; 7 = *Psidium araca*; and 8 = *Metrosideros polymorpha* + *Metrosideros excelsa* + *Eugenia koolauensis* + *Syzygium jambos* + *Rhodomytrus tomentosa*.

Puccinia psidii populations in South America are not geographically structured. Although sample numbers are low, several host species in South America appear to be infected by a unique genotype of *P. psidii* that only infects that host species. This could represent recent host shifts by *P. psidii* in South America and the existence of host-specific genotypes of *P. psidii* in South America, which could also indicate the early stages of speciation in *P. psidii*.

Hawaiian isolates were all of the same multilocus genotype, regardless of host of isolation or geographic location. Furthermore, this genotype is not found in South America suggesting that South America is not the source of the introduction to Hawaii.

The apparent wide host range of *P. psidii* in Hawaii contrasts sharply with the high fidelity and narrow host range that is observed in South America. The wide host range of the *P. psidii* genotype found in Hawaii may indicate that this genotype represents a serious invasive pathogen risk if introduced to other global locations. Inoculation tests are needed to better understand the host range of the Hawaiian genotype of *P. psidii*.

The introduction of any new *P. psidii* genotypes into Hawaii represents a potential invasive pathogen risk for Myrtaceous trees. Furthermore, potential recombination among genotypes could create new genotypes with unknown risks. For these reasons, it seems prudent to avoid the introductions of new *P. psidii* genotypes into Hawaii and elsewhere, and studies should continue to better assess invasive risks posed by diverse genotypes.

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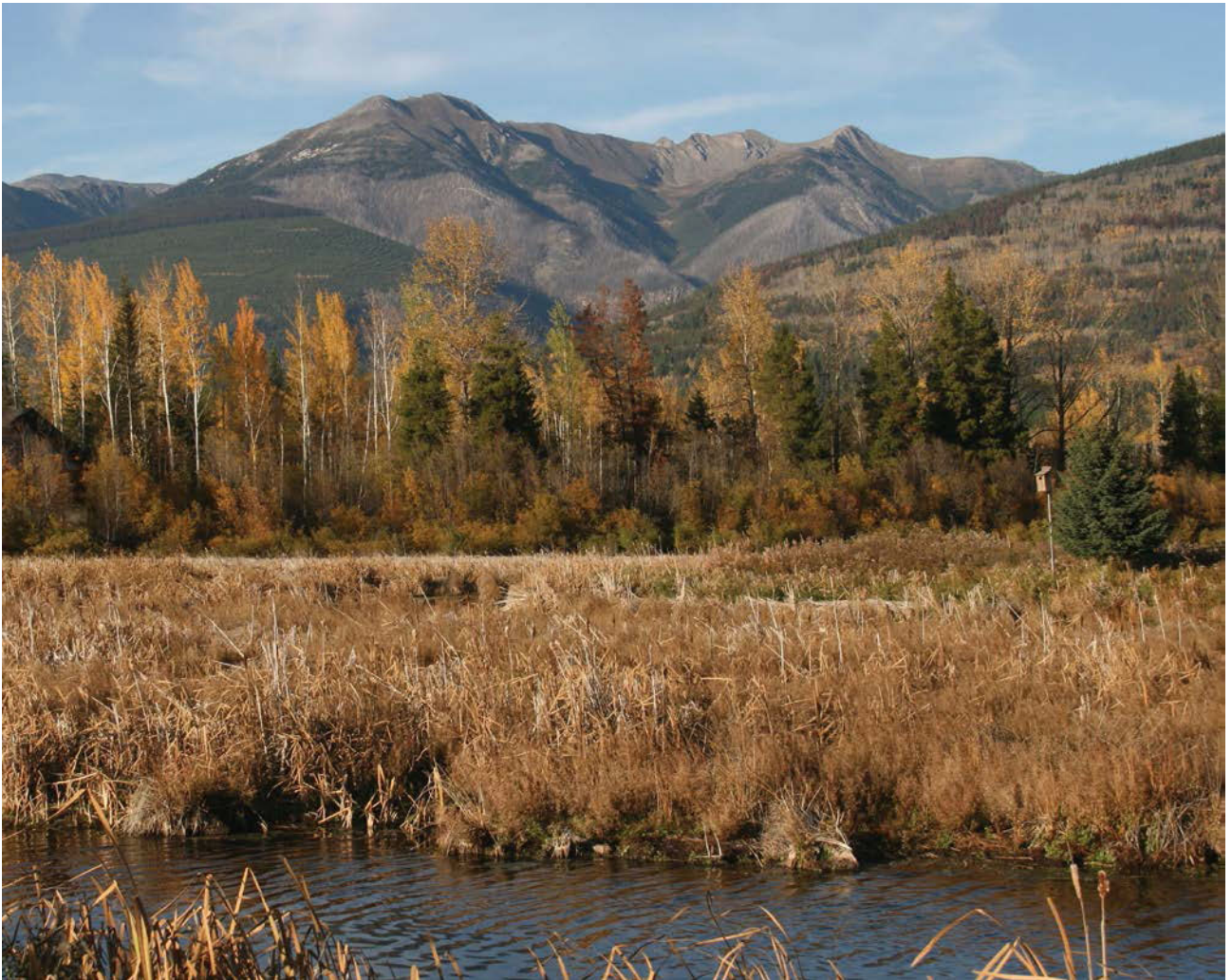
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