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Gene Flow And Genetic Diversity Of Austropuccinia Psidii (G. Winter) Beenken Populations In Brazil, South Africa, New Zealand And Uruguay

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Abstract
Phytopathogens of forest species can negatively impact various ecosystems worldwide. The rust disease caused by Austropuccinia psidii (G. Winter) Beenken has a broad geographical distribution and exhibits significant genetic variability among its populations, attacking various species within the Myrtaceae family. This study addressed the genetic diversity of A. psidii through the analysis of 5 microsatellite loci for 192 samples collected in South Africa, New Zealand, Brazil, and Uruguay, subdivided into 15 subpopulations according to the host species. Results of expected heterozygosity (He) ranged from 0.322 to 0.620. The principal component analysis revealed genetic clusters, differentiating subpopulations in Brazil and Uruguay from those in South Africa and New Zealand. Wright's F-statistics (F_{ST}) were calculated, showing values close to 0 for New Zealand subpopulations, indicating the possibility of a single population. The number of migrants (Nm) was estimated by Wright's method using F_{ST} values, generally exhibiting values below 1.0 between geographically distant populations and values above 1.0, reaching up to 50.594, between geographically close subpopulations, suggesting gene flow. This study highlighted genetic differences among A. psidii populations sampled in South Africa, New Zealand, Brazil, and Uruguay.

Keywords: *Rust Disease, Heterozygosity, Microsatellite Markers.*

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I. Introduction

Phytopathogens are organisms with the potential to negatively impact various ecosystems on the planet. Particularly, pathogens affecting forest essences can lead to epidemics, resulting in the alteration of the biological structure of the ecosystem (STEWART et al., 2018; FENSHMAN and RADFORD-SMITH, 2021). In this way, they can reduce wood production in forest plantations (SILVA et al., 2017) and affect the quality and quantity of fruits through constant attacks on plant material (HEIM et al., 2018; WINZER et al., 2020).

The fungus *Austropuccinia psidii* (G. Winter) Beenken (syn. = *Puccinia psidii*), the causal agent of myrtle rust, is classified in the kingdom Fungi, phylum Basidiomycota, class Pucciniomycetes, order Puccinales, family Sphaerophragmiaceae, genus Austropuccinia, and species *A. psidii* Sphaerophragmiaceae (BEENKEN, 2017). It is a pathogenic agent that induces rust in more than 500 species within the Myrtaceae family (MCTAGGART et al., 2020; NAROUEI-KHANDAN et al., 2020).

This pathogen was first described infecting *Psidium guajava* L. (guava) in Brazil (WINTER, 1884; GLEN et al., 2007). It has a geographical distribution in countries across the African continent (MCTAGGART et al., 2020), Oceania (SOEWARTO et al., 2018), Southeast Asia (DU PLESSIS et al., 2019), Asia (DU PLESSIS et al., 2017), Central America (MACLACHAN, 1936), and North America (STEWART et al., 2018; ESPERÓN-RODRÍGUEZ et al., 2018).

The occurrence of the disease is recorded at temperatures between 18 and 25 ºC, with 23 ºC being the optimal growth temperature for *A. psidii*. In addition to this factor, the pathogen requires high relative air humidity with prolonged periods of leaf wetness due to drizzle or dew and long nights to carry out infection and colonization of susceptible host tissues (ALFENAS et al., 2009). Environmental conditions directly influence the infection process, increasing the aggressiveness of the pathogen under favorable conditions (CHOCK, 2020).

There are several studies demonstrating the phenotypic and/or genotypic variability of *A. psidii*, either through host specialization or molecular tools (MARLATT and KIMBROUGH, 1980; CASTRO, 1983; COELHO et al., 2001; APARECIDO et al., 2003; ZHONG et al., 2011). Although the evolutionary history of *A. psidii* lacks a definitive conclusion, there is a hypothesis of host jump, suggesting a "leap" of the pathogen from its original host to other taxonomically distant hosts (STUKENBROCK and MCDONALD, 2008).

The expansion of this host range may have been crucial for the development and evolutionary success of *A. psidii*. It is known that host plant populations can drive modifications in pathogen populations due to selection pressure in coevolutionary avirulence/resistance mechanisms (CASELA, 2005). Thus, if the pathogen is unable to colonize the host plant, it will not survive. Additionally, genetic variability is influenced by evolutionary forces that can act separately or in concert, including mutation, selection, genetic drift, and gene flow (FUTUYMA, 2009; HARTL and CLARK, 2010).

Mutation occurs randomly in the genome and generates genetic variability, which can be beneficial, deleterious, or neutral. The presence of mutation is a very real possibility for *A. psidii*, as evidenced by the discovery of various transposable elements (TEs) in its genome (TSUI, 2015). Thus, mutation can be one of the mechanisms that play a role in the selection and evolution of the species.

Genetic drift is a process that occurs randomly, impacting allele frequencies through the drastic reduction of individuals in a specific population (WRIGHT, 1931). Small population size and inbreeding can accentuate the effects of genetic drift (TAMBARUSSI et al., 2015).

Gene flow can be defined as the migration of genes between populations, allowing them to exchange alleles, increasing genetic variability within these populations and introducing new genes and combinations (SLATKIN, 1989). In the absence of gene flow, populations may accumulate cumulative genetic differences and, over time, may become distinct species (FUTUYAMA, 2009; HARTL and CLARK, 2010).

Microsatellite molecular markers can be used in studies involving genetic analyses in various living organisms due to their high degree of information (BORÉM and CAIXETA, 2009).

Thus, this study aims to estimate the genetic diversity of *A. psidii* subpopulations using five microsatellite loci from samples collected among 18 taxa of the Myrtaceae family in Brazil, Uruguay, South Africa, and New Zealand. The hypothesis is that there is a difference between the populations present in South Africa, New Zealand, Brazil, and Uruguay.

II. Methodology

Origin of Austropuccinia psidii samples

The sample data and genotyping of microsatellite data in the present work are the same as those used by Graça et al. (2013) which were obtained from the international database DRYAD under the CC0 1.0 Universal Public Domain Dedication license and by McTaggart et al. (2020) which were obtained from the table in the work itself.

In total, there are 18 host species divided into 15 subpopulations that total 192 isolates, sampled around the globe in Brazil, Uruguay, South Africa and New Zealand (Table 1).

Table 1 – List of samples used in the work, collected in different hosts, their respective locations, identifications (ID), and authors.

Authors	Hosts	Locations	No. of samples	ID
Graça et al. (2013)	*Eucalyptus* spp.	Brazil and Uruguay(1)	70	Pop1
	Syzigium jambos	Brazil	4	
	Psidium guajava	Brazil	63	Pop2
	Psidium araca	Brazil	2	
	Syzigium cumini	Brazil	4	Pop3
	Myrciaria cauliflora	Brazil	3	Pop4
	Eugenia uniflora	Brazil	2	
McTaggart et al. (2020)	*Lophomyrtus bullata*	New Zealand	3	Pop5
	Eugenia capensis	South Africa	8	Pop6
	Myrtus communis	South Africa	3	Pop7
	Eugenia erythrophylla	South Africa	4	Pop8
	Metrosideros excelsa	New Zealand	4	Pop9
	Metrosideros kermadecensis	New Zealand	2	Pop10
	Eugenia natalitia	South Africa	6	Pop11
	Eugenia simii	South Africa	2	Pop12
	Eugenia umtamvunensis	South Africa	4	Pop13
	Eugenia verdoorniae	South Africa	5	Pop14
	Melaleuca viminalis	South Africa	3	Pop15
Total samples: 192				

Source: Graça et al. (2013); McTaggart et al. (2020)

Note: Only one sample was taken from a *Eucalyptus* spp. host in Uruguay.

Data analysis

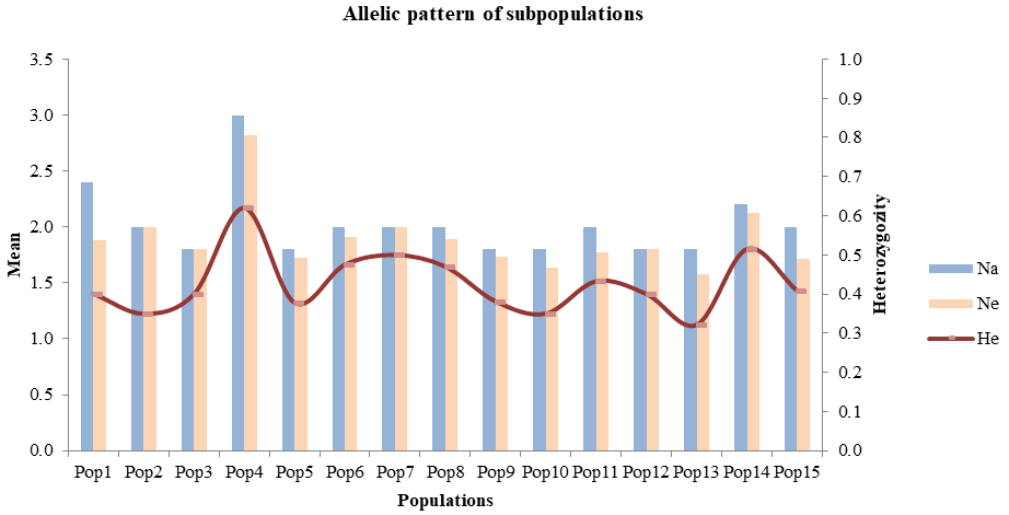
Microsoft Excel 2015 software was used to filter the genotyping data for the 192 samples for five loci, namely PpSSR 12, PpSSR 14, PpSSR 18, PpSSR 102 and PpSSR 161, grouping 15 subpopulations of *A. psidii* in relation to the host for the samples described by McTaggart et al. (2020), while for the samples by Graça et al. (2013), 6 population-genetic models of gene flow were previously tested in the software Migrate-N 3.6.11 (BEERLI and PALCZEWSKI, 2010), of which the one that presented the best probabilistic results grouped the host species *Eucalyptus* spp. and *Syzigium jambos* (L.) Alston (Pop1), *P. guajava* and *Psidium araca* Raddi (Pop2), Syzigium cumini (L.) Skeels (Pop3), *Myrciaria cauliflora* (Mart.) O.Berg (syn = *Plinia cauliflora* (Mart.) Kausel) and *Eugenia uniflora* L. (Pop4) (PORTELA, 2023).

Using Microsoft Excel 2015 software and the Genetic Analysis in Excel – GenAlEx (PEAKALL and SMOUSE, 2006) add-on package, analyzes of diversity indices (He), descriptive statistics of diversity (Total number of alleles – Na; Total effective number of alleles – Ne), principal components analysis (PCA), Wright's F statistics (1951) F_{ST} and Number of Migrants $Nm = 1/4\,(1/F_{ST} - 1)$.

III. Results

Among populations, the average total number of alleles ranged from 1.80 (Pop3; Pop5; Pop9; Pop10; Pop12; Pop13) to 3.00 (Pop4), while the average total number of effective alleles ranged from 1.58 (Pop13) to 2.82 (Pop4) for the 15 A. psidii subpopulations sampled (Figure 1).

Figure 1 – Allelic pattern of the 15 subpopulations of *A. psidii* sampled in Brazil and Uruguay (Pop1, Pop2, Pop3 and Pop4), South Africa (Pop6, Pop7, Pop8, Pop11, Pop12, Pop13, Pop14 and Pop15) and New Zealand (Pop5 and Pop10), with five loci sampled.

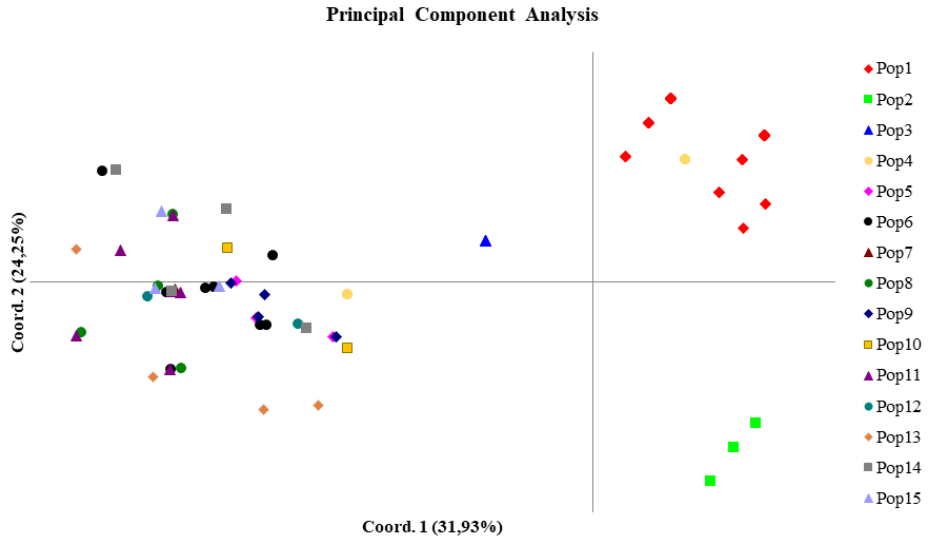


Source: Graça et al. (2013); McTaggart et al. (2020)
Legend: Total number of alleles in the subpopulation (Na); Total effective number of alleles (Ne); Expected heterozygosity (He).

The expected heterozygosity ranged from 0.322 (Pop13) to 0.620 (Pop4), being higher in subpopulation 4 (Pop4), composed of hosts of the species *M. cauliflora* and *E. uniflora*, which presented the highest mean values of total alleles and total effective alleles, while subpopulation 13 (Pop13), composed of the host of the species *Eugenia umtamvunensis* A. E. Van Wyk, presented the lowest mean values of total alleles and total effective alleles.

The principal components analysis (PCA) quantified 25 axes, of which only the two with the highest percentage of variation were considered for the graph. In the Principal Components analysis (Figure 2), the abscissa axis (Coord. 1 – x axis) represents 31.93% of the data variation while the ordinate axis (Coord. 2 – y axis) represents 24.25% of the variation of the data.

Figure 2 – Principal Component Analysis for the 15 subpopulations of *A. psidii* sampled in Brazil and Uruguay, South Africa, and New Zealand, with five loci sampled.



Source: Graça et al. (2013); McTaggart et al. (2020).
Legend: Brazil and Uruguay (Pop1, Pop2, Pop3 and Pop4), South Africa (Pop6, Pop7, Pop8, Pop11, Pop12, Pop13, Pop14 and Pop15) and New Zealand (Pop5 and Pop10). The identification of subpopulations (ID) is shown in Table 1.

The lowest F_{ST} value was 0.005 and was found between the Pop5 and Pop9 subpopulations, while the highest F_{ST} value was 0.504 between the Pop2 and Pop10 subpopulations of *A. psidii*. Among the subpopulations present in South Africa, F_{ST} values were below 0.127 (between Pop8 and Pop13). Similarly, F_{ST} values among New Zealand subpopulations were low, with the highest being just 0.068 between Pop5 and Pop10 (Table 2).

Table 2 – Results of the F_{ST} statistics for the 15 subpopulations of *A. psidii* sampled in Brazil and Uruguay, South Africa and New Zealand, with five *loci* sampled.

	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Pop7	Pop8	Pop9	Pop10	Pop11	Pop12	Pop13	Pop14	Pop15
Pop1	-														
Pop2	0,229	-													
Pop3	0,301	0,363	-												
Pop4	0,148	0,219	0,250	-											
Pop5	0,440	0,460	0,325	0,289	-										
Pop6	0,291	0,332	0,366	0,184	0,376	-									
Pop7	0,296	0,340	0,333	0,183	0,343	0,013	-								
Pop8	0,335	0,365	0,323	0,206	0,334	0,039	0,015	-							
Pop9	0,437	0,462	0,320	0,286	0,005	0,375	0,342	0,332	-						
Pop10	0,456	0,504	0,309	0,287	0,068	0,390	0,357	0,347	0,075	-					
Pop11	0,354	0,393	0,332	0,225	0,343	0,086	0,035	0,033	0,341	0,357	-				
Pop12	0,305	0,341	0,467	0,210	0,477	0,056	0,067	0,103	0,475	0,490	0,129	-			
Pop13	0,408	0,374	0,453	0,254	0,466	0,112	0,111	0,127	0,463	0,479	0,154	0,145	-		
Pop14	0,272	0,346	0,308	0,166	0,325	0,035	0,026	0,051	0,322	0,318	0,064	0,092	0,170	-	
Pop15	0,333	0,402	0,376	0,222	0,387	0,032	0,050	0,071	0,386	0,403	0,114	0,116	0,168	0,064	-

Source: The author, based on Graça et al. (2013); McTaggart et al. (2020).
Legend: Brazil and Uruguay (Pop1, Pop2, Pop3 and Pop4), South Africa (Pop6, Pop7, Pop8, Pop11, Pop12, Pop13, Pop14 and Pop15) and New Zealand (Pop5 and Pop10). The identification of subpopulations (ID) is shown in Table 1.

The number of migrants Nm is dependent on the variable F_{ST}, the highest value found between the Pop5 and Pop9 subpopulation (50.594), while the lowest value was found between the Pop2 and Pop10 subpopulation (0.246) (Table 3).

Table 3 – Result of the Number of migrants *Nm* for the 15 subpopulations of *A. psidii* sampled in Brazil and Uruguay, South Africa and New Zealand, with five loci sampled.

	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Pop7	Pop8	Pop9	Pop10	Pop11	Pop12	Pop13	Pop14	Pop15
Pop1	-														
Pop2	0,842	-													
Pop3	0,581	0,439	-												
Pop4	**1,443**	0,892	0,750	-											
Pop5	0,319	0,294	0,518	0,615	-										
Pop6	0,609	0,502	0,433	**1,111**	0,414	-									
Pop7	0,594	0,486	0,500	**1,115**	0,478	**19,437**	-								
Pop8	0,496	0,435	0,523	0,965	0,499	**6,181**	**16,156**	-							
Pop9	0,322	0,291	0,531	0,623	**50,594**	0,417	0,482	0,503	-						
Pop10	0,298	0,246	0,560	0,621	**3,453**	0,391	0,451	0,470	**3,066**	-					
Pop11	0,457	0,387	0,502	0,860	0,479	**2,663**	**6,869**	**7,332**	0,483	0,451	-				
Pop12	0,570	0,483	0,286	0,940	0,274	**4,240**	**3,500**	**2,178**	0,276	0,260	**1,682**	-			
Pop13	0,363	0,418	0,302	0,734	0,286	**1,986**	**2,006**	**1,712**	0,290	0,272	**1,376**	**1,473**	-		
Pop14	0,671	0,473	0,563	**1,253**	0,520	**6,855**	**9,509**	**4,653**	0,528	0,535	**3,677**	**2,459**	**1,222**	-	
Pop15	0,500	0,372	0,414	0,876	0,396	**7,622**	**4,774**	**3,287**	0,398	0,371	**1,949**	**1,897**	**1,238**	**3,663**	-

Source: The author, based on Graça et al. (2013); McTaggart et al. (2020).

Legend: Brazil and Uruguay (Pop1, Pop2, Pop3 and Pop4), South Africa (Pop6, Pop7, Pop8, Pop11, Pop12, Pop13, Pop14 and Pop15) and New Zealand (Pop5 and Pop10). The identification of subpopulations (ID) is shown in Table 1.

IV. Discussion

The present work highlighted the presence of variability among the 15 subpopulations of *A. psidii* that were sampled in Brazil, Uruguay, South Africa and New Zealand, for the five loci considered for the analyzes (PpSSR 12, PpSSR 14, Pp SSR 18, PpSSR 102 and PpSSR 161).

Heterozygosity is a measure indicating genetic diversity in the population (LEWONTIN, 1974). The expected heterozygosity (He) assesses population variability, with observed values in this study for *A. psidii* subpopulations ranging from 0.322 (Pop13) to 0.620 (Pop4). Faradilla et al. (2022) evaluated 7 loci from 28 samples isolated from 3 hosts in Indonesia, obtaining He values ranging from 0.268 to 0.381. Graça et al. (2013) reported He values of 0.355 for *A. puccinia* populations isolated from *Eucalyptus* spp. and He = 0.247 for populations isolated from *P. guajava*. This demonstrates that the variability of the *A. psidii* population reduces when there is geographical or host range limitation for the pathogen. This is crucial, as there are always uncertainties regarding the influence of an *A. psidii* population occurring in a specific host group that could initiate an epidemic in another group. These results suggest variability among these populations, helping to explain the lower likelihood of a population existing in *P. guajava*, for example, causing an epidemic in *Eucalyptus* sp.

Furthermore, it is important to note that part of the database evaluated by Graça et al. (2013) was used in the present study. However, we employed a smaller number of loci to align assessments with subpopulations previously studied by McTaggart et al. (2020).

In the principal component analysis (Figure 2), it was possible to observe the groupings of subpopulations collected in hosts in Brazil and Uruguay (Pop1, Pop2, Pop3, and Pop4), concentrated to the right of the graph. The subpopulations of *A. psidii* collected in New Zealand and South Africa are grouped more to the left. However, a sample collected in Brazil from the Pop4 subpopulation is positioned close to the grouping of South African and New Zealand subpopulations.

The differences observed can be explained by the coevolution of the plant-pathogen interaction, as one of the mechanisms of pathogenesis involves the secretion of effectors that manipulate the host for the pathogen's advantage. Genes encoding such effectors are among the fastest-evolving genes in pathogen genomes (SÁNCHEZ-VALLET et al., 2018). This occurs due to the so-called "arms race" in the coevolutionary process between the pathogen and host (HAWKINS, 2019). As the host range in Brazil and Uruguay differs from that in South Africa and New Zealand, this may be one of the explanations.

Moreover, evolutionary forces such as selection, genetic drift, migration, and mutation should be considered, acting as factors that alter genetic variability and the species' ability to adapt to the environment (RYDLEY, 2007). When addressing diseases, ecological interactions involve the characteristics of the fungus, the host, and vectors, along with the dynamic interaction among them (AGRIOS, 2005).

Graça et al. (2013) explained that the genetic structure of *A. psidii* in Brazil is highly influenced by the host species. Balbinott et al. (2022) differentiated 27 species of the Myrtaceae family using a database available on NCBI with 20 chloroplast protein-coding genes, grouping them in a dendrogram to study phylogeny. Among the compiled species, some are included in the present study, such as *P. guajava*, *M. cauliflora*, *Eucalyptus* spp.,

and *S. cumini*, which showed phylogenetic differences when considering the 20 studied genes. This helps explain the groupings formed in the principal component analysis (Figure 2), where differentiation between subpopulations sampled in Brazil and Uruguay can be observed.

Values of the number of migrants (Nm) are related to their F_{ST}, showing an inversely proportional relationship. In other words, the smaller the F_{ST} (Table 2), the greater the number of migrants (Nm) (Table 3). Nm serves as an indicator of gene flow between subpopulations, and higher values indicate greater homogeneity among subpopulations. F_{ST} values can be interpreted as a measure of differentiation between subpopulations, and when close to zero, they may indicate that the subpopulations are unstructured (FRANKHAM et al., 2002).

The matrix with Nm values (Table 3) comprises 105 pairwise values for the 15 subpopulations. Considering that the estimators used assume symmetric and fixed gene flow over generations (WRIGHT, 1951), there are 35 values above 1 migrant per generation. The lowest F_{ST} and Nm values were 0.005 and 50.594, respectively, between Pop5 and Pop9, which includes A. psidii samples from New Zealand on hosts *Lophomyrtus bullata* Burret and *Metrosideros excelsa* Banks ex Gaertn. The highest F_{ST} and Nm values were 0.504 and 0.246, respectively, between Pop2 and Pop10, comprising *A. psidii* samples in Brazil on hosts *P. guajava* and *P. araca* (Pop2) and *A. psidii* samples in New Zealand on hosts *Metrosideros kermadecensis*.

In general, most Nm values remained below 1 when analyzing pairs of subpopulations from different geographical regions (Table 3). Similarly, when examining pairings within similar geographical regions, Nm reached relatively high values. For example, Pop5 and Pop9 (Nm = 50.594), representing samples from New Zealand on hosts *L. bullata* and *M. excelsa*, showed high similarity between both subpopulations, possibly indicating the same population (F_{ST} = 0.001). Samples from South Africa also displayed high Nm values, ranging from 1.222 between Pop13 and Pop14 to 19.437 between Pop6 and Pop7. Among the subpopulations in Brazil (Pop1, Pop2, Pop3, and Pop4), only Pop1 and Pop4 exhibited an Nm greater than 1.0 (Nm = 1.443), while the greatest differentiation was observed between Pop2 and Pop3 (Nm = 0.439), indicating low gene flow.

The high Nm values among subpopulations in New Zealand and among subpopulations in South Africa can be explained by the geographical proximity of the sampled hosts. The Nm values between populations from New Zealand and South Africa were low, ranging from 0.274 between Pop5 and Pop12 to 0.535 between Pop10 and Pop14.

Stewart et al. (2017), studying 6 loci from 226 *A. psidii* samples collected in North America, Central America, and South America, subdivided into 9 genetic clusters, obtained F_{ST} values ranging from 0.06 for subpopulations in Costa Rica, Jamaica, Mexico, Puerto Rico, Hawaii (sample C1), and the United States (Florida – sample C4) on various hosts, up to 0.46 between subpopulations in Brazil on hosts *P. guajava* (sample C5) and *S. cumini* (sample C6). These results differ from the findings in this study when considering geographical location, especially when analyzing samples C1 and C4, which have relatively distant samples with various geographical barriers and low F_{ST}, supporting the results of the samples analyzed on hosts in Brazil, where the F_{ST} value was 0.46, indicating low gene flow.

Considering the significant geographical distance between Brazil and Uruguay, South Africa, and New Zealand, along with the oceanic geographical barrier separating the continents of these respective countries, it is plausible to assume that over time and generations of *A. psidii*, peripatric speciation may occur. This process could create new genetic groups with accumulated differences, as speciation occurs when there is reproductive isolation. This is evident in this study among populations from Brazil, South Africa, and New Zealand, which showed Nm values less than 1.0 when comparing pairings between their subpopulations.

However, it is essential to be conservative when discussing the speciation process, considering that *A. psidii* is a fungus first reported in Brazil in 1884 (WINTER, 1884), whereas the first report in New Zealand was in 1979 (KOLMER, 2005), and in South Africa, it was in 2013 (ROUX et al., 2013). This timeframe may be insufficient to categorically assert that speciation has already occurred.

Another point to consider is that Wright's theoretical estimator (F_{ST}) uses allele frequencies, and these frequencies are consequences of part of the evolutionary past of *A. psidii*. assuming that all subpopulations have the same size or that there are infinite subpopulations, and gene flow occurs symmetrically. As the Nm value is related to the (F_{ST}) value, it is assumed that this value remains fixed (WRIGHT, 1951; BEERLI and FENSENSTEIN, 2001).

Among the factors that may have affected the results in this study, one can mention the small number of samples within some subpopulations, in contrast to others with more than 50 isolates, and the failure to amplify certain loci for certain individuals. However, the study was conducted without major issues, and important results regarding the genetic variability of *A. psidii* were obtained.

Santini et al. (2018) argue that a significant portion of the spread of plant disease epidemics caused by phytopathogens is human induced. They emphasize that this process has intensified with the technological advancement of civilizations, representing a negative aspect of globalization. On the other hand, the paper discusses the geopolitical role of countries in adopting preventive measures against the spread of these pathogens, citing examples such as the creation of legislation, checkpoints, and quarantines.

Further complementary studies on the population genetics of *A. psidii* could be conducted to stratify differences even more. Such studies could serve as a basis to assess the invasion risks of *A. psidii* for these subpopulations, considering different ecological behaviors. Although the rust fungus has a broad range of hosts from different genera within the Myrtaceae family and a wide geographical distribution in regions with climate conditions similar to those in Brazil, its dynamics as an invader in other continents is likely to be different.

V. Conclusion

This work highlighted genetic differences between *A. psidii* populations sampled in South Africa, New Zealand, Brazil and Uruguay. It was also possible to observe similarities between the subpopulations of *A. psidii* in South Africa and between the subpopulations of *A. psidii* in New Zealand, just as it was possible to observe a certain difference between the subpopulations of *A. psidii* sampled in Brazil and Uruguay.

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