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Austropuccinia Psidii In Evolutionary Perspective: Exploring Diversity, Gene Flow, And Effective Size Through Coalescence Theory

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Abstract:
A Forest-based activities play a significant socio-economic role in the country. Currently, Brazil is a leader in productivity rankings for forest plantations, however, this has been affected by constant attacks from pathogens causing economic damage to these crops. An example is the rust in Eucalyptus spp., caused by Austropuccinia psidii (G. Winter) Beenken, which has a wide geographical distribution, and the pathogen shows great genetic variability among its populations, also attacking other species of the Myrtaceae family. In this regard, genetic-population and evolutionary aspects of A. psidii populations were studied. These data refer to microsatellite loci of A. psidii isolates, obtained from fungal isolates from a sample of 148 plants, which originated these markers deposited in DRYAD. The model used in the estimates was of four A. psidii subpopulations grouped based on seven hosts from the Myrtaceae family, being SPOP1 (Eucalyptus spp. +Syzygium jambos), SPOP2 (Psidium guajava + Psidium guineense), SPOP3 (Syzygium cumini), and SPOP4 (Myrciaria cauliflora and Eugenia uniflora). These subpopulations were formed with collections made by the authors of the data deposit in the states of Bahia, Espírito Santo, Mato Grosso do Sul, Minas Gerais, Rio de Janeiro, Paraná, Santa Catarina, São Paulo, Rio Grande do Sul, and Uruguay. The effective population size and gene flow between the subpopulations were also estimated, through Bayesian inference method, based on coalescence theory implemented in the MIGRATE-N software. In addition to descriptive statistics and graphs of the allelic population patterns, paired estimates of F_{ST} and intra and intergroup differentiation were made by molecular variance analysis (AMOVA) considering the genetic groups of four subpopulations and the seven hosts in a hierarchical manner. The gene flow (Nm) between pairs of populations was low (all < 1 unit) and ranged from 0.04 to 0.67. The estimates of the effective population size (Ne) were 119, 111, 189, and 1315 for SPOP1, SPOP2, SPOP3, and SPOP4, respectively. The AMOVA showed that the percentage of variation was 39.43% among subpopulations, 16.29% among hosts within a subpopulation, and 44.28% within hosts. The F_{ST} analogs were Φ_{CT} = 0.394 (Among Subpopulations); Φ_{SC} = 0.269 (Among Hosts within Subpopulation); Φ_{ST} = 0.556 (Within Hosts), in addition to high paired F_{ST} values ranging from 0.355 to 0.560. The results obtained add knowledge about the understanding of the population genetics of A. psidii, proposing plausible scenarios in relation to the evolutionary and demographic past, as well as the processes that gave rise to the observed variability. Different subpopulations may pose unique invasive threats, underscoring the need for genetic conservation in commercial forests to develop resistant plant genotypes against new A. psidii subpopulations.

Keywords*: population genetics; evolution; speciation*

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I. Introduçtion

Forestry in Brazil, essential for GDP and employment, expanded forest plantations between 2010 and 2020, with an emphasis on eucalyptus, covering 6.97 million hectares (IBÁ, 2020). The country has been leading in forest productivity since 2022, due to edaphoclimatic conditions and technological advances in

forestry. The average productivity of Brazilian eucalyptus is 35.3 m³.ha-1.year-1, more than double that of the 1970s (IBÁ, 2020).

However, high productivity techniques associated with increasingly intensive cycles and a genetic base of similar genetic materials, make eucalyptus forests vulnerable to phytopathogens, such as myrtle rust, caused by the fungus *Austropuccinia psidii*, resulting in economic damage (FERREIRA and MILANI, 2002; ALFENAS et al., 2009). This pathogen also affects other economic species of the Myrtaceae family, such as araçá, guava, and jaboticaba (COUTINHO et al., 1998; FERREIRA, 1989; GALLI, 1980).

A. psidii, a biotrophic parasite of the basidiomycota phylum, is native to South America but has been reported in various parts of the world (MOON et al., 2012). Its infection process is influenced by environmental conditions, affecting young sprouts and tender tissues (FERREIRA, 1989; FURTADO et al., 2009). Infection leads to tissue necrosis, with possible reinfections under favorable conditions (FERREIRA and MILANI, 2002). To control A. psidii, the use of resistant materials is prioritized, in addition to escape strategies and chemical control (ALFENAS et al., 2009; FURTADO et al., 2009; AUER et al., 2010; MASSON et al., 2011).

This study aims to infer genetic and evolutionary aspects of *A. psidii* populations, using coalescence theory. It seeks to understand the pathogen's geographical and host structuring, gene flows, effective population size, and their implications in phytopathology.

II. Material and methods

The data used in this research were obtained from the Dryad international open-access research data repository, under the CC0 1.0 Universal Public Domain Dedication license, published by Graça et al. (2013).

In the study, subpopulations of *A. psidii* were used, grouped according to seven hosts, from samples collected by Graça et al. (2013) from Bahia, Espírito Santo, Mato Grosso do Sul, Minas Gerais, Rio de Janeiro, Paraná, Santa Catarina, São Paulo, and Rio Grande do Sul, as well as an individual from Uruguay (Figure 1).

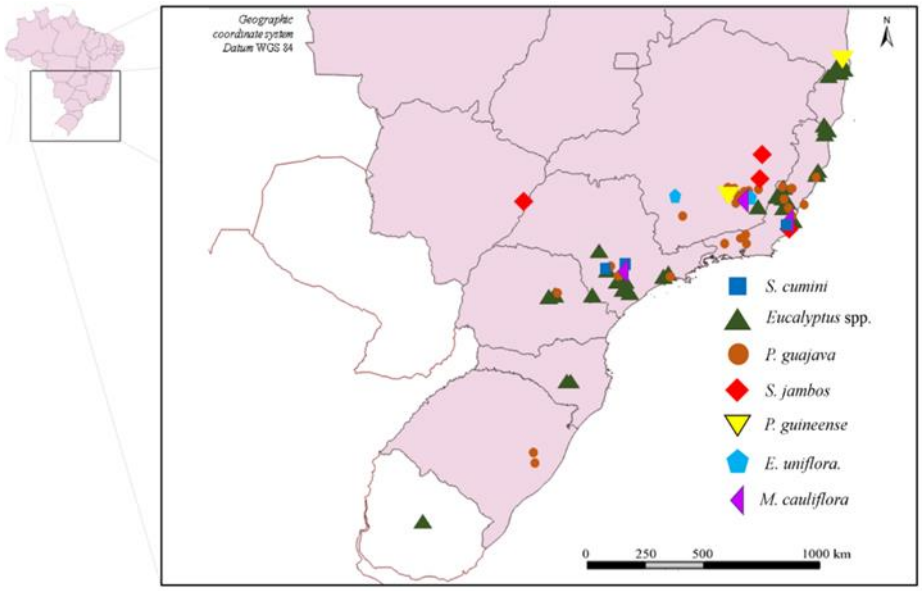


Figure 1: Sampling locations of *Austropuccinia psidii* isolates associated with the Myrtaceae family in Brazil and Uruguay. The species are *Eucalyptus* spp., *Eugenia uniflora*, *Myrciaria cauliflora*, *Psidium guajava*, *Psidium guineense*, *Syzygium jambos*, *Syzygium cumini*..
Source:The author, based on Graça (2013).

Graça et al. (2013) collected uredinial pustules from 148 plants of *Eucalyptus* spp., *P. guajava*, *S. jambos*, *P. guineense*, *S. cumini*, *M. cauliflora*, and *E. uniflora*, where all sampled individuals were georeferenced.

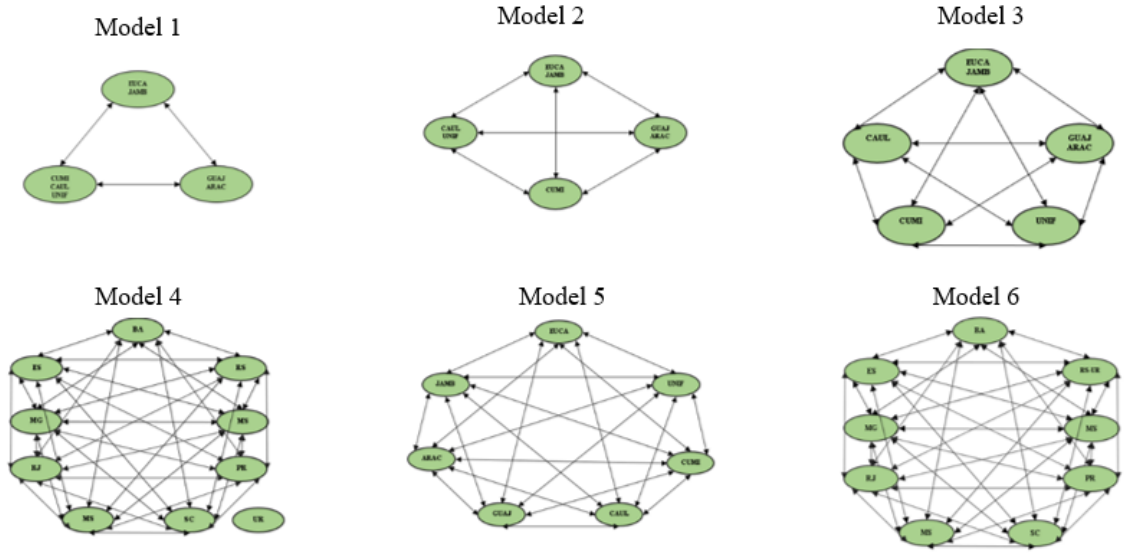
The samples were genotyped using 10 microsatellite loci (PpSSR012, PpSSR014, PpSSR018, PpSSR022, PpSSR087, PpSSR102, PpSSR146, PpSSR161, PpSSR178, and PpSSR195.

The number of samples collected in each state and botanical taxon were different, this occurred due to the fact that *A. psidii* infects only young and tender tissues, and its development is dependent on environmental, microclimatic conditions, the phenological stage of the host, and its organs. Information about DNA extraction and genotyping can be accessed in the works of Graça et al. (2013).

Six different demographic and connectivity models among the populations were tested (Figure 2). These models sought to elucidate whether populations are in panmixia or if they are genetically structured and if

there are indications of isolation by distance. The comparison of the models was carried out by estimating the probability of the model (equation 1). The models responsible for the highest proportion of likelihood are the most probable, estimated from the marginal probabilities obtained by thermodynamic integration in the Migrate-N 3.6.11 software (BEERLI and PALCZEWSKI, 2010).

Figure 2: Graphical scheme of the simplified representation of the six genetic-population models of gene flow proposed by the author and tested in the Migrate-N software



Source: Authors (2023)

The arrows represent gene flow in the model. EUCA, JAMB, GUAJ, ARAC, CUMI, CAUL, and UNIF respectively represent the subpopulations grouped by the species *Eucalyptus* spp., *S. jambos* , *P. guajava, P. guineense, S. cumini, M. cauliflora* and *E. uniflora;* BA, ES, MG, SP, RJ, MS, SC, PR, RS and UR are respectively the geographical subpopulations of Bahia, Espírito Santo, Minas Gerais, São Paulo, Rio de Janeiro, Mato Grosso do Sul, Paraná, Santa Catarina, Rio Grande do Sul, and Uruguay.

The probability of the model is given by the division of the marginal probability by the sum of the marginal probabilities of all the models used.

$Prob(\text{model}_i) = \frac{mL\text{model}(i)}{\sum mL\text{models}(all)}$	(1)

$Prob(\text{model}_i)$ = the he probability of model *i* in relation to the other models;
$mL\text{model}(i)$ = the marginal probability of the model (*i*);
$\sum mL\text{models}(all)$ = sum of the probabilities of all the models used.

To assess the effective size and asymmetric gene flow between populations, the Bayesian inference method based on coalescence theory implemented in the MIGRATE-N software (BEERLI and PALCZEWSKI, 2010) was used. The parameters effective population size scaled by mutation rate.(Θ_i) nd migration rate scaled by the mutation rate (M_{ij}) are estimated simultaneously:

	$\Theta_i = xN_e$	(2)

x= a multiplier that depends on the ploidy and inheritance of the data (x=4 for nuclear diploids);
N_e = the effective population size;
μ = mutation rate.

	$M_{ij} = \frac{mij}{\mu}$	(3)

mij = migration rate from group i to group j;
μ = mutation rate.

From these values, the effective number of migrants per generation $(xN_i m_{ji})$, was obtained, which can be estimated by:

	$xN_i m_{ji} = \Theta_i * M_{ij}$	(4)

The *a priori* distribution of the parameters chosen was uniform, with minimum, maximum, and delta values respectively of 0, 100, and 10. The estimation process with 5,000,000 iterations, with a burn-in period of 10,000, using the static heating scheme with four chains of different temperatures (1.0; 1.5; 3.0 and 100,000.0) (BEERLI and PALCZEWSKI, 2010). After the completion of each analysis, the area under the likelihood curve, performed by thermodynamic integration, was calculated using the Bezier curve approximation, which allows a better approximation with a small number of heated chains (BEERLI and PALCZEWSKI, 2010). The mutation rate used varied only between loci, and a mutation rate of $5x10^{-5}$ close to those described by Bhargava and Fuentes (2010) was assumed.

The genetic groups of the subpopulations named according to the host were grouped based on the results of models made in the MIGRATE-N software as SPOP1 (*Eucalyptus* spp. + *S. jambos*), SPOP2 (*P. guajava* + *P. guineense*), SPOP3 (*S. cumini*) and SPOP4 (*M. cauliflora* and *E. uniflora*).

Diversity analyses were conducted in relation to each subpopulation group, with estimates made at different levels considering the genetic groups and hosts in a hierarchical manner and with four populations, estimating F_{ST}, diversity indices (*He*), descriptive statistics of diversity (Total number of alleles - Na; Total effective number of alleles Ne) in the GenAlEx 6.5 software (PEAKALL and SMOUSE 2006 and 2012) and intra- and inter-group differentiation was estimated by molecular variance analysis (AMOVA) considering the genetic groups of four subpopulations and the hosts in a hierarchical manner using the Arlequin ver. 3.5 program (EXCOFFIER; LISCHER, 2010). Frequencies and descriptive statistics of diversity at the total and host level have already been previously published by Graça (2013) and thus, were not estimated in this work.

III. Results and Discussion

Model 2 (Figure 2) emerged as the most suitable for inferences, showing the highest probability based on equation 1 and model probability criteria. This model grouped the subpopulations by host, including SPOP1 (*Eucalyptus* spp. + *S. jambos*), SPOP2 (*P. guajava* + *P. guineense*), SPOP3 (*S. cumini*) and SPOP4 (*M. cauliflora* and *E. uniflora*). This model also predicts the possibility of gene flow between each of these subpopulations (Figure 2).

Estimates of the M_{ij} parameters were made, along with the 95% confidence interval for this parameter (Table 1), where M_{ij} represents the migration rate scaled by the mutation rate ($M_{ij} = m_{ij} /\mu$).

The estimated Mij of incoming migrants into the SPOP1 population from the SPOP2, SPOP3, and SPOP4 populations was 1.060, 1.750 and 2.870, respectively. The SPOP2 population received immigrants from the SPOP1, SPOP3, and SPOP4 populations, with rates of 0.790, 1.790 and 1.740, respectively. For the SPOP3 population, the M_{ij} migration rates from SPOP1, SPOP2, and SPOP4 were 0.760, 0.790 and 1.540, respectively. For the SPOP4 population, the immigration rates from the SPOP1, SPOP2, and SPOP3 populations were 0.590, 0.690, and 1.030, respectively (Table 1).

Table 1: Estimates of migration rates, scaled by the mutation rate (M_{ij}), considering *A. psidii* isolates associated with the seven host species of the Myrtaceae family in Brazil and Uruguay, for the average of the ten microsatellite loci used

M_{ij}		Receive			
		SPOP1	**SPOP2**	**SPOP3**	**SPOP4**
		EUCA	GUAJ	CUMI	CAUL
		JAMB	ARAC		UNIF
Donate	**SPOP1**	*	**0.79**	**0.76**	**0.59**
	EUCA		(0.13-3.27)	(0.00-3.00)	(0.00-3.47)
	JAMB				
	SPOP2	**1.06**	*	**0.79**	**0.69**
	GUAJ				
	ARAC	(0.00-3.27)		(0.00-2.67)	(0.00-3.40)
	SPOP3	**1.75**	**1.79**	*	**1.03**
	CUMI				
		(0.00-2.07)	(0.00-2.60)		(0.00-2.93)
	SPOP4	**2.87**	**1.74**	**1.54**	*
	CAUL				
	UNIF	(0.00-3.33)	(0.06-4.87)	(0.00-3.00)	

Source: Authors

$M_{ij} = (m_{ij}/\mu)$ where m_{ij} is the migration rate between locality *i* and *j*, and μ is the mutation rate; EUCA, JAMB, GUAJ, ARAC, CUMI, CAUL e UNIF are respectively the A. psidii subpopulations grouped by the species *Eucalyptus* spp., *S. jambos, P. guajava, P. guineense, S. cumini, M. cauliflora* e *E. uniflora.*

The model selection was made by comparing the likelihoods (BEERLI, 2012), in which the scenarios of the models are summed and then the models are proportioned to the whole for comparison. Therefore, the model with the highest proportion of likelihood is predicted as the most probable.

It is highlighted that the present study is the first report of the use of SSR markers addressing the parameters of gene flow and effective population size, based on coalescence theory for population studies of *A. psidi*i.

The estimates of the effective number of migrants in each generation ($N_i m_{ji}$), received by the SPOP1 subpopulation were 0.063, 0.104 and 0.171, originating respectively from SPOP2, SPOP3, and SPOP4. For the SPOP2 subpopulation, the migrants received were 0.044, 0.099 and 0.096 respectively from SPOP1, SPOP3, and SPOP4. The SPOP3 subpopulation received 0.072, 0.075 and 0.075, coming from SPOP1, SPOP2, and SPOP4. In the SPOP4 subpopulation, the migrants received were 0.388, 0.454 and 0.677, originating from SPOP1, SPOP2, and SPOP3 (Table 2).

The migration rate scaled by the mutation rate (M_{ij}) comes from the relationship (m_{ij}/μ), where m_{ij} is the population's immigration rate, dependent on the mutation rate. This signifies the importance of the migration process in relation to the mutation rate in introducing variability into the populations studied.

These results should be interpreted cautiously as they can ambiguously indicate either high gene flow or a low mutation rate. This can lead to difficulties in realistic interpretation without prior knowledge of mutation rates. Depending on the values of μ in the studied populations, different migration rates can have the same impact on population structure, and similar migration rates can have different impacts (BEERLI, 1998). The most commonly used parameter for migration is the effective number of migrants per generation ($N_i m_{ji}$), which is easily derived from the algebraic manipulation of the expression $xN_i m_{ji}$=$\Theta_i * M_{ij}$.

The values related to the effective number of migrants per generation (N_m ou $N_i m_{ji}$) in all subpopulations were relatively low (all < 1 unit), ranging from 0.044 (SPOP1→ SPOP2) to 0.677 (SPOP3 → SPOP4). Interestingly, in some cases, the host species from which the *A. psidii* samples used in this study were collected are geographically close, yet do not show sufficient gene flow, indicating genetic variability among the strains. In situations where gene flow exceeds one unit, the effect can be sufficient to partially prevent the differentiation of allele frequencies by genetic drift and keep the populations as a single evolutionary unit.

Table 2: Effective number of migrants per generation (N_m or $N_i m_{ji}$), estimated for the *A. psidii* subpopulations associated with host species of the Myrtaceae family in Brazil and Uruguay, and considering the 95% confidence interval.

$N_i m_{ij}$		Receive			
		SPOP1	**SPOP2**	**SPOP3**	**SPOP4**
		EUCA	GUAJ	CUMI	CAUL
		JAMB	ARAC		UNIF
Donate	**SPOP1**	*	**0.044**	**0.072**	**0.388**
	EUCA		(0.01-3.23)	(0.00-2.55)	(0.00-2.95)
	JAMB				
	SPOP2	**0.063**	*	**0.075**	**0.454**
	GUAJ				
	ARAC	(0.00-2.78)		(0.00-2.27)	(0.00-2.89)
	SPOP3	**0.104**	**0.099**	*	**0.677**
	CUMI				
		(0.00-1.76)	(0.00-2.21)		(0.00-2.49)
	SPOP4	**0.171**	**0.096**	**0.075**	*
	CAUL				
	UNIF	(0.00-2.83)	(0.04-4.14)	(0.00-2.55)	

Source: Authors

The values are the effective number of migrants per generation ($N_i m_{ji}$), obtained by $xN_i m_{ji} = \Theta_i * M_{ij}$ and below are the 95% confidence intervals. The abbreviations EUCA, JAMB, GUAJ, ARAC, CUMI, CAUL, and UNIF are respectively the *A. psidii* subpopulations grouped by the species *Eucalyptus* spp., *S. jambos, P. guajava, P. guineense, S. cumini, M. cauliflora* and *E. uniflora*.

Some hypotheses can explain this differentiation between geographically close species, relating it to reproductive isolation and reproductive biology. The reproductive cycle of *A. psidii* is not yet fully elucidated. Initially, Ruiz et al. (1989) suggested that the spermagonium and aecium occur in an unknown, alternate host, similar to heteroecious rusts. This would imply a barrier since each set of hosts for the fungus to complete its

cycle would act as a geographical barrier, but this is not a consensus. Coutinho et al. (1998) suggested that the life cycle occurs on a single host without the production of spermagonium and aecium.

Graça et al. (2013) and Machado et al. (2015) did not find evidence to support sexual reproduction as the main means of reproduction for their datasets. According to these authors, *A. psidii* reproduces mainly through the formation of urediniospores by successive mitotic cycles, and they consider the genotypic diversity observed in populations of *A. psidii* as a product of mutation and not sexual reproduction. However, McTaggart et al. (2018) conducted experiments showing that *A. psidii* completes both asexual and sexual parts of its life cycle on a single host (autoecious) and that meiotic recombination produces basidiospores capable of infecting Myrtaceae species.

McTaggart et al. (2020) tested the hypothesis that reproduction by basidiospores occurs in Myrtaceae infections in New Zealand and South Africa. They found strong evidence of the role of the sexual cycle in diversity, finding the presence of a sexual stage and high genotypic diversity within an invasive population.

Thus, we can assume that subpopulations are subject to both types of reproduction (sexual and asexual) and suppose scenarios where speciation processes are occurring to explain why there is no gene flow between geographically close individuals, even though it is possible.

Another hypothesis is that speciation leads to the formation of new genetic groups (synonyms of lineage, race, strains, species, etc.), involving reproductive isolation between groups from an ancestral population, which accumulate genetic differences among themselves.

It is plausible to suppose that sympatric speciation may be occurring, which happens without geographical separation. In this, populations of the same species live in the same place, but there is no crossing due to incompatibility resulting from chromosomal rearrangements or allopolyploidy, or through mutations that favor individuals with a certain genotype to interbreed among themselves to the detriment of others (KLEIN, 2017).

One possibility is the occurrence of a tetrapolar mating system (also called bifactorial), preventing interbreeding between incompatible populations (NIEUWENHUIS et al., 2013). Over time, differences will accumulate and cause speciation. However, it is important to note that sympatric speciation is one of the rarest forms of speciation. Tetrapolar mating is not necessarily reproductive isolation, but a mechanism involving two different mating *loci*. In this mechanism, only cells with different alleles at the loci can mate to produce viable offspring, thereby increasing variability and reducing inbreeding (NIEUWENHUIS et al., 2013; MAIA et al., 2015).

Granados et al. (2017) studied the genotypic diversity of *A. psidii* associated with *Corymbia* spp., *Eucalyptus* spp., *Psidium* spp., and *Syzygium* spp. in Colombia, along with collections from Australia, Brazil, Indonesia, Paraguay, and South Africa. They used seven microsatellite markers on 58 samples across 15 genera within Myrtaceae. Among samples from Colombia, they detected two lineages of *A. psidii*, one being an unknown genotype in P. guajava, different from those sampled in Brazil, and the pandemic biotype found in Pacific countries like Australia, Hawaii, and Indonesia. To explain the difference between *A. psidii* genotypes in *P. guajava* between Brazil and Colombia, they cited the process of allopatric speciation. The Amazon rainforest could act as a 4700 km barrier, preventing gene flow between populations.

The results regarding the patterns of subpopulation groupings in this study and the geographic edaphoclimatic conditions can also be explained by the possibility of parapatric speciation. In this mode, there is an environmental gradient that selects divergent phenotypes without populations losing contact with each other, as there is an extensive area of *A. psidii* occurrence in the samples used in this study encompassing the states of Bahia, Espírito Santo, Mato Grosso do Sul, Minas Gerais, Rio de Janeiro, Paraná, Santa Catarina, São Paulo, and Rio Grande do Sul, as well as an individual from Uruguay. Most of the occurrence areas are located within the Atlantic Forest Biome, however, there are edaphoclimatic and ecosystem variations along the range of occurrence, where consequently the host species and environmental gradients vary along their geographical distribution.

Due to the interaction between natural selection and gene flow, if the magnitude of selection pressure is high, even in scenarios with intense gene flow, it is possible to maintain distinct populations. If the selection pressure decreases, gene flow will rapidly homogenize allele frequencies in distinct populations. However, in theory, a strong selective force will maintain heterogeneity between two populations, even with gene flow (RIDLEY, 2009; HARTL and CLARCK, 2010).

Scenarios with peripatric speciation are also possible, in this process, individuals from the original population disperse (KLEIN, 2017). It usually occurs through the founder effect, causing their genotypic and phenotypic composition to increasingly diverge from the original.

Regarding the host, it is known that there is a process called host jump for some phytopathogens. In this process, pathogens establish themselves in new genetically distinct host groups, leading to isolation from the original genetic pool, genetic differentiation, and speciation (CHOI and THINES, 2015). Initially, it was thought

possible that *A. psidii* had moved from *P. guajava* to *Eucalyptus* spp., the host jump, a hypothesis rejected by Graça et al. (2013).

However, McTaggart et al. (2016), studying the evolutionary timeline of various species in the order Pucciniales, including several *Puccinia* spp., assert that 'host jumping' has shaped the diversity of existing rust-causing fungal species. Another evidence that contradicts Graça et al. (2013) and demonstrates the importance of host jumping for *A. psidii*, is the introduction of the pathogen in Australia, where it colonized a variety of endemic Myrtaceae (CARNEGIE and LIDBETTER, 2012; CARNEGIE et al., 2016). Indeed, host jumping is a survival form without which the species would be eliminated due to coevolutionary processes with the hosts (THINES, 2019). This process is plausible to explain the genetic diversity of *A. psidii* observed in the present study.

This observed genetic diversity may indicate that speciation of this fungus is occurring. Another point supporting the hypotheses that the subpopulations are in speciation processes is the emergence of new lineages of *A. psidii* in Brazil (GRAÇA et al., 2011; ALMEIDA et al., 2021). The perception of this variability is not new, however, it was previously attributed to physiological factors of the fungus (CASTRO et al., 1983), considering it a variability related only to pathogen virulence factors (XAVIER, 2002).

Possibly this is linked to the considerable increase in areas occupied by commercial plantations of *Eucalyptus* spp., successive cycles, and the time that *eucalyptus* has been cultivated, along with the fragmentation of the Atlantic Forest, which may have served as a barrier between *A. psidii* populations occupying neighboring habitat patches, a hypothesis based on genetic differences.

Concerning the association pattern between *A. psidii* and the hosts obtained in this work, the results reinforce the relationship of coevolutionary processes with selection factors, genetic drift, migration, and mutation. Evolutionary processes are capable of affecting the variability and adaptability of the species in its environment (RYDLEY, 2007). However, when it comes to diseases, ecological interactions become multifactorial and involve aspects of the fungus, environment, host, and vectors, covering all aspects of this dynamic (AGRIOS, 2005).

The evolutionary theory of the 'Red Queen Hypothesis' (VAN VALEN, 1973) explains coevolution and the constant 'arms race' between plant-pathogen. Situations in which two competing species evolve in such a way that competition remains stable. The name comes from the book 'Through the Looking-Glass' by Lewis Carroll, which says, *'here, in this country Alice, you have to run as fast as you can to stay in the same place...*'.

Coevolutionary changes that have occurred in the gene pools of the host and the involved pathogen, with a significant impact on adaptive value, will unbalance this scale (BARBIERI and CARVALHO, 2001). This situation, from a phytopathological point of view, is concerning, especially in the context of commercial plantations. In these plantations, the selection of genotypes is done artificially (without reciprocal selection pressures), based on traits of interest such as reduced bark content, straightness of the trunk, reduced lignin content, etc., not always considering plant resistance to pathogens. Since *A. psidii* is a biotrophic fungus, coevolution has a greater impact on the plant-pathogen interaction compared to fungi that obtain their nutrition from dead organisms (BARBIERI and CARVALHO, 2001).

The importance of proper genetic conservation in commercial species is evident, aiming to ensure the existence of resistant genotypes for use in breeding programs. This strategy considers the possibility that resistance could be overcome or reduced by the emergence of new pathogen genotypes capable of adapting to the defenses present in cultivated genotypes. Maintaining genetically dissimilar and interspecific materials in germplasm banks can also aid in the breeding program. According to Schulze-Lefert and Panstruga (2011), the non-host resistance to a particular pathogen is a function of the phylogenetic distance to the pathogen's host. This could be an interesting strategy for *Eucalyptus* spp., given the wide variety of species, some of which are compatible and capable of hybridization.

Stewart et al. (2018) studied *A. psidii* using microsatellite markers in over two hundred samples collected from Brazil, Costa Rica, Jamaica, Mexico, Puerto Rico, Uruguay, and the United States, covering 18 host species associated with *A. psidii*. Within the study, 9 genetic groupings were obtained, with five of these groupings made with samples collected in Brazil, which formed clusters similar to the subpopulation groupings used in this research.

Table 3: Estimates of effective population size and $\Theta_i = 4N_e\mu$ for *A. psidii* subpopulations associated with host species of the Myrtaceae family in Brazil and Uruguay, considering a 95% confidence interval.

Subpopulation	**Effective population size**	$\boldsymbol{\Theta_i = 4N_e\mu}$
SPOP1	**119,26**	0,238
EUCA		

JAMB			
SPOP2	**110,78**	0,221	
GUAJ			
ARAC			
SPOP3	**189,25**	0,378	
CUMI			
SPOP4	**1315,08**	2,63	
CAUL			
UNIF			

Where $\boldsymbol{N_e}$ is the effective population size; $\boldsymbol{\mu}$ is the mutation rate per generation; the abbreviations EUCA, JAMB, GUAJ, ARAC, CUMI, CAUL and UNIF are respectively the *A. psidii* subpopulations grouped by the species *Eucalyptus* spp., *S. jambos, P. guajava, P. guineense, S. cumini, M. cauliflora* and *E. uniflora*.
Source: Authors

It is understood that the fungus-plant association observed in the present work, the physiological and biological similarities, follow this logic. It is assumed that it becomes more accessible for the pathogen to interact with a related host as opposed to an unrelated host. For example, *S. jambos* has its origin center in South Asia, regions like India, Malaysia, and nearby islands, before being cultivated in Brazil (LORENZI, 2006). Being closer to Australia, the center of origin of *Eucalyptus* spp., it is assumed to have greater similarity to these plants than to P. *guajav*a. This justifies the grouping of *S. jambos* populations with *Eucalyptus* spp. observed by us and by Stewart et al. (2018), which could be indicative of coevolution in the region of occurrence.

This elucidates issues addressed in the past by phytopathologists as physiological effects and/or virulence variation of *A. psidii* isolates (CASTRO et al., 1983; XAVIER, 2002, and the literature cited by them). What is actually a coevolutionary issue between the pathogen and the host.

A careful analysis of the formed groupings reaffirms all the reasoning made. The *A. psidii* samples collected from the species *P. guajava* and *P. guineense* were grouped in the same subpopulation, both hosts being of the same botanical genus, native trees of South America with a wide range of natural dispersion, having physiological, anatomical, and morphological similarities, as well as ecological aspects and climatic requirements. *Eugenia uniflora* and *Myrciaria cauliflora*, although not of the same genus, both have close phylogenetic relations in the Myrtaceae family, with the same place of origin and edaphoclimatic conditions, and mainly occur in the Atlantic Forest.

The estimated effective population size (N_e) in the subpopulations were 119.26 for SOPO1, 110.78 for SPOP2, 189.25 for SPOP3, and 1315.08 for SPOP4 (Table 3). Due to the reduced effective size, the effects caused by genetic drift may be more pronounced in SPOP1, SPOP2, and SOPO3 compared to SOPO4, such as increased variance between populations, decreased diversity within populations, contributing to the fixation or loss of alleles.

One of the reasons for a high N_e in SPOP4, compared to the others, might be the subpopulation being formed from isolates from host species of two different genera. However, recent studies have investigated the relationship between the effective population size of a pathogen and the number of infected hosts (ROSENBERG and NORDBORG 2002; KOELLE et al., 2011; DEARLOVE and WILSON, 2013). There is no direct association between N_e and the number of infected hosts, due to the complexity of interactions (VOLZ et al., 2017).

Effective population size is a measure of genetic representativeness and is generally lower than the number of individuals in the population (census), especially in cases like *A. psidii*, due to ecological, biological, and reproductive system peculiarities. It is important to note this because mutations, despite being random, are influenced by the number of organisms in the population. Large pathogen populations have a higher number of mutants than small populations (MCDONALD and LINDE, 2002).

Possibly the *A. psidii* subpopulations will have different ecological behaviors and represent distinct invasive threats, indicating the need for studies that can measure the invasive risks presented by the different subpopulations.

Based on the grouping Model 1, a molecular variance analysis (AMOVA) was conducted considering the hierarchical structure between subpopulations, among hosts within subpopulation, and within hosts (Table 4).

The percentage of variation was respectively 39.43% among subpopulations, 16.29% among hosts within subpopulation, and 44.28% within hosts. Analogous estimators to F_{ST}, called Φ-statistics, were generated and estimated as $\Phi_{CT} = 0.394$ (Among Subpopulations); $\Phi_{SC} = 0.269$ (Among Hosts within Subpopulation); $\Phi_{ST} = 0.556$ (Within Hosts).

Table 4: Molecular variance analysis (AMOVA) for the species *A. psiddi* considering hierarchical structure using ten microsatellite loci employed in the research.

Source of Variation	Degrees of Freedom	Sum of Squares	Variance Component	Percentage of Variation
Among Subpopulations	3	317.597	1.38833	**39.43%**
Among Hosts within Subpopulation	3	20.554	0.57338	**16.29%**
Within Hosts	289	450.593	1.55914	**44.28%**
Total	295	788.743	3.52086	100

Estimates made in the software Arlequin 3.5 by Excoffier and Lischer (2010) based on Weir and Cockerham (1984); Excoffier, Smouse, and Quattro (1992) and Weir (1996).
Source: Authors

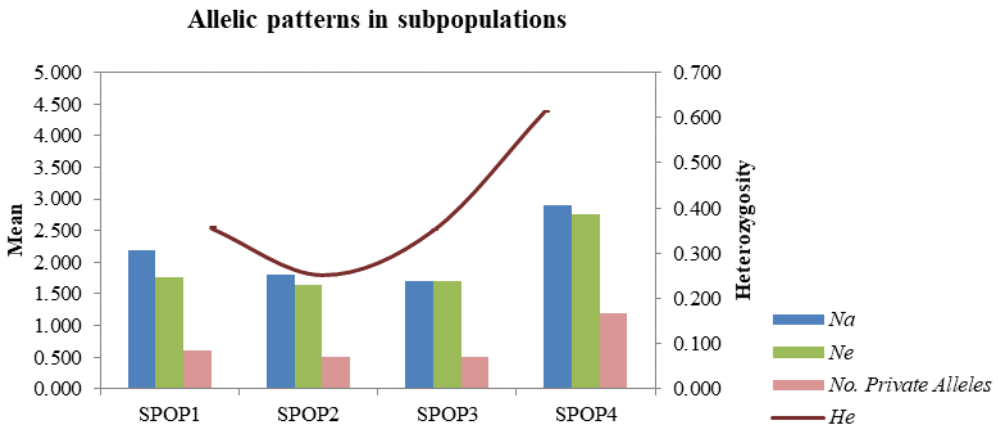
The variability among the four *A. psidii* subpopulations sampled in Brazil and Uruguay was demonstrated by the presented results. Regarding genetic structuring, the molecular variance analysis (AMOVA) using ten loci revealed that the majority of variation is found within populations of the same host species.

Maia (2009) studied isolates of *H. vastatrix*, the causal agent of coffee rust, divided into three populations based on hosts, including cultivars of *Coffea arabica*, *Coffea canephora*, and derivatives of Timor Hybrid (HDT) and Icatu. The AMOVA showed that the greatest genetic variance (99.56%) is found within hosts. Among the factors that may explain this, it is noted that mechanisms of genetic variability, along with dispersal of inoculum, promote great genetic variability within the population, aiming at the homogenization of allelic frequencies in pathogen populations.

Graça et al. (2013) report that the genetic structure of *A. psidii* in Brazil is highly influenced by the host species it parasitizes. However, Ferrarezi et al. (2022), based on studies done on mating genes and homeodomains, raise the theoretical hypothesis that *A. psidii* populations structured by host range could cross with universal hosts, consequently generating implications for biosecurity.

Descriptive allelic patterns obtained for subpopulations SPOP1, SPOP2, SPOP3, and SPOP4 are shown in figure 4. The estimated values of expected heterozygosity were *He*= 0.355, 0.252, 0.350, and 0.612, respectively for SPOP1, SPOP2, SPOP3, and SPOP4.

Figure 4: Allelic patterns among *A.psidii* subpopulations based on 10 microsatellite loci. Including average number of different alleles (*Na*), average number of effective alleles (*Ne*), average number of private alleles (*No. private alleles*), and expected heterozygosity (*He*) for the four population groups considered in the study. Being SPOP1 (*Eucalyptus* spp. + *S. jambos*), SPOP2 (*P. guajava* + *P. guineense*), SPOP3 (*S. cumini*) and SPOP4 (*M. cauliflora* e *E. uniflora*).



Source: Authors

Paired F_{ST} analogous values were estimated for each subpopulation (Table 5) Excoffier and Lischer (2010). The obtained F_{ST} values were 0.560 (SPOP1 with SPOP2), 0.468 (SPOP1 with SPOP3), 0.419 (SPOP1 with SPOP4), 0.608 (SPOP2 with SPOP3), 0.565 (SPOP2 with SPOP3) and 0.355 (SPOP3 with SPOP4).

Faradilla et al. (2021) studied the variability of *A. psidii* with 28 samples collected from three different hosts (*Syzygium polyanthum*, *Syzygium myrtifolium*, and *Melaleuca cajuputi*), using seven SSR loci in Java, Indonesia. They obtained values that varied between *He*=0.381 in *S. polyanthum*; *He*=0.286 in *M. cajuputi*, and

He=0.268 for *S. myrtifolium*. These values are similar to the average results obtained, except for SPOP4, which had values above those observed in this work.

One factor that may be linked to this is that *A. psidii* originates from South America, and only on a relatively recent chronological scale was it introduced to Indonesia, allowing speculation that a founder effect occurred, where habitat colonization is started by a few members of the original population, potentially resulting in reduced genetic variation compared to the original population (FUTUYMA, 1992).

In three of the subpopulations considered in this study, the genetic diversity or expected average heterozygosity was higher than the observed heterozygosity, except in SPOP4.

Proportionally high frequencies of private alleles associated with isolates collected from a particular host species may indicate an absence of gene flow. One possibility is the occurrence of the tetrapolar mating system (also called bifactorial), preventing interbreeding between incompatible populations (NIEUWENHUIS et al., 2013; FERRAREZI et al., 2022)

Table 5: F_{ST} values below the diagonal. Probability values based on 9999 permutations are shown above the diagonal.

Subpopulations	**SPOP1**	**SPOP2**	**SPOP3**	**SPOP4**
	EUCA	GUAJ	CUMI	CAUL
	JAMB	ARAC		UNIF
SPOP1				
EUCA		*p < 0,01*	*p < 0,01*	*p < 0,01*
JAMB				
SPOP2				
GUAJ	**0,560**		*p < 0,01*	*p < 0,01*
ARAC				
SPOP3				
CUMI	**0,468**	**0,608**		*p < 0,01*
SPOP4				
CAUL	**0,419**	**0,565**	**0,355**	
UNIF				

SPOP1 (*Eucalyptus* spp. + *S. jambos*), SPOP2 (*P. guajava* + *P. guineense*), SPOP3 (*S. cumini*) e SPOP4 (*M. cauliflora* e *E. uniflora*).
Source: Authors

Stewart et al. (2018), studying nine distinct genetic groups of *A. psidii* using six SSR loci with samples from Costa Rica, Jamaica, Mexico, Puerto Rico, and USA-Hawaii, USA-California; Brazil, Jamaica, and Uruguay obtained *He* values ranging from 0.17 to 0.51. Regarding the level of differentiation between the groups considered, they obtained an average F_{ST} value of 0.471, with paired F_{ST} values varying from 0.06 (between Jamaica, Mexico, Puerto Rico, Hawaii sample C1, and United States Florida – sample C4) to 0.46 (among isolates from Brazil).

These values corroborate the hypothesis of the absence of gene flow between subpopulations that occur geographically close, in a way that contributes to greater heterogeneity among the groups analyzed.

IV. Conclusion

Inference of parameters regarding effective size, gene flow, and diversity was key to better understanding the population genetics of *A. psidii*.

No gene flow above one unit was observed between subpopulations, even in geographically proximate areas. Nevertheless, this enabled inferences and the proposal of scenarios related to their evolutionary and demographic histories, as well as factors involved in the evolution of *A. psidii*. The acquired data can underpin integrated disease management and planning, highlighting the importance of genetic improvement. *A. psidii* subpopulations might exhibit varying ecological behaviors and represent distinct invasive threats, necessitating studies to evaluate the invasive risks posed by different subpopulations.

This underscores the importance of proper genetic conservation in commercial species to ensure the availability of resistant plant genotypes for breeding programs, given the possibility that resistance may be overcome or reduced by the emergence of new *A. psidii* strains capable of adapting to defenses in currently cultivated genotypes.

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