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Microsatellite Analysis Reveals Genetic Variation in 'Yerba Mate' Trees from Argentina

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INTRODUCTION & AIM

Ilex paraguariensis A. St. Hil., commonly known as 'yerba mate', is a native southern South American tree with significant socioeconomic importance in the region. Its leaves and stems are used to prepare the popular "mate" infusion, valued for its nutraceutical benefits and stimulating effects.

Our purpose is to investigate the population dynamics and genetic structure of wild *I. paraguariensis* germplasm found in a remnant ombrophilous mixed forest in San Antonio, Misiones, Argentina. These unexplored genetic resources hold great potential for the future of ´yerba mate´ production. Therefore, in this preliminary study we characterize the genetic diversity by describing the variability of nuclear microsatellites.

Messi drinking 'mate' after winning the world cup.

A typical gourd (Cucurbitaceae) filled with 'yerba mate' (Aquifoliaceae) and a metal straw (´bombilla´).

Collecting samples at CAMB.

METHODS

Leaf samples were gathered at the 'Campo Anexo M. Belgrano' (CAMB, San Antonio, Misiones), covering a total area of 15.5 km². The 11 sites sampled (named as subpopulations) are separated 1,5 km from each other (Fig. 1). Genomic DNA from 113 individual plants, was extracted with commercial kits; five nuclear microsatellite loci[1] were PCR amplified and initially checked through denaturing PAGE and silver nitrate staining[2]. Then, the genotyping was carried out with fluorescent tags[3] in a Sanger sequencing fragment analyses service[4]. Allelic size determination was estimated using GeneMarker[4]. The genotypic matrix was analysed in GenAlEx[5]. Specimens showing more than 40% missing data were excluded from subsequent analyses. Genetic distances were calculated using the Nei's index (1972)[6] in *poppr*[7] package of R; a UPGMA tree was constructed in *ape*[8] of R and visualised with FigTree 1.4.4[9]. The relationship between genetic and geographic distances was evaluated with the Mantel test in *vegan*[10] of R.

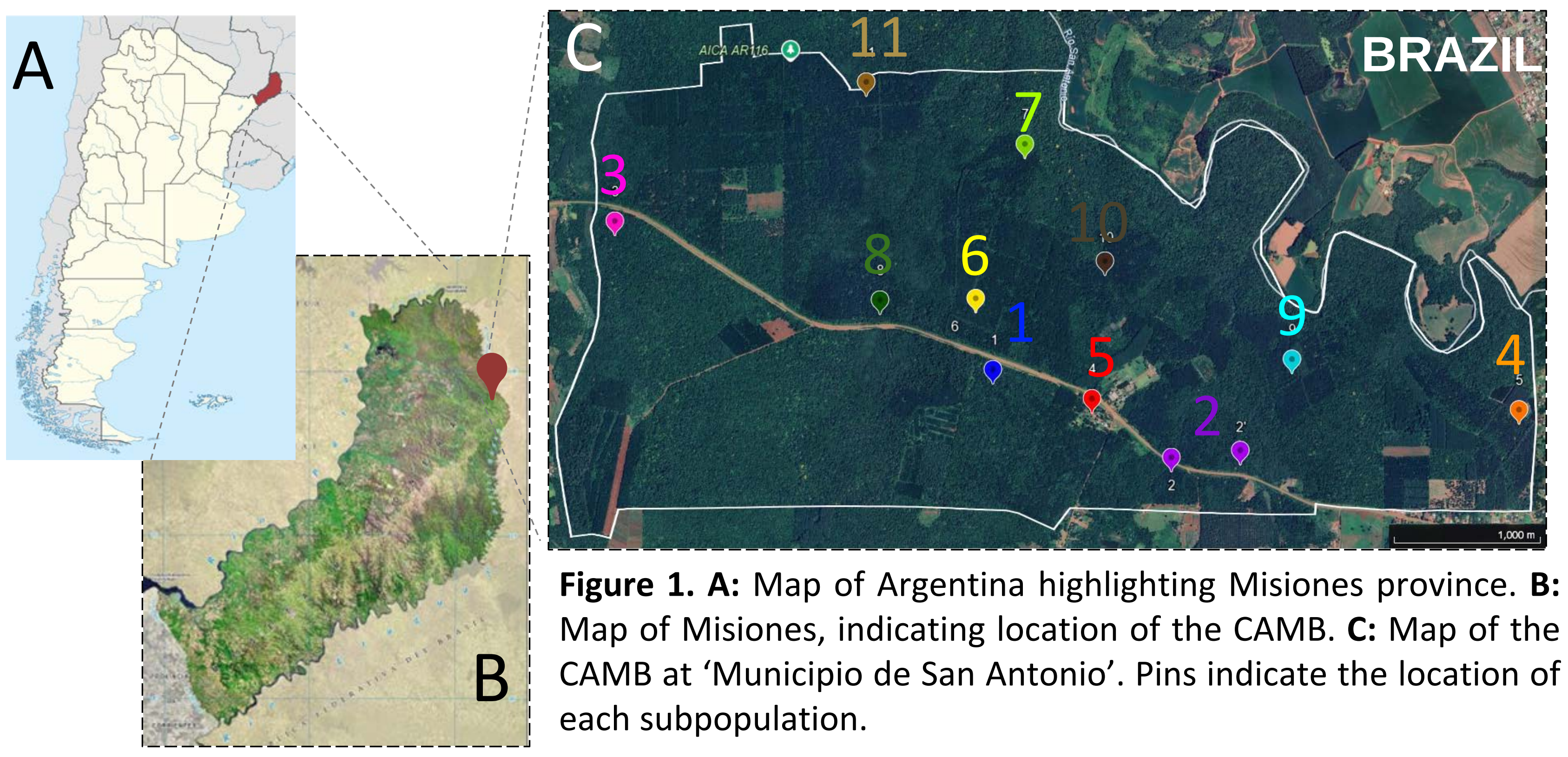


Figure 1. A: Map of Argentina highlighting Misiones province. **B:** Map of Misiones, indicating location of the CAMB. **C:** Map of the CAMB at 'Municipio de San Antonio'. Pins indicate the location of each subpopulation.

RESULTS & DISCUSSION

Locus	Na	Ho	He	F	I	md	MAF
Ipg_10	10	0.716	0.703	-0.018	1.521	34.5%	0.459
Ipg_19	6	0.515	0.529	0.027	1.082	8.8%	0.660
Ipg_28	9	0.532	0.618	0.140	1.267	1.8%	0.568
Ipg_37	6	0.356	0.375	0.052	0.787	20.4%	0.778
Ipg_46	9	0.453	0.719	0.370	1.468	6.2%	0.401
TOTAL	**40**	**0.514**	**0.589**	**0.114**	**1.225**	**14.33%**	**-**

Table 1. Genetic variability of ´yerba mate´ plants (**N=113**) by locus. **Na:** number of distinct alleles, **Ho:** observed heterozygosity, **He:** expected heterozygosity, **F:** fixation index, **I:** Shannon information index. **md:** missing data, **MAF:** major allele frequency.

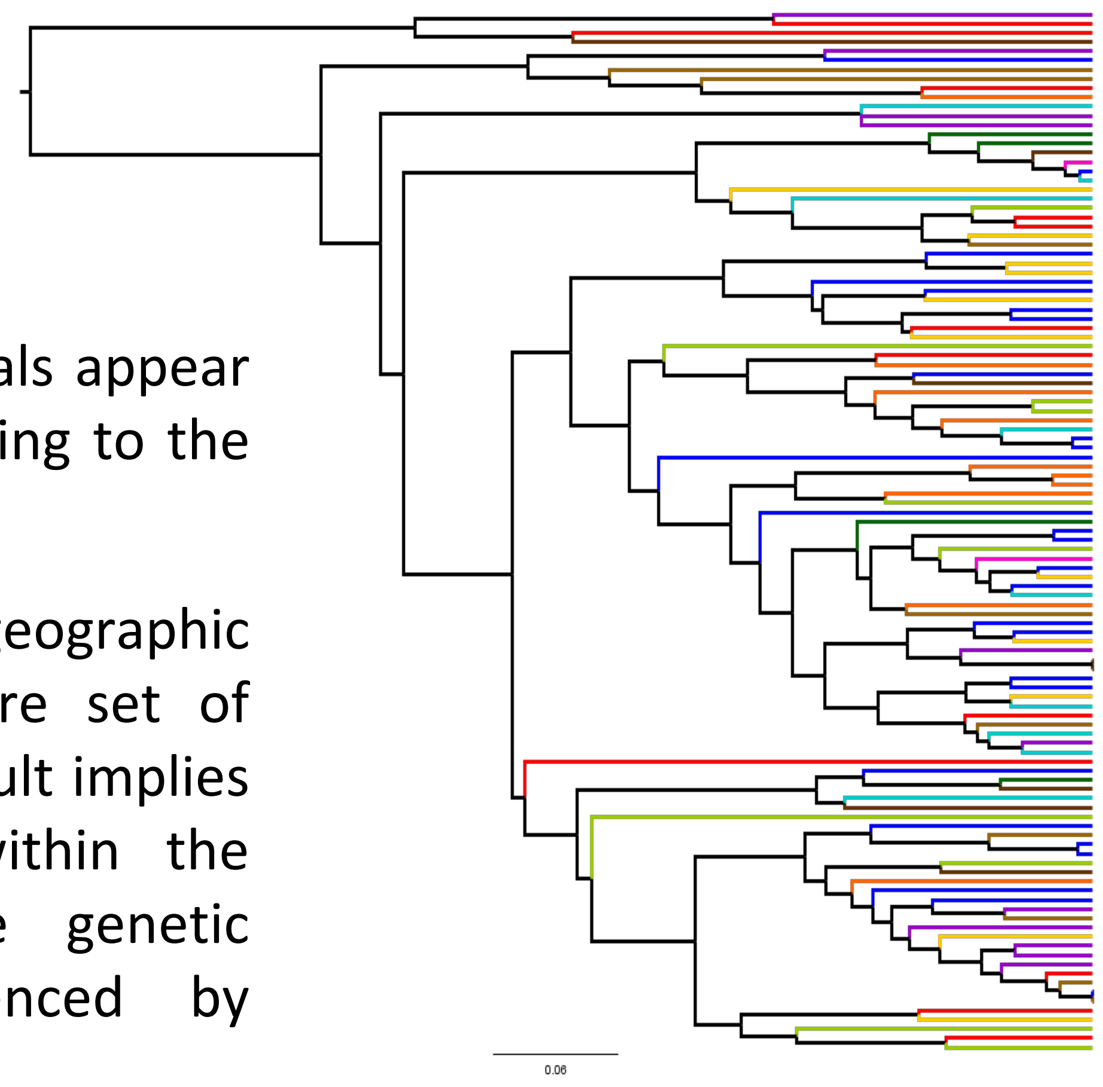
Current dataset has 14.3% of missing data (range= 1.8-34.5%; **Table 1**). Still, a total of 40 alleles and 72 genotypes were recorded, with an average of 8 alleles per locus (range= 6-10). All SSRs were found to be polymorphic (1% level). Average Ho is slightly lower than av He, which it may indicate some degree of inbreeding or could be due to genetic drift effects, requiring further investigation. Average fixation index (F= 0.11) indicates very low differentiation between subpopulations. The Shannon index (I= 1.22) shows that genetic diversity is moderate in CAMB. The heterozygosity obtained here is intermediate to those estimated for Brazilian natural populations (He=0.42[11], He=0.60[1], He=0.41[12]) and almost double that recorded in Uruguay (He=0.31[13]).

Source	df	SS	MS	Est. Var.	Per.
Among groups	10	40.16	4.02	0.12	7%
Within groups	215	332.36	1.55	1.55	**93%**
TOTAL	**225**	**372.52**		**1.67**	100%

Table 2. Analysis of molecular variance (AMOVA). **df:** degrees of freedom, **SS:** Sum of Squares, **MS:** Mean Square, **Est. Var.:** Estimated Variance, **Per.:** Percentage of Variation. Fst = 0.074; p=0.0001.

The AMOVA reveals that most of the genetic variation is held within the subpopulations (**Table 2**). A similar trend was detected for Brazilian (>85%) and Uruguayan (>65%) wild populations [13]. This implies that individuals within the same population are genetically similar, indicating genetic homogeneity.

Figure 2. UPGMA dendrogram for 113 ´yerba mate´ plants. Each subpopulation is indicated by the color of the branch (see Figure 1C for color reference).



In the UPGMA **(Fig. 2)**, all the individuals appear intermingled, and no groupings according to the subpopulation are retrieved.

No correlation between genetic and geographic distances was detected for the entire set of samples (r = 0.038; p = 0.151). This result implies that there is a high gene flow within the metapopulation and therefore, the genetic variation patterns are not influenced by geographical distance.

CONCLUSIONS

- The nSSRs examined to date have revealed substantial genetic variation among wild Argentinian 'yerba mate' trees. This aligns with the diversity found in Brazilian populations, which is reasonable given that Argentina, Brazil and Paraguay constitute the species' center of origin. Uruguay represents the tail of the species' natural range, and thus, its lower genetic diversity was expected.
- While current data suggest limited differentiation among the subpopulations, further research may reveal greater variation.
- Present results suggest that the entire CAMB might function as a metapopulation. This could be attributed to either insufficient data or a lack of genetic structure.

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