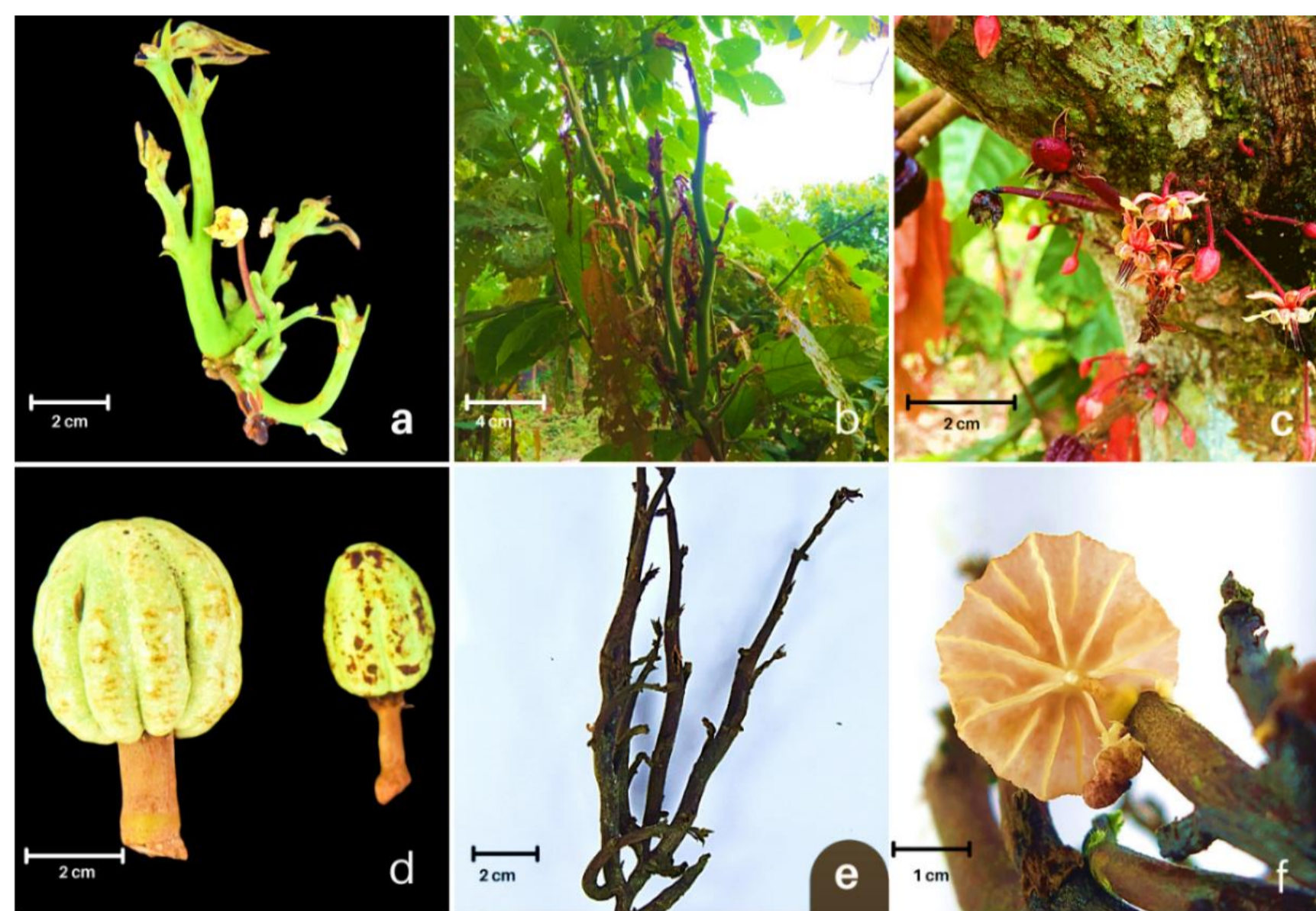


## Introduction

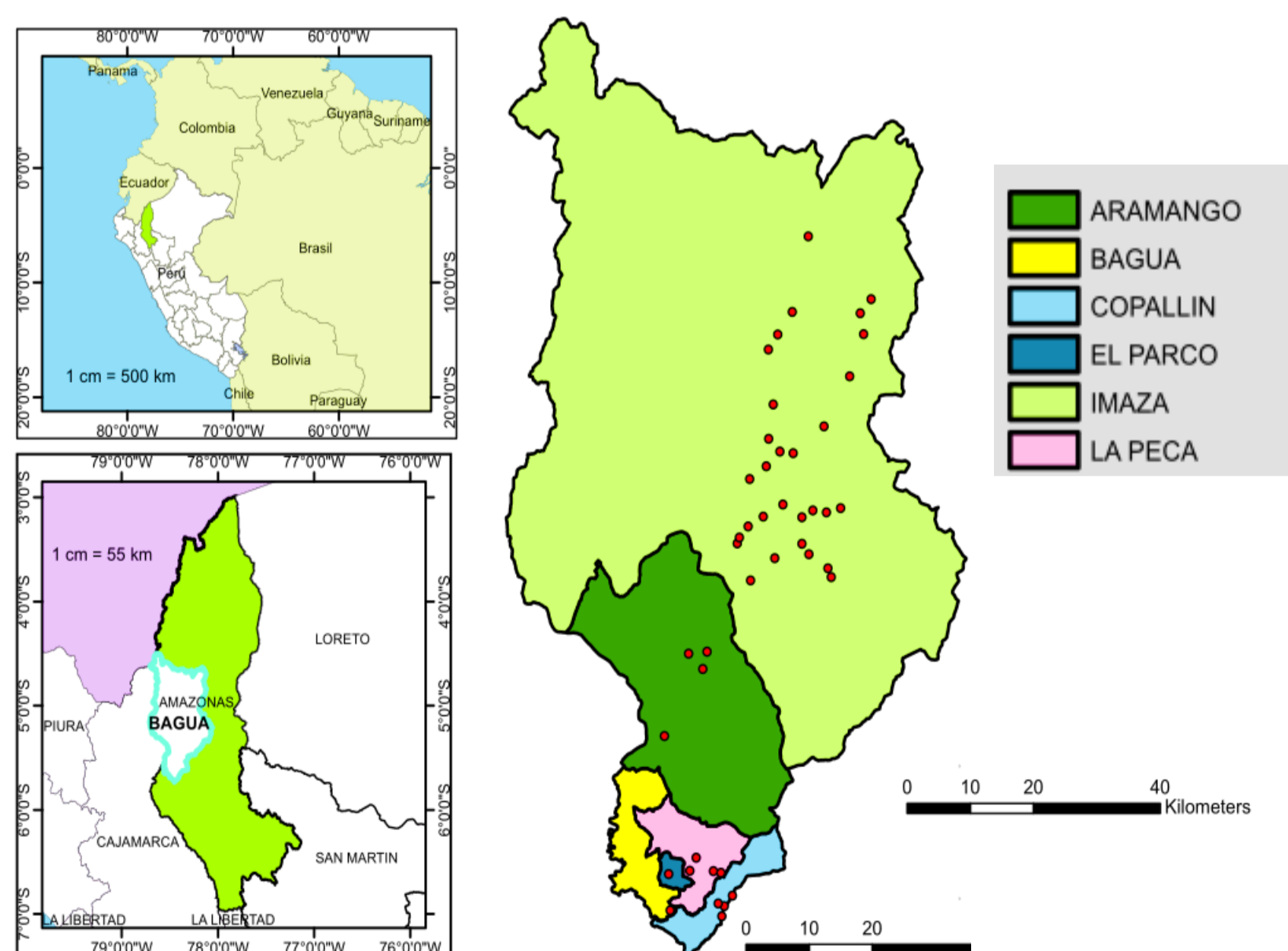
*Moniliophthora perniciosa* (Marasmiaceae family of fungi) causes witches' broom (Figure 1), one of cacao's most important diseases in the Americas<sup>1</sup>. In this study, we evaluated the *in vitro* characteristics and the population genetics dynamics of the pathogen in the Peruvian Upper Amazon, i.e., in the center of diversity and domestication of the crop. We aimed to investigate *M. perniciosa* invasion routes within the country and its reproductive biology.



**Figure 1.** Symptoms and signs of witches' broom disease of cacao. **a.** Young stem with hypertrophy. **b.** Typical witches' broom. **c.** Floral cushion with thickened peduncles. **d.** Fruit deformation. **e.** Necrotic witches' broom. **f.** Fruiting bodies (basidiocarps) of *M. perniciosa*.

## Methods

We collected fifty samples from six cacao growing districts in the province of Bagua, department of Amazonas, Peru (Figure 2): three with more intensive cacao cultivation in not remote areas, and three where cacao plots are more dispersed and are managed by people in native communities in remote Amazonian areas.



**Figure 2.** Geographic distribution of the fifty *M. perniciosa* samples collected in this study.

In each district, we evaluated the incidence levels of witches' broom disease based on the examination of twenty trees per plantation. We contrasted these results with weather historical records in this area<sup>2</sup>. One sample of diseased cacao tissue per plantation was brought to the lab for further processing. Samples were isolated in pure culture and their macro and micro characteristics were evaluated. Isolates were molecularly genotyped with eleven microsatellite markers previously reported in the literature<sup>3,4</sup>, and the multilocus dataset was analyzed with R package *poppr*<sup>5</sup>.

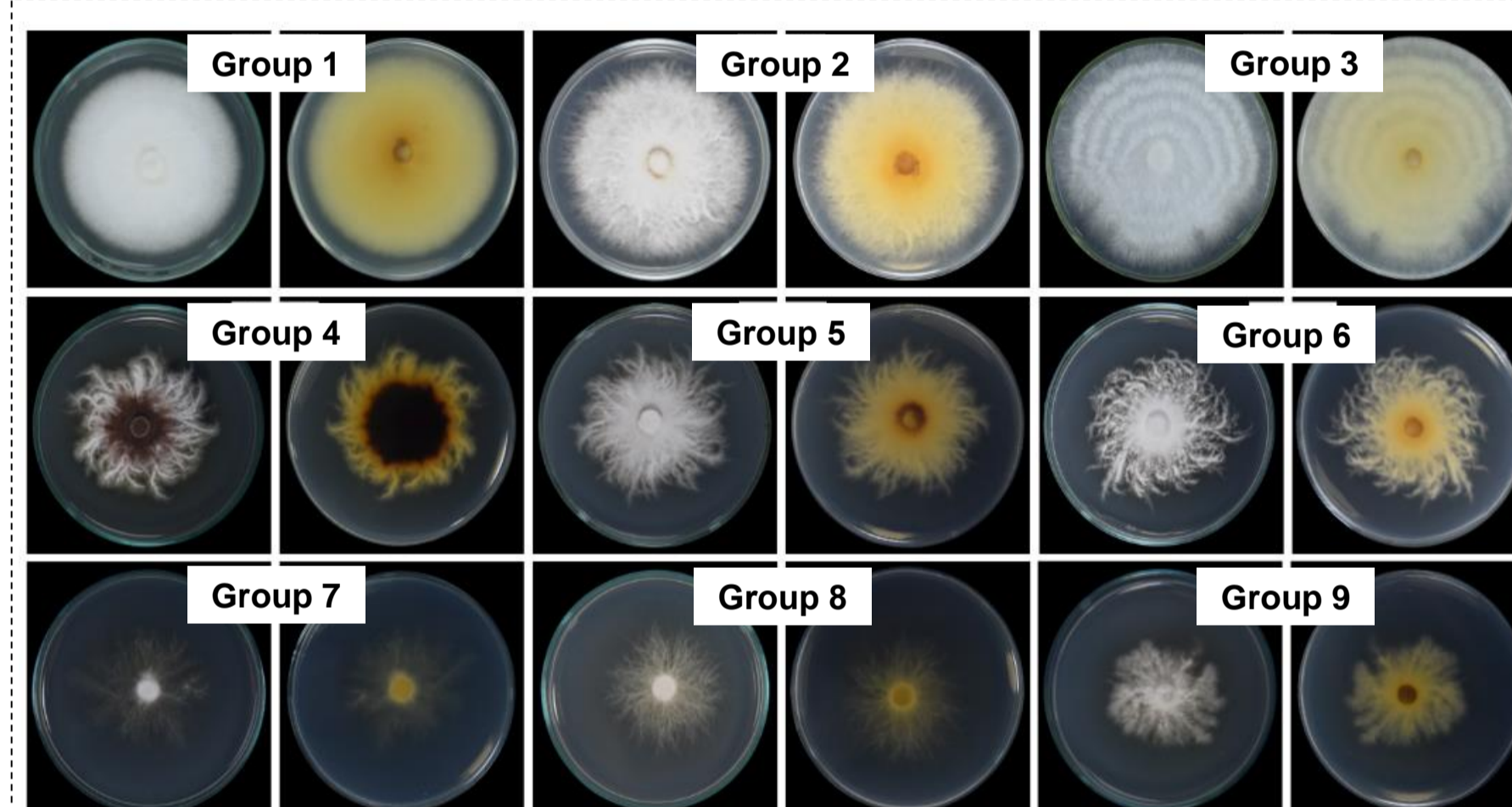
## Results

Incidence levels of witches' broom in the six districts evaluated varied from 10 to 98%, which were correlated with the historical records of precipitation and humidity (Table 1)

**Table 1.** Incidence of witches' broom disease found in the six districts of the Bagua province, Amazonas department,

District	Humidity <sup>2</sup>	Precipitation (mm/year) <sup>2</sup>	Annual T <sup>o2</sup>	Incidence
Aramango	Very humid	1748.7	24.3°C	98%
Imaza	Very humid	3013.3	24.7°C	74%
Copallín	Very humid	1748.7	24.3°C	80%
La Peca	Very humid	1748.7	24.3°C	72%
El Parco	Dry	673.5	26.3°C	10%
Bagua	Dry	673.5	26.3°C	30%

We identified nine morphological patterns of mycelial growth (groups 1–9; Figure 3), which were related to the abundance or scarcity of clamp connection-bearing hyphae and culture growth rate, and had no relation with the origin of isolates.



**Figure 3.** Colony morphology groups of *Moniliophthora perniciosa* found in this study at 25 days of growth on potato dextrose medium.

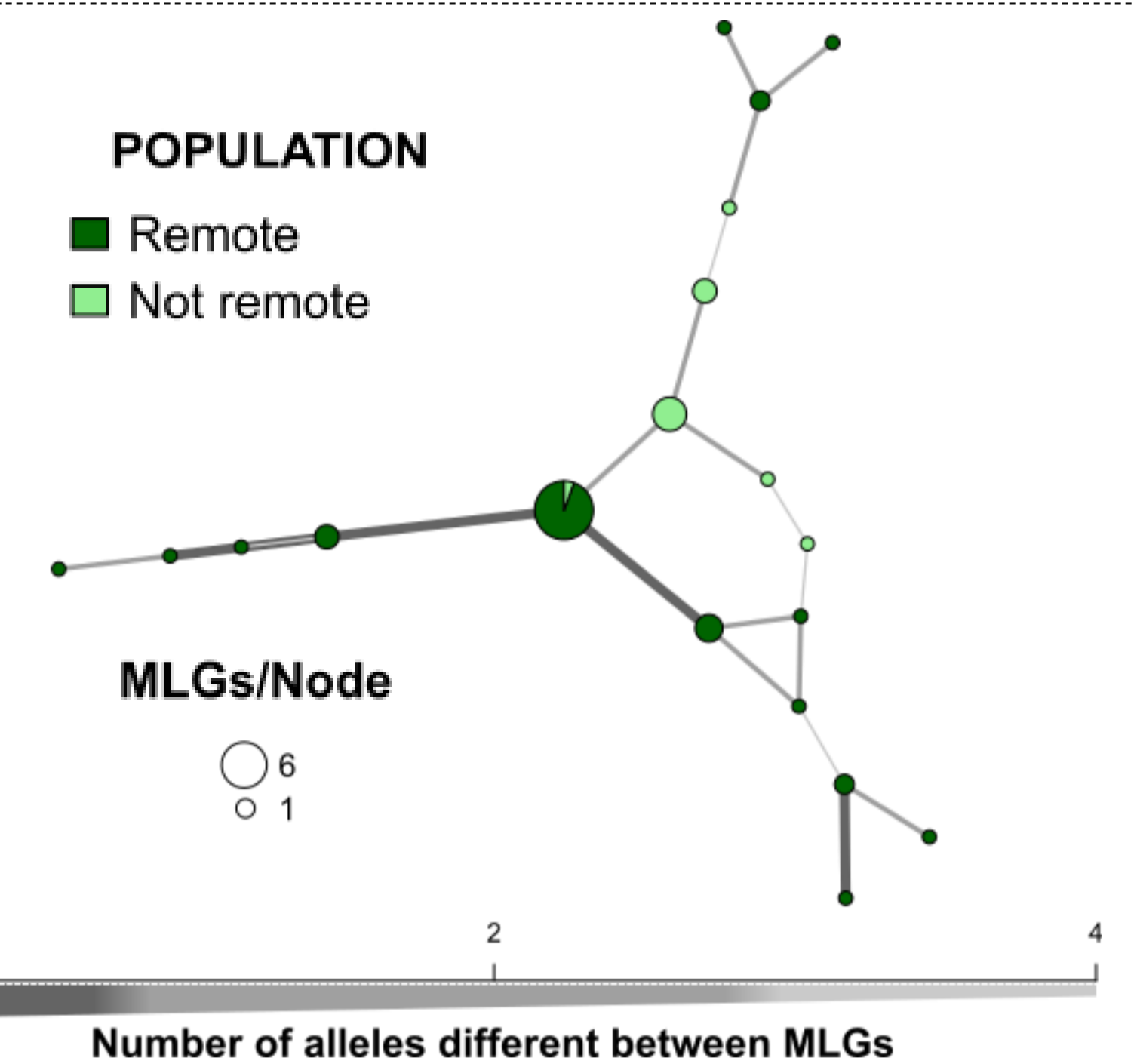
Conversely, the microsatellite genotyping analysis revealed that the population genetics dynamic of *M. perniciosa* is shaped by origin, i.e., the area where samples were collected (Table 2). Samples collected closer to urban areas were less diverse than those from remote areas deeper into the Amazon, supported by the gene (*Hexp*) and genotypic (*H*) diversity indexes (Table 2).

**Table 2.** Diversity analysis of *Moniliophthora perniciosa* from remote and not remote areas in Amazonas, Peru.

Location	Population	N	MLG	<i>Hexp</i>	<i>H</i>
Remote	Condorcanqui	3	3	0.25	1.1
Remote	Imaza	30	10	0.23	2.3
Remote	Aramango	4	4	0.18	1.39
Not remote	Copallin	4	2	0.06	0.69
Not remote	Bagua – El Parco – Utcubamba	4	3	0.17	1.1
Not remote	La Peca	5	3	0.19	1.1
<b>Total</b>		<b>50</b>	<b>19</b>	<b>0.22</b>	<b>2.84</b>

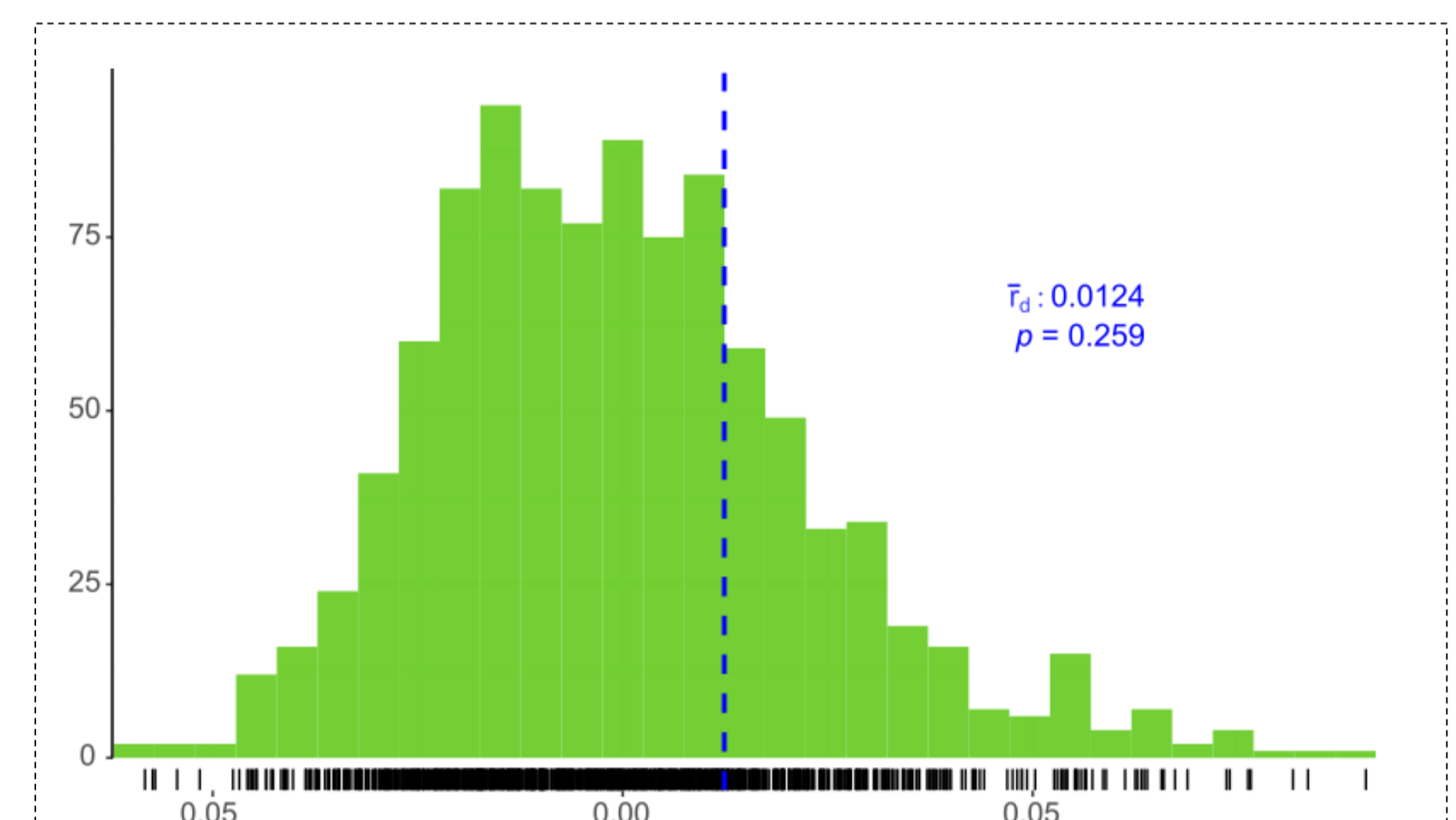
N = number of isolates; MLG = multilocus genotype; *Hexp* = Nei gene diversity index; *H* = Shannon genotypic diversity index

Additionally, clustering and minimum spanning network analyses revealed that all genotypes found in not remote areas are derived from the genotypes found in remote districts, i.e., the pathogen was introduced from areas located deeper in the Amazon (Figure 4)



**Figure 4.** Minimum spanning network analysis of *Moniliophthora perniciosa* samples from Amazonas, Peru. Each node represents a multilocus genotype (MLG). The size of the node responds to the number of isolates per MLG.

The standardized index of association analysis with clone correction values ( $\bar{r}_d = 0.0124$ ) was non significantly different than zero ( $p = 0.259$ ), indicating the existence of linkage equilibrium and the occurrence of genetic recombination through outcrossing in *M. perniciosa* (Figure 5).



**Figure 5.** Histogram based on 1000 random permutations of the clone-corrected microsatellite allele dataset of *M. perniciosa* samples to calculate the standardized index of association ( $r_d$ ) as an indicator of random mating and outcrossing

## Concluding Remarks

- Witches' broom disease of cacao has an incidence above 70% in districts with high levels of precipitation, revealing the importance of the adoption of cultural practices among farmers to secure economic income.
- High levels of cultural and genetic diversity were found in *M. perniciosa* isolates from Northern Peru.
- *Moniliophthora perniciosa* invaded Peru via Amazonian routes. A similar invasion pattern took place in Brasil<sup>1</sup>.
- Cacao-infecting *M. perniciosa* ("C" biotype) has been considered primarily homothallic<sup>6</sup>. Here we provide strong evidence for an outcrossing reproductive strategy.

## References

1. Díaz-Valderrama et al. (2020). *Phytopathology* 110(10), 1604-1619.
2. Vargas (2010). <http://www.iiap.org.pe/upload/publicacion/PUBL510.pdf>
3. Silva et al. (2008). *Molecular Ecology Resources*, 8(4), 783-785
4. Gramacho et al. (2007). *Molecular Ecology*, 7, 153-155
5. Kamvar et al. (2014). *PeerJ* (1), 1-14
6. Kues & Navarro-González (2010). *J Basic Micro.*, 50(5), 442-451.

## Funding

1. 30-2019-FONDECYT- BM-INC-INV
2. PE501078997-2022-PROCIENCIA