

Supplementary Material

P. vivax

AmpliSeq - SNP (40)

N = 81

- NJ 2019 (48)
- NJ 2020 (20)
- Mazan (13)

MS (16)

N = 62

- NJ 2019 (38)
- NJ 2020 (18)
- Mazan (6)

AmpliSeq + MS (16) - SNP (40)

N = 62

- NJ 2019 (38)
- NJ 2020 (18)
- Mazan (6)

AmpliSeq + MS (16) - SNP (40)

N = 51

- NJ 2019 (27)
- NJ 2020 (18)
- Mazan (6)

AmpliSeq + MS (15) - SNP (40)

N = 51

- NJ 2019 (27)
- NJ 2020 (18)
- Mazan (6)

Samples with
%missingness
> 25%

Markers with
%missingness
> 25%

Pop	MLG - SNP	MLG - MS
NJ 2019	35	45
NJ 2020	25	32
Mazan	8	9
Total	68	86

P. falciparum

AmpliSeq - SNP (28)

N = 86

- NJ 2019 (17)
- NJ 2020 (47)
- Mazan (10)
- SE (12)

MS (7)

N = 90

- NJ 2019 (17)
- NJ 2020 (49)
- Mazan (10)
- SE (14)

AmpliSeq + MS (7) - SNP (28)

N = 86

- NJ 2019 (17)
- NJ 2020 (47)
- Mazan (10)
- SE (12)

AmpliSeq + MS (7) - SNP (28)

N = 80

- NJ 2019 (14)
- NJ 2020 (45)
- Mazan (9)
- SE (12)

AmpliSeq + MS (7) - SNP (26)

N = 80

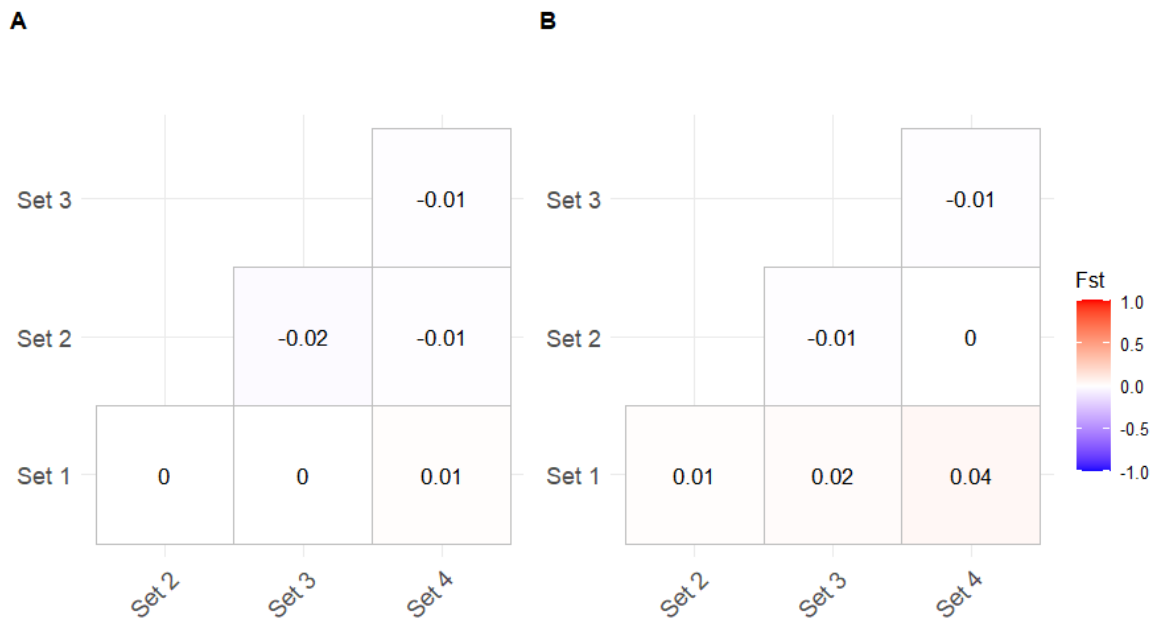
- NJ 2019 (14)
- NJ 2020 (45)
- Mazan (9)
- SE (12)

Samples with
%missingness
> 25%

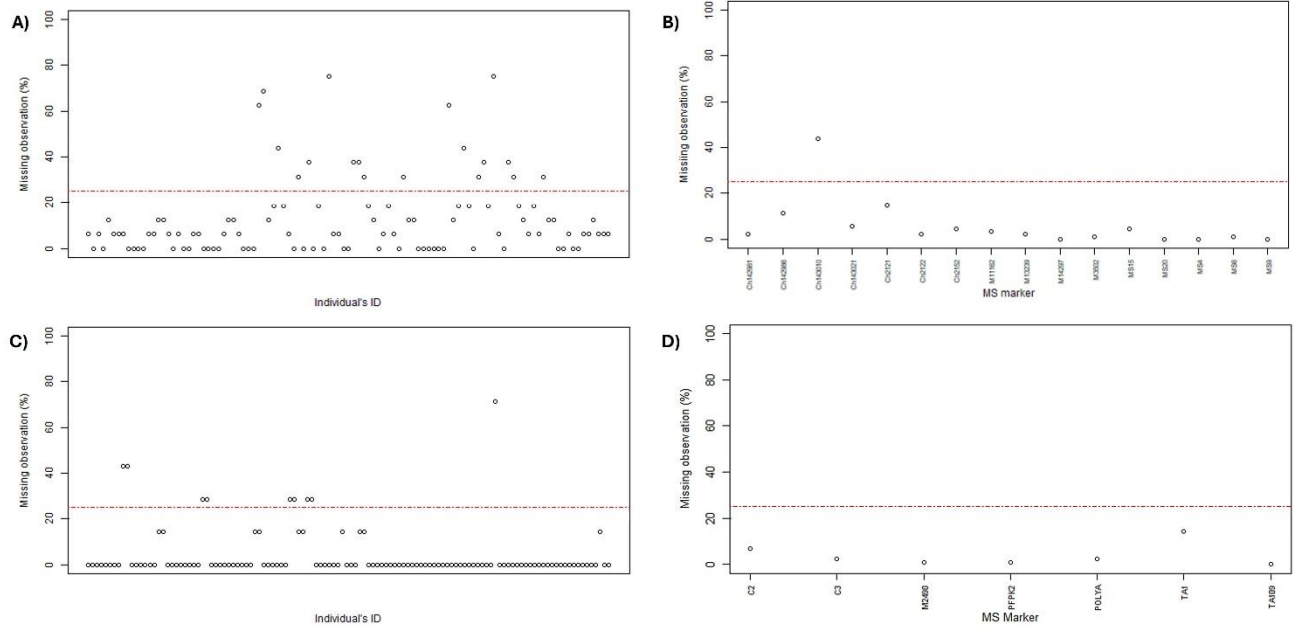
Markers with
%missingness
> 25%

Pop	MLG - SNP	MLG - MS
NJ 2019	22	18
NJ 2020	72	65
Mazan	9	10
SE	14	12
Total	117	105

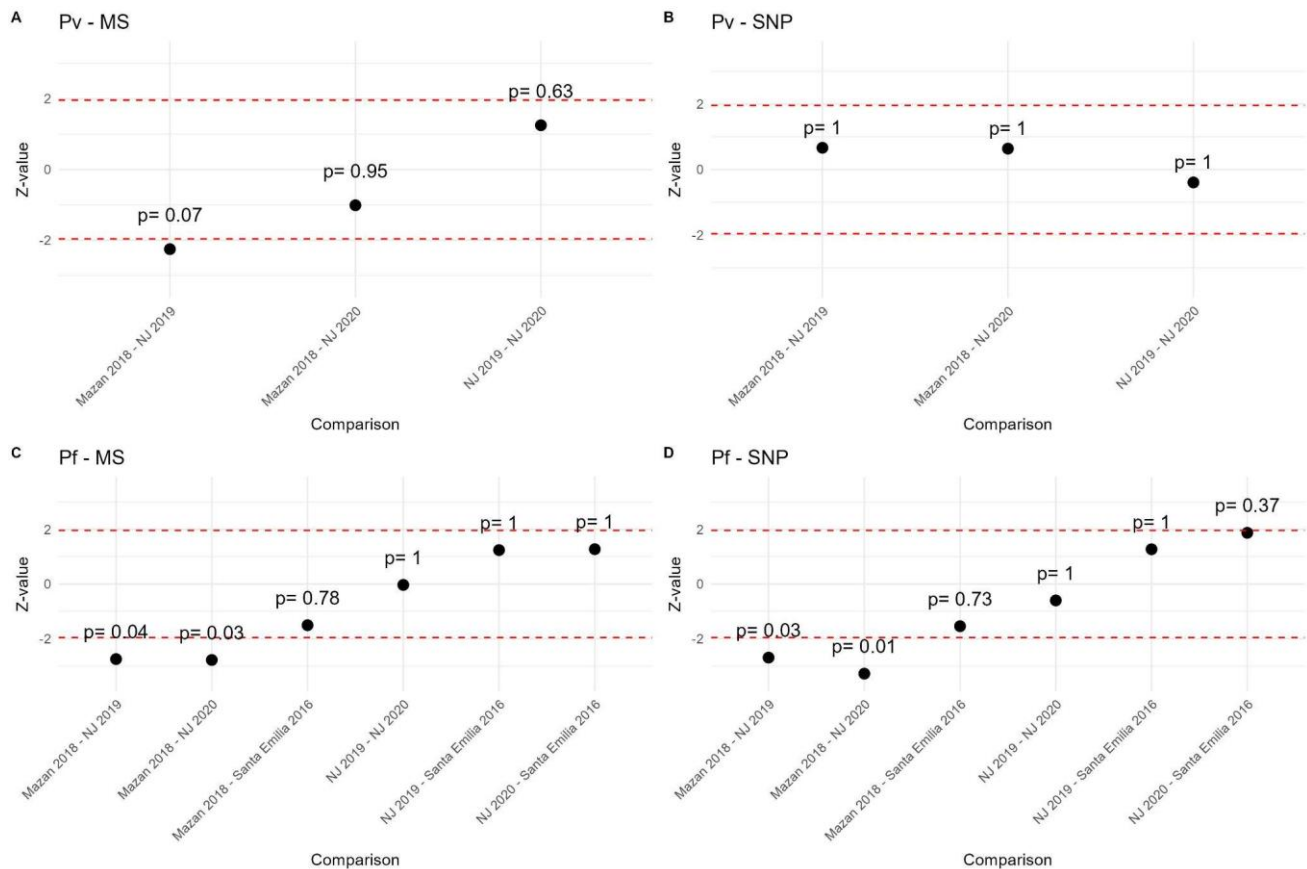
Supplementary Figure S1. Flowchart describing number and source of Pv and Pf samples and inclusion criteria. The tables at the right show the number of haplotypes included in the analyzes. MS: microsatellite, NJ: Nueva Jerusalem, SE: Santa Emilia, Pop: population, MLG: N° of multi-locus genotypes.



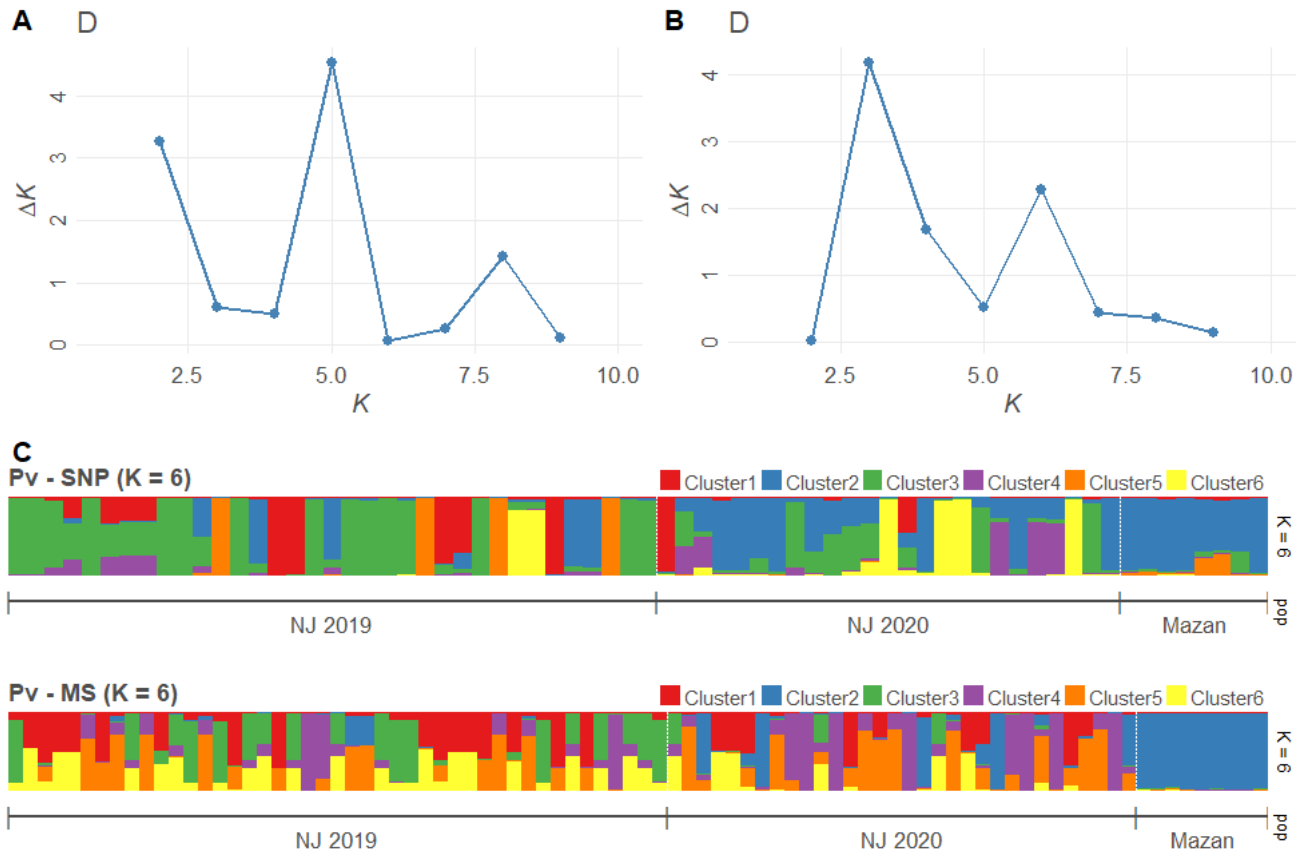
Supplementary Figure S2. Inclusion of secondary MLG. Heatmap of pairwise F_{ST} values between sets containing different type of MLG. Set 1: samples with only one MLG. Set 2: dominant MLG of all samples. Set 3: dominant MLG of all samples and secondary MLG of samples with in only one secondary allele. Set 4: all dominant and secondary MLG of all samples



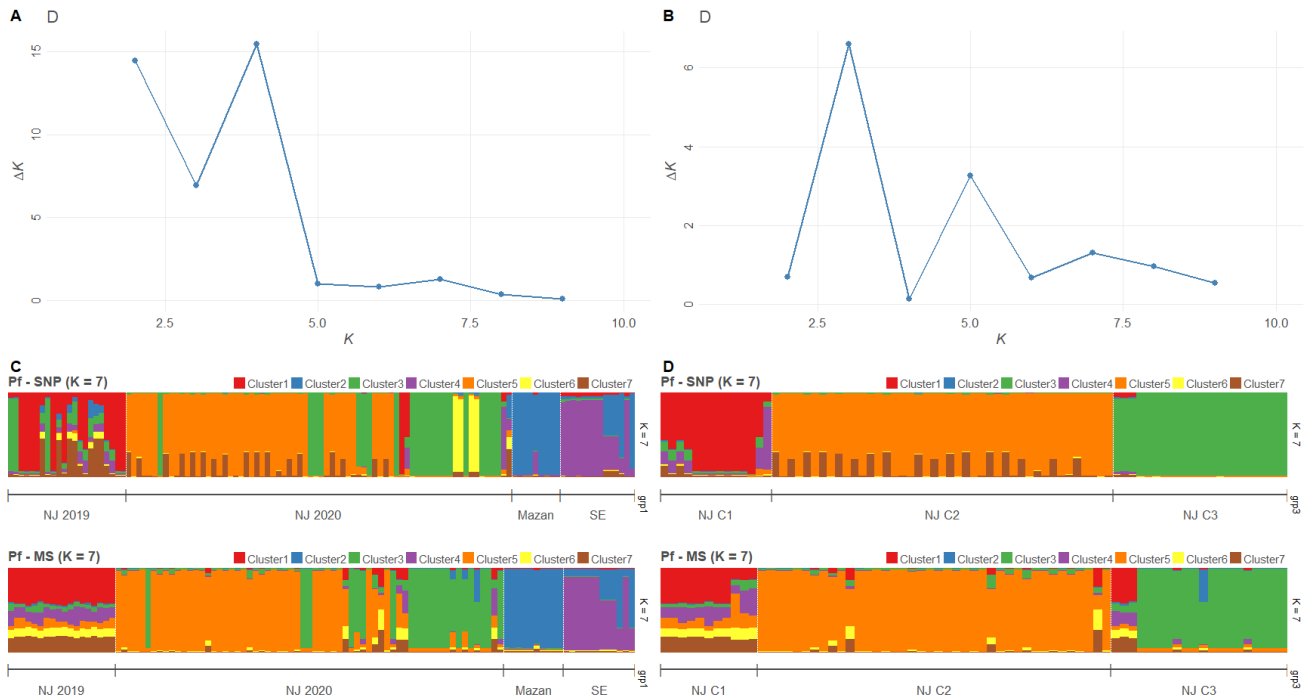
Supplementary Figure S3. MLG and MS markers excluded from the analysis. The red dashed line indicates missing rate threshold of 25%. The x-axis corresponds to the individual MLG identification (ID) (A and C) or the names of MS markers (B and D). (A) MLG from MS genotyping in Pv (A) and Pf (B) populations. MS panels for Pv (B) and Pf (D).



Supplementary Figure S4. Dunn's post hoc comparisons of genetic diversity (expressed as expected heterozygosity, H_e) across Pv and Pf populations. The red dashed lines represent z-values of -1.96 and 1.96, corresponding to a 5% significance level. The black circles indicate the z-scores calculated for each paired comparison, with the associated adjusted p-values displayed alongside each dot. The post hoc tests were performed using H_e values obtained with (A) MS panel in Pv, (B) SNP barcode for Pv, (C) MS panel in Pf, (D) SNP barcode for Pf. No significant differences were found in Pv with any panel. In Pf, only the comparisons between Mazan and NJ (2019 & 2020) were significant across both panels.



Supplementary Figure S5. Evanno plots of the STRUCTURE analysis in Pv samples using (A) SNP or (B) MS. (C) Clustering analysis by STRUCTURE at K = 7 using SNP or MS in Pv populations by location.



Supplementary Figure S6. Evanno plots of the STRUCTURE analysis in Pf samples using (A) SNP or (B) MS. Clustering analysis by STRUCTURE at $K = 7$ using SNP or MS in (C) Pf populations by location or (D) Pf samples in NJ by NJ by previous classification according to Cabrera-Sosa et al. (2024).

Supplementary Table

Supplementary Table S1. Pv and Pf sample size and collection data used in this study from different previous projects.

Project (SIDISI code)	Area	Year of collection	Type of collection	<i>P. vivax</i>			<i>P. falciparum</i>		
				Sample size - AmpliSeq	Sample size - MS	Final Sample size	Sample size - AmpliSeq	Sample size - MS	Final Sample size
VLIR- TEAM (102725)	NJ	2019	ACD	48	38	38	17	17	17
		2020	PCD	20	18	16	47	49	47
ICERM 2.0 (101518)	Mazan	2018	Population- based survey	13	6	6	10	10	10
	SE	2016	ACD & PCD	-	-	-	12	14	12

NJ: Nueva Jerusalen, SE: Santa Emilia, ACD: active case detection, PCD: passive case detection

Supplementary Table S2. Fixed positions in the SNP barcodes for Pv and Pf populations.

Fixed positions in Pv Peru SNP barcode
PvP01_03_v1_114702
PvP01_07_v1_959564

Fixed positions in Pf Peru SNP barcode
Pf3D7_03_v3_849476
Pf3D7_05_v3_921893
Pf3D7_06_v3_636044
Pf3D7_07_v3_782111
Pf3D7_08_v3_803172
Pf3D7_10_v3_1172712
Pf3D7_10_v3_341106
Pf3D7_11_v3_1505533
Pf3D7_12_v3_1552084
Pf3D7_14_v3_1381943
