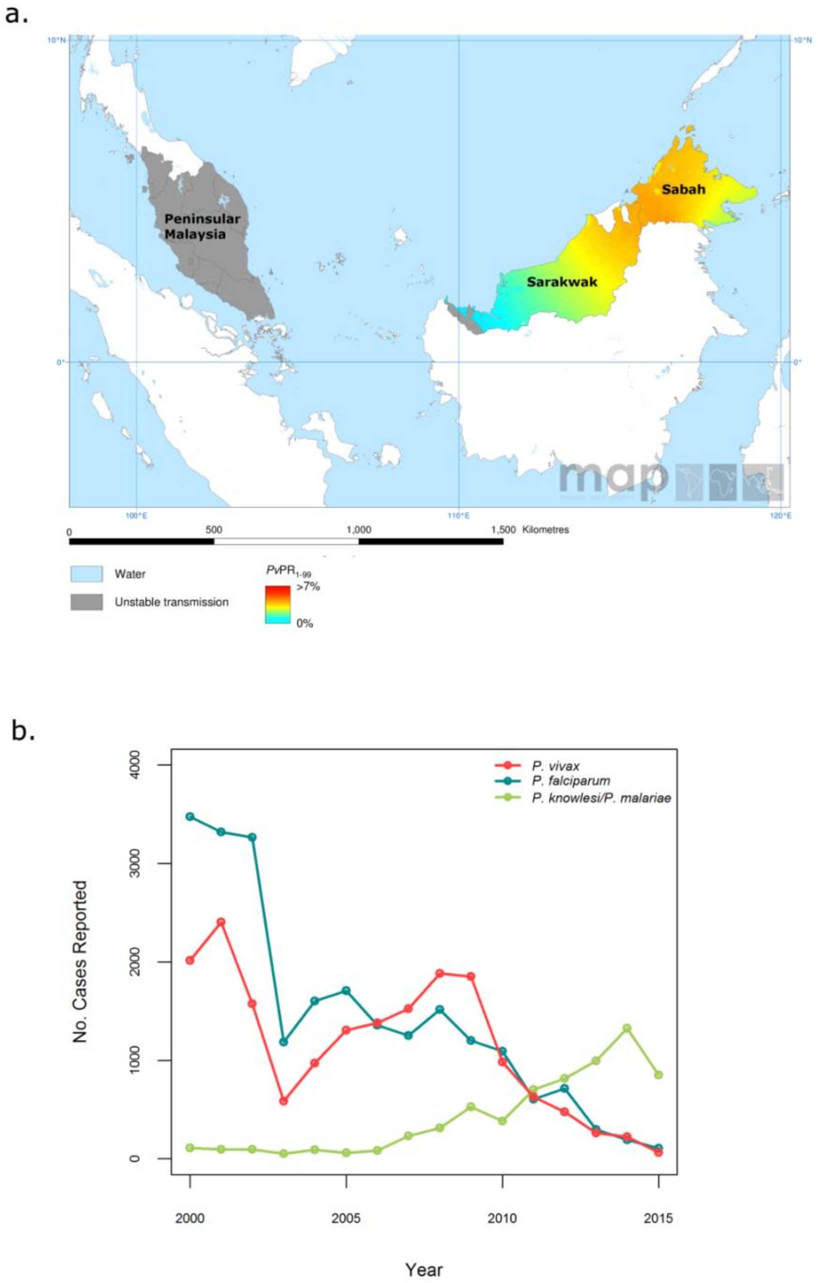
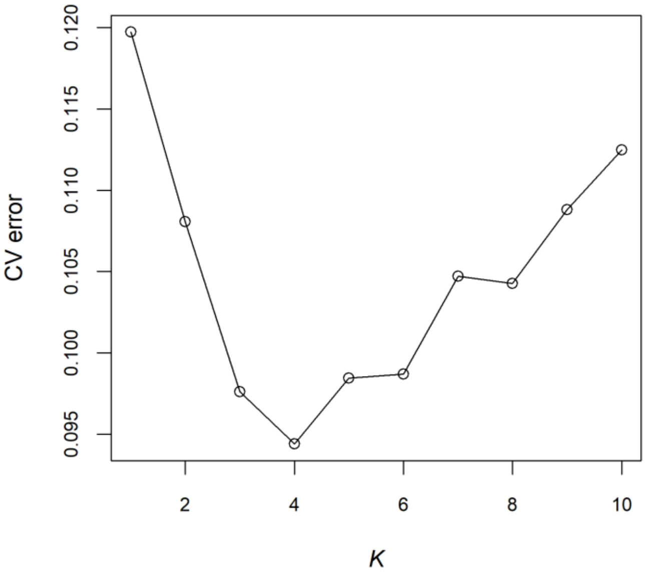
Supplementary Information

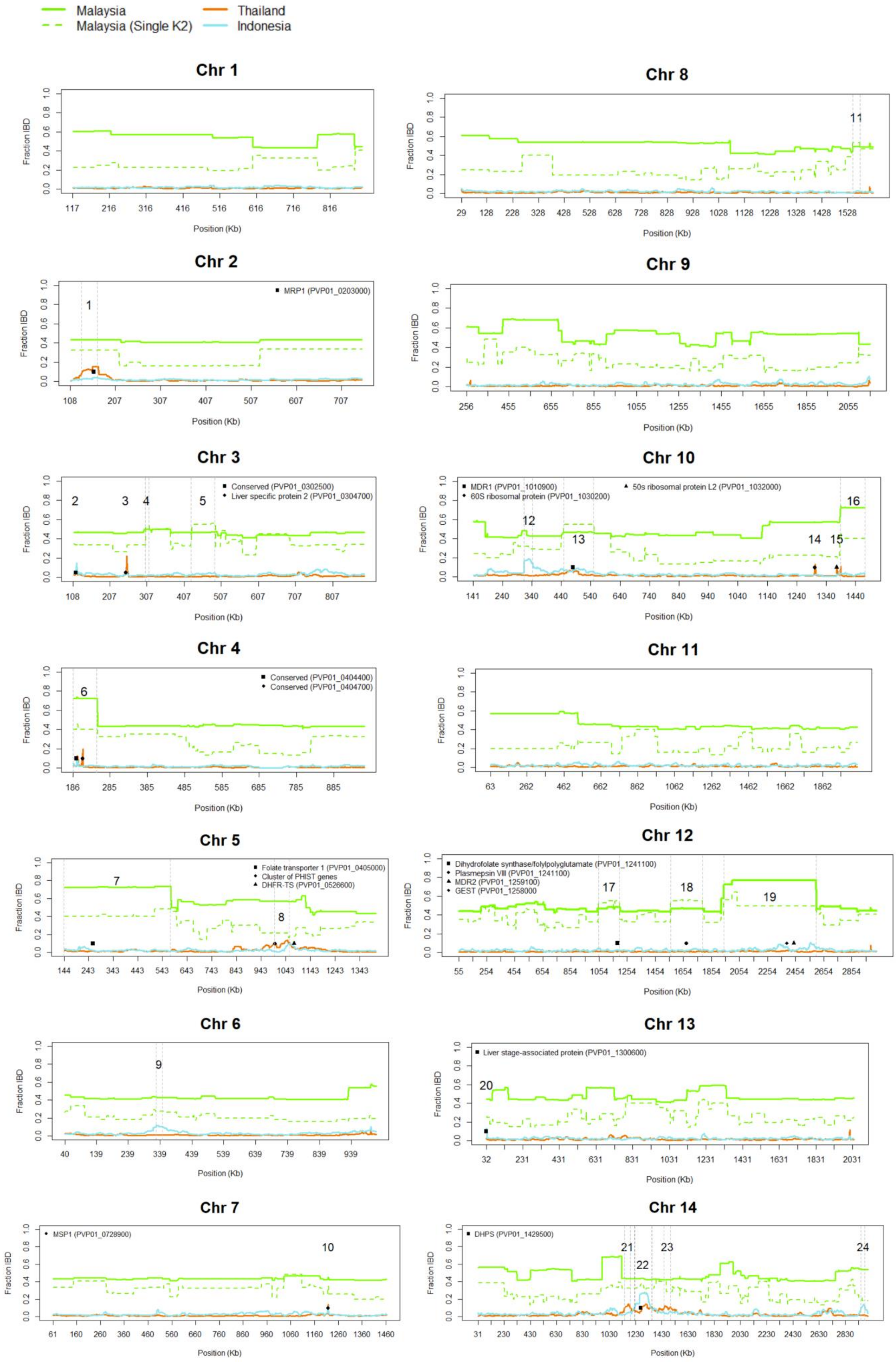
Supplementary Figures



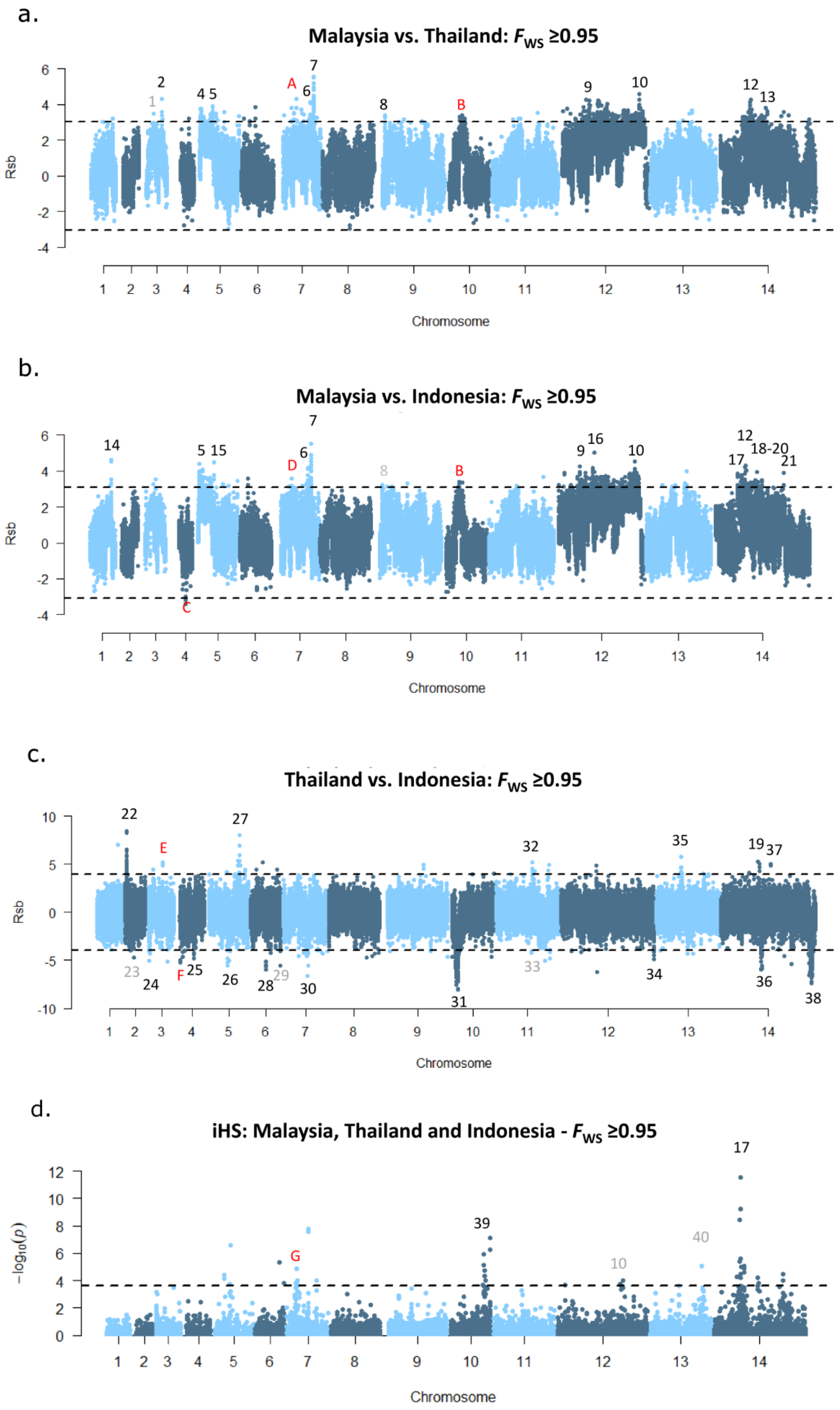
Supplementary Figure 1. *P. vivax* prevalence and incidence in Sabah. Panel a) provides a *P. vivax* prevalence map for Malaysia generated by the Malaria Atlas Project. The colour scales reflect the model-based geostatistical point estimates of the annual mean *P. vivax* parasite rate in the 1-99 year age range (*Pv*$PR_{1\text{-}99}$) within the stable spatial limits of transmission in 2010. All Malaria Atlas Project maps are available to users under the Creative Commons Attribution License CCAL 3.0. [http://www.map.ox.ac.uk/about-map/open-access/]. Panel b) illustrates the annual number of reported cases of *P. vivax*, *P. falciparum* and *P. knowlesi*/*P. malariae* in Sabah between 2000 and 2015. The data was provided by the Sabah Department of Health. Based on population size data provided by the Malaysian Department of Statistics, the annual *P. vivax* incidence (per 1000 population) was approximately 0.78 in 2000, 0.32 in 2010 and 0.02 in 2015.



Supplementary Figure 2. Admixture cross-validation errors for estimates of *K* 1 to 10 in the country-wide sample set. Analysis was performed on the full data set (*n*=259 samples). The lowest cross-validation error was observed at *K* = 4 (CV=0.094).


Malaysia
Malaysia (Single K2)
Thailand
Indonesia
Fraction IBD
Position (Kb)
0.0 0.2 0.4 0.6 0.8 1.0
Chr 1
117 216 316 416 516 616 716 816
Chr 2
1
MRP1 (PVP01_0203000)
108 207 307 407 507 607 707
Chr 3
2 3 4 5
Conserved (PVP01_0302500)
Liver specific protein 2 (PVP01_0304700)
108 207 307 407 507 607 707 807
Chr 4
6
Conserved (PVP01_0404400)
Conserved (PVP01_0404700)
186 285 385 485 585 685 785 885
Chr 5
7 8
Folate transporter 1 (PVP01_0405000)
Cluster of PHIST genes
DHFR-TS (PVP01_0526600)
144 243 343 443 543 643 743 843 943 1043 1143 1243 1343
Fraaction IBD
Chr 6
9
40 139 239 339 439 539 639 739 839 939
Chr 7
10
MSP1 (PVP01_0728900)
61 160 260 360 460 560 660 760 860 960 1060 1160 1260 1360 1460
Chr 8
11
29 128 228 328 428 528 628 728 828 928 1028 1128 1228 1328 1428 1528
Chr 9
256 455 655 855 1055 1255 1455 1655 1855 2055
Chr 10
12 13 14 15 16
MDR1 (PVP01_1010900)
60S ribosomal protein (PVP01_1030200)
50s ribosomal protein L2 (PVP01_1032000)
141 240 340 440 540 640 740 840 940 1040 1140 1240 1340 1440
Chr 11
63 262 462 662 862 1062 1262 1462 1662 1862
Chr 12
17 18 19
Dihydrofolate synthase/folylpolyglutamate (PVP01_1241100)
Plasmepsin VIII (PVP01_1241100)
MDR2 (PVP01_1259100)
GEST (PVP01_1258000)
55 254 454 654 854 1054 1254 1454 1654 1854 2054 2254 2454 2654 2854
Chr 13
20
Liver stage-associated protein (PVP01_1300600)
32 231 431 631 831 1031 1231 1431 1631 1831 2031
Chr 14
21 22 23 24
DHPS (PVP01_1429500)
31 230 430 630 830 1030 1230 1430 1630 1830 2030 2230 2430 2630 2830

Supplementary Figure 3. Fraction of sample pairs that are IBD along each chromosome in Malaysia, Thailand and Indonesia. Each plot presents the fraction of pairwise samples that are IBD for each population at 1 Kb intervals along the accessible (mappable) regions of the given chromosome. Analyses were conducted on monoclonal samples (F_{WS}≥0.95). In Malaysia, analyses were conducted on all samples excluding the 3 putatively imported cases (All) and additionally with single representation of the K2 strain (Single K2). Markedly higher levels of IBD were observed in the Malaysian samples than the Indonesian and Thai samples across the genome. Regions supported by a minimum of two adjacent positions among the top 5% IBD fractions in Malaysia and the top 1% fractions in each of Thailand and Indonesia are numbered from 1-24, with the boundaries of regions exceeding 10 Kb demarked by dashed grey lines. Gene annotations are provided where regions encompass a single gene or a biologically plausible drug resistance candidate. Region 1 (chr2, 126-161 Kb) spans 6 genes; region 2 (chr3, 116-117 Kb) spans 1 gene; region 3 (chr3, 251-252 Kb) spans 1 gene; region 4 (chr3, 303-313 Kb) spans 4 genes; region 5 (chr3, 427-491 Kb) spans 22 genes; region 6 (chr4, 186-250 Kb) spans 13 genes; region 7 (chr5, 144-576 Kb) spans 104 genes; region 8 (chr5, 997-1058 Kb) spans 16 genes; region 9 (chr6, 327-347 Kb) spans 3 genes; region 10 (chr7, 1216-1218 Kb) spans 1 gene; region 11 (chr8, 1550-1577 Kb) spans 6 genes; region 12 (chr10, 314-343 Kb) spans 4 genes; region 13 (chr10, 449-551 Kb) spans 20 genes; region 14 (chr10, 1302-1303 Kb) spans 1 gene; region 15 (chr10, 1376-1377 Kb) spans 1 gene; region 16 (chr10, 1389-1472 Kb) spans 19 genes; region 17 (chr12, 1051-1199 Kb) spans 37 genes; region 18 (chr12, 1566-1792 Kb) spans 48 genes; region 19 (chr12, 1942-2599 Kb) spans 153 genes; region 20 (chr13, 32-33 Kb) spans 1 gene; region 21 (chr14, 1150-1191 Kb) spans 9 genes; region 22 (chr14, 1224-1356 Kb) spans 24 genes; region 23 (chr14, 1449-1499 Kb) spans 19 genes; and region 24 (chr14, 2949-2978 Kb) spans 2 genes.



Supplementary Figure 4. Genome-wide scans to identify regions with extended haplotype homozygosity using monoclonal samples. Panels a), b) and c) present Manhattan plots of the *Rsb* index for the given populations, and panel d) presents the *iHS p-value* for the pooled populations. Analyses were conducted on monoclonal samples ($F_{WS} \geq 0.95$). The dashed black lines demark the top 0.5% SNPs with the most significant *p-values*. The dashed grey line in panel d) demarks the top 1% SNPs. Regions with ≥3 SNPs and with 1-2 SNPs within 80 Kb of one another and overall SNP density < 10 Kb per SNP amongst the top 0.5% SNPs that were also identified in the low complexity samples ($F_{WS} \geq 0.60$) are numbered in black and grey respectively. Details on these regions are provided in Figure 6. Seven new multi-SNP signals observed in the monoclonal dataset are labelled with red letters A-G. Signals A, B, E, F and G are reflected by 1-2 SNPs in the low-complexity data and hence are represented in Supplementary Tables 6 and 7. Signal C encompasses a conserved gene (PVP01_0415400), and signal D is an intergenic region with no clear candidates. Overall, there are no major differences in the monoclonal and low-complexity datasets.

Supplementary Tables

Supplementary Table 1. Demographic summary of the patients with successfully genotyped samples

Year	No. patients	Median age (Range)	% Males	Median parasite density, μL^{-1} (Range)
2010	24	23 (13-63)	83%	5,642 (929-57,000) ^{8}
2011	32	20.5 (4-79)	72%	3,152 (120-10,160) ^{10}
2012	18	30.5 (4-71)	72%	2,124 (179-5,700) ^{7}
2013	26	11 (2-42)	58%	5,279 (249-24,400)
2014	105	17 (2-68)	69%	3,882 (91-140,500)
2015	7	32 (2-70)	71%	6,767 (77-36250)
2010-2015	**212**	**19 (2-79)**	**70%**	**3,995 (77-140,500)** ^{25}

Superscript denotes number of patients with missing data

Supplementary Table 2. Temporal trends in within-host and population level diversity using the STR genotyping data

Year	No. samples	No. unique MLGs	No. low complexity samples (%)*	No. polyclonal samples (%)	Mean MOI, median (range)	Median *R*s (range)	Median *R*s of unique MLGs (range)	Median *R*s of low complexity samples (range)
2010	24	17	22 (92%)	12 (50%)	1.54, 1.5 (1-3)	4.98 (3.94-8.67)	4.89 (3.90-7.49)	4.93 (3.75-8.73)
2011	32	23	26 (81%)	9 (28%)	1.34, 1 (1-3)	6.51 (4.40-8.65)	6.02 (4.17-7.63)	6.11 (4.45-7.93)
2012	18	16	17 (94%)	3 (17%)	1.17, 1 (1-2)	8.00 (3.89-10.20)	6.67 (3.59-7.59)	7.88 (3.88-9.38)
2013	26	12	25 (96%)	4 (15%)	1.15, 1 (1-2)	3.91 (3.00-5.91)	4.64 (3.00-6.15)	3.60 (2.99-5.94)
2014	105	16	104 (99%)	8 (8%)	1.08, 1 (1-2)	2.51 (2.21-4.22)	4.46 (3.48-6.21)	2.37 (2.16-4.14)

*No. samples with a maximum of 1 multi-allelic locus

Supplementary Table 3. Temporal trends in linkage disequilibrium using the STR genotyping data

Year	**No. samples with complete MLGs**	**I^{S}_{A} on all complete MLGs**	**No. unique MLGs [1]**	**I^{S}_{A} unique MLGs**	**No. low complexity samples [2]**	**I^{S}_{A} low complexity MLGs**
2010	16	0.486 **	11	0.230 **	15	0.529 **
2011	27	0.478 **	18	0.116 **	21	0.613 **
2012	13	0.167 **	11	0.019 *	12	0.179 **
2013	21	0.536 **	9	0.258 **	21	0.536 **
2014	103	0.366 **	15	0.285 **	102	0.373 **

[1] No. unique MLGs in the samples with complete genotyping data. [2] No. samples with a maximum of one multi-allelic locus with complete MLGs. (Monte Carlo simulation, *$p < 0.05$. **$p < 0.01$).

Supplementary Table 4. Demographic details of Individuals with ‘outbreak’ *P. vivax* strains relative to other cases

	K2 (MLG38)	**[s] Other high frequency MLGs**	**[b] Low frequency MLGs**
Cases, *n*	68	52	67
Age in years, median (range)	15 (2-68)	18 (3-45)	24 (2-79)
Males, *n* (%)	42 (62%)	38 (73%)	49 (73%)
Parasite density (μl^{-1}), median (range)	4,180 (77-140,500)	3,690 (91-42,520) [5]	4,186 (120-57,000) [15]
Unknown occupation (missing data), *n* (%)	0/68 (0%)	1/52 (2%)	4/67 (11%)
Farmer, rubber tapper, forestry or plantation worker	20/68 (29%)	22/51 (43%)	23/63 (37%)
Student, *n* (%)	25/68 (37%)	14/51 (27%)	10/63 (16%)
Other occupation, *n* (%)	10/68 (15%)	4/51 (8%)	21/63 (33%)
Unemployed, *n* (%)	13/68 (19%)	11/51 (22%)	9/63 (14%)

[a] MLGs observed in 5 or more Independent cases (excluding K2 (MLG38)), including MLGs 39, 43, 44, 45, 46, 49, 50, 52, 55. [b] MLGs observed in less than 5 Independent cases. [5-20] Number of Individuals with missing data.

Supplementary Table 5. European Nucleotide Archive run accession codes for the new *P. vivax* sequence data from Malaysia

Sample ID	Run accession	URL
PY0002-C	ERR054089	[https://www.ebi.ac.uk/ena/data/view/ERR054089]
PY0004-C	ERR152414	[https://www.ebi.ac.uk/ena/data/view/ERR152414]
PY0005-C	ERR152415	[https://www.ebi.ac.uk/ena/data/view/ERR152415]
PY0018-C	ERR527337	[https://www.ebi.ac.uk/ena/data/view/ERR527337]
PY0019-C	ERR527363	[https://www.ebi.ac.uk/ena/data/view/ERR527363]
PY0023-C	ERR1138855	[https://www.ebi.ac.uk/ena/data/view/ERR1138855]
PY0024-C	ERR1138856	[https://www.ebi.ac.uk/ena/data/view/ERR1138856]
PY0026-C	ERR1138857	[https://www.ebi.ac.uk/ena/data/view/ERR1138857]
PY0027-C	ERR1138858	[https://www.ebi.ac.uk/ena/data/view/ERR1138858]
PY0034-C	ERR1138861	[https://www.ebi.ac.uk/ena/data/view/ERR1138861]
PY0035-C	ERR1138862	[https://www.ebi.ac.uk/ena/data/view/ERR1138862]
PY0042-C	ERR1138864	[https://www.ebi.ac.uk/ena/data/view/ERR1138864]
PY0044-C	ERR1138865	[https://www.ebi.ac.uk/ena/data/view/ERR1138865]
PY0045-C	ERR1138866	[https://www.ebi.ac.uk/ena/data/view/ERR1138866]
PY0048-C	ERR1138867	[https://www.ebi.ac.uk/ena/data/view/ERR1138867]
PY0050-C	ERR1138868	[https://www.ebi.ac.uk/ena/data/view/ERR1138868]
PY0051-C	ERR1106842	[https://www.ebi.ac.uk/ena/data/view/ERR1106842]
PY0053-C	ERR1138869	[https://www.ebi.ac.uk/ena/data/view/ERR1138869]
PY0054-C	ERR1138870	[https://www.ebi.ac.uk/ena/data/view/ERR1138870]
PY0055-C	ERR1138871	[https://www.ebi.ac.uk/ena/data/view/ERR1138871]
PY0056-C	ERR1138872	[https://www.ebi.ac.uk/ena/data/view/ERR1138872]
PY0057-C	ERR1138873	[https://www.ebi.ac.uk/ena/data/view/ERR1138873]
PY0058-C	ERR1106843	[https://www.ebi.ac.uk/ena/data/view/ERR1106843]
PY0060-C	ERR1138875	[https://www.ebi.ac.uk/ena/data/view/ERR1138875]
PY0061-C	ERR1138876	[https://www.ebi.ac.uk/ena/data/view/ERR1138876]
PY0067-C	ERR1138879	[https://www.ebi.ac.uk/ena/data/view/ERR1138879]
PY0068-C	ERR1106846	[https://www.ebi.ac.uk/ena/data/view/ERR1106846]
PY0072-C	ERR1138881	[https://www.ebi.ac.uk/ena/data/view/ERR1138881]
PY0073-C	ERR1138882	[https://www.ebi.ac.uk/ena/data/view/ERR1138882]
PY0074-C	ERR1138883	[https://www.ebi.ac.uk/ena/data/view/ERR1138883]
PY0075-C	ERR1138884	[https://www.ebi.ac.uk/ena/data/view/ERR1138884]
PY0076-C	ERR1138885	[https://www.ebi.ac.uk/ena/data/view/ERR1138885]
PY0085-C	ERR1475425	[https://www.ebi.ac.uk/ena/data/view/ERR1475425]
PY0087-C	ERR1475427	[https://www.ebi.ac.uk/ena/data/view/ERR1475427]
PY0088-C	ERR1475395	[https://www.ebi.ac.uk/ena/data/view/ERR1475395]
PY0089-C	ERR1475451	[https://www.ebi.ac.uk/ena/data/view/ERR1475451]
PY0090-C	ERR1475441	[https://www.ebi.ac.uk/ena/data/view/ERR1475441]
PY0092-C	ERR1475396	[https://www.ebi.ac.uk/ena/data/view/ERR1475396]
PY0093-C	ERR1475397	[https://www.ebi.ac.uk/ena/data/view/ERR1475397]
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PY0096-C	ERR1475399	[https://www.ebi.ac.uk/ena/data/view/ERR1475399]

PY0099-C	ERR1475418	[https://www.ebi.ac.uk/ena/data/view/ERR1475418]
PY0100-C	ERR1475419	[https://www.ebi.ac.uk/ena/data/view/ERR1475419]
PY0101-C	ERR1475420	[https://www.ebi.ac.uk/ena/data/view/ERR1475420]
PY0103-C	ERR1475429	[https://www.ebi.ac.uk/ena/data/view/ERR1475429]
PY0105-C	ERR1475430	[https://www.ebi.ac.uk/ena/data/view/ERR1475430]
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PY0120-C	ERR1475434	[https://www.ebi.ac.uk/ena/data/view/ERR1475434]