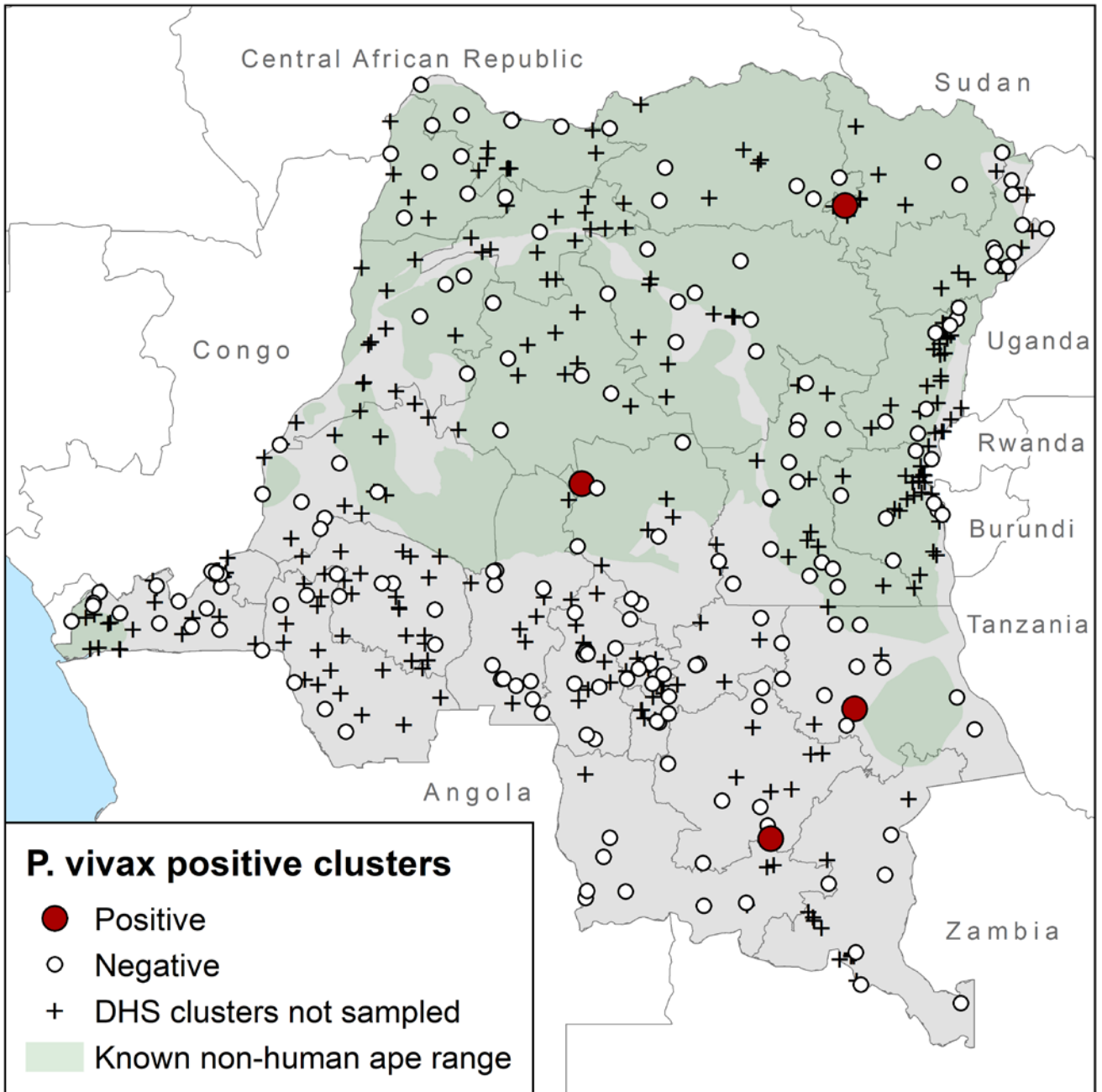


The following are supplemental materials and will be published online only



Supplemental Figure 1: Map of the Democratic Republic of the Congo with known non-human ape range (green), clusters not selected in the screen (black), clusters that screened positive for *P. vivax* (red), and clusters that screened negative for *P. vivax* (white). Shapefiles for the Democratic Republic of the Congo were downloaded from the Database of Global Administrative Areas (<http://www.gadm.org/>) and plotted with ArcGIS (version 10.4.1). The non-human ape boundaries were defined by data from the International Union for Conservation of Nature (<http://www.iucnredlist.org/>).

| Ref. | Primer | Sequence | Recipe | Thermocycler Conditions |
|--|-----------------------|--|---|--|
| Singh <i>et al.</i> 1999 ²⁹ | rPLU 1 | 5' – TCAAAGATTA AGCCATGCAA GTGA | Round 1 1. 12.5 µL of HotStarTaq Master Mix (Qiagen©, Venlo, Netherlands) 2. 400 nM of forward and reverse primer 3. 5 µL of template DNA | 1. 95°C for 15 minutes 2. 35 cycles of 94°C for 1 minute, 50°C for 1 minute, and 72°C for 1 minute 3. Final extension of 72°C for 10 minutes |
| | rPLU 5 | 5' – CCTGTTGTTGC CTTAAACTCC | | |
| | rViv 1 | 5' – CGCTTCTAGCT TAATCCACAT AACTGATAC | Round 2 1. 12.5 µL of HotStarTaq Master Mix (Qiagen©, Venlo, Netherlands) 2. 400 nM of forward and reverse primer 3. 1 µL of round-1 PCR product | |
| | rViv 2 | 5' – ACTTCCAAGC CGAAGCAAAG AAAGTCCTTA | | |
| Veron <i>et al.</i> 2009 ³⁰ | Pv-1 | 5' – CGCTTCTAGCT TAATCCACAT TAACTG | 1. 12.5 µL of FastStart Universal Probe (Roche©, Indianapolis, IN) 2. 1000 nM of forward and reverse primer 3. 200 nM Pv-probe (VIC-MGB) 4. 2 µL of template DNA | 1. 95°C for 10 min 2. 40 cycles of 95°C for 15 sec 3. 60°C for 1 minute |
| | Pv-2 | 5' – AATTTACTCA AAGTAACAAG GACTTCCAAG | | |
| | Pv-probe (VIC-MGB) | 5' – CGCATTTTGCT ATTATGT | | |
| Ménard <i>et al.</i> 2010 ⁶ | Duffy Outer Forward * | 5' – GTGGGGTAAG GCTTCCTGAT | Round 1 1. 400 nM of each primer 2. 0.25 µL of FastStart High Fidelity Taq (Enzyme Blend; Roche©, | 1. 95°C for 2 min 2. 40 cycles of 95°C for 30 seconds, 64°C for 30 seconds, and 72°C for 5 minutes |
| | Duffy Outer Reverse | 5' – CAGAGCTGCG AGTGCTACCT | | |

| | | | | |
|--|-----------------------|----------------------------------|--|---|
| | | | Indianapolis, IN) 3. 2.5 µL of 10x FastStart High Fidelity reaction buffer with 18 mM MgCl ₂ 4. 250 µM of dNTPs 5. 3µL of template DNA | 3. Final extension of 72°C for 5 minutes |
| | Duffy Outer Forward * | 5' – GTGGGGTAAG GCTTCCTGAT | Round 2 1. 360 nM of each primer 2. 0.25 µL of FastStart High Fidelity Taq (Enzyme Blend; Roche, Indianapolis, IN) 3. 2.5 µL of 10x FastStart High Fidelity Reaction Buffer, 1 mM of MgCl ₂ 4. 250 µM of dNTPs 5. 3 µL of first-round PCR product as the template | 1. 95°C for 2 min 2. 40 cycles of 95°C for 30 seconds, 61°C for 30 seconds, and 72°C for 5 minutes 3. Final extension of 72°C for 5 minutes |
| | Duffy GATA1 Reverse | 5' – CAAACAGCAG GGGAAATGAG | | |

Supplemental Table 1: The adapted protocols used for diagnostic PCR, confirmatory qPCR, and Duffy-genotyping with associated PCR recipes and thermocycler conditions. *Hemi-nested PCR protocol (same primer).

| Sample | Sequence |
|--------|---|
| D9U3K | CTTTGTGCGCATTGCTATTATGTGTTCTTTAATTAAAATGATTCTTTTAAGGACTTTCTTTGCTTCGGCTTGGAAGTAA |
| I4G3V | TTTGTGCGCATTGCTATTATGTGTTCTTTAATTAAAATGATTCTTTTAAGGACTTTCTTTGCTTCGGCTTGGA |
| N4M7L | TTTGTGCGCATTGCTATTATGTGTTCTTTAATTAAAATGATTCTTTTAAGGACTTTCTTTGCTTCGGCTTGGA |
| Q8J6O | TTGTGCGCATTGCTATTATGTGTTCTTTAATTAAAATGATTCTTTTAAGGACTTTCTTTGCTTCGGCTTGGA |
| O6Y4X | TGCTATTATGTGTTCTTTAATTAAAATGATTCTTTTAAGGACTTTCTTTGCTTC |
| M2B5U | ATTATGTGTTCTTTAATTAAAATGATTCTTTTAAGGACTTTCTTTGCTTCGG |
| P8A6E | TTATGTGTTCTTTAATTAAAATGATTCTTTTAAGGACTTTCTTTGCTTCGGCTTGGA |
| X3M5S | TTATGTGTTCTTTAATTAAAATGATTCTTTTAAGGACTTTCTTTGCTTCGGCTTGGA |
| B5A7N | TTATGTGTTCTTTAATTAAAATGATTCTTTTAAGGACTTTCTTTGCTTCGGCTTGGAAGTA |
| Z6W9M | TTAATTAAAATGATTCTTTTAAGGACTTTCTTTGCTTCGGCTTGGA |
| J6V8T | AATTAAAATGATTCCTTTTAAGGACTT |
| T6B8J | TTTAAGGACTTTCTTTGCTTCGGCTTGGA |

Supplemental Table 2: Sanger sequences for the *P. vivax* 18s rRNA gene by sample. The ends of the sequences were trimmed for low quality bases using the default settings in Geneious® 10.1.3 (Biomatters Limited, Auckland, New Zealand).