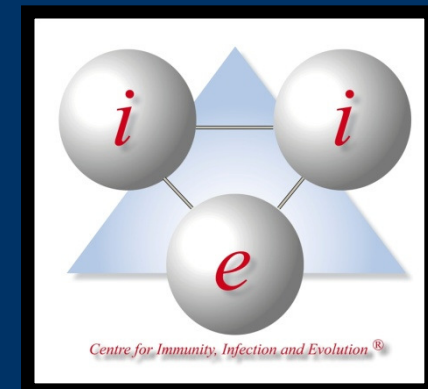


Comparative genomics of ape malaria parasites and the emergence of human malaria

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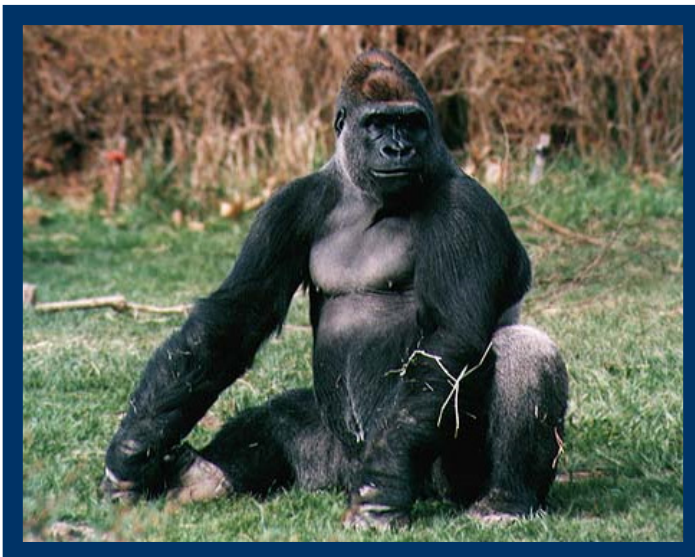
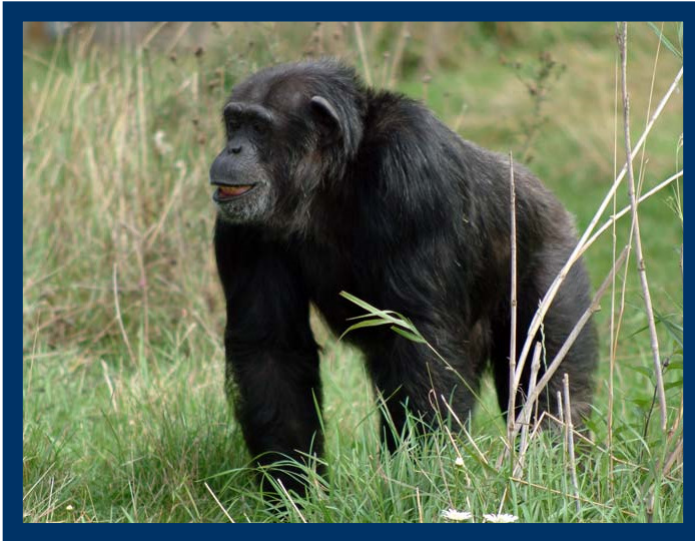
Background



Beatrice Hahn
University of Pennsylvania

2013 National Academy of Sciences USA

Samples from wild apes (for SIV)

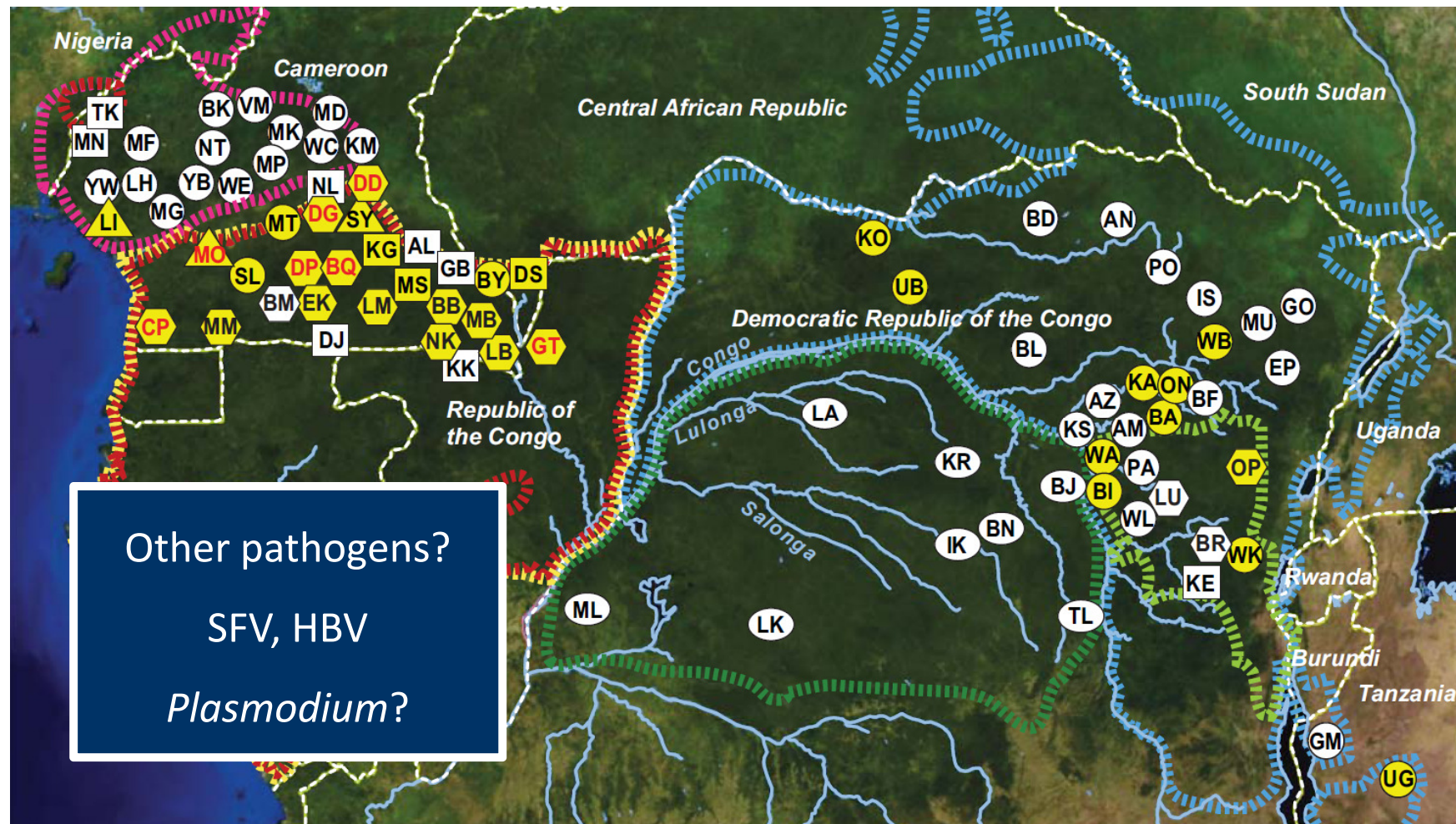


Faecal samples (in RNAlater)
from which:

- host DNA
 - mtDNA – species
 - amelogenin (X vs Y)
 - microsatellites
- viral antibodies
- viral nucleic acids

Sample sites

Chimpanzees, Bonobos, Gorillas (>5000 samples)



Other pathogens?
SFV, HBV
Plasmodium?

The malaria parasites: *Plasmodium*

Plasmodium species are protozoa

(Phylum Apicomplexa)

- transmitted by *Anopheles* mosquitoes

Plasmodium species infecting humans:

<i>P. falciparum</i>	Africa, Asia
<i>P. vivax</i>	Asia
<i>P. malariae</i>	Africa
<i>P. ovale</i>	Africa
<i>P. knowlesi</i>	SE Asia (monkeys)

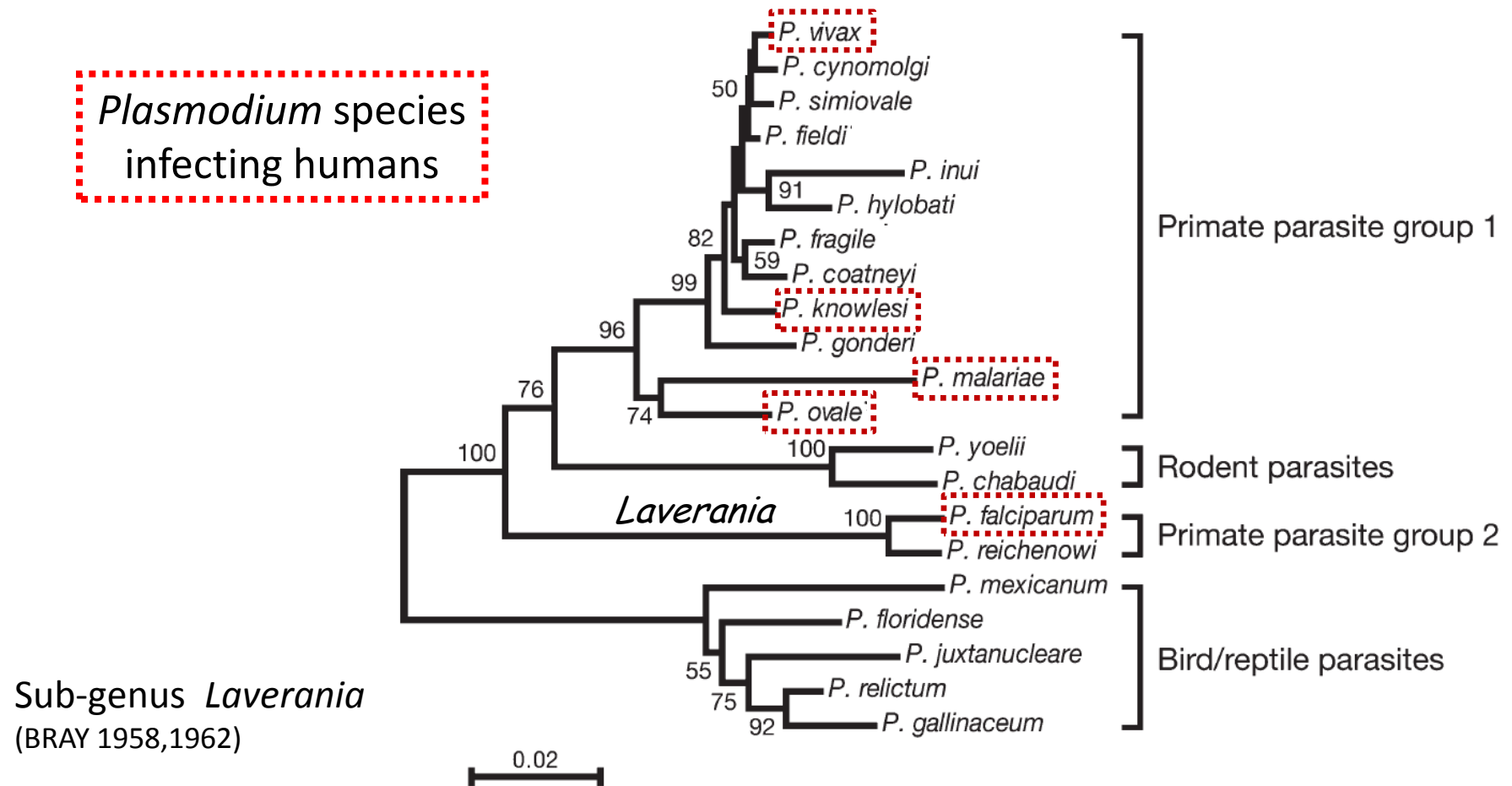
Other *Plasmodium* species known infecting other mammals, and birds and reptiles

Plasmodium falciparum



Anopheles mosquitoes

Phylogeny of malaria parasites



What might we find in apes?

Ape *Plasmodium* species:

Parasites from chimpanzees and gorillas

Eduard Reichenow in Cameroon ~1917

Saul Adler, Donald Blacklock

in Sierra Leone ~1922

- three morphologically distinct species:

Human parasite

P. falciparum

P. malariae

P. vivax OR *P. ovale*

Ape parasite

P. reichenowi

P. rhodaini

P. schwetzi



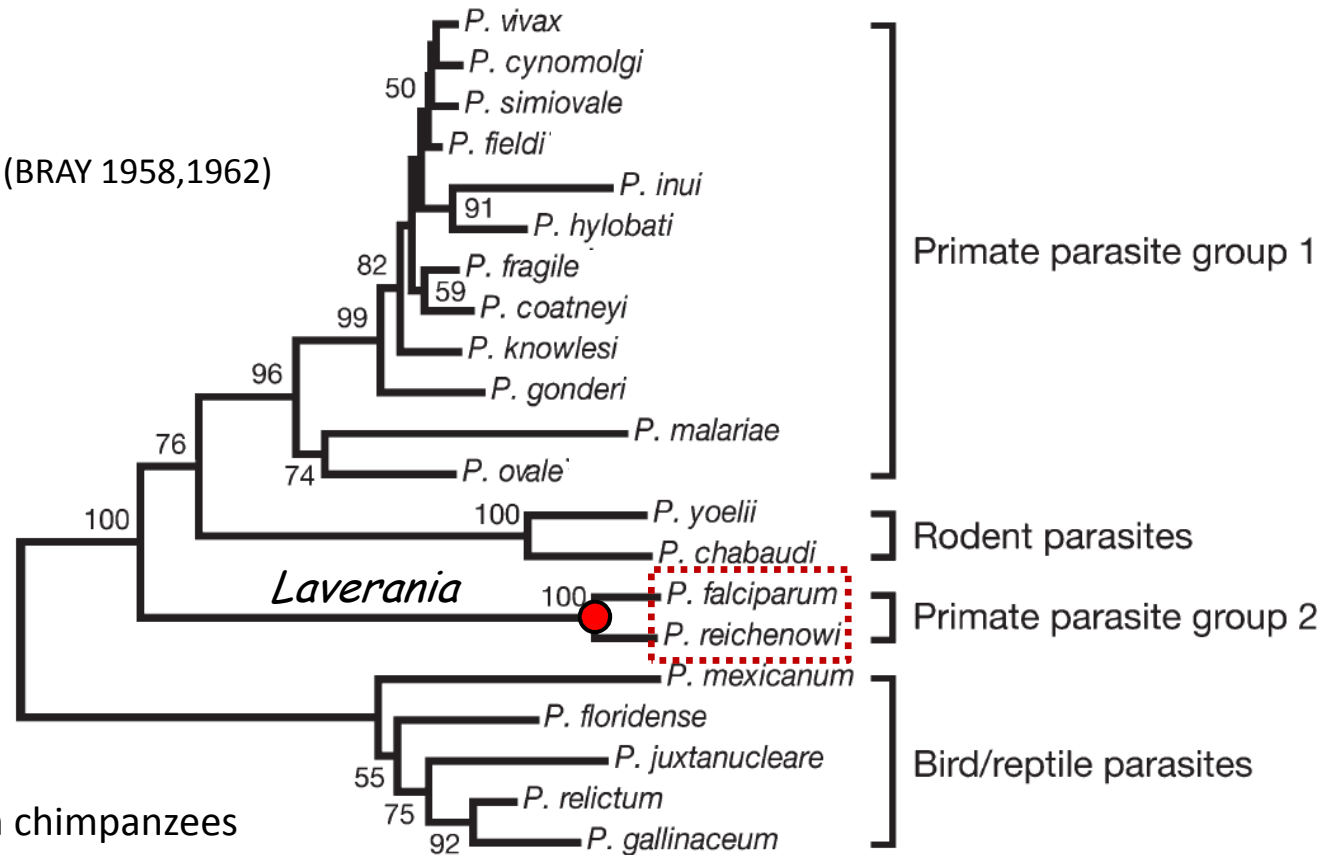
ONE isolate in culture

no samples

no samples

Origin of *Plasmodium falciparum* ?

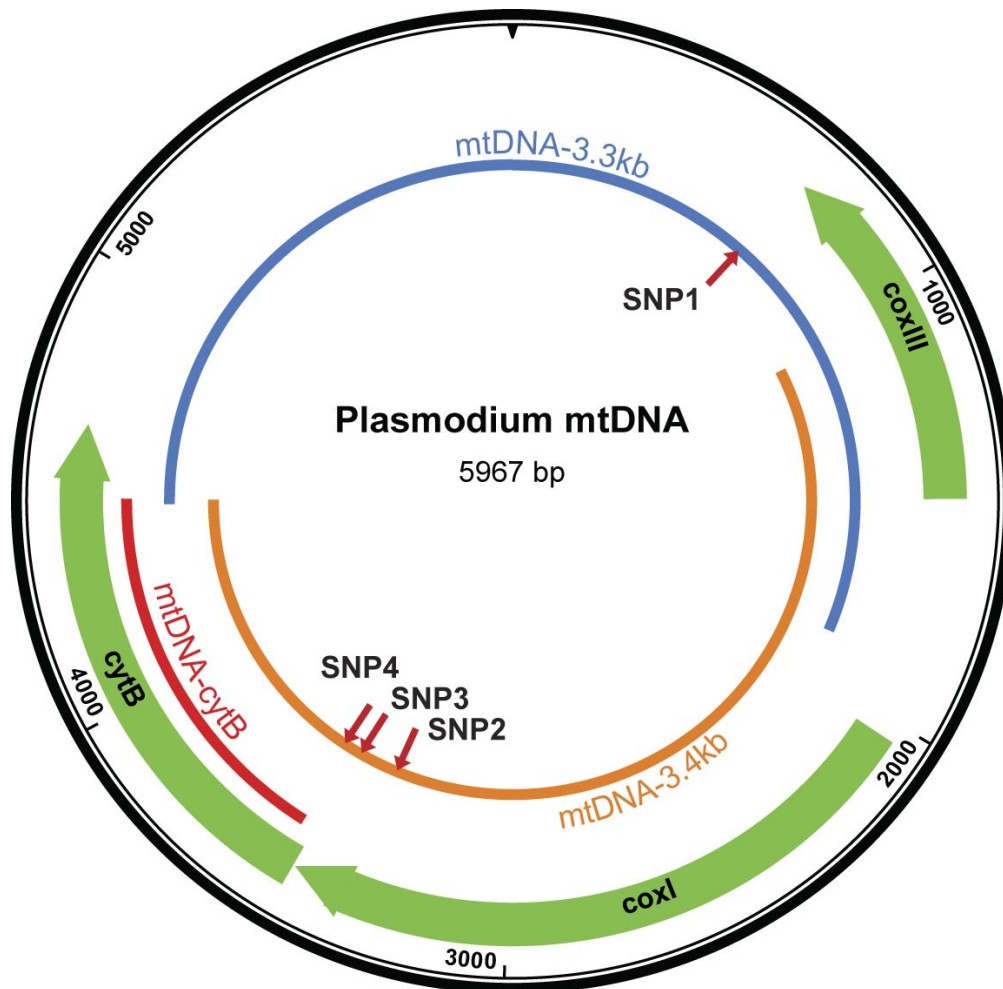
Sub-genus *Laverania* (BRAY 1958,1962)



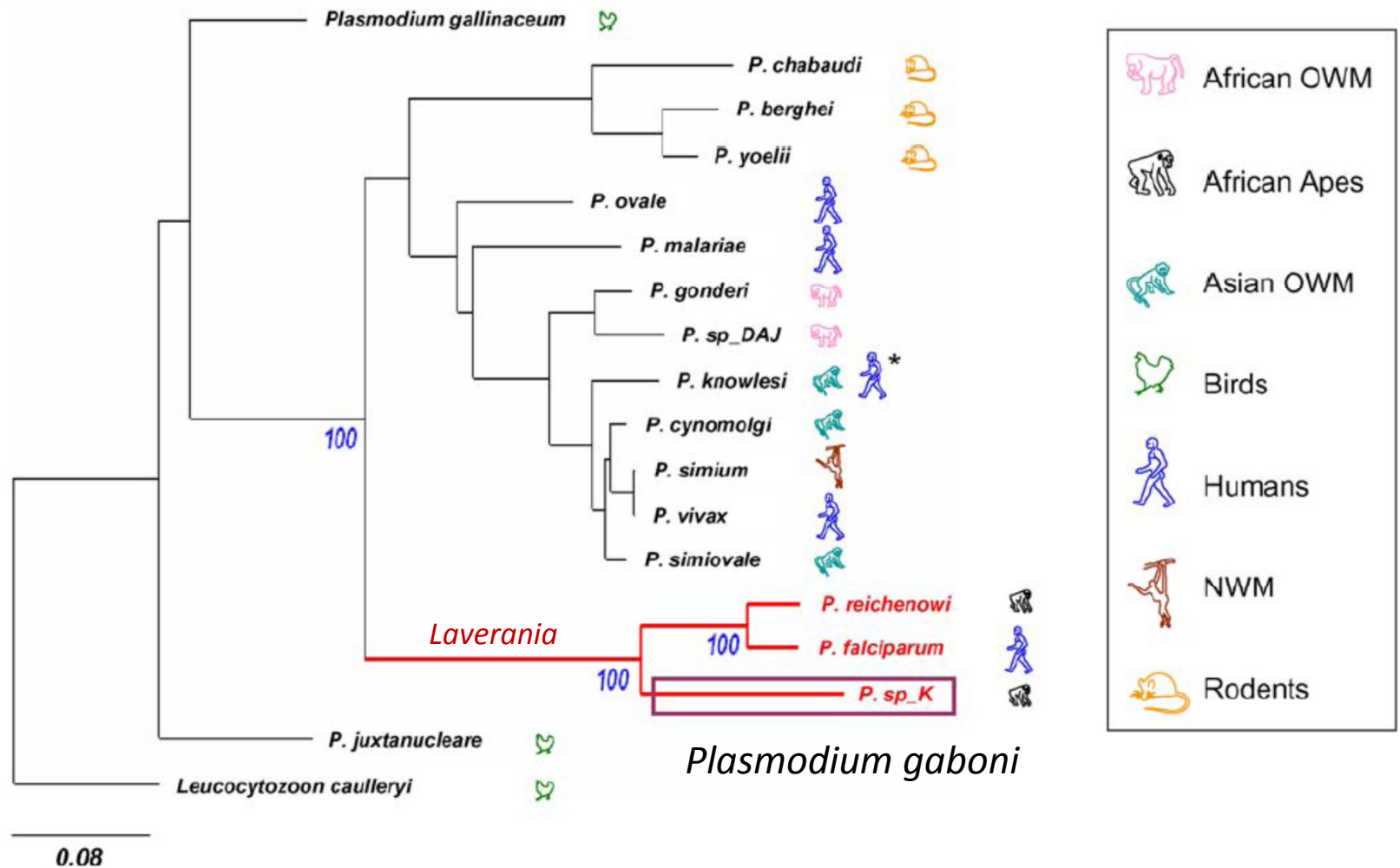
Plasmodium reichenowi from chimpanzees

- co-divergence?
- common ancestor ● at ~7 Myr ago ?

Plasmodium sequence amplification

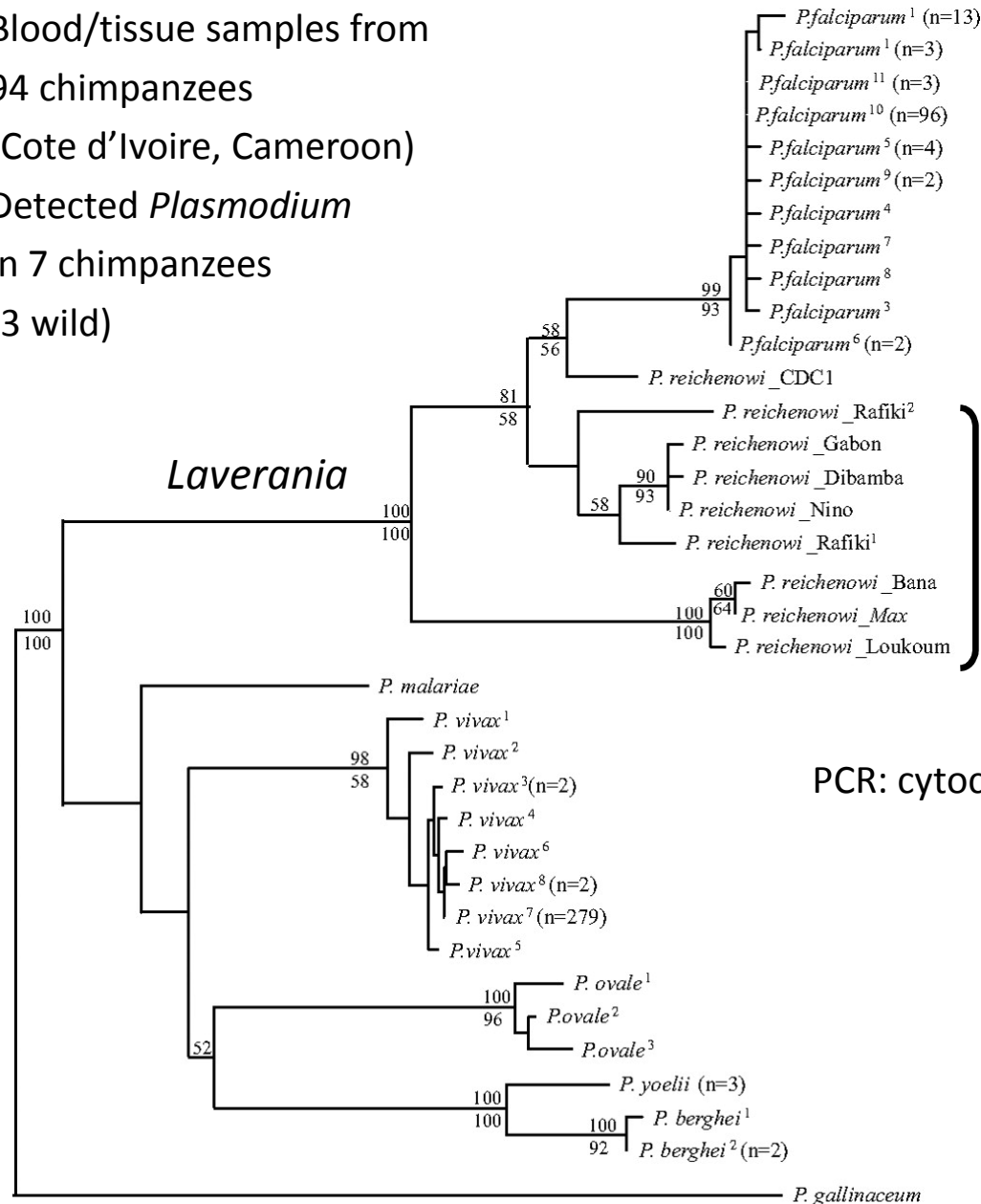
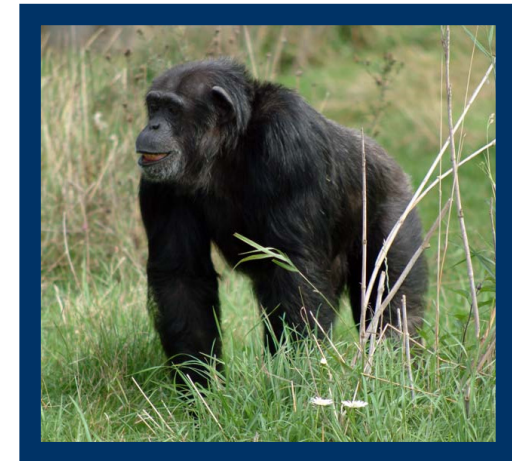


- Plasmodium mitochondrial genome**
 - 950 bp *cytB* gene
 - 3.4 kb half genome
 - 3.3 kb half genome
- apicoplast genome**
 - 390 bp *clpC* gene
- nuclear genome**
 - 780 bp *ldh* gene



Blood samples from 17 chimpanzees (Gabon) – wild-born pets
 Detected *Plasmodium* (PCR and microscopy) in 2 chimpanzees
 PCR: full length mtDNA (6 kb) for one, *cytB* (866bp) for second

Blood/tissue samples from
94 chimpanzees
(Cote d'Ivoire, Cameroon)
Detected *Plasmodium*
in 7 chimpanzees
(3 wild)

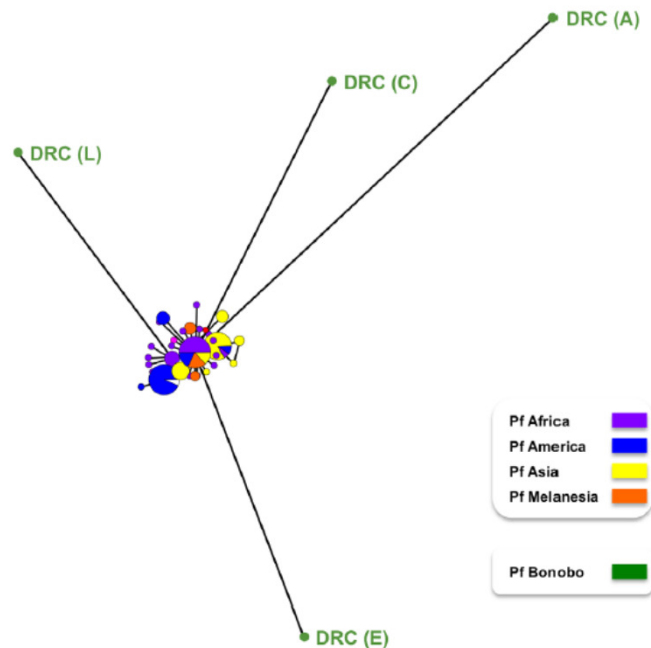


new samples from
chimpanzees

PCR: cytochrome b (mtDNA)

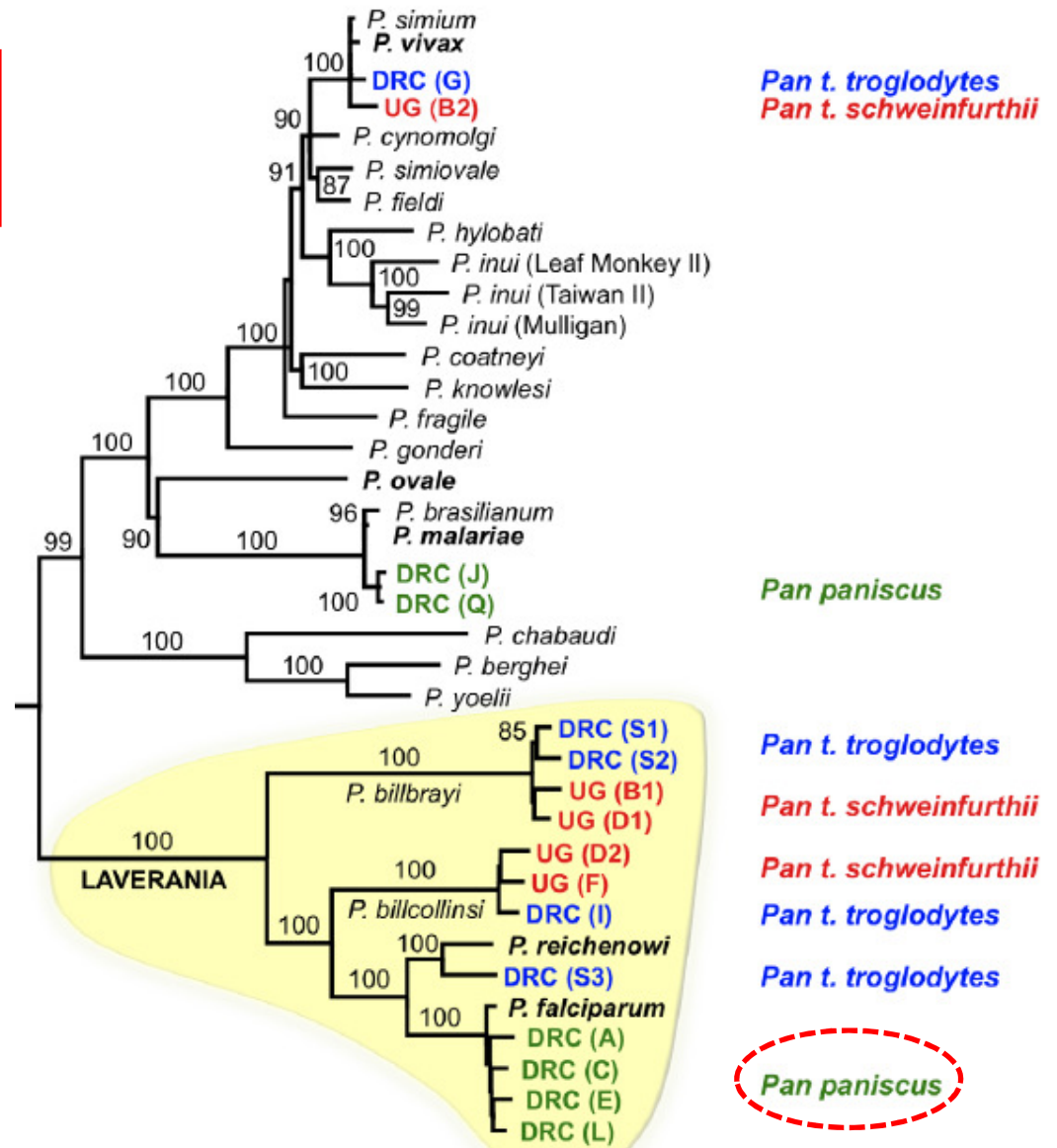
“The origin of malignant malaria”
= chimpanzees

“The origin of malignant malaria”
= bonobos

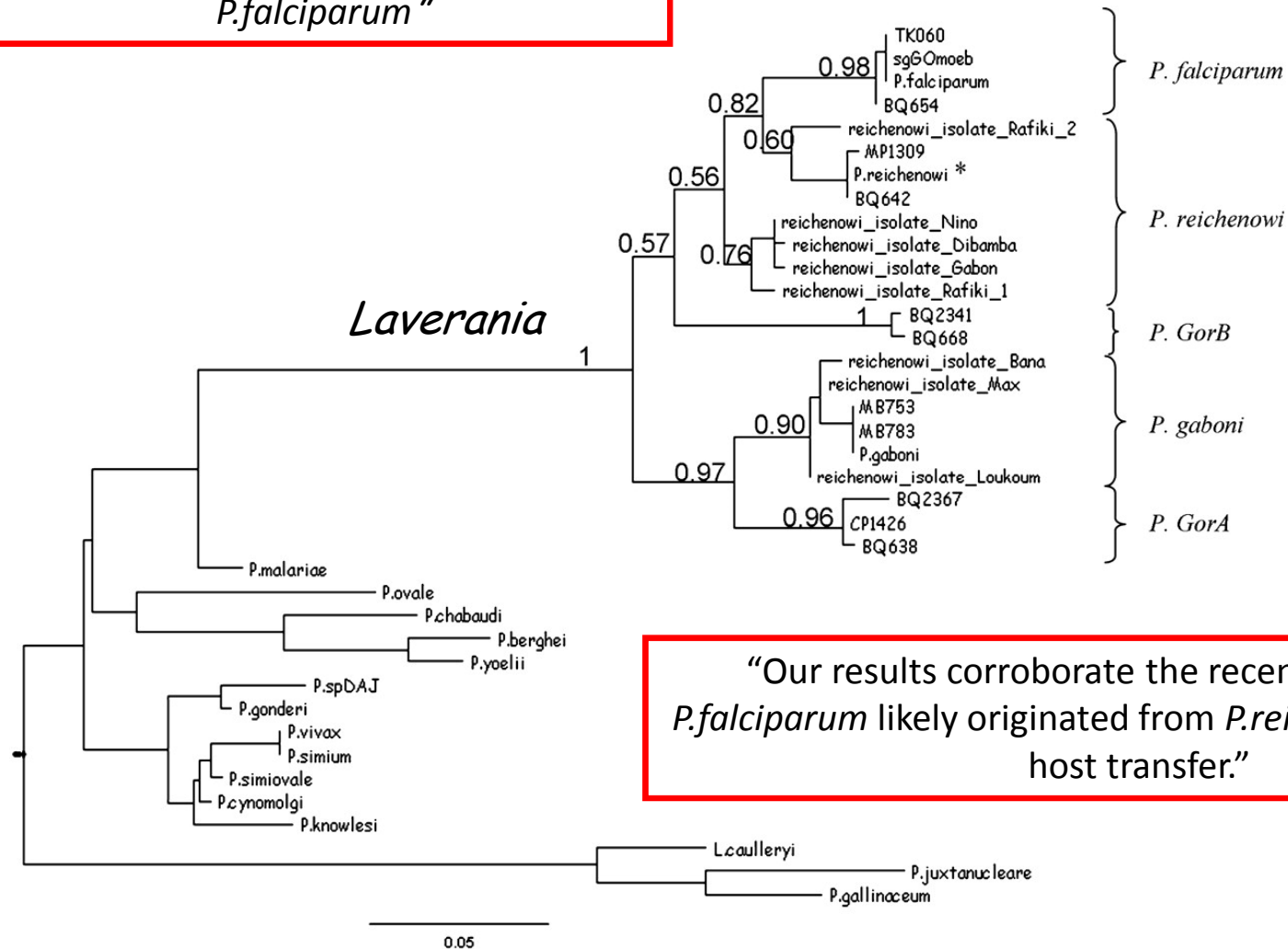


Bonobos from Kinshasa sanctuary

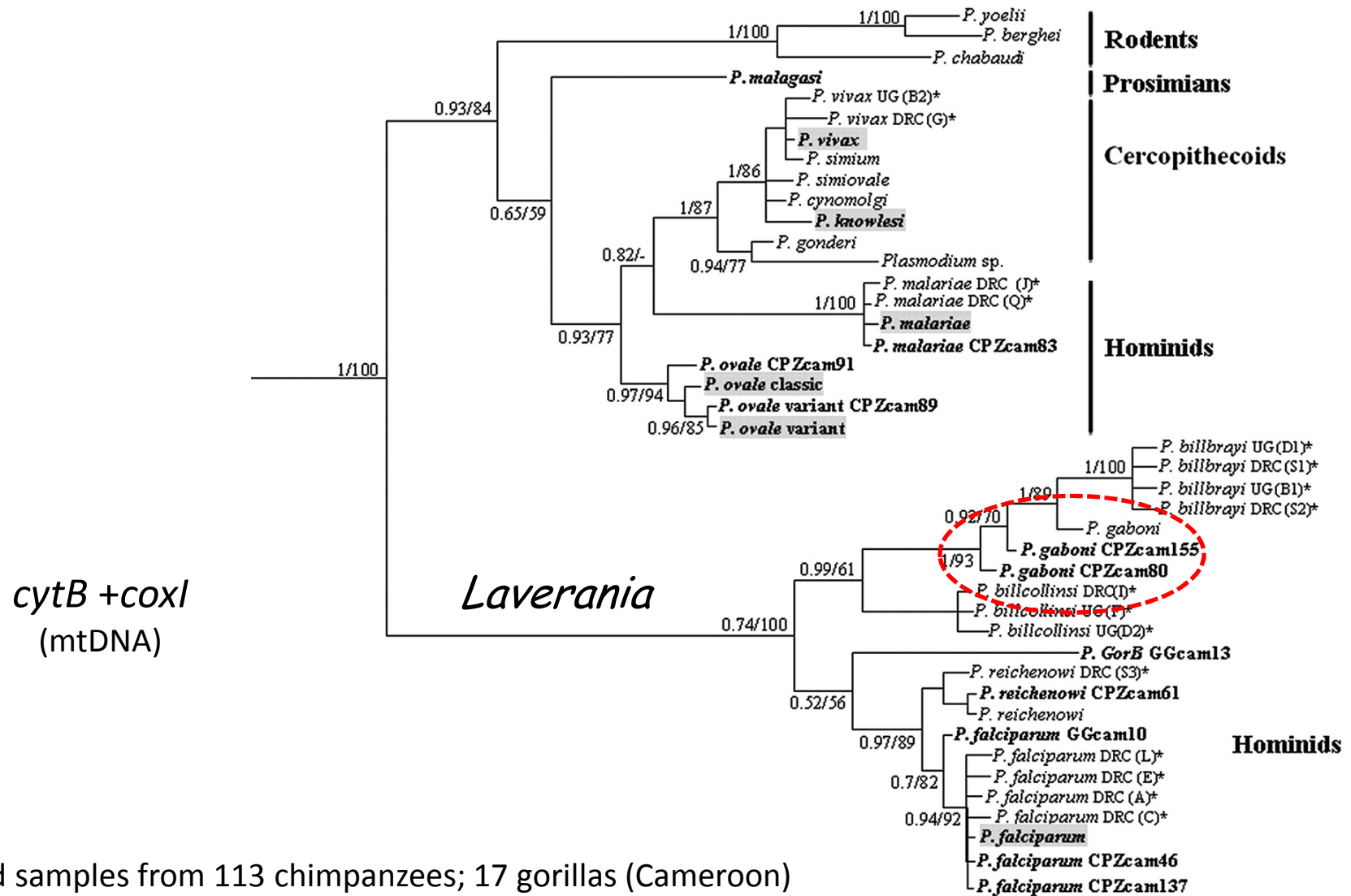
Bonobo *Plasmodium* had drug-resistance mutations



“... gorillas are naturally infected by
P.falciparum”



“Our results corroborate the recent proposal that *P.falciparum* likely originated from *P.reichenowi* following a host transfer.”



Blood samples from 113 chimpanzees; 17 gorillas (Cameroon)

Plasmodium sequences from: 12 chimpanzees; 2 gorillas;

Plasmodium from apes

- CONFUSION:
- How many *Plasmodium* (*Laverania*) species ?
 - Which are natural hosts ?
 - Origin of *Plasmodium falciparum* ?

PROBLEMS:

1. Limited sample sizes
2. Mostly captive apes
3. PCR errors
 - chimaeric sequences

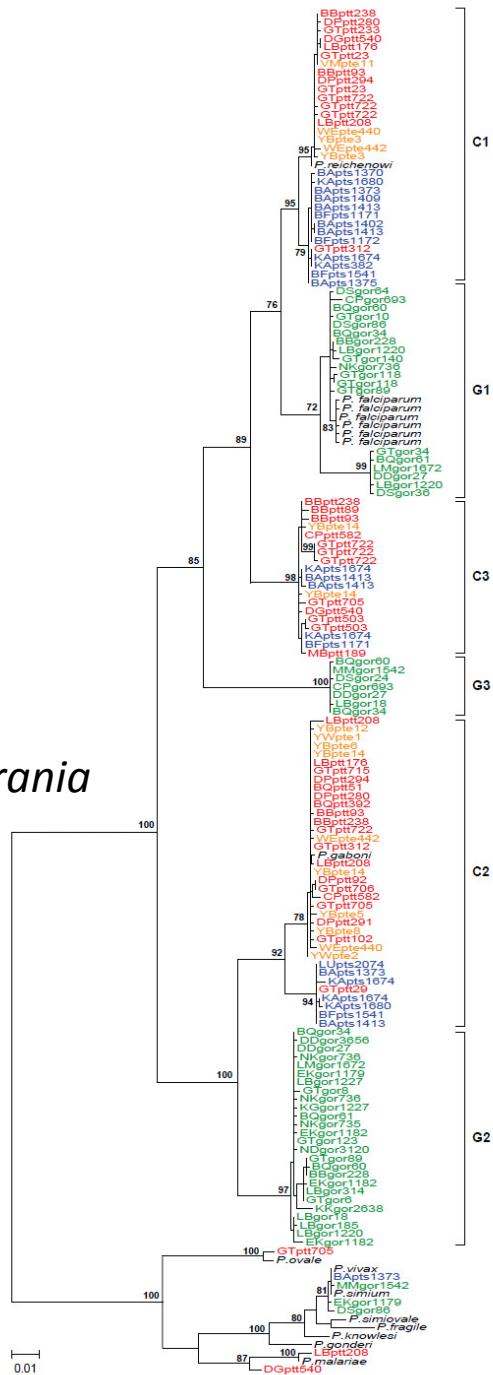
SOLUTIONS:

1. Faecal samples from wild apes
2. Single genome amplification (SGA)

cytB

[also
apDNA
& nDNA]

Laverania



Sequences from:

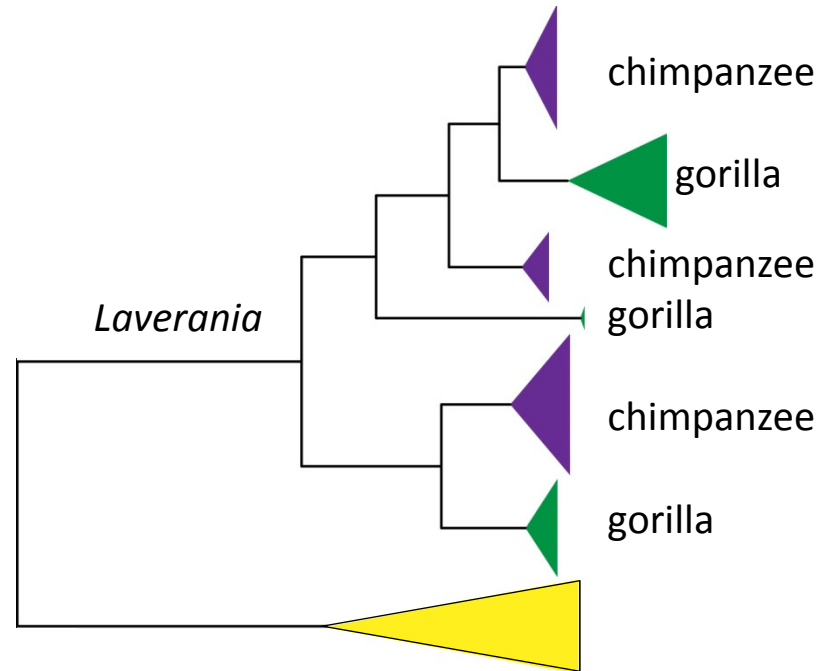
Gorilla

Nigerian chimpanzees

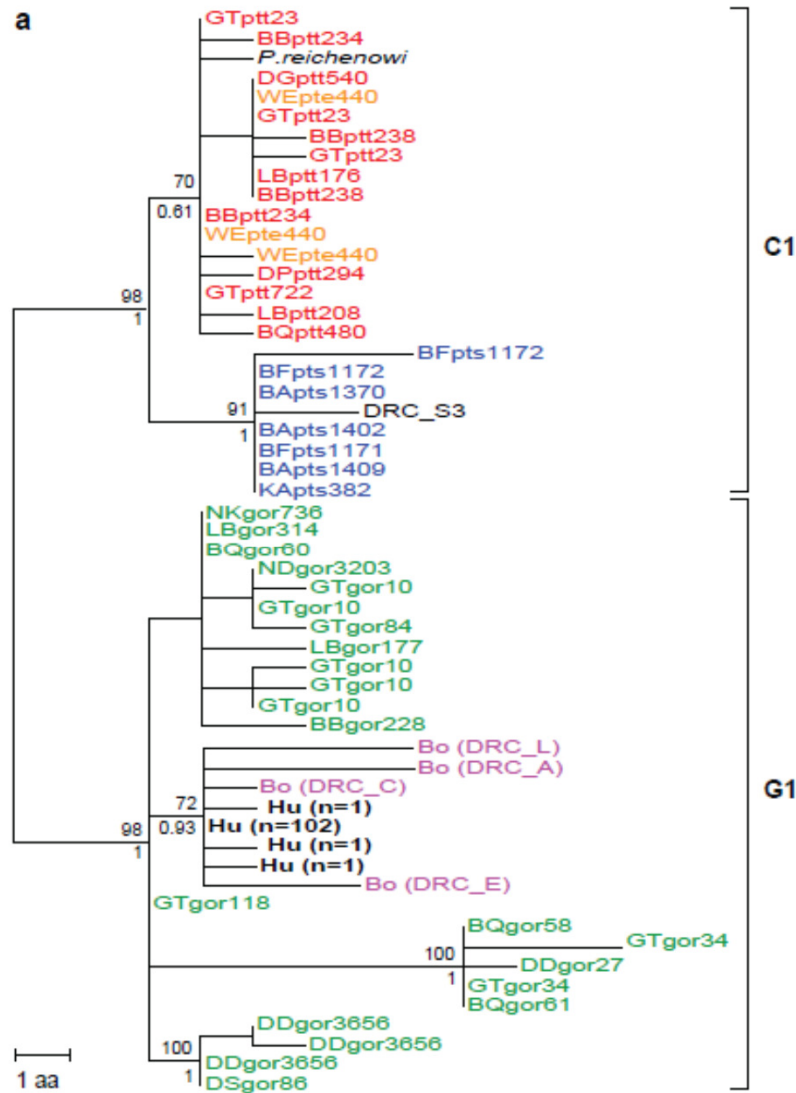
Central chimpanzees

Eastern chimpanzees

Others (incl. humans)



Origin of *Plasmodium falciparum*



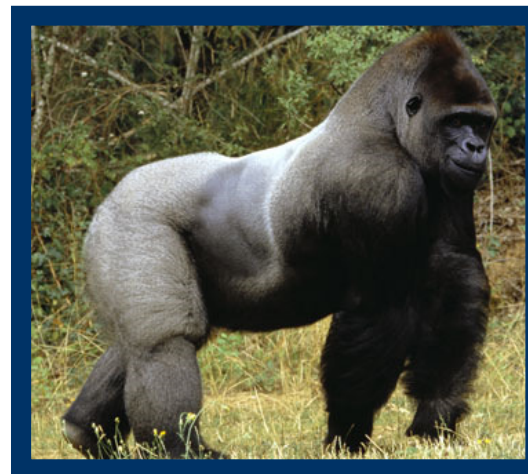
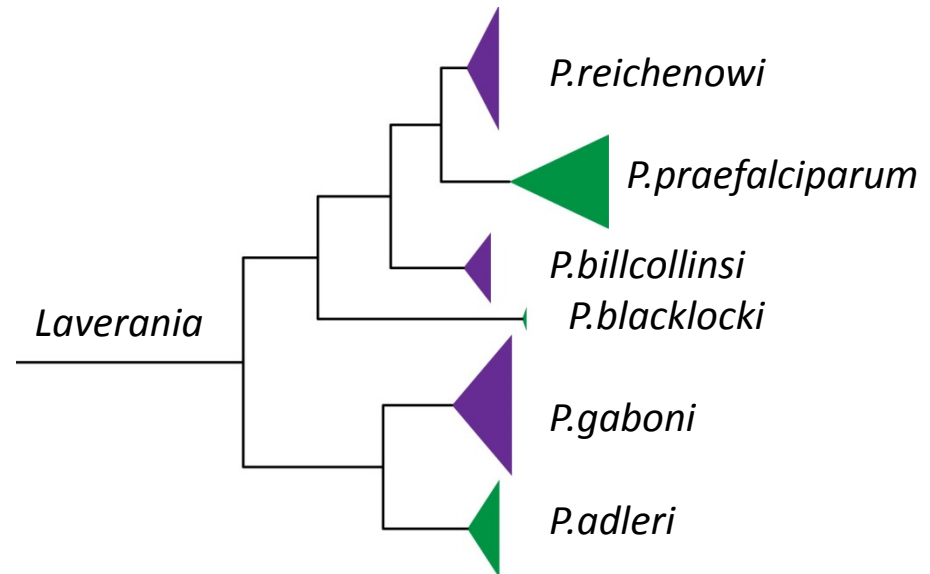
Human *Plasmodium falciparum*
from Western gorillas
(*Gorilla gorilla*)

Similar strains found in
(captive) bonobos, chimps and a monkey
are of immediate human origin

Laverania genome sequences

Origin of *Plasmodium falciparum*
genomic features?

Determinants of host specificity
among sympatric Chimpanzee
and Gorilla parasites ?



Selective Whole Genome Amplification

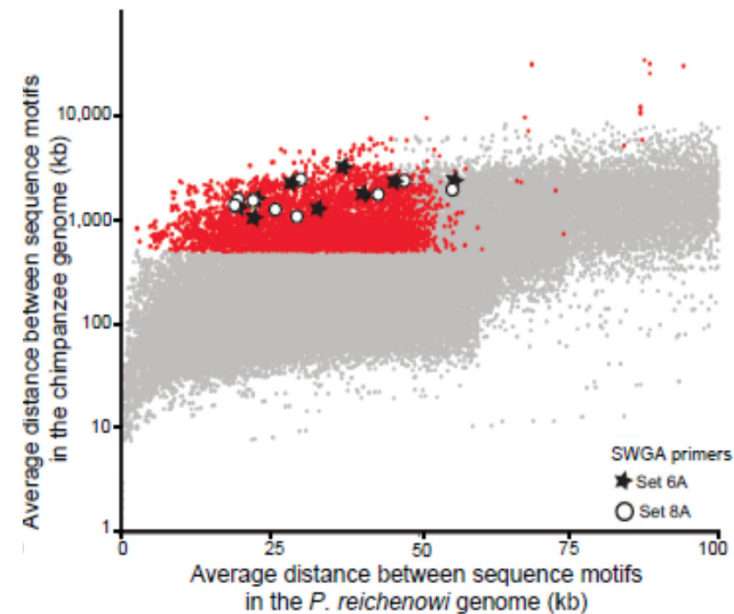
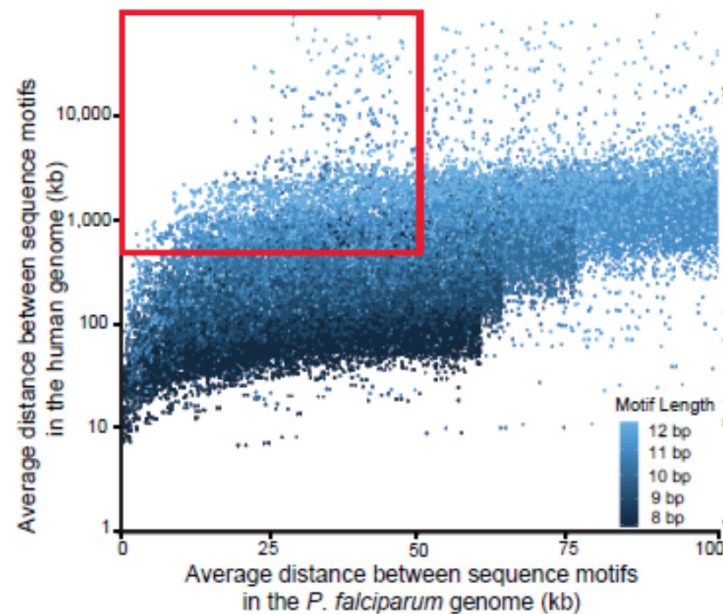
SWGA = Selective Whole Genome Amplification

LEICHTY & BRISSON (2014)

*Msp*II, *Fsp*EI digestion

Phi29 polymerase

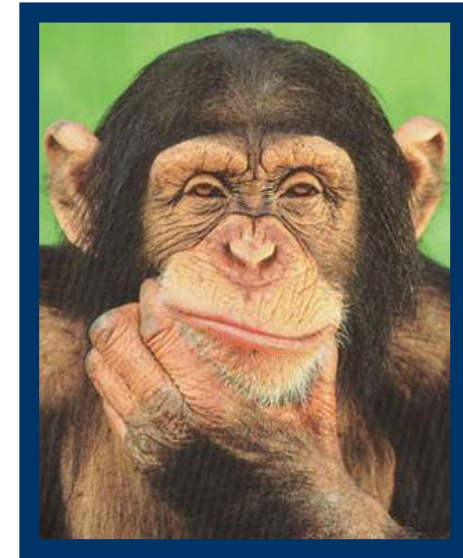
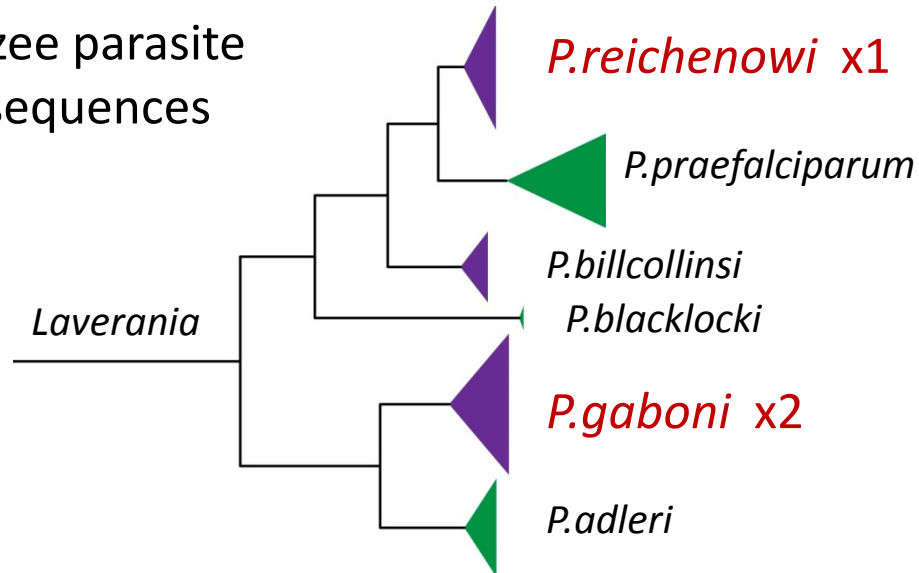
Primer selection: identify motifs that are common in *Plasmodium falciparum* but rare in the human genome



➔ 70,000-fold enrichment

Selective Whole Genome Amplification

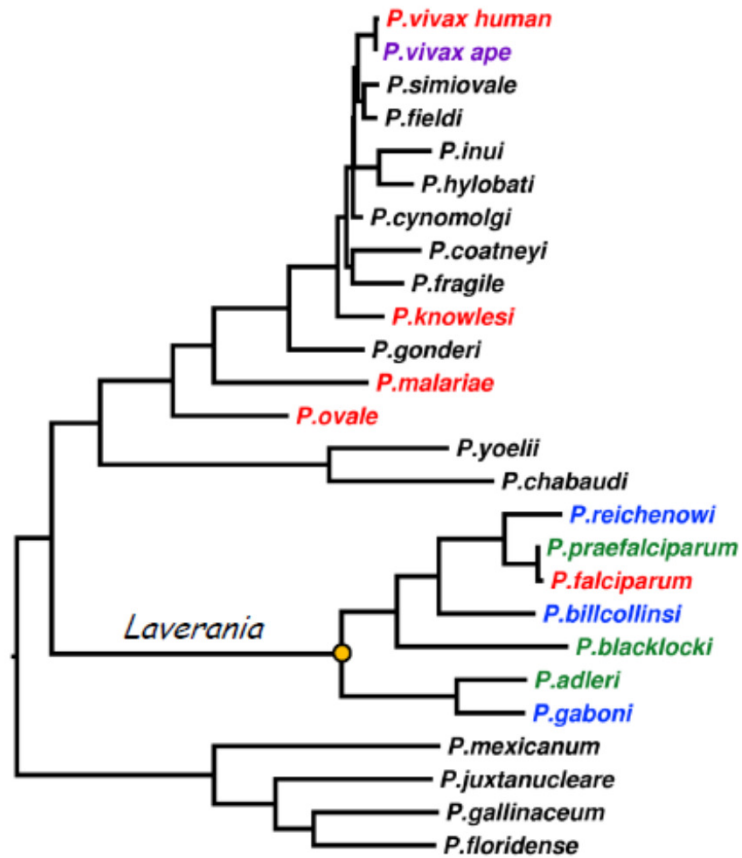
Chimpanzee parasite genome sequences



SESH SUNDARARAMAN

	<i>P.reichenowi</i>	<i>P.gaboni</i>	<i>P.gaboni</i>
Sample	SY57	SY75	SY37
Assembly	18.9 Mb	18.5 Mb	15.3 Mb
Core genes	4670	4689	4071
	=98.3%	=98.7%	=85.7%
Subtelomeric genes	235	222	108
	=23.8%		

FIKK kinase gene family



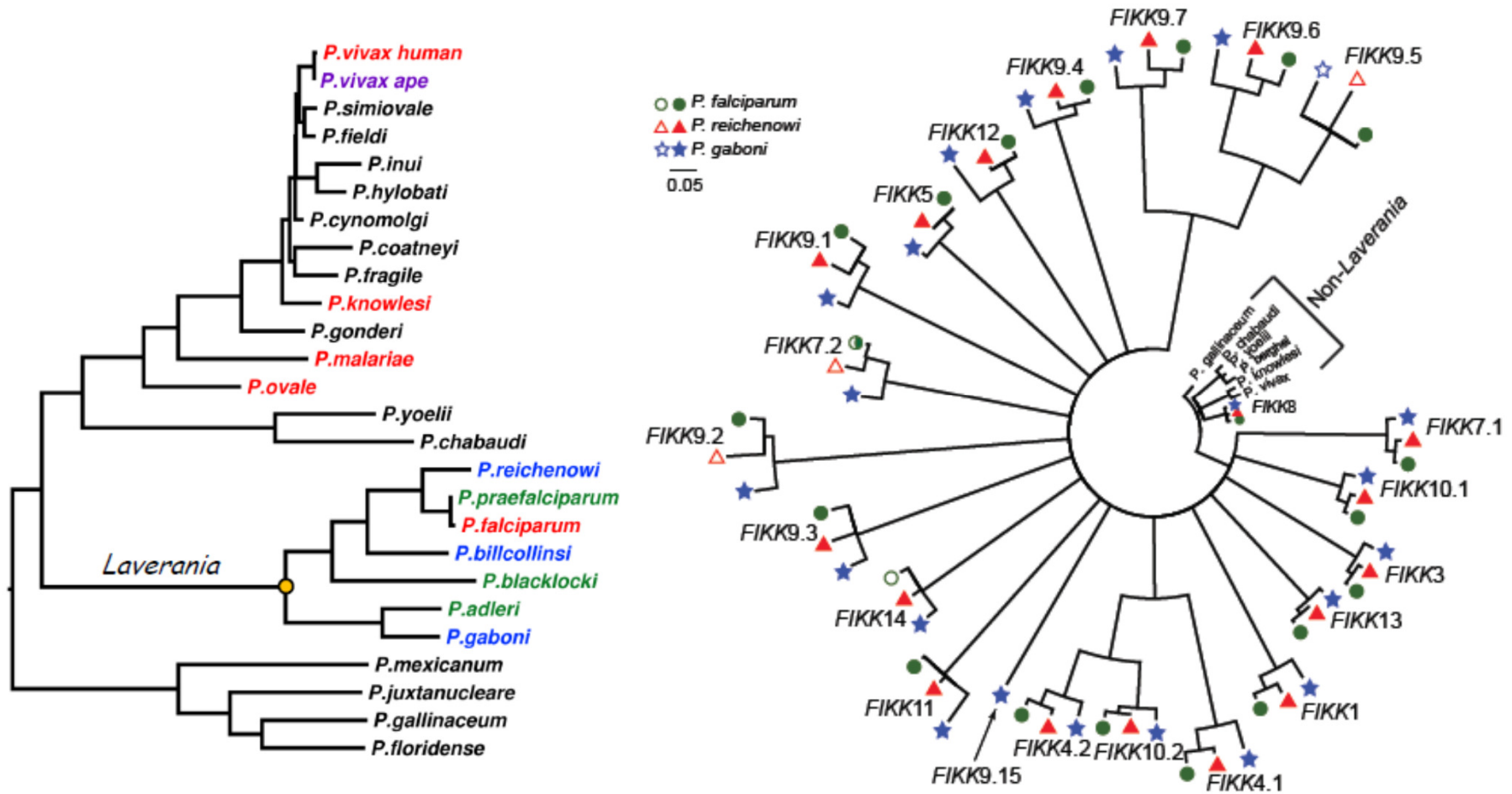
FIKK = Phe:Ile:Lys:Lys

One gene in *P. vivax*, *P. knowlesi*
& rodent malaras

20 genes in *P. falciparum*

FIKK kinase gene family

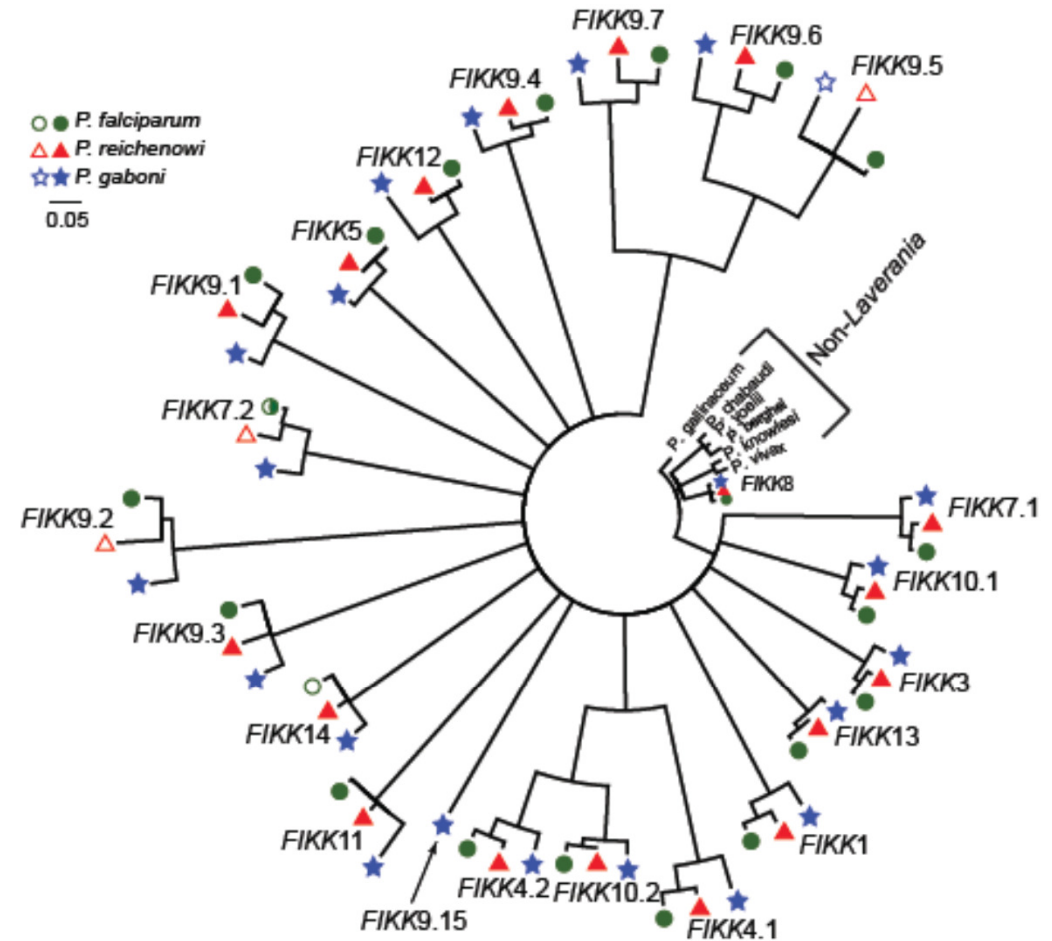
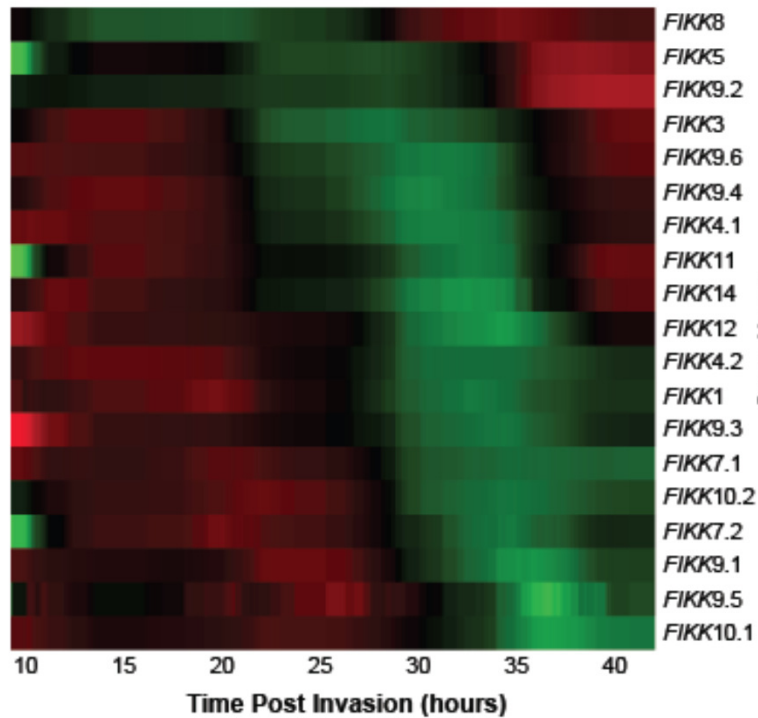
FIKK = Phe:Ile:Lys:Lys



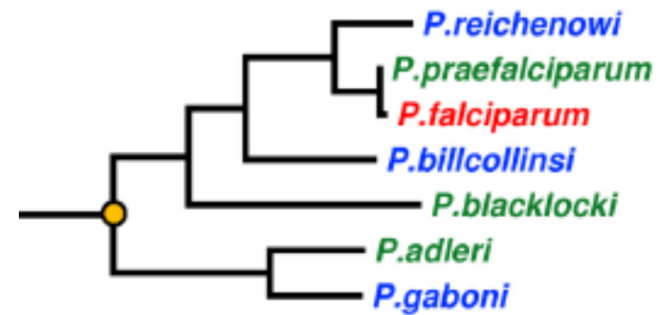
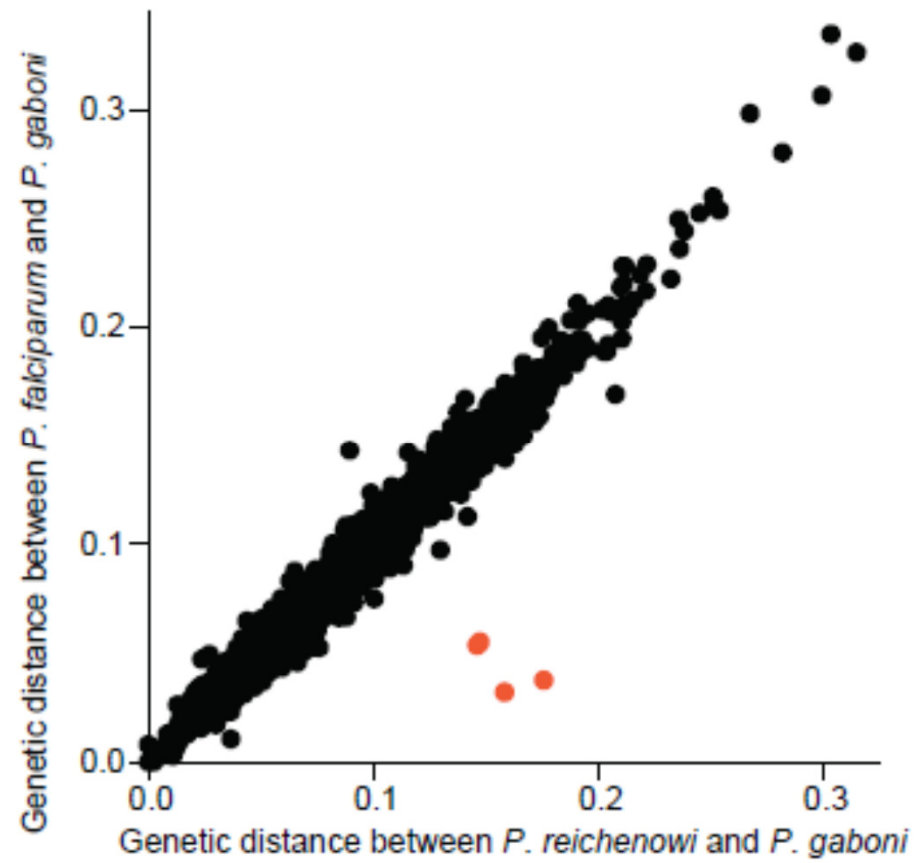
FIKK kinase gene family

FIKK = Phe:Ile:Lys:Lys

Expression profile
(*P. falciparum*)

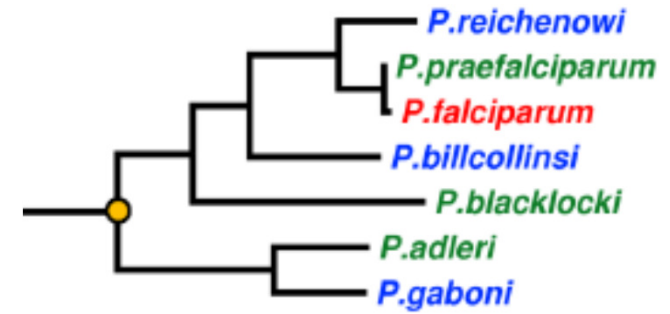


Interspecies divergence

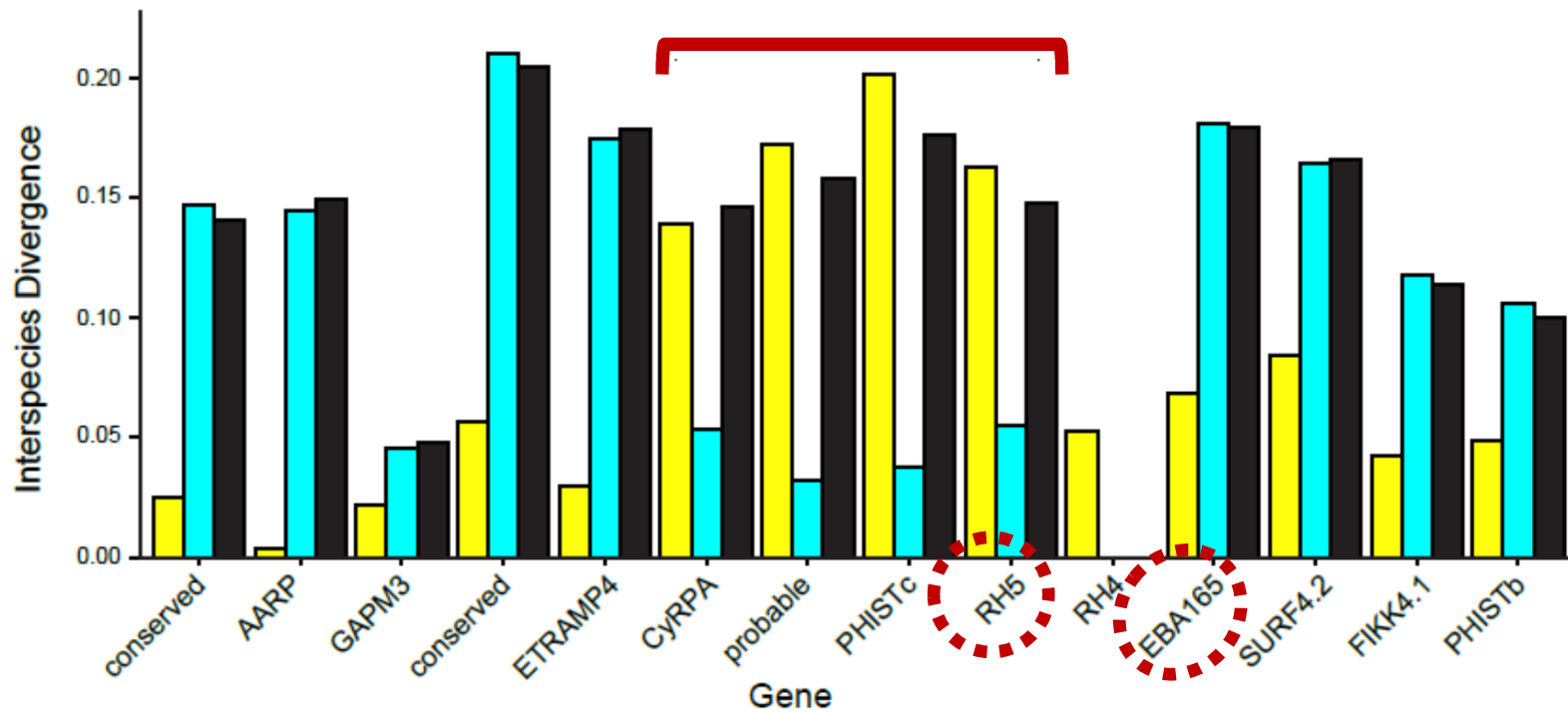


Chromosome 4

- P. falciparum* v *P. reichenowi*
- P. falciparum* v *P. gaboni*
- P. reichenowi* v *P. gaboni*

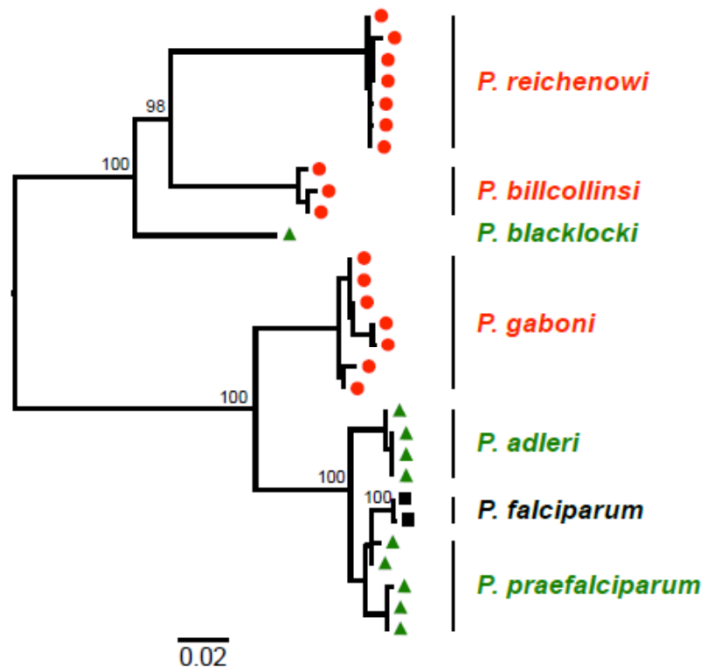


a



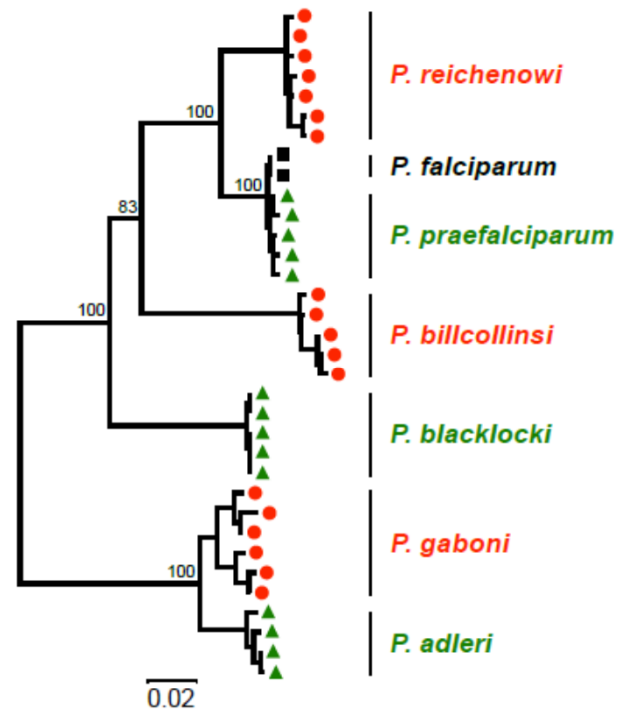
Horizontal gene transfer

RH5



unusual topology

EBA165



standard topology

Chromosome 4

- *P. falciparum* v *P. reichenowi*
- *P. falciparum* v *P. gaboni*
- *P. reichenowi* v *P. gaboni*

Rh5 : parasite ligand essential for blood stage growth

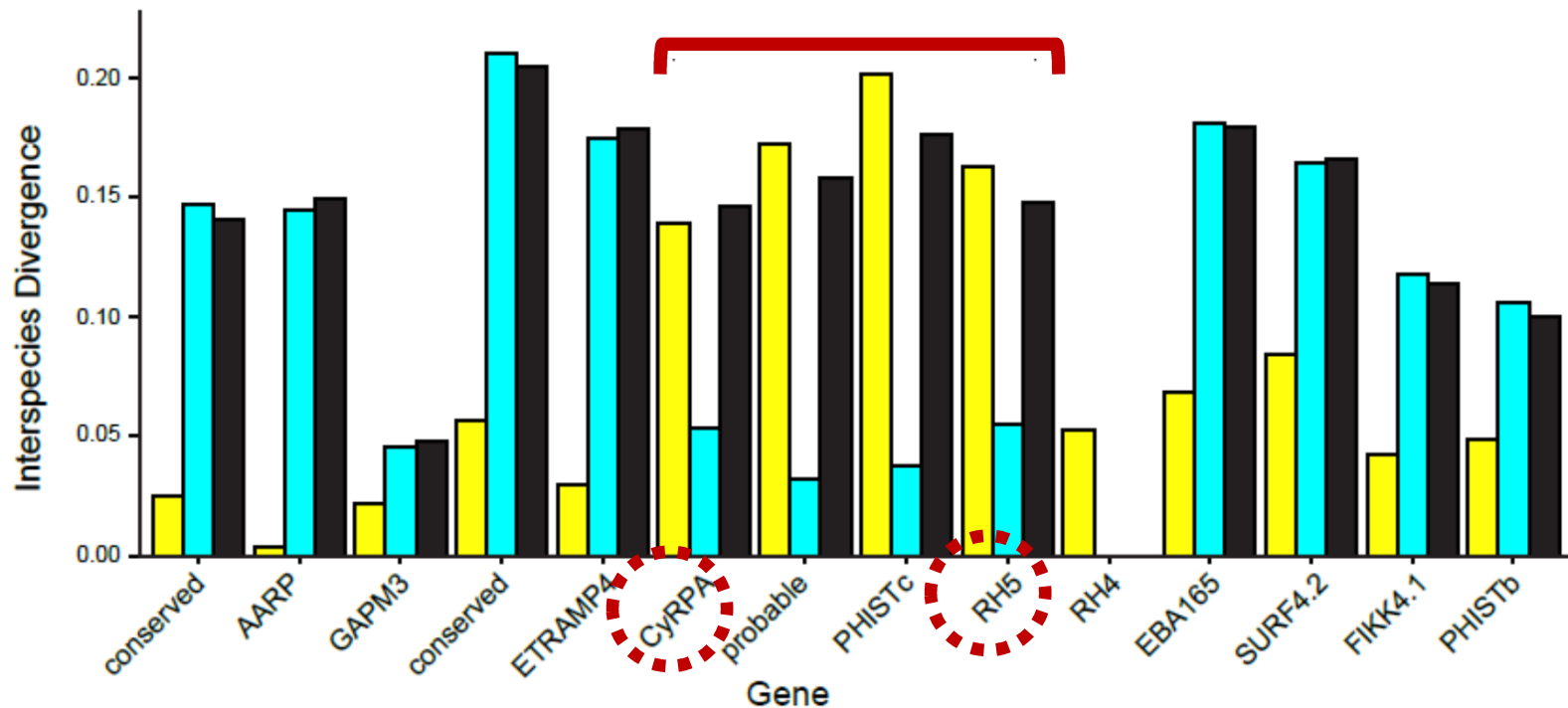
CROSNIER *et al.* (2011) Nature 480:534

- Rh5 binds human basigin
- binding essential for erythrocyte invasion


REDDY *et al.* (2015) PNAS 112:1179

- **CyRPA** also essential

a

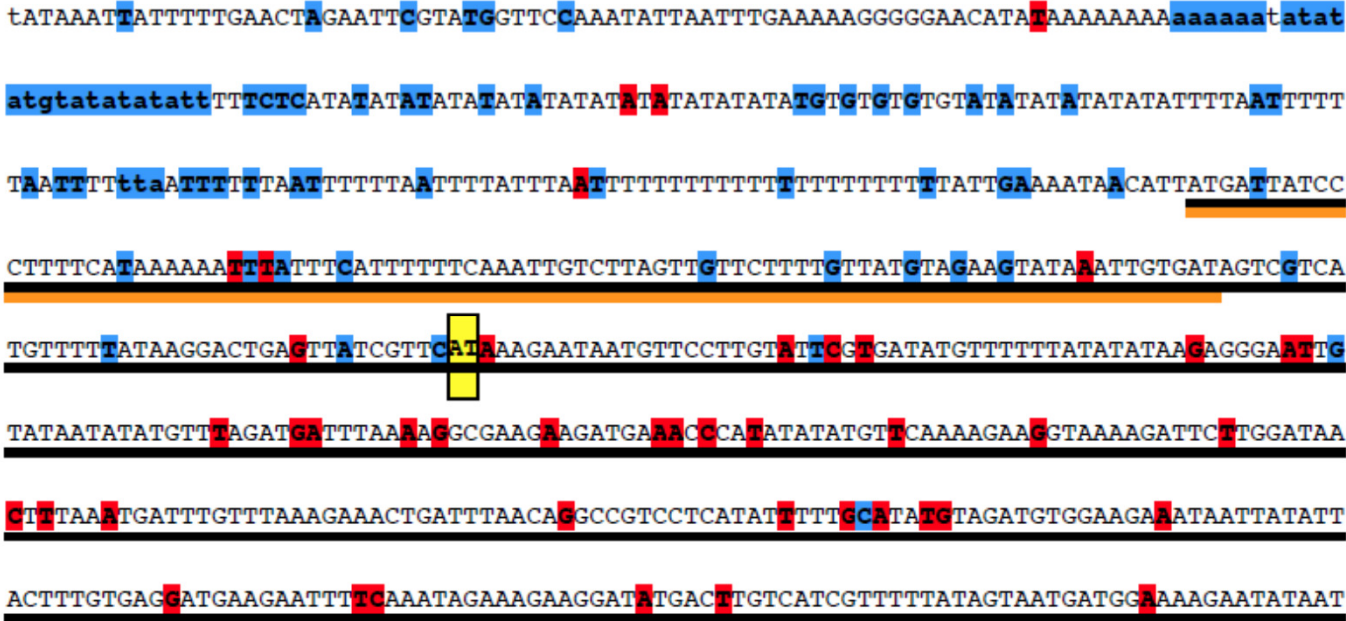


Horizontal gene transfer

 *P. falciparum* = *P. reichenowi* (*P. gaboni* different)

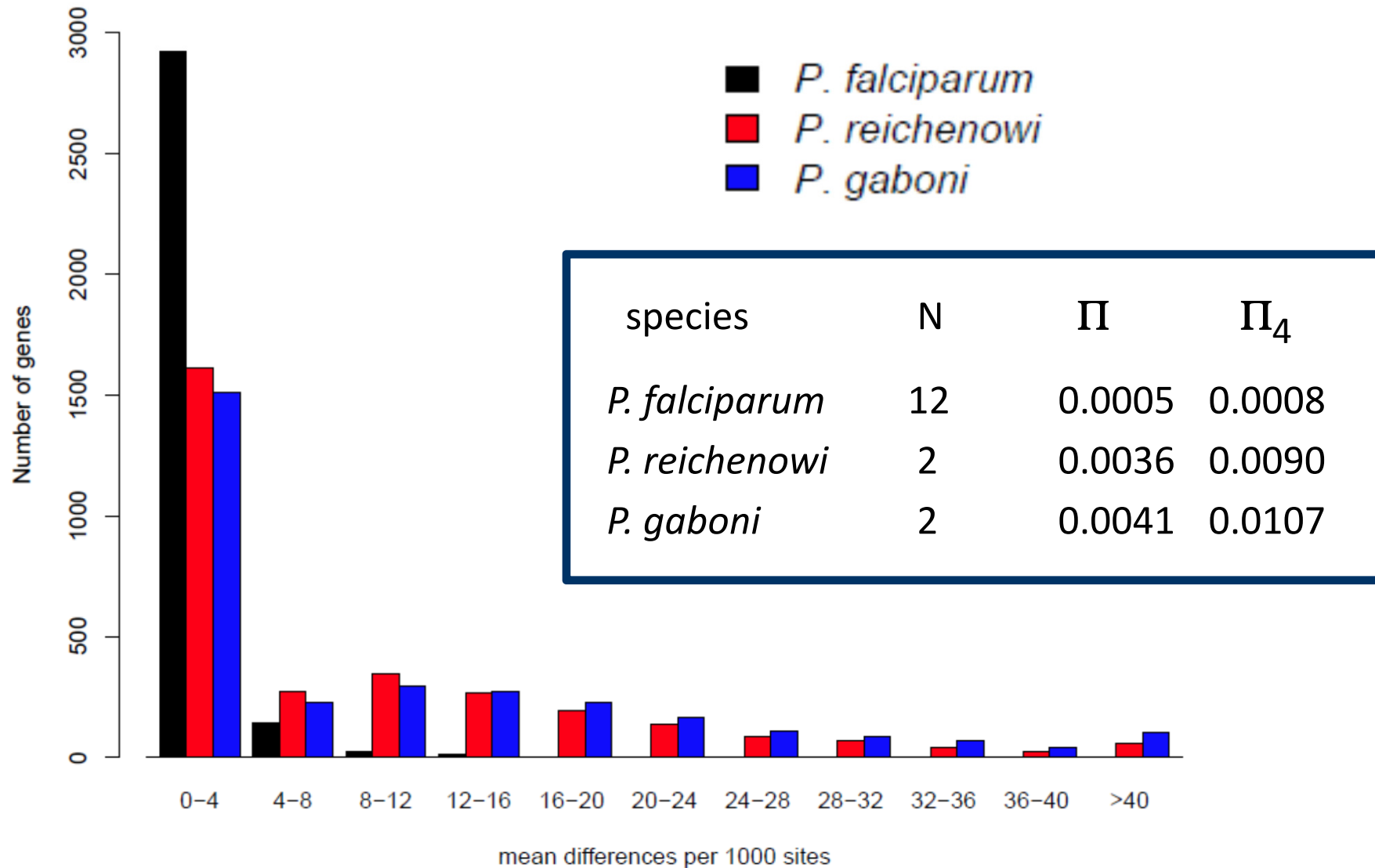
 *P. falciparum* = *P. gaboni* (*P. reichenowi* different)

CyRPA gene



— CyRPA protein coding region
 — signal sequence

Within species diversity



Timescale of *P. falciparum* in humans ?

➔ Transmission dynamics too weak to maintain parasite
in hunter-gatherer populations [CARTER & MENDIS 2002]

➔ Anti-*P.falciparum* mutations in humans are young

HbC mutation offers resistance to *P.falciparum* malaria
arose <5 kyr ago [WOOD *et al.* 2005]

G6PD A- allele offers resistance to *P.falciparum* malaria
arose ~ 4-12 kyr ago [TISHKOFF *et al.* 2001]



Richard Carter

NEAFSEY *et al.* (2012) Nature Genetics

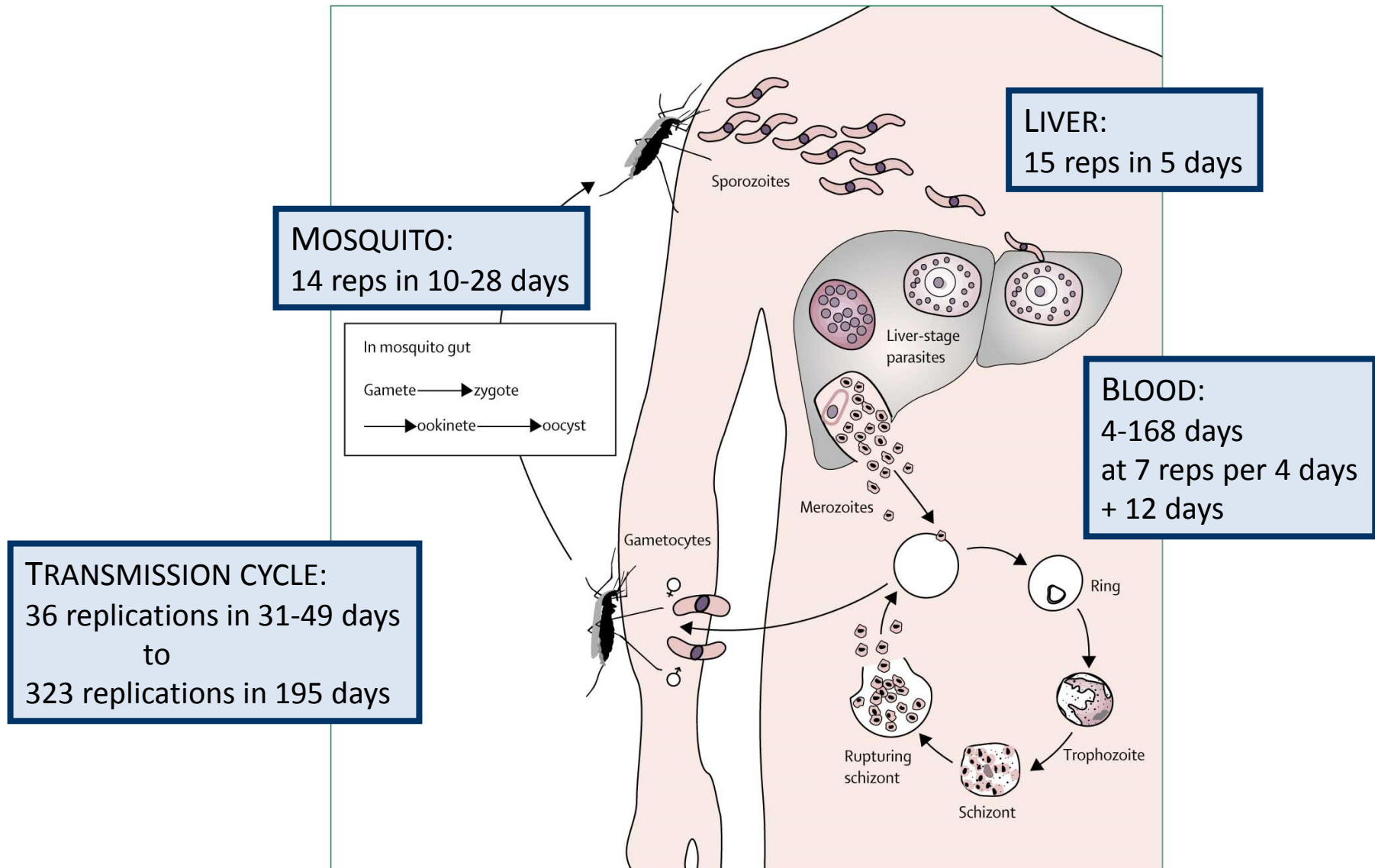
max diversity among 5 genome sequences: 9.59×10^{-4} subs/site
(4-fold degenerate sites)

“commonly accepted mutation rate for eukaryotes”: 2.2×10^{-9} /site/yr

➔ 218 kyr (452 kyr)

“similar to a mutation rate estimate for Plasmodium”: 2.1×10^{-9} /site/replication

Replication rate of *Plasmodium falciparum*



Conclusions

Plasmodium in African apes

Chimpanzees and gorillas have:

- very high prevalence of *Plasmodium*
- mostly multiply infected
(similar to humans in areas of hyper-endemicity)

Six species of *Laverania* parasites in wild apes:
three in gorillas, three in chimpanzees

Plasmodium falciparum

- originally transmitted to humans from gorillas
- perhaps very recently ?



Comparative genomics of *Laverania* species:

- Unique features of *Laverania* genomes
- HGT in the precursor of *P. falciparum*
- Greatly reduced diversity in *P. falciparum*

Acknowledgements

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University of Montpellier

Martine Peeters

Ahidjo Ayouba ++



Sanger Center, Cambridge

Julian Rayner

Nagasaki University

Richard Culleton

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David B. Morgan

University of New Mexico

Martin N. Muller

University of Minnesota

Emily Wroblewski
Joann Schumacher-Stankey
Michael L. Wilson
Anne Pusey
Lilian Pintea

Jane Goodall Institute/ Gombe National Park

Jane Goodall
Shadrack Kamenya

Sanaga-Yong Sanctuary

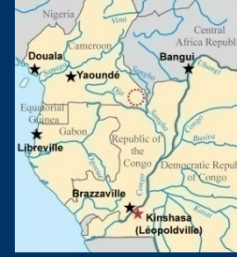
Sheri Speede

VaccineApe

Peter D. Walsh
Philip J. Kranzusch

University of Kisangani

Bosco Ndjango



Comparative genomics of ape malaria parasites and the emergence of human malaria

Paul Sharp



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