

The African origin of *Plasmodium vivax*

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Rougeron and colleagues recently reviewed the origin of the two major human malaria parasites, *Plasmodium falciparum* and *P. vivax* (Rougeron et al. 2021). They supported their arguments with an evolutionary tree intended to depict the relationships among mammal-infecting *Plasmodium* species. However, the phylogeny they presented is based on outdated analyses, and some (erroneous) guesswork. More recent analyses of larger datasets have yielded different results, which are key for correctly interpreting the origins of *P. vivax*.

The phylogeny shown by Rougeron et al. (2021) in Fig. 1, with relevant aspects repeated in Fig. 3, is described as ‘mostly based on the results of Galen et al. (2018)’. Galen et al. (2018) were interested in the higher order relationships among *Plasmodium* and other genera of Haemosporidian parasites and used a dataset of 21 proteins conserved across this broad range of species. More recent analyses by several groups have focused on *Plasmodium* species infecting mammals, using 30 protein sequences encoded by the apicoplast genome (Arisue et al. 2019), or more than 1000 protein sequences encoded by the nuclear genome (Rutledge et al. 2017, Sharp et al. 2020); these studies have yielded results that differ from the phylogeny shown in Fig. 1 of Rougeron et al. (2021) in important ways.

First, in Fig. 1, *P. fragile* from macaques, is shown as the basal lineage of the clade including *P. gonderi*, *P. vivax*, and various species infecting Southeast Asian primates. This is curious, since the phylogeny in Galen et al. (2018) as well as many analyses by others (e.g. Arisue et al. 2019, Sharp et al. 2020) have consistently identified *P. gonderi* (from African monkeys) as clearly the basal lineage of this clade. Moreover, all of these phylogenies place *P. fragile* with *P. knowlesi* and *P. coatneyi*, within the radiation of other species infecting Southeast Asian primates.

Second, in Fig. 1, the *P. vivax* group, i.e. *P. vivax* together with closely related ‘*P. vivax*-like’ (from apes) and *P. simium* (a recent transmission from humans to South American monkeys), is shown as lying within the radiation of species infecting Southeast Asian primates. This relationship was previously inferred from small numbers of mitochondrial sequences (Mu et al. 2005, Hayakawa et al. 2008), but is clearly outdated. More recent analyses place *P. vivax* basal to the clade of Southeast Asian parasites (Arisue et al. 2019, Sharp et al. 2020).

Finally, in Fig. 1 *P. carteri* is shown, tentatively, as clustering outside a clade containing the *P. vivax* group plus *P. simiovale*, *P. fieldi*,

and *P. cynomolgi*. Again, this is inconsistent with the literature, since all published phylogenies where the position of *P. carteri* has strong support depict this species as the closest relative of the *P. vivax* group (Loy et al. 2017, 2018).

These discrepancies have important implications for the origin of *P. vivax* in humans. In early studies of limited sequence data, *P. vivax* appeared to emerge from within the radiation of numerous species infecting primates in Southeast Asia, leading to a widespread belief that the human parasite originated via an ancient transmission from monkeys in that part of the world (Escalante et al. 2005, Mu et al. 2005, Hayakawa et al. 2008). However, this did not explain why people across central Africa have an extremely high frequency of the Duffy-negative mutation, which largely prevents infection by *P. vivax*. The discovery in Africa of very closely-related *P. vivax*-like parasites in apes (Liu et al. 2010, 2014, Prugnolle et al. 2013), and of another closely-related species, *P. carteri*, were clearly inconsistent with the Asian hypothesis. Instead, these findings indicated that the common ancestor of *P. carteri* and the *P. vivax* group existed in Africa. Indeed, given that (i) all five major lineages within the mammalian *Plasmodium* radiation appear to have originated in Africa (Sharp et al. 2020), (ii) the earliest divergence on the lineage leading to *P. vivax* involves two parasites (*P. gonderi* and *Plasmodium* sp. DAJ-2004) that are only found in African monkeys, and (iii) a later split separates the African *P. vivax*-related clade (including *P. carteri*) from the clade of parasite species infecting Southeast Asian primates, the only tenable interpretation is that the ancestor of the parasites infecting primates in Asia migrated out-of-Africa only after the divergence of the ancestor of the *P. vivax*-related clade. Since *P. vivax*-like can infect humans (Prugnolle et al. 2013), it is also evident that these parasites most likely circulated among all ape species in sub-Saharan Africa, including humans; then, after the spread of the Duffy-negative mutation in humans across Africa, the only *P. vivax* remaining in humans resulted from a lineage that had escaped out-of-Africa (Sharp et al. 2020).

Rougeron et al. (2021) concluded that the origin of *P. vivax* ‘remains unclear’, suggesting that there is still a debate between the out-of-Africa and out-of-Asia scenarios. However, current phylogenetic evidence is inconsistent with the out-of-Asia hypothesis. The other compelling evidence comes from genetic diversity. Daron et al. (2021) found that levels of genetic diversity among human *P. vivax* strains decline with the distance from Southeast

Asia, and Rougeron et al. (2021) have taken this finding as support for the out-of-Asia scenario. However, this claim ignores the fact that *P. vivax*-like strains in African apes exhibit eight times more genetic diversity than human *P. vivax* (Gilabert et al. 2018, Loy et al. 2018). Scenarios, whereby an ancestor of *P. vivax* was transmitted from monkeys to humans in Southeast Asia, spread across Asia to reach apes in sub-Saharan Africa, and then underwent a bottleneck in humans are far-fetched (Prugnolle et al. 2013), and fail to explain the origin of *P. carteri*.

In conclusion, current phylogenetic evidence indicates that the lineage leading to *P. vivax* never left Africa, until it migrated out-of-Africa recently with humans (Sharp et al. 2020). The phylogenetic relationships presented by Rougeron et al. (2021) are erroneous, and as a consequence they obfuscate the progress made by the field in elucidating the origins of human malaria parasites.

Conflict of interest statement. None declared.

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