REVIEW

The primate malaria parasites *Plasmodium malariae*, *Plasmodium brasilianum* and *Plasmodium ovale* spp.: genomic insights into distribution, dispersal and host transitions

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Abstract

During the twentieth century, there was an explosion in understanding of the malaria parasites infecting humans and wild primates. This was built on three main data sources: from detailed descriptive morphology, from observational histories of induced infections in captive primates, syphilis patients, prison inmates and volunteers, and from clinical and epidemiological studies in the field. All three were wholly dependent on parasitological information from blood-film microscopy, and *The Primate Malarias* by Coatney and colleagues (1971) provides an overview of this knowledge available at that time. Here, 50 years on, a perspective from the third decade of the twenty-first century is presented on two pairs of primate malaria parasite species. Included is a near-exhaustive summary of the recent and current geographical distribution for each of these four species, and of the underlying molecular and genomic evidence for each. The important role of host transitions in the radiation of *Plasmodium* spp. is discussed, as are any implications for the desired elimination of all malaria species in human populations. Two important questions are posed, requiring further work on these often ignored taxa. Is *Plasmodium brasilianum*, circulating among wild simian hosts in the Americas, a distinct species from *Plasmodium malariae*? Can new insights into the genomic differences between *Plasmodium ovale curtisi* and *Plasmodium ovale wallikeri* be linked to any important differences in parasite morphology, cell biology or clinical and epidemiological features?

Keywords: *Plasmodium malariae*, *Plasmodium brasilianum*, *Plasmodium ovale curtisi*, *Plasmodium ovale wallikeri*, Host transitions

Background

In *The Primate Malarias* (1971), by Coatney et al. [1], detailed species comparisons are presented based on descriptive morphology of both blood and mosquito stages, the geographic distribution of each parasite and certain features readily measurable in induced human infections, including the estimated duration of the liver-stage, time to symptoms and fever periodicity. Much of this work was performed in prison inmates in Georgia, USA. In this paper, fifty years since, the focus on the geographic, genomic and genetic characteristics of four primate malaria species—one currently regarded as zoonotic in South American monkeys, *Plasmodium brasilianum*, and three malaria parasites of *Homo sapiens*, namely *Plasmodium malariae*, *Plasmodium ovale curtisi* and *Plasmodium ovale wallikeri*. An exhaustive bibliography of reported identification of these species since 1890, across the globe and in different primate hosts, will also be presented.
Over the last two decades, the analytical techniques of evolutionary biology and the task of reconstructing phylogenetic relationships within the genus have benefited greatly from the explosion in genomic data available for malaria parasites, and the now well-established practice of non-invasive faecal sampling of parasite genomic material from the faeces of wild primates [2]. This wealth of data provides new understanding of diversity both within and among the primate-infecting *Plasmodium* species, and points to the importance of transitions into new primate hosts. These transitions are gateways to the radiation of parasite species, but also act as genetic bottlenecks, as evidenced by reduced diversity among parasites in the new host [2, 3].

Among the homophilic species considered of clinical importance, a range of life history and transmission strategies are evident, and each of these strategies have their equivalent counterparts among the parasites of living simian hosts, and those of *Pan* and *Gorilla*. Thus, the majority of evolution leading to these diverse life histories occurred in the parasite lineages of non-human primates in the evolutionary past. However, as with *Plasmodium knowlesi*, the zoonotic potential of *P. brasilianum* shows that host transition can be a dynamic process operating over an extended time period, rather than a singular event, and understanding this in the present is essential to maintain effective malaria elimination strategies world-wide.

**Plasmodium brasilianum**

**History & discovery**

The first report of *P. brasilianum* is based on a finding in the blood of a bald uakari (*Cacajao calvus*) imported from the Brazil Amazonas region to Hamburg, Germany in 1908 [4]. Initial studies reported that *P. brasilianum* closely resembles *P. malariae*, and to be a relatively common parasite of New World monkeys in Panama and Brazil (reviewed in [1]).

**Distribution and known non-human primate hosts**

Historically, natural infections of *P. brasilianum* were reported in various primates in Central and Southern America—Panama, Colombia, Venezuela, Peru, and Brazil. The spectrum of primate hosts (incl. sequence confirmed reports) is given in Table 1 [5–12], indicating that *P. brasilianum* has promiscuous host-specificity compared to other malaria parasites. Moreover, natural infections in humans have been reported from Venezuela [13].

**Genomic studies of Plasmodium brasilianum**

*Plasmodium brasilianum* is a parasite thought to be closely related to *P. malariae*, and blood-stage infections of the two species present a morphologically identical picture, with discrimination determined by the host, monkey or human, respectively. The few molecular epidemiological studies reported so far have shown that *P. brasilianum* and *P. malariae* infections are almost indistinguishable genetically. Sequencing studies of the gene coding for the circumsporozoite protein (csp) appear not to differentiate the identity of the two parasites [14–16]. Similar, studies involving the merozoite surface protein-1 (msp1), the ssrRNA small subunit (18S) of ribosomes and the mitochondrial gene cytochrome b (cytb), have identified sequences that were 100% identical or that had only a few randomly distributed single nucleotide position differences [7, 13, 15–18]. Further, the close genetic resemblance of these parasites has been observed across studies in Brazil, Venezuela, Costa Rica, Peru, Colombia and French Guiana from infected humans, monkeys and mosquitoes [7–9, 11, 12, 15–18]. Under conditions of close contact, as shown in Yanomami people and monkeys species in the Venezuelan Amazon, both humans and non-human primates shared quartan parasites without any host specificity that are genetically identical in target candidate genes [13].

A small study using microsatellite genotyping showed that in 14 *P. malariae* isolates from infected individuals from the Brazilian Atlantic forest, all isolates had identical haplotypes, while in one mosquito sample from the same region a different haplotype was found [19]. In the same study, three *P. brasilianum* isolates from non-human primates sampled from a different region (Amazonia) were analysed, and diverse haplotypes were observed. Unfortunately, across all such studies to date only a small number of samples have been compared at only a few genetic loci. To understand the degree of similarity among *P. brasilianum* and *P. malariae* parasites, a comprehensive analysis of whole genome sequencing data is necessary, using many more parasites obtained from different hosts, across a range of geographic regions. Only one draft reference genome of *P. brasilianum* is available [20]. Similarly, only a few genomes are available for *P. malariae*, sourced from Africa and Asia, and none from South America [8, 20–22]. The apicoplast and mitochondrion genomes of *P. brasilianum* are indistinguishable from those of the *P. malariae* reference genome [20, 23], but further comparative analysis of nuclear genomes is needed to fully understand the status of these two species. This is made difficult by the scarcity of whole genome data, so it remains an open question whether these parasites are variants of a single species that is naturally adapted to both human and New World monkey hosts, and freely circulates between them. Related to this, it is also difficult to infer the direction of the cross-species transfer. Nevertheless, the similarity of these parasites suggests that monkeys can act as...
Table 1  Non-human primate host spectrum of *Plasmodium brasilianum* (modified after Coatney 1971)

| Host                                      | Host Distribution                                                                 | GenBank ID  | References |
|-------------------------------------------|-----------------------------------------------------------------------------------|-------------|------------|
| Black howler (*Alouatta caraya*)          | Argentina, Bolivia, Brazil, Paraguay                                             |             | [5]        |
| Brown howler (*Alouatta guiraiba*; Syn.: *A. fusca*) | Atlantic Forest—Brazil, Argentina                                                |             | [1]        |
| Northern brown howler (*Alouatta guiraiba*) | Brazil                                                                            |             | [5]        |
| Southern brown howler (*Alouatta guiraiba clamitans*) | Brazil, Argentina                                                                | MF573323    | [6]        |
| Mantled howler (*Alouatta palliata*)       | Colombia, Costa Rica, Ecuador, Guatemala, Honduras, Mexico, Nicaragua, Panama, Peru | KU999995    | [1]        |
| Red howler (*Alouatta seniculus*)          | Venezuela, Colombia, Ecuador, Peru, Brazil, French Guyana                         | AF138878    | [7]        |
| Guatemalan black howler (*Alouatta pigra*; Syn.: *Alouatta villosa*) | Belize, Guatemala, Mexico                                                       |             | [1]        |
| Gray-handed night monkey (*Aotus griseimembra*) | Colombia, Venezuela                                                              |             | [8]        |
| Black-handed night monkey (*Aotus nigriceps*) | Brazil, Bolivia and Peru                                                       | KC906732    | [9]        |
| White-bellied spider monkey (*Ateles betzebuth*) | Colombia, Ecuador, Venezuela, Peru                                               |             | [5]        |
| Peruvian spider monkey (*Ateles chamek*)   | Peru, Brazil, Bolivia                                                           | KC906714    | [9]        |
| Black-headed spider monkey (*Ateles fusciceps*) | Colombia, Ecuador, Panama                                                       |             | [1]        |
| Geoffroy’s spider monkey (*Ateles Geoffroyi*) | Central America incl. parts of Mexico, Colombia                                  |             | [1]        |
| Nicaraguan spider monkey (*Ateles Geoffroyi Geoffroyi*) | Nicaragua, Costa Rica                                                          |             | [1]        |
| Hooded spider monkey (*Ateles Geoffroyi Griscens*) | Panama, Colombia                                                                |             | [1]        |
| Brown spider monkey (*Ateles Hybrida*)     | Colombia, Venezuela                                                             |             | [8]        |
| Red-faced spider monkey (*Ateles paniscus*) | northern Brazil, Suriname, Guyana, French Guiana and Venezuela                  |             | [5]        |
| Southern muriqui (*Brachyteles arachnoides*) | Brazilian states Paraná, São Paulo, Rio de Janeiro, Espírito Santo, Minas Gerais |             | [5]        |
| Bald uakari (*Cacajao calvus*)              | Brazil, Peru                                                                     |             | [5]        |
| Red bald-headed uakari (*Cacajao calvus rubicundus*) | Brazil                                                                       |             | [5]        |
| Masked titi (*Callicebus personatus*)       | Brazil                                                                            |             | [5]        |
| White-headed marmoset (*Callithrix Geoffroyi*) | Brazil (Amazonas)                                                               |             | [10]       |
| Collared titi (*Cheracebus torquatus*; Syn.: *Callicebus torquatus*) | Brazil                                                                         |             | [5]        |
| White-fronted capuchin (*Cebus albifrons*)  | Bolivia, Brazil, Colombia, Venezuela, Ecuador, Peru, Trinidad and Tobago        |             | [1]        |
| Colombian white-faced capuchin (*Cebus Capucinus*) | Colombia, Ecuador                                                               |             | [1]        |
| Panamanian white-faced capuchin (*Cebus Imitator*) | Honduras, Nicaragua, Costa Rica, Guatemala, Belize, Panama                    |             | [1]        |
| Varied white-fronted capuchin (*Cebus Versicolor*) | Colombia                                                                      |             | [8]        |
| White-nosed saki (*Chiroptes Albinasus*)   | Brazil, Bolivia                                                                  |             | [5]        |
| Red-backed bearded saki (*Chiroptes Chiropotes*) | North of the Amazon River and East of the Branco River, in Brazil, Venezuela and the Guianas | KC906730    | [9]        |
| Black bearded saki (*Chiroptes Satanas*)   | Brazil                                                                            |             | [5]        |
| Gray woolly monkey (*Lagothrix Cana*)       | Bolivia, Brazil, Peru                                                           | KC906726    | [9]        |
| Brown woolly monkey (*Lagothrix Lagatricha*) | Colombia, Ecuador, Peru, Brazil                                                  |             | [5]        |
| Brown-mantled tamarin (*Leontocebus Fuscicolis*; Syn.: *Saguinus Fuscicolis*) | Bolivia, Brazil, Peru                                                          |             | [11]       |
| Golden-headed lion tamarin (*Leontopithecus Chrysomelas*) | Brazil                                                                        |             | [10]       |
| Golden lion tamarin (*Leontopithecus Rosalida*) | Brazil                                                                        |             | [10]       |
| Santarem marmoset (*Mico Humeralifer*)      | Brazil                                                                            |             | [10]       |
| Gray’s bald-faced saki (*Pithecia Irrorata*) | Colombia, Bolivia, Brazil                                                      | KC906717    | [9]        |
| Monk saki (*Pithecia Monachus*)              | Brazil, Peru, Ecuador, Colombia                                                  |             | [5]        |
| White-faced saki (*Pithecia Pithecia*)      | Brazil, French Guiana, Guyana, Suriname, Venezuela                              |             | [5]        |
| Brown titi (*Plecturocebus Brunneus*; Syn.: *Callicebus Brunneus*) | Brazil, Peru and Bolivia                                                        |             | [9]        |
| Chestnut-bellied titi (*Plecturocebus Caligatus*; Syn.: *Callicebus Caligatus*) | Brazil                                                                         | JX045640    | [12]       |
| Red-bellied titi (*Plecturocebus Moloch*)    | Brazil                                                                            | KC906723    | [9]        |
| Hershkovitz’s titi (*Plecturocebus Dubius*; Syn.: *Callicebus Dubius*)            | Bolivia, Brazil, Peru                                                          | JX045642    | [12]       |
reservoirs of \textit{P. malariae} / \textit{P. brasilianum}, and this must be considered in control and eradication programmes.

\textbf{Plasmodium malariae}

\textbf{History & discovery; epidemiology and disease}

As Collins and Jeffery relate [24], \textit{P. malariae} was named by Grassi and Feletti in 1890, following the observations of Golgi in 1886, who noted the existence of malaria parasites with either 48 h or 72 h cycles of fever, the latter subsequently being recognized as characteristic of \textit{P. malariae} infections. This slow-growing species is widely distributed across the tropics and sub-tropics, with often asymptomatic infections characterized by low parasitaemia and a recognized ability to persist in a single host for years or decades [25, 26]. There is evidence that \textit{P. malariae} can survive combination therapies used for treating acute \textit{P. falciparum} malaria, and may present as a post-treatment recrudescence in \textit{P. falciparum} patients [27–29]. Clinical malaria caused by \textit{P. malariae} rarely progresses to severe, complicated or life-threatening illness, although the literature contains consistent reports of mortality due specifically to either glomerulonephritis or severe anaemia in small children with chronic infections [30].

\textbf{Distribution and abundance}

\textit{Plasmodium malariae} is a cosmopolitan parasite distributed in sub-Saharan Africa, South-East Asia, western Pacific islands, and Central and South America [24]. Formerly this parasite was also present in the southern parts of the USA, Argentina, Bhutan, Brunei, South Korea, Morocco, Turkey, and parts of Europe where malaria was eradicated [31–33]. The distribution of this parasite is variable and patchy, and limited to particular mosquito vectors (sporogony needs a minimal temperature of 15 °C), yet autochthonous \textit{P. malariae} cases have been documented from much of the tropics and sub-tropics (Fig. 1; Table 2) [34–143].

Assessment of the abundance of \textit{P. malariae} is difficult because this parasite has been neglected by researchers, and studies differ (e.g. symptomatic patients vs. population studies; Table 2). Some epidemiological studies reported a high prevalence (15–30%) in Africa, Papua New Guinea, and the Western Pacific, in contrast to scanty observations (1–2%) from Asia, the Middle East, Central and Southern America [144]. However, with the advent of molecular diagnostic techniques this parasite species has been reported more frequently, being found in regions where it was not previously thought be present (e.g. Bangladesh), more commonly observed in mixed infections with \textit{P. falciparum} [24], and identified as recrudescent infections in historical cases from areas such as Greece, formerly endemic for malariae malaria, but since having eliminated contemporary transmission of the disease [145].

\textbf{Genomic studies of \textit{Plasmodium malariae}}

Large-scale genomic studies of the neglected malaria parasites and zoonotic species have been difficult to date, limited by infections having low parasite densities and being mixed with other \textit{Plasmodium} species, thereby making it difficult to obtain sufficient parasite DNA to perform whole genome sequencing. For \textit{P. malariae}, the first partial genome using next-generation sequencing

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\begin{table}[h]
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\begin{tabular}{|c|c|c|c|}
\hline
Host & Host Distribution & GenBank ID & References \\
\hline
Emperor tamarin (\textit{Saguinus imperator}) & Bolivia, Brazil, Peru & KY709306 & [11] \\
Golden-handed tamarin (\textit{Saguinus midas}) & Brazil, Guyana, French Guiana, Suriname & & [5] \\
Geoffroy’s tamarin (\textit{Saguinus geoffroyi}) & Panama, Colombia & & [11] \\
Martins’s tamarin (\textit{Saguinus martinsi}; both subspecies: \textit{Saguinus martinsi martinsi}, \textit{Saguinus martinsi ochraceous}) & Brazil & & [10] \\
Black tamarin (\textit{Saguinus niger}) & Brazil & & [11] \\
Tufted capuchin (\textit{Sapajus apella}) & Brazil, Venezuela, Guyanas, Colombia, Ecuador, Bolivia, Peru & KC906715 & [9] \\
Blond capuchin (\textit{Sapajus flavius}) & Brazil & KX618476 & ** \\
Large-headed capuchin (\textit{Sapajus macrocephalus}; Syn.: \textit{Sapajus apella macrocephalus}) & Bolivia, Brazil, Colombia, Ecuador, Peru & & [5] \\
Robust tufted capuchin (\textit{Sapajus robustus}) & Brazil & & [5] \\
Golden-bellied capuchin (\textit{Sapajus xanthosternos}) & Brazil & & [5] \\
Black-capped squirrel monkey (\textit{Saimiri boliviensis}) & Amazon basin in Bolivia, western Brazil, and eastern Peru & & [5] \\
Common squirrel monkey (\textit{Saimiri sciureus}) & Brazil, Colombia, Ecuador, French Guiana, Guyana, Peru, Suriname, Venezuela & JX045641 & [12] \\
Bare-eared squirrel monkey (\textit{Saimiri ustus}) & Brazil, Bolivia & KC906728 & [9] \\
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\end{tabular}
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**Unpublished: Bueno et al.
was produced from CDC Uganda I strain DNA [22, 146]. A subsequent study generated a more complete reference using long-read sequencing technology from DNA of the *P. malariae* isolate PmUG01, from an Australian traveler infected in Uganda [22, 23]. Additional genomic data from short-read Illumina data of travelers’ isolates from Mali, Indonesia and Guinea, and one patient in Sabah, Malaysia, were also reported by Rutledge et al. Analysis of these genomes revealed that around 40% of the 33.6 Mb genome (24% GC content), particularly in subtelomeric chromosome regions, is taken up by multigene families, as seen in *P. ovale* species [22, 25]. The *P. malariae* genome displays some unique characteristics, such as the presence of two large families, the fam-l and fam-m genes, with almost 700 members [22, 23]. Most of these genes encode proteins with a PEXEL export signal peptide and many encode proteins with structural homology to Rh5 of *P. falciparum*, the only known protein that is essential for *P. falciparum* red blood cell invasion [147]. These observations suggest that the fam-l and fam-m gene products may also have an important role in binding to host ligands. Other gene families, such as the *Plasmodium* interspersed repeat (*pir*) loci that are present in many species in the genus, including in *Plasmodium vivax* (~1,500 *vir* genes), are present in the *P. malariae* genome. Of the 250 *mir* genes identified, half are possible pseudogenes. Products of the *pir* genes are predicted to be exported to the infected erythrocyte surface and may have a role in cell adhesion. Like *pir* genes, SURFIN proteins are also encoded in the *P. malariae* genome at around 125 loci, much greater than the number present in *P. falciparum* (ten) or *P. vivax* (two). Another unique feature of the *P. malariae* genome is the presence of 20 copies, in a single tandem array, of the P27/25 gene, a sexual-stage cytoplasmic protein with a possible role in maintaining cell integrity. P27/25 is encoded by a single copy gene in all other species evaluated to date [23, 25].

The sequences of an additional eighteen *P. malariae* genomes from Africa and Asia have recently been reported [21]. These were derived directly from patient isolates, using a selective whole genome DNA amplification (SWGA) approach to increase the relative abundance of parasite DNA sequence reads relative to host reads. A total of 868,476 genome-wide SNPs were identified, filtered to 104,583 SNPs after exclusion of the hyper-variable subtelomeric regions. Phylogenetic analysis showed a clear separation of isolates sourced from Africa and Asia, similar to observations from the analysis of sequence data from the circumsporozoite (*pmcsp*) gene [148]. Many non-synonymous SNPs in orthologs of *P. falciparum* drug resistance-associated loci (*pmdhfr*, *pmdhps* and *pmmdr1*) were detected [21, 52], but their impact on drug efficacy remains unknown. Thus, to date, there are no validated molecular markers of drug resistance in *P.
| Country         | Region                        | Diagnostic Technique | Prevalence                                      | References                                                                 |
|-----------------|-------------------------------|----------------------|-------------------------------------------------|---------------------------------------------------------------------------|
| Afghanistan      | Jalalabad                     | PCR                  | 0.3% (1/306)                                    | Mikhail et al. 2011                                                       |
|                 | Laghman District              | Microscopy           | 1 case                                          | Ramachandra 1951                                                          |
|                 | Chardhi                       | Microscopy           | 1.4% (1/71 infants)                             | Ramachandra 1951                                                          |
| Angola           | Bengo province                | PCR                  | 8.1% of malaria positives; 1.3% general         | Fancony et al. 2012                                                       |
|                 | Luanda                        | PCR                  | 1.2% (1/81 symptomatic)                         | Pembele et al. 2015                                                       |
| Bangladesh       | Bandarban                     | PCR                  | 2.7% (60/2246); 8% of 746 malaria positives; 4.3% of symptomatic patients | Fuehrer et al. 2014                                                       |
| Belize           |                               | MoH official data    | 0.04% of malaria positives (1990–2008)          | Bardach et al. 2015                                                       |
| Benin            |                               | PCR                  | 8.3% (12/144)                                   | Doderer-Lang et al. 2014                                                  |
| Botswana         | Tutume                        | PCR                  | 0.6% (2/320 asymptomatic)                       | Motshoge et al. 2016                                                      |
|                 | Francistown                   | PCR                  | 0.5% (1/195 asymptomatic)                       | Motshoge et al. 2016                                                      |
|                 | Kweneng East                  | PCR                  | 0.4% (3/687 asymptomatic)                       | Motshoge et al. 2016                                                      |
| Brazil           |                               | MoH official data    | 0.08% (1990–2008)                               | Bardach et al. 2015                                                       |
|                 | Apiacás—Mato Grosso State     | PCR                  | 11.9% (59/497)                                  | Scopel et al. 2004                                                        |
|                 | Amazon Region                 | PCR                  | 33.3% (42/126 malaria positives)                | Cunha et al. 2021                                                         |
|                 | Espírito Santo                | PCR                  | 2.3% (2/92)                                     | de Alencar et al. 2018                                                   |
|                 | Kossi District                | PCR                  | 0.1% (1/695 pregnant)                           | Williams et al. 2016                                                     |
| Burkina Faso     |                               | PCR                  | 2.1–13.4% prevalence (decreasing from 2000–2011) | Geiger et al. 2013                                                        |
|                 | Bassy and Zanga               | PCR                  | 7.4% (8/108) of Pf positives                    | Culleton et al. 2008                                                      |
|                 | Laye                          | Microscopy           | 0.9–13.2% (children)                            | Gnémé et al. 2013                                                         |
| Burma/Myanmar    | Kachin State                  | PCR                  | 0.1% (3/2598)                                   | Li et al. 2016                                                            |
|                 | northern Myanmar              | Microscopy           | 0.04 (2/5585)                                   | Wang et al. 2014                                                          |
| Burundi          | Karuzi                        | Microscopy           | 6.7% (228/3393)                                 | Protopopoff et al. 2008                                                  |
|                 | Northern Imbo Plain           | Microscopy           | 5% (23/459 malaria positives)                   | Nimpaye et al. 2020                                                      |
| Cambodia         |                               | PCR                  | –                                               | Khim et al. 2012                                                          |
|                 |                                | PCR                  | 2.1% (33/1792)                                  | Durnez et al. 2018                                                        |
|                 | 2007 Cambodian National Malaria Survey | PCR | 0.2% (17/7707)                                      | Lek et al. 2016                                                          |
| Cameroon         | Yaoundé region                | PCR                  | –                                               | Khim et al. 2012                                                          |
|                 | Yawoundé region               | PCR                  | –                                               | Tahar et al. 1998                                                         |
|                 | Adamawa region                | PCR                  | 17.7% (of 1367)                                 | Feufack-Donfack et al. 2021                                              |
|                 | Yaoundé region                | PCR                  | 12% (of 122 asymptomatic children)              | Roman et al. 2018                                                         |
| Central African Republic | Dzanga-Sangha Protected Area | PCR                  | 0.2% (2/95 asymptomatic)                        | Mapua et al. 2018                                                         |
|                 | Dzanga-Sangha region          | PCR                  | 11.1% (of 540 symptomatic)                      | Bylicka-Szczepanska et al. 2021                                           |
| Chad             |                               | Microscopy           | 1 case (infant; mixed with Pf)—imported case in the Netherlands | Terveer et al. 2016                                                       |
| China            | Yunnan                        | PCR                  | 1% (1/103)                                      | Li et al. 2016                                                            |
| Colombia         | Colombia’s Amazon department  | PCR                  | 38.65% (of 1392 symptomatic)                     | Nino et al. 2016                                                          |
|                 |                              | MoH official data    | 0.03% (1990–2008)                               | Bardach et al. 2015                                                       |
|                 | Colombian Amazon trapezium    | PCR                  | 43.2% (862/1995 symptomatic)                     | Camargo et al. 2018                                                       |
| Comores          | Grande Comore                 | PCR                  | 0.62% (1/159)                                   | Papa Mze et al. 2016                                                      |
Table 2 (continued)

| Country           | Region                                      | Diagnostic Technique | Prevalence                                                                 | References                                      |
|-------------------|---------------------------------------------|----------------------|-----------------------------------------------------------------------------|------------------------------------------------|
| Congo DRC         | Kinshasa province                          | PCR                  | 39% asymptomatic and 7% symptomatic (of malaria positives)                  | Nundu et al. 2021 [64]                         |
|                   |                                             | PCR                  | 3.7% (mixed with Pf of malaria positives)                                   | Kiyonga Aimeé et al. 2020 [65]                 |
|                   |                                             | PCR                  | 1.5% (1/65; mixed with Pf; asymptomatic children)                           | Podgorski et al. 2020 [66]                    |
| Republic of Congo |                                             | PCR                  | 4.9% (7/142; 6 mixed with Pf; symptomatic)                                   | Kavunga-Membo et al. 2018 [67]                |
| Costa Rica        |                                             | PCR                  | 0.9% (8 of 851)                                                            | Culleton et al. 2008 [46]                     |
| Cote d'Ivoire     |                                             | PCR                  | 4 cases                                                                    | Calvo et al. 2015 [68]                        |
|                   | Yamoussoukro                                | PCR                  | 1.6% (7/438) febrile; 2.3% (8/346) afebrile                                | Ehounoud et al. 2021 [69]                     |
| Dominican Republic| MoH official data                           | 0.02% (1990–2008)    |                                                                             | Bardach et al. 2015 [31]                      |
| El Salvador       | MoH official data                           | 0.01% of malaria positives (1990–2008); free of malaria since 2021       |                                                                             | Bardach et al. 2015 [31]                      |
| Equatorial Guinea | Bioko Island (Ureka, Bareso, Sacriba)       | PCR                  | 10–31% (asymptomatic < 10 years)                                          | Guerra-Neira et al. 2006 [70]                 |
|                   | Bioko Island                                | PCR                  | 15.3% (9/59; blood donors)                                                  | Schindler et al. 2019 [71]                    |
| Eritrea           | Eritrean migrants                           | PCR                  | 0.7% (of 146)                                                              | Schlagenhauf et al. 2018 [72]                 |
| Ethiopia          | Southern Ethiopia Ormo Nada                 | PCR                  | 2 mono and 2 mixed with Pf                                                  | Mekonnen et al. 2014 [73]                     |
|                   | Amhara Regional State                       | PCR                  | 0.3% (1/359)                                                               | Getnet et al. 2015 [74]                       |
| French Guyana     | MoH official data                           | 1.39% of malaria positives (1990–2008)                                   |                                                                             | Bardach et al. 2015 [31]                      |
|                   |                                             | PCR                  | Case (GenBank: AF138881)                                                    | Fandeur et al. 2000 [7]                       |
| Gabon             | Franceville                                 | PCR                  | 2.5% (4/162); febrile children                                             | Maghendji-Nzondo et al. 2016 [75]             |
|                   | Lambarene                                   | PCR                  | 0.5% (1/206)                                                               | Culleton et al. 2008 [46]                     |
|                   | Fougamou and villages in the surroundings   | PCR                  | 23% (193/834)                                                              | Woldearegai et al. 2019 [76]                  |
| Gambia            | Microscopy                                  | rarely               |                                                                             | [http://www.rollbackmalaria.org/files/files/countries/Gambia.pdf](http://www.rollbackmalaria.org/files/files/countries/Gambia.pdf) (accessed: July 25th, 2017) |
| Ghana             | Kwahu-South                                 | PCR                  | 12.7% (18/142)                                                             | Owusu et al. 2017 [77]                        |
|                   |                                             | PCR                  | 12.8% (45/352) coinfections with Pf                                        | Culleton et al. 2008 [46]                     |
|                   |                                             | PCR                  | 28% (76/274) school children                                               | Dinko et al. 2013 [27]                        |
| Guatemala         | MoH official data                           | 0.01% of malaria positives (1990–2008)                                   |                                                                             | Bardach et al. 2015 [31]                      |
| Guinea            |                                             | PCR                  |                                                                             | Khim et al. 2012 [52]                         |
|                   |                                             | Microscopy           | 0.3% (2/724) in young infants, 12.0% (90/748) in children 1–9 years of age, and 5.8% (43/743) in children 10–15y. 97% (131/135) mixed with Pf | Ceesay et al. 2015 [78]                       |
| Guinea-Bissau     |                                             | PCR                  |                                                                             | Tanomsing et al. 2007 [79]                    |
|                   | Antula                                      | PCR                  | 18% (60) in 1995, 4% (of 71) in 1996                                       | Arez et al. 2003 [80]                         |
| Guyana            | Georgetown                                 | PCR                  | 3 PCR confirmed cases                                                      | Baird et al. 2002 [81]                        |
|                   |                                             | MoH official data    | 0.03% of malaria positives (1990–2008)                                     | Bardach et al. 2015 [31]                      |
| Haiti             |                                             | PCR                  | Imported to Jamaica                                                        | Lindo et al. 2007 [82]                        |
| Country | Region | Diagnostic Technique | Prevalence | References |
|---------|--------|----------------------|------------|------------|
| India   | various | PCR GenBank ID: KUS10228 rare | Krishna et al. unpublished | [83] |
|         | Odisha | PCR 9.1% (10/110) mono; 10.9% (12/110) mixed; febrile malaria positives | Pati et al. 2017 | [84] |
| Indonesia | Papua | PCR | Tanomsing et al. 2007 | [79] |
|         | Flores—Ende District | PCR 1.9% (of 1509) | Kaisar et al. 2013 | [85] |
|         | North Sumatra | PCR 3.4% of 3731 participants; 2.9–11.5% of malaria positives | Lubis et al. 2017 | [29] |
| Iran    | Baluchestan | PCR 1.4% (2/140) | Adel and Ashgar 2008 | [86] |
| Kenya   | Lake Victoria basin Western Kenya | PCR 5.3% (35/663) of asymptomatic infections and 3.3% (8/245) of clinical cases | Lo et al. 2017 | [87] |
|         | Kisi district | PCR 11.6% (84 of 722) | Culleton et al. 2008 | [46] |
| Laos    | northern provinces | PCR 0.05% (3/5082); 7.7% of PCR positives for malaria; 2 mono+1 mixed Pv | Lover et al. 2018 | [88] |
| Liberia | Far | microscopy 39% | Björkman et al. 1985 | [89] |
|         | PCR | 3 cases imported to China | Cao et al. 2016 | [90] |
| Madonna | Ampasimpotsy | PCR 2.1% (12/559 malaria positives) | Khim et al. 2012 | [54] |
| Malawi  | Dedza and Mangochi | PCR 1 case imported to China | Cao et al. 2016 | [90] |
|         | Malaysia Borneo | PCR 9.4% of 2918 | Bruce et al. 2011 | [92] |
|         | Sabah | PCR 2.8% (1/47) | Lee et al. 2009 | [93] |
|         | Peninsular Malaysia | PCR 0.6% (8/1366); 7 mono+1 mixed with Pf | William et al. 2014 | [94] |
|         | PCR | 18% (20/111) of malaria positives; 16 mono; 1 with Pf and 3 with Pk | Vythilingam et al. 2008 | [95] |
| Mali    | PCR | 14/603; 3 mono, 10 Pf mix, 1 Pf, PoC mix; pregnant | Khim et al. 2012 | [52] |
|         | Northern Mali | PCR 9.4–22.5% of malaria positives— asymptomatic | Koita et al. 2005 | [96] |
| Mauritania | Boghe-Sahelian zone | Microscopy 0.03% (1/3445 children); 0.7% (1/143 malaria positives) | Ouldabdallah Moukah et al. 2016 | [97] |
|         | Hodh Elgharbi (Sahelian zone) | Microscopy 1.1% (4/378) of malaria positives febrile patients; 0.3% (4/1161) in febrile patients | Ould Ahmedou Salem et al. 2016 | [98] |
| Mayotte | Mayotte Island | Microscopy 4% of all malaria positive cases | Maillard et al. 2015 | [99] |
| Mozambique | Manchiana and Ilha Josina | PCR Manchiana: 19.3% (27/140); Ilha Josina: 28.7% (54/188) | Marques et al. 2005 | [100] |
| Namibia | Bushmanland | Microscopy rare | mentioned in Noor et al. 2013 | [101] |
| Niger   | south-eastern | Microscopy 1.7% of malaria positives | Doudou et al. 2012 | [102] |
| Nigeria | Ibadan area | PCR 11.7% (69/590), children; mainly mixed infections | May et al. 1999 | [103] |
|         | Eboyi State | PCR 6.67% mono; 2% mixed with pf of 150 HIV positive patients | Nnoso et al. 2015 | [104] |
| Country         | Region                        | Diagnostic Technique | Prevalence                                                                 | References                      |
|-----------------|-------------------------------|----------------------|-----------------------------------------------------------------------------|---------------------------------|
| Laifa           |                               | PCR                  | 0.7% (7/960)—3 mono and 4 mixed Pf, asymptomatic children                   | Oyedj et al. 2017 [105]         |
| Ibadan          |                               | PCR                  | 66% (352/530) of malaria positive asymptomatic adolescents (ages 10–19 years), mainly mixed | Abdulraheem et al. 2021 [106]   |
| Pakistan        |                               | PCR, Microscopy      | 1 case imported to China                                                    | Cao et al. 2016 [90]            |
|                 |                               |                      | 0.4% (2/521) hospitalized patients                                          | Beg et al. 2008 [107]           |
| Panama          |                               | MoH official data    | 0.01% of malaria positives (1990–2008)                                     | Bardach et al. 2015 [31]        |
|                 |                               |                      | Eradicated?—Last case in 1972                                               | Hurtado et al. 2020 [108]       |
| Papua New Guinea| East Sepik Province           | PCR                  | 4.62% (100/2162), 75 mono and 25 mixed                                      | Mehlotra et al. 2000 [109]      |
|                 |                               | PCR                  | Oro (0.7%); Eastern Highlands (0.2%); Madang (1.5%); New Ireland (1.3%); East New Britain (0.3%); Bougainville (0.1%) | Hetzel et al. 2015 [110]        |
| Peru            | south-east Amerindian population | Microscopy          | above 80% of all malaria infections                                         | Sulzer et al. 1975 [111]        |
|                 |                               | MoH official data    | 0.02% of malaria positives (1990–2008)                                     | Bardach et al. 2015 [31]        |
| Philippines     | Palawan                       | Microscopy           | 0–0.5%                                                                      | Oberst et al. 1988 [112]        |
|                 | Mindanao                      | PCR                  | 0.03% (1/2639) asymptomatic                                                | Dacuma et al. 2021 [113]        |
| Rwanda          | Rukara Health Centre          | PCR                  | 1% (1/99)                                                                  | Culleton et al. 2008 [46]       |
| Sao Tome/Principe| Principe                      | Microscopy           | 11 cases                                                                    | Lee et al. 2010 [114]           |
| Saudi Arabia    | Western regions               | Microscopy           | 0.5% (48/9255 malaria positives)                                            | Amer et al. 2020 [115]          |
| Senegal         | Kedougou                      | PCR                  | GenBank ID: KX417705                                                        | unpublished                     |
|                 | southeastern Senegal          | PCR                  | 3.3% of 122 asymptomatic participants                                      | Badiane et al. 2021 [116]       |
| Sierra Leone    | Moyamba District              | Microscopy           | 2.1% Pm mono                                                               | Gbakima et al. 1994 [117]       |
|                 | Bo                            | PCR                  | 0.4% (2/534) febrile patients                                              | Leski et al. 2020 [118]         |
| Somalia         | Imported to USA—marines       | Microscopy           | 5% of all malaria positives reviewed in Oldfield et al. 1993                | [119]                           |
| South Sudan     | Jonglei State                 | Microscopy           | 6 of 392; 7.7% of malaria positives                                        | Newton et al. 1994 [120]         |
| Sudan           | Gezira                        | Microscopy           | 38 of 1987; 4.1% of malaria positives                                       | Omer et al. 1978 [121]          |
|                 | East Sudan                    | PCR                  | case report                                                                 | Imirzaloglu et al. 2006         |
|                 | Red Sea State                 | microscopy           | 1.1% (3/283 malaria positives)                                             | Ageep 2013 [123]                |
| Suriname        | MoH official data             |                     | 5.25% of malaria positives (1990–2008)                                     | Bardach et al. 2015 [31]        |
|                 |                               | MoH official data    | 12% of 86 Pf positives                                                     | Peek et al. 2004 [124]          |
| Swaziland       |                               | PCR                  | 0.02% (1/4028)                                                             | Hisiang et al.2012 [125]        |
| Tanzania        | Zanzibar                      | PCR                  | 24—14 mono and 10 mixed Pf                                                 | Xu et al. 2015 [126]            |
|                 |                               |                      | 0.5% (3/594) febrile patients but Pf-RDT negative                          | Baltzell et al. 2013 [127]      |
|                 | Kibiti District               | PCR                  | 2.4% in 2016 (11.3–16.2% in the 1990’s)                                     | Yman et al. 2019 [128]          |


**Table 2** (continued)

| Country       | Region                     | Diagnostic Technique | Prevalence                                                                 | References                                      |
|---------------|----------------------------|----------------------|-----------------------------------------------------------------------------|-------------------------------------------------|
| Thailand      | Kanchanaburi Province      | PCR                  | Various GenBank entries (e.g. EF206337)                                     | Tanomsing et al. 2007 [79]                      |
|               |                            | MoH                  | 0.2% (2/812)                                                               | Yorsaeng et al. 2019 [129]                     |
|               |                            |                      | 2012: 0.3% (48/16196 malaria positives)                                    | Summarized in Yorsaeng et al. 2019 [129]       |
|               |                            |                      | 2013: 0.5% (80/14740 malaria positives)                                     |                                                  |
|               |                            |                      | 2015: 0.2% (26/12637 malaria positives)                                     |                                                  |
|               |                            |                      | 2016: 0.2% (26/15451 malaria positives)                                     |                                                  |
| Vanuatu       | Imported to Australia      | Microscopy           | 0.57% (6 cases)                                                            | Bragonier et al. 2002 [130]                     |
|               |                            |                      | 0.6% (3/501 malaria positives from East Timor; 1 mono and 2 mixed)         | Elmes 2010 [131]                               |
| Togo          |                            | PCR                  | GenBank ID:AB354570                                                        | Khim et al. 2012 [52]                          |
|               |                            |                      | 4.8% (48/1000) blood donors                                               | Dorkenoo et al. 2016 [132]                     |
|               |                            |                      | 31.2% of all malaria positives                                             | Hayakawa et al. 2008 [133]                     |
|                |                            |                      |                                                                           | Murphy et al. 2020 [134]                       |
| Timor-Leste   |                            | Microscopy           | 0.57% (6 cases)                                                            |                                                 |
|               |                            |                      | 0.6% (3/501 malaria positives from East Timor; 1 mono and 2 mixed)         |                                                 |
|               |                            |                      |                                                                           |                                                 |
| Yemen         | Taiz-region                | Microscopy           | 0.06% (1/1638) asymptomatic                                                | Al-Eryani et al. 2016 [138]                    |
|               | highlands                  | Microscopy           | 0.2% (1/455) symptomatic                                                   | Al-Mekhlafi et al. 2011 [139]                  |
|               |                            |                      | 1.3% (1/78) Plasmodium positives                                           |                                                 |
| Vietnam       |                            | PCR                  | Various, e.g. KM016331                                                      | Lalremruata et al. 2015 [13]                   |
|               |                            |                      | 11.8% (75/630); 25 mixed infections                                        | Lalremruata et al. 2015 [13]                   |
|               |                            | MoH official data     | 0.09% of malaria positives (1990–2008)                                     | Bardach et al. 2015 [31]                       |
|                | Yanomami villages         | PCR                  | Various; e.g. KM016331                                                      |                                                 |
|                |                            |                      | 11.8% (75/630); 25 mixed infections                                        |                                                 |
|                |                            |                      |                                                                           |                                                 |
| Zambia        | Nchelenge District         | Microscopy           | 0.6% (5/782) Children < 10 years; 2.1%, (5/236) of malaria positives       | Nambozi et al. 2014 [140]                      |
|               | Western and Southern       | PCR                  | 1.7% (5/304); 2 mono and 3 mixed Pf                                       | Sitali et al. 2019 [141]                       |
|               | Province                   |                      |                                                                           |                                                 |
|               | Choma District, Southern   | PCR                  | 0.2% of 3292 participants; 2 Pf + 5 Pf + 4 Pf; low transmission area       | Laban et al. 2015 [142]                        |
|               | Province                   |                      |                                                                           |                                                 |
|                |                            |                      |                                                                           |                                                 |
|                |                            | Microscopy           | 1.8% of 51,962; 8.3% of malaria infections (1972–1981)                    | Taylor and Mutambu 1986 [143]                  |

*Note: P. malariae* parasites although, as noted above, prophylaxis breakthrough, treatment failures and emergence following treatment for other species have been reported [26–29, 149].

In the wider *Plasmodium* species context, phylogenetic analysis has shown that *P. malariae* isolates group with malariae-like species that infect monkeys and non-human primates [2, 23]. *Plasmodium malariae* parasites also cluster closer to *P. ovale* spp., but in separate clades, and more generally in a clade with *P. vivax*, *P. knowlesi* and *Plasmodium cynomolgi* that is distant from the Laverania sub-genus exemplified by

P. falciparum and Plasmodium reichenowi [2, 150]. Given the range of primate hosts that are infected by P. malariae, P. brasilianum and their close relatives, further genomic studies are needed to tease out the two main questions raised by the studies so far:

- Should P. brasilianum, as is currently circulating in South America, and P. malariae be considered distinct, non-recombining species?
- What is the extent of the radiation of P. malariae-like species in the great apes?

**Plasmodium ovale curtisi and Plasmodium ovale wallikeri**

**History & discovery**

First identified in Liverpool by Stephens in 1918, the index case of ovale malaria was a British army private, returning to the UK in 1918 following deployment in “East Africa”, and having reported an episode of symptomatic malaria in December, 1916 [151]. This soldier’s blood films were examined over several months, with no mention of any treatment being offered, during which time the presence of fimbriated, oval infected red cells was noted as a key feature, together with a 48 h fever periodicity. This “new parasite of man” (sic) was thus characterized as a benign tertian infection and named *Plasmodium ovale* in the primary paper, published in 1922. Some additional detailed description of the parasite and its presentation was published by Stephens and Owen in 1927 [152].

For much of the twentieth century, ovale malaria remained a minor entrant in parasitology textbooks, including Coatney et al. [1], until the advent of molecular diagnostic studies in the 1990s began to uncover evidence of genetic dimorphism [153], leading to a series of papers in the first decade of the twenty-first century examining the impact of this dimorphism on molecular and antigen-based diagnosis [154–158]. A multi-centre effort to gather 51 geographically diverse parasite isolates and generate sequencing data across seven genetic loci was then able to demonstrate that ovale malaria was the result of infection by either of two non-recombining, sympatric sibling parasite species, which were named *P. ovale curtisi* and *P. ovale wallikeri* [159]. In the decade that followed, various molecular tools were developed to distinguish the two ovale species, and there was an explosion of our understanding of the contribution of the newly recognized parasites to malaria burden across the tropics.

**Distribution and abundance**

Although the original identification of *P. ovale* sensu lato (s.l.) by Stephens was in a British soldier who contracted malaria in “East Africa”, the species was subsequently recognized as highly endemic in West Africa (especially Nigeria). Coatney et al. described the distribution of the species as extending to the East African Coast, and as far south as Mozambique [1]. Outside Africa, ovale malaria was sporadically reported from Papua New Guinea, Indonesian islands and some South-East Asian countries [144]. However, with the introduction of molecular diagnostic tools and recognition and widespread acceptance of the two sympatric species, *P. o. curtisi* (former “classic” type) and *P. o. wallikeri* (former “variant” type) [159], a much more complex understanding of these parasites has developed. Molecular diagnostics have greatly facilitated the confirmation of the presence of ovale malaria parasites in much of Africa and Asia, including countries where it was not previously known to be present (e.g. Bangladesh, Afghanistan, Angola) [35–37, 160–162], and in non-human primates [163]. However, it remains generally accepted that these parasites are not endemic in the Americas [159].

Infections with ovale malaria parasites are often asymptomatic and parasite densities low, leading to difficulties in accurate microscopic diagnosis and some uncertainties as to distribution in the recent past. Given the presence of intra-erythrocytic stippling on thin films, and the irregular shapes adopted by ovale-infected cells, there is some morphological similarity to *P. vivax*, which exacerbates diagnostic difficulties. This also influenced early phylogenetic thinking; Coatney and colleagues write that “from the vivax-like stem developed a morphologically similar species, *P. ovale*, that was capable of surviving in (African) hominids...” (1). Moreover, mixed infections with other human malaria parasites are very common. Double infections of *P. ovale curtisi* and *P. ovale wallikeri* in the same individual have also been reported (e.g. Angola, Bangladesh) [36, 161], confirming the lack of recombination between the two species. However, reported prevalence estimates vary widely among various studies, reflecting different study designs and blood sample collection strategies (e.g. asymptomatic vs. febrile patients). The known distribution of *P. ovale* ssp., *P. o. wallikeri* and *P. o. curtisi* is presented in Fig. 2, and a detailed listing of reports identifying these species, including GenBank accession ID where relevant, is given in Table 3 [27, 36, 48, 58, 72, 76, 83, 90, 97, 102, 106, 116, 118, 137, 156, 159, 166–217].
Genomic studies of *P. o. curtisi* and *P. o. wallikeri*

In the period since the two genetically distinct forms of *P. ovale* spp. were recognized, there have been a limited number of studies that have explored the differences between them. A study in UK travellers with ovale malaria by Nolder and colleagues could not identify any robust features of morphology that can distinguish *P. o. curtisi* from *P. o. wallikeri* [168], but were able to provide evidence of a significant difference in the distribution of relapse periodicity: the former species displayed a geometric mean latency of 85.7 days (95% CI 66.1 to 111.1, N = 74), compared to the significantly shorter 40.6 days (95% CI 28.9 to 57.0, N = 60) of the latter. This contrasts with the earlier observation of Chin and Coatney, who conducted studies of experimentally infected volunteers whose initial infections (all with the same “West African strain”) were treated with quinine or chloroquine before extended follow-up for evidence of *P. vivax*-type relapse [218]. These authors concluded that “These results leave little doubt that ovale malaria is a relapsing disease, but there appears to be no definite relapse pattern...” Subsequent studies in European travellers, a group in which super-infection is absent as a potential confounder, have confirmed this difference in latency period between *P. ovale curtisi* and *P. ovale wallikeri* [168, 219, 220]. These studies were also consistent in finding that *P. ovale wallikeri* is associated with low platelet counts and thus more likely to elicit clinical thrombocytopenia, and more likely to be correctly identified by immunochromatographic lateral flow tests that detect the LDH antigen, which fail to identify > 90% of *P. ovale curtisi* infections, a reflection of differences in the amino acid sequence of LDH in the two species [158, 159].

Given the absence of distinguishing morphological characters, despite reliable differences in some clinical and diagnostic features, there has been increasing attention to characterisation of the genomic organisation of the two sibling species as a route to better understanding their divergence from each other, and to describe the level of within-species diversity. Initial efforts were based on direct sequencing of PCR-amplified loci, and gave a general picture of fixed differences in both synonymous and non-synonymous substitutions between the species in almost every coding region examined, but very little intra-species genetic diversity [159–161, 185, 210, 211]. This was also true of genes related to sexual stage development, which had been examined for evidence of
Table 3  Geographic distribution and prevalence of \textit{P. ovale} sp., \textit{P. ovale wallikeri} and \textit{P. ovale curtisi} (Sequences submitted to GenBank as \textit{P. ovale} were assigned to species level post hoc)

| Country               | Type              | Diagnostic Technique | Prevalence                                      | References                                      |
|-----------------------|-------------------|----------------------|-------------------------------------------------|------------------------------------------------|
| Afghanistan           | \textit{P. ovale curtisi} | PCR                 | Imported to Switzerland                         | Nguyen et al. 2020 [162]                       |
|                       | \textit{P. ovale curtisi} | Sequence            | GenBank: FJ409571; FJ409567                      | Duval et al. 2009 [163]                        |
|                       | \textit{P. ovale wallikeri} | Sequence            | GenBank: MG588149; imported to China            | Zhou et al. Unpublished –                     |
|                       | \textit{P. ovale wallikeri} | PCR                 | 0.3% (11/3316) 3 mono + 8 mixed; 2% (11/541) malaria positives | Fançony et al. 2013 [36]                       |
|                       | \textit{P. ovale curtisi} | PCR                 | 0.3% (11/3316) 4 mono + 7 mixed; 2% (11/541) malaria positives | Fançony et al. 2013 [36]                       |
| Angola                | \textit{P. ovale curtisi} | Sequence            | GenBank: FJ409571; FJ409567                      | Duval et al. 2009 [163]                        |
|                       | \textit{P. ovale wallikeri} | Sequence            | GenBank: MG588149; imported to China            | Zhou et al. Unpublished –                     |
|                       | \textit{P. ovale wallikeri} | PCR                 | 0.3% (11/3316) 3 mono + 8 mixed; 2% (11/541) malaria positives | Fançony et al. 2013 [36]                       |
|                       | \textit{P. ovale curtisi} | PCR                 | 0.3% (11/3316) 4 mono + 7 mixed; 2% (11/541) malaria positives | Fançony et al. 2013 [36]                       |
| Bangladesh            | \textit{P. ovale curtisi} | Sequence            | 0.26% (1/379) symptomatic; 0.45% (10/1867) incl. asymptomatic participants; Mono—36.4% | Fuehrer et al. 2012 [161]                       |
|                       | \textit{P. ovale wallikeri} | Sequence            | 0.79% (3/379) symptomatic; 0.53% (12/1867) incl. asymptomatic participants; Mono—46.1% | Fuehrer et al. 2012 [161]                       |
| Benin                 | \textit{P. ovale wallikeri} | Sequence            | GenBank: GQ183063; EU266604                      | Sutherland et al. 2010 [159]                   |
|                       | \textit{P. ovale wallikeri} | PCR                 | 1 isolate in meta-analysis                      | Bauffe et al. 2012 [164]                       |
|                       | \textit{P. ovale curtisi} | PCR                 | 2 isolates in meta-analysis                     | Bauffe et al. 2012 [164]                       |
| Botswana              | \textit{P. ovale curtisi} | PCR                 | 1.85% (30/1614); 11 mono and 19 mixed           | Motshoge et al. 2021 [165]                     |
| Brunei                | \textit{P. ovale sp.}    | PCR                 | 1 case imported to China                        | Cao et al. 2016 [90]                           |
| Burkina Faso          | \textit{P. ovale curtisi} | PCR                 | 3 isolates                                      | Calderaro et al. 2012 [166]                    |
|                       | \textit{P. ovale wallikeri} | PCR                 | Imported to Germany                             | Frickmann et al. 2019 [167]                    |
| Burma/Myanmar         | \textit{P. ovale curtisi} | Sequence            | Various: e.g. KX672039; AB182496                | Win et al. 2004; Li et al. 2016 [48, 156]      |
|                       | \textit{P. ovale wallikeri} | Sequence            | Various: e.g. AB182497                          | Win et al. 2004 [48]                           |
| Burundi               | \textit{P. ovale wallikeri} | PCR                 | 1 isolate, imported to UK                       | Nolder et al. 2013 [168]                       |
|                       | \textit{P. ovale curtisi} | Sequence            | GenBank: e.g. FJ409571                          | Duval et al. 2009 [163]                        |
|                       | \textit{P. ovale wallikeri} | Sequence            | GenBank: e.g. FJ409566                          | Duval et al. 2009 [56]                         |
| Cameroon              | \textit{P. ovale curtisi} | Sequence            | Imported to Singapore; GenBank: e.g. KP050401   | Chavatte et al. 2015 [170]                     |
|                       | \textit{P. ovale wallikeri} | Sequence            | GenBank: e.g. FJ409566                          | Duval et al. 2009 [56]                         |
|                       | \textit{P. ovale wallikeri} | Sequence            | Various GenBank: e.g. FJ409571; KP050465        | Duval et al. 2009; Chavatte et al. 2015 [163, 170] |
|                       | \textit{P. ovale wallikeri} | Sequence            | 1.1% (1/95) asymptomatic; 4.3% (1/23) of malaria positives; GenBank: MG241227 | Mapua et al. 2018 [58]                         |
| Chad                  | \textit{P. ovale curtisi} | PCR                 | 1 isolate in meta-analysis                      | Bauffe et al. 2012 [164]                       |
|                       | \textit{P. ovale wallikeri} | PCR                 | 1 isolate in meta-analysis                      | Bauffe et al. 2012 [164]                       |
|                       | \textit{P. ovale curtisi} | PCR                 | Imported to China                               | Zhou et al. 2019 [172]                         |
|                       | \textit{P. ovale wallikeri} | PCR                 | Imported to China                               | Zhou et al. 2019 [172]                         |
| China (Yunnan)        | \textit{P. ovale curtisi} | Sequence            | GenBank: KX672045; certified malaria free since 2021 | Li et al. 2016 [48]                           |
| Comoros               | \textit{P. ovale curtisi} | PCR                 | 7 isolates                                      | Bauffe et al. 2012 [164]                       |
|                       | \textit{P. ovale wallikeri} | PCR                 | 11 isolates                                     | Bauffe et al. 2012 [164]                       |
| Congo DRC             | \textit{P. ovale curtisi} | Sequence            | GenBank: e.g. FJ409567                          | Duval et al. 2009 [163]                        |
Table 3 (continued)

| Country               | Type              | Diagnostic Technique | Prevalence                                      | References                            |
|-----------------------|-------------------|----------------------|-------------------------------------------------|---------------------------------------|
|                       | P. ovale wallikeri | Sequence             | 1% (2/198) children < 5 years; GenBank: KT867772 | Gabrielli et al. 2016 [173]            |
| Congo Republic of the | P. ovale curtisi  | Sequence             | Imported to China; GenBank: MT430962              | Chen et al. 2020 [174]                |
|                       | P. ovale curtisi  | PCR                  | 4 clinical cases                                  | Oguke et al. 2011 [175]               |
|                       | P. ovale wallikeri| PCR                  | 2 clinical cases                                  | Oguke et al. 2011 [175]               |
| Cote d'Ivoire         | P. ovale curtisi  | Sequence             | GenBank: e.g. FJ409567; KP050411                  | Duval et al. 2009; Chavatte et al. 2015 [163, 170] |
|                       | P. ovale wallikeri| Sequence             | GenBank: e.g. GU723538                            | Sutherland et al. 2010 [159]          |
| Djibouti              | P. ovale sp.      | Rarely, 1 case in 2018/19 season |                                                      | de Santi et al. 2021 [176]           |
| East Timor (Timor-Leste)| P. ovale sp.    | Present according to WHO, Documented in West Timor |                                                      | Gundelfinger 1975 [177]              |
|                       | P. ovale curtisi  | Sequence             | GenBank: JF505386                                  | Unpublished                           | –                                    |
|                       | P. ovale wallikeri| Sequence             | GenBank: e.g.: KP050469                            | Chavatte et al. 2015 [170]            |
|                       | P. ovale curtisi  | PCR                  | Bioko Island—0.9–1.4% ovale in total population  | Oguke et al. 2011 [175]               |
|                       | P. ovale wallikeri| PCR                  | Bioko Island—0.9–1.4% ovale in total population  | Oguke et al. 2011 [175]               |
| Eritrea               | P. ovale sp.      | 1 case—imported to Germany |                                                      | Roggelin et al. 2016 [178]           |
|                       | P. ovale sp.      | 2.7% (4/146)—imported to Europe |                                                      | Schlagenhauf et al. 2018 [72]        |
| Ethiopia              | P. ovale curtisi  | Sequence             | 0.7% (2/300) of symptomatic patients; 1.1% (2/184) of malaria positives, GenBank: e.g. KF536874 | Alemu et al. 2013 [179]              |
|                       | P. ovale wallikeri| Sequence             | 2.3% (7/300) of symptomatic patients; 3.8% (7/184) of malaria positives, GenBank: e.g. KF536874 | Alemu et al. 2013 [179]              |
| Gabon                 | P. ovale curtisi  | Sequence             | GenBank: e.g. FJ409571; MG867083                   | Duval et al. 2009; Groger et al. 2019 [163, 180] |
|                       | P. ovale wallikeri| Sequence             | GenBank: e.g.: KJ170104; MG869598                 | Groger et al. 2019 [180]              |
|                       | P. ovale curtisi  | PCR                  | Rural Gabon—8.9% of malaria positives, 7 of 74 mono infection | Woldearegai et al. 2019 [76]         |
|                       | P. ovale wallikeri| PCR                  | Rural Gabon—4.6% of malaria positives, 1 of 38 mono infection | Woldearegai et al. 2019 [76]         |
| Gambia, The           | P. ovale wallikeri| PCR                  | 0.16% (1/604) pregnant                            | Williams et al. 2016 [44]            |
|                       | P. ovale curtisi  | Sequence             | GenBank: e.g.: GU723554                            | Sutherland et al. 2010 [159]          |
|                       | P. ovale wallikeri| Sequence             | GenBank: e.g.: KP725067                            | Oguke and Sutherland 2015             |
|                       | P. ovale curtisi  | PCR                  | Ashanti Region, 4% (15/284) malaria positives     | Heinemann et al. 2020 [182]          |
|                       | P. ovale wallikeri| PCR                  | Ashanti Region, 3% (12/284) malaria positives     | Heinemann et al. 2020 [182]          |
|                       | P. ovale curtisi  | PCR                  | 27 cases—Children 5–17                            | Dinko et al. 2013 [27]               |
|                       | P. ovale wallikeri| PCR                  | 7 cases—Children 5–17                              | Dinko et al. 2013 [27]               |
| Guinea                | P. ovale curtisi  | Sequence             | GenBank: e.g. FJ409571                             | Duval et al. 2009 [181]              |
|                       | P. ovale curtisi  | PCR                  | Imported to France                                 | Joste et al. 2021 [183]              |
| Country       | Type              | Diagnostic Technique                  | Prevalence                                                                 | References                        |
|--------------|-------------------|---------------------------------------|----------------------------------------------------------------------------|-----------------------------------|
|              | *P. ovale wallikeri* | PCR                                  | Imported to China and France                                               |                                   |
| Guinea-Bissau|                   |                                       |                                                                             |                                   |
|              | *P. ovale curtisi* | Sequence                             | GenBank: e.g.: EU266611                                                    | Sutherland et al. 2010 [159]       |
|              | *P. ovale wallikeri* | PCR                                  |                                                                             | Saralamba et al. 2019 [185]       |
| India        | *P. ovale curtisi* | Sequence                             | GenBank: e.g.: KUS10234; KP050460                                          | Chavatte et al. 2015; Krishna et al. 2017 [170, 186] |
|              | *P. ovale wallikeri* | Sequence                             | Mono infection, Bastar division of Chhattisgarh state, GenBank: KM873370    | Chaturvedi et al. 2015 [83]        |
|              | *P. ovale curtisi* | Sequence                             | Mono infection, Bastar division of Chhattisgarh state, GenBank: KM288710    | Chaturvedi et al. 2015 [83]        |
| India        | *P. ovale curtisi* | Sequence                             | Sumatra,—GenBank: e.g.: KP050463                                            | Chavatte et al. 2015 [170]         |
|              | *P. ovale wallikeri* | Sequence                             | GenBank: e.g.: AB182497                                                    | Win et al. 2004 [167]              |
|              | *P. ovale wallikeri* | Sequence                             | GenBank: e.g.: KM494987                                                    | Miller et al. 2015 [186]           |
|              |                   |                                       |                                                                             |                                   |
| Laos         | *P. ovale curtisi* | Sequence                             |                                                                             |                                   |
|              | *P. ovale wallikeri* | Sequence                             |                                                                             |                                   |
|              | *P. ovale sp.*    | PCR                                  | 0.04% (1/2409) partici-pants                                               | Iwagami et al. 2018 [189]          |
|              |                   |                                       |                                                                             |                                   |
| Liberia      | *P. ovale curtisi* | Sequence                             |                                                                             |                                   |
|              | *P. ovale wallikeri* | Sequence                             |                                                                             |                                   |
|              | *P. ovale sp.*    | PCR                                  | 0.17% (1/585) asymptomatic; 5.3% (1/91) of malaria positives; primers rOVA1/rOVA2 | Noordin et al. 2020 [191]          |
|              | *P. ovale curtisi* | Sequence                             | Pahang; GenBank: MK351321                                                   | unpublished –                     |
|              | *P. ovale sp.*    | PCR                                  | 0.4% (2/457) malaria positives                                             | Yusof et al. 2014 [192]            |
| Malawi       | *P. ovale curtisi* | PCR                                  | 2 isolates                                                                 | Oguigue and Sutherland 2015 [181]  |
|              | *P. ovale wallikeri* | PCR                                  | 2 isolates                                                                 | Oguigue and Sutherland 2015 [181]  |
| Malaysia     | *P. ovale sp. (P. ovale curtisi)* | PCR                                  | 0.17% (1/585) asymptomatic; 5.3% (1/91) of malaria positives; primers rOVA1/rOVA2 | Noordin et al. 2020 [191]          |
|              | *P. ovale curtisi* | Sequence                             |                                                                             |                                   |
|              | *P. ovale sp.*    | PCR                                  |                                                                             |                                   |
| Mauritania   | *P. ovale sp.*    | Microscopy                           | Asymptomatic; Sahelian zone 0.47% (5/1056); Saharan zone 0.47% (2/1059); Sahel-Saharan zone 0.37% (5/1330) | Ouldabdallah Moukah et al. 2016 [97] |
|              |                   |                                       |                                                                             |                                   |
| Mayotte      | *P. ovale curtisi* | PCR                                  | Imported to France                                                          | Joste et al. 2021 [183]            |
| Mozambique   | *P. ovale curtisi* | Sequence                             | GenBank: e.g. GU723517                                                     | Sutherland et al. 2010 [159]       |
|              | *P. ovale curtisi* | PCR                                  | Imported to China                                                           | Cao et al. 2016 [90]              |
| Country            | Type                      | Diagnostic Technique | Prevalence                                                                 | References                                                                 |
|--------------------|---------------------------|----------------------|-----------------------------------------------------------------------------|----------------------------------------------------------------------------|
| Namibia            | *P. ovale curtisi*        | PCR                  | 0.31% (of 952) children < 9 years                                          | Rojo-Marcos et al. 2014; Joste et al. 2021 [183, 193]                      |
|                  | *P. ovale wallikeri*      | PCR                  | Imported to France and Spain                                               | Hopey et al. 2016; Joste et al. 2021 [183]                                  |
| Niger              | *P. ovale sp.*            | Microscopy           | 1 case                                                                     | Haiek et al. 2012 [102]                                                    |
| Niger              | *P. ovale curtisi*        | PCR                  | Imported to France                                                          | Joste et al. 2021 [183]                                                    |
| Niger              | *P. ovale wallikeri*      | PCR                  | Imported to France                                                          | Joste et al. 2021 [183]                                                    |
| Nigeria            | *P. ovale curtisi*        | Sequence             | GenBank: e.g.: GU723534, KP050374                                           | Sutherland et al. 2010; Chavatte et al. 2015 [159, 170]                   |
| Nigeria            | *P. ovale wallikeri*      | Sequence             | GenBank: e.g.: GU723579                                                     | Sutherland et al. 2010                                                    |
| Nigeria            | *P. ovale sp.*            | PCR                  | 24% of malaria positives                                                   | Abdulraheem et al. 2019                                                   |
| Nigeria            | *P. ovale curtisi*        | PCR                  | 1.1% (4/365) malaria positive children                                     | Oyedeji et al. 2021                                                       |
| Nigeria            | *P. ovale curtisi*        | PCR                  | Imported to China, France and Spain                                        | Cao et al. 2016; Joste et al. 2021; Rojo-Marcos et al. 2014 [90, 183, 193] |
| Pakistan           | *P. ovale sp.*            | PCR                  | Imported to China                                                           | Cao et al. 2016                                                            |
| Papua New Guinea   | *P. ovale curtisi*        | Sequence             | GenBank: e.g.: AF145337                                                     | Mehlotra et al. 2002                                                       |
| Papua New Guinea   | *P. ovale wallikeri*      | Sequence             | GenBank: e.g.: EU266603                                                     | Sutherland et al. 2010                                                    |
| Philippines        | *P. ovale sp.*            | PCR                  | Rare, Palawan only until 1977                                               | Cabrera and Arambulo 1977                                                  |
|                   | *P. ovale sp.*            | PCR                  | Palawan—0.3% (2/613)                                                        | Reyes et al. 2021                                                          |
| Rwanda             | *P. ovale wallikeri*      | PCR                  | Imported to France                                                          | Joste et al. 2021                                                          |
| Rwanda             | *P. ovale wallikeri*      | Sequence             | GenBank: e.g.: FJ409570                                                     | Duval et al. 2009                                                          |
| Rwanda             | *P. ovale sp.*            | PCR                  | 4.9% (531/1089) school-children                                            | Sifft et al. 2016                                                          |
| Sao Tome and Principe | *P. ovale curtisi*        | Sequence             | GenBank: e.g.: GQ231520                                                     | Sutherland et al. 2010                                                    |
| Sao Tome and Principe | *P. ovale wallikeri*      | Sequence             | GenBank: e.g.: EU266603                                                     | Sutherland et al. 2010                                                    |
| Senegal            | *P. ovale curtisi*        | Sequence             | GenBank: e.g.: KX417703 unpublished                                          | –                                                                           |
| Senegal            | *P. ovale wallikeri*      | Sequence             | GenBank: e.g.: KX417699 unpublished                                         | –                                                                           |
| Senegal            | *P. ovale sp.*            | PCR                  | 4.91% (6/122)                                                              | Badiane et al. 2021                                                       |
| Sierra Leone       | *P. ovale curtisi*        | Sequence             | GenBank: e.g.: GU723523                                                     | Sutherland et al. 2010                                                    |
| Sierra Leone       | *P. ovale wallikeri*      | Sequence             | GenBank: e.g.: GU723571                                                     | Sutherland et al. 2010                                                    |
| Sierra Leone       | *P. ovale curtisi*        | PCR                  | Imported to France                                                          | Joste et al. 2021                                                          |
| Sierra Leone       | *P. ovale wallikeri*      | PCR                  | Imported to France                                                          | Joste et al. 2021                                                          |
| Sierra Leone       | *P. ovale sp.*            | PCR                  | 0.4% (2/534) febrile patients                                               | Leski et al. 2020                                                          |
| Solomon Islands    | *P. ovale wallikeri*      | PCR                  | Imported to USA (military)                                                 | Echevery et al. 2016; Echevery et al. 2017 [202, 203]                      |
| Somalia            | *P. ovale sp.*            | PCR                  | 0.05% (1/1914)                                                             | Russell et al. 2021                                                       |
| Somalia            | *P. ovale sp.*            | PCR                  | Imported to USA (military)                                                 | CDC 1993                                                                  |
| South Africa       | *P. ovale sp.*            | PCR                  | Imported to China                                                           | Cao et al. 2016                                                            |
| South Sudan        | *P. ovale sp.*            | Microscopy           | Bor, 1.2% of 392                                                            | Omer et al. 1978                                                           |
| South Sudan        | *P. ovale sp.*            | PCR                  | Imported to France                                                          | Leski et al. 2020                                                          |

**Table 3 (continued)**

*Note: The table continues with additional rows, each detailing specific diagnostic techniques, prevalence rates, and references for various countries.*
| Country  | Type              | Diagnostic Technique | Prevalence                                                                 | References                        |
|---------|-------------------|----------------------|-----------------------------------------------------------------------------|-----------------------------------|
| Sri Lanka | *P. ovale curtisi* | PCR                  | 1 isolate in meta-analysis; Sri Lanka malaria free since 2016               | Bauffe et al. 2012 [164]          |
| Sudan   | *P. ovale* sp.    | Microscopy           | New Halfa, 2% of 190 malaria positives                                       | Himeidan et al. 2005 [206]        |
|         | *P. ovale* sp.    | Microscopy           | Khartoum, 0.32% of 3791 participants                                         | El Sayed et al. 2000 [207]        |
| Tanzania | *P. ovale curtisi*| PCR                  | Imported to China                                                            | Cao et al. 2016 [90]              |
|         | *P. ovale wallikeri* | PCR                | 1 isolate                                                                   | Calderaro et al. 2013 [208]       |
|         | *P. ovale wallikeri* | PCR                | 2 cases, Imported to France                                                  | Joste et al. 2021 [183]           |
|         | *P. ovale* sp.    | PCR                  | Zanzibar; 16.2% (30/185) malaria PCR positives; 10 mono + 20 mixed infections | Cook et al. 2015 [209]            |
| Thailand | *P. ovale curtisi*| Sequence            | GenBank: e.g.: KC137349; KF018432                                           | Putaporntip et al. 2013; Tanomsing et al. 2013 [210, 211] |
|         | *P. ovale wallikeri* | Sequence          | GenBank: e.g.: GQ231519; KC137344; KF018430                                  | Sutherland et al. 2010; Putaporntip et al. 2013; Tanomsing et al. 2013 [159, 210, 211] |
|         | *P. ovale* sp.    | PCR                 | 0.3% (4/1347) asymptomatic participants; 4 mixed infections                 | Baum et al. 2016 [212]            |
| Togo    | *P. ovale* sp.    | 2.8%                 | Gbary et al. 1988                                                            |                                   |
|         | *P. ovale* sp.    | 2% of malaria positives | MSPS 2017                                                                 |                                   |
|         | *P. ovale curtisi*| PCR                 | 12 cases, Imported to France                                                 | Joste et al. 2021 [183]           |
|         | *P. ovale wallikeri* | PCR                | 14 cases, Imported to France                                                 | Joste et al. 2021 [183]           |
| Uganda  | *P. ovale curtisi*| Sequence            | GenBank: e.g.: GU723521                                                     | Sutherland et al. 2010 [159]       |
|         | *P. ovale wallikeri* | Sequence          | GenBank: e.g.: GU723573; KP050464                                           | Chavatte et al. 2015; Sutherland et al. 2010 [159, 170] |
|         | *P. ovale curtisi*| PCR                 | Apac District; Buliisa District, Mayuge District                             | Oguike et al. 2011 [175]          |
|         | *P. ovale wallikeri* | PCR                | Apac District; Buliisa District, Mayuge District                             | Oguike et al. 2011 [175]          |
|         | *P. ovale* sp.    | PCR                 | 0–6.7% of all malaria; 0–4.3% of population                                  | Oguike et al. 2011 [175]          |
|         | *P. ovale* sp.    | PCR                 | Imported to China                                                            | Cao et al. 2016 [90]              |
| Vietnam | *P. ovale curtisi*| Sequence            | GenBank: e.g.: GU723523                                                     | Sutherland et al. 2010 [159]       |
|         | *P. ovale wallikeri* | Sequence          | GenBank: e.g.: AF387041                                                     | Unpublished –                     |
|         | *P. ovale* sp.    | PCR                 | 0.8% (19/2303) of population                                                 | Nguyen et al. 2012 [137]          |
| Yemen   | *P. ovale* sp.    | Microscopy          | 1 symptomatic case, Beni-Hussan village                                      | Al-Maktari and Bassioune 1999 [215]|
| Zambia  | *P. ovale wallikeri* | PCR                | 1 case                                                                      | Nolder et al. 2013 [168]          |
|         | *P. ovale wallikeri* | LAMP               | eastern Zambia                                                              | Hayashida et al. 2017 [216]       |
|         | *P. ovale curtisi*| LAMP                | eastern Zambia                                                              | Hayashida et al. 2017 [216]       |
|         | *P. ovale* sp.    | LAMP                | 10.6% in asymptomatic participants                                          | Hayashida et al. 2017 [216]       |
|         | *P. ovale* sp.    | PCR                 | Western province (cross-sectional survey); 12.4% (32/259); 6 mono + 26 mixed infections | Stali et al. 2019 [141]          |
| Zimbabwe| *P. ovale wallikeri* | Sequence          | GenBank: e.g.: FJ409570                                                     | Duval et al. 2009 [163]           |
a mating barrier between the two species [181]. Whole genome analysis would clearly be very informative, but very few draft genomes of either species are available due to the difficulty in obtaining parasite DNA from these typically very low parasitaemia infections. The first partial genomes to become available were assembled from Illumina short-read sequencing of two isolates of *P. o. wallikeri* from Chinese workers returning from West Africa, as well as one *P. o. curtisi* isolate also from a Chinese worker returning from West Africa and the genome of the chimpanzee-propagated Nigeria I strain [1, 22, 24]. Subsequently, three partial genomes of *P. o. curtisi* from two patients that tested positive for *P. falciparum* in Ghana and one mixed infection from Cameroon, together with two *P. o. wallikeri* genomes obtained from individual patients in Cameroon, were also assembled [23].

Analysis of the *P. ovale* spp. genomes published to date has estimated a total genome length for both species of ~35 Mbp (29% GC content), with 40% being sub-telomeric [22, 23]. Differences in total length (maximum observed 38Mbp) were observed between isolates, primarily due to differences in the estimated size of expansion of the *ocir/owir* gene families. These species have considerably more *pir* genes (1500–2000), than other human plasmodium parasites (~300) [25]. A larger number of *surfin* genes have also been identified, with >50 present in *P. o. curtisi* and >125 in *P. o. wallikeri*. The variant protein isoforms expressed by members of these gene families may be important for interactions with multiple host ligands and, as they are likely to be antigenically variant, their expansion is thought to have been driven by host immune pressure. Expansion of reticulocyte binding-like proteins (RBP), involved in red blood cell invasion, has been observed in both ovale genomes (13–14 genes), gene copy numbers similar to *P. vivax*, while in other species only ~2–8 copies have been identified. An expansion of the *Plasmodium* ookinete surface protein P28 appears to be a specific feature of both *P. ovale* spp, as only one copy appears to exist in the genomes of other human-infecting species in the genus.

All the available data confirm that there is a close genetic relationship between the two species, supported by phylogenetic analysis that show *P. o. curtisi* and *P. o. wallikeri* grouping together in the same clade in all studies to date [2, 23, 159]. However, many differences between the two taxa have been observed when comparing *surfin*, *pir* and *rhp* genes, as isoforms with identical sequences have been observed between isolates of the same species, but these families are far more divergent in between-species comparisons of the few *P. o. curtisi* and *P. o. wallikeri* genomes assembled so far. Significant dimorphism has previously been reported in candidate genes across larger datasets from Asian and African isolates [159–161, 175, 185, 210, 211]. For example, specific analysis of nucleotide sequences of five protein-coding regions, likely involved in life cycle sexual stages and so potentially contributing to mating barriers, found that intra-species variation was minimal at each locus, but clear dimorphism were detected when comparing *P. o. curtisi* to *P. o. wallikeri* [181]. Similar results were observed across three vaccine candidate surface proteins in samples collected from Thailand and countries in Africa [185], and in multi-locus sequence analyses reported in a large study of both species in Bangladesh [161]. To better understand the intra- and inter -genetic diversity of these species, more complete reference genomes are needed, as well as a much greater number of isolates undergoing whole genome sequencing across geographic regions.

**Table 3 (continued)**

| Country | Type | Diagnostic Technique | Prevalence | References |
|---------|------|----------------------|------------|------------|
|         | *P. ovale* sp. | <2% of malaria positives | Taylor 1985 | [217] |

**Likely origin of these two closely-related, sympatric and non-recombining species**

The question as to how two non-recombining sibling species have ended up co-circulating in the same mammalian hosts, transmitted by the same arthropod vectors, has attracted some attention, as has the difficulty in estimating when the two lineages diverged, and in which primate hosts [2, 3, 23, 25, 159]. A thorough summary of the current thinking can be found therein, but the most parsimonious explanation for the current co-circulation of *P. o. curtisi* and *P. o. wallikeri*, in what appears to be perfect sympatry, can be paraphrased from reference 26: pre-ovale parasites in an unknown non-human primate host underwent an initial host transition into hominids some millions of years before the present. This new lineage thus began from a single event, representing an extreme genetic bottleneck, and developed apart from the progenitor stock. Substantial genetic drift occurred, while the two parasite lineages were partitioned in different hosts, a form of allopatry. When a second transition into hominid hosts occurred, again through an extreme genetic bottleneck, both lineages now shared the same hosts, but there was insufficient genetic similarity for
fertilisation, meiotic pairing and recombination to occur. However, as the two new species shared almost all features of biology and life history, they together flourished in settings where conditions were favourable and appropriate vectors abundant, and both perished where conditions were harsh. This provides a plausible scenario to explain the contemporary observation that P. o. curtisi and P. o. wallikeri are now always found co-circulating in the same host and vector populations. Considering these observations, and the irrefutable evidence assembled since 2010 that the ovale parasites represent two distinct sibling species, it is clear that the trinomial nomenclature currently in use is not fit for purpose. Some of the arguments around this can be found in Box 2 of reference 26; to resolve this situation, the current authors and collaborators have developed a proposed solution in which two new binomials are utilized in place of the current nomenclature (manuscript in preparation). In the meantime, correspondence on this topic is most welcome.

As to the evolutionary origins of the ovale parasites, despite twentieth century phylogenetic analyses in general favouring kinship with P. vivax [1, 221], genomic sequencing and elucidation of nuclear protein-coding, ribosomal RNA-coding, and mitochondrial genes have more recently placed these species distant from the vivax clade, which includes P. cynomolgi, P. knowlesi and other SE Asian parasites of simian hosts. Rather a position closer to P. malariae [159], Lemuroidea [222], or perhaps the rodent parasite clade [23], have also been put forward. As more genomic information becomes available for P. o. curtisi and P. o. wallikeri the kinship of these species, and therefore identification of their closest contemporary relatives, should become clearer.

Concluding remarks
Multi-population genomic studies of the neglected malaria parasites considered here are essential to provide insights into the biology underlying mechanisms of infection, disease progression and adaptation to different hosts. Many questions, for these and other Plasmodium species, remain answered, including the ability of some species to form dormant stages in the liver (hypnozoites) as observed for P. vivax and P. ovale species, and suggested as also possible for P. malariae [26], and the regulation of the blood stage cycles that can differ among species (e.g., P. malariae has a quartan cycle, a quotidian cycle is observed for P. knowlesi, while the other primate species all follow a tertian cycle).

Although genomics studies of these parasites have been difficult, the development of new assays such as SWGA allow the whole genome sequencing of parasite DNA from clinical samples [21], and have therefore opened up new opportunities to understand genomic diversity. Sequencing developments, such as real-time selective sequencing using Nanopore technology, will favour the selection of parasite DNA molecules for sequencing while excluding human molecules [223]. Phenotypic studies of important characters such as drug susceptibility are challenging for these species [224], but the recently developed strategy of “orthologue exchange” now permits detailed in vitro studies of gene function for every species, using transgenic lines with P. falciparum or P. knowlesi as the recipient parasite cell. These and future advances can support the large-scale and cost-effective genomic studies of neglected malaria that are now needed. The resulting gains in knowledge will greatly assist the design of species-specific diagnostics, treatments, and surveillance tools, thereby supporting malaria elimination goals.

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Declarations
Competing interests
The authors declare they have no competing interests.

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