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## Opinion

The two parasite species formerly known as  
*Plasmodium ovale*Georges Snounou <sup>1,\*</sup> Paul M. Sharp <sup>2</sup> and Richard Culleton <sup>3,\*</sup>

*Plasmodium ovale* was the last of the exclusively human malaria parasites to be described, in 1922, and has remained the least well studied. Beginning in 1995, two divergent forms of the parasite, later termed ‘classic’ and ‘variant’, were described. By 2010, it was realised that these forms are two closely related, but genetically distinct and non-recombining species; they were given the names *Plasmodium ovale curtisi* and *Plasmodium ovale wallikeri*. Since then, substantial additional data have confirmed that the two parasites are indeed separate species, but the trinomial nomenclature has often led to confusion about their status, with many authors describing them as subspecies. We hereby formally name them *Plasmodium ovalecurtisi* and *Plasmodium ovalewallikeri*.

### A *Plasmodium ovale* primer

Among the *Plasmodium* parasites that infect humans, *P. ovale* has proven to be a paragon of discretion. First, it is morphologically highly similar to, and sometimes mistaken for, *Plasmodium vivax*. Second, it causes a relatively mild, low-grade, and often self-resolving infection. Third, it is frequently present as the minor partner in mixed species infections, often going undetected. Consequently, the identification of three *Plasmodium* species infecting humans – namely *Plasmodium vivax* (Grassi and Feletti 1890), *Plasmodium malariae* (Grassi and Feletti 1892), and *Plasmodium falciparum* (Welch 1897) – preceded that of *P. ovale* (Stephens 1922) by more than two decades, during which time it remained unnoticed by some of the most experienced malariologists. In 1900 Charles Craig, a volunteer surgeon with the US army, noted and accurately described a peculiar type of **tertian parasite** (see [Glossary](#)) in six soldiers returning from the Philippines, but assumed it to be a variant of *P. vivax* [1]. Similarly, tertian parasites with peculiar morphological characteristics consistent with *P. ovale* were observed in Ghana from 1913 to 1916 [2].

The honour of naming *P. ovale* fell to Stephens who spotted parasites with ‘peculiar forms’ in a soldier returning from West Africa whom he had treated for malaria at the Liverpool School of Tropical Medicine in 1918 [3]. Given that the broad similarities to *P. vivax* exceeded the rather subtle morphological differences that many thought as mere variations, acceptance of this species was less than enthusiastic. Definitive proof of a separate species status was provided by Yorke and Owen [4] and then James *et al.* [5,6] through detailed observations of the first erythrocyte- and **sporozoite**-induced ovale malaria infections in neurosyphilitic patients. Craig confirmed that the parasite he had first seen in 1900 was identical to that described by Stephens [7]. Armed with this knowledge, malariologists increasingly reported ovale cases from various endemic regions, though to date, none from the Americas, an unexpected and as yet unexplained geographical restriction. Nonetheless, *P. ovale* has been reported mainly in a *P. falciparum*-dominated sub-Saharan Africa as a

### Highlights

We provide a historical review of the discovery and characterisation of *Plasmodium ovale*, and the recent discovery that their population consists of two genetically distinct types.

Current evidence indicates that *Plasmodium ovale curtisi* and *Plasmodium ovale wallikeri* are two separate species; thus, the use of trinomial names is leading to confusion in the field. We rename these as *Plasmodium ovalecurtisi* and *Plasmodium ovalewallikeri*.

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third minor species (the two others being *P. vivax* and *P. malariae*). Indeed, following the discovery in 1976 of **Duffy negativity**, a trait highly prevalent across sub-Saharan Africa, as a resistance factor for *P. vivax* [8], any parasites with **Schüffner's dots** observed in that region have been recorded as *P. ovale*. However, recent molecular evidence concerning the common occurrence of *P. vivax* in sub-Saharan Africa render Schüffner's dots an invalid pathognomonic trait for *P. ovale* in this region [9]. The advent of sensitive molecular techniques [10] for the specific detection of parasites has since shown that the prevalence of *P. ovale* in Africa is not insignificant and that it is not very rare in the remaining endemic regions of the world, except the Americas [11].

However, very little is known about the epidemiology or the biology of *P. ovale* [12], making it arguably the least investigated of the parasites of humans (with *P. malariae* a close second). The sum of our knowledge of its natural course of infection, pathology, transmission, or immunity, derives almost exclusively from observations made on infections induced for **malaria therapy** of **neurosyphilis** or in volunteers, and from rare case reports. The malaria therapy data were gathered mainly in England and in the USA. The observations in England were made with five strains originating from West Africa and one from the **Belgian Congo**, which were used consecutively between 1931 and 1945 to induce infections in several hundred neurosyphilitics [13]. Then, in the mid-1950s, a Liberian strain was used at the London School of Hygiene and Tropical Medicine to infect six volunteers (the authors of the article) to obtain the sporozoites needed to investigate the *P. ovale* pre-erythrocytic stages [14]. Around the same time, in the USA, 150 neurosyphilitics were inoculated with the Donaldson strain of *P. ovale*, derived from a soldier who acquired it in the Philippines or Admiralty Islands, and 27 were inoculated with the London School's Liberian strain; a summary of these infections was presented retrospectively by Collins and Jeffery [15]. Finally, in the mid-1960s, a West African strain derived from a **relapse** suffered by William Chin was characterised through experimental infections of a handful of volunteers [16,17].

The above observations yielded the following picture. The *P. ovale* strains used could be transmitted by mosquito species from Africa, Southeast Asia, and North America. The **extrinsic period** is about 14–15 days. The **pre-patent period** following sporozoite inoculation is variable, with a mean close to 15 days. In naive individuals, most of the infections resolve spontaneously within a couple of weeks following clinical onset, though low-level parasitaemia may persist for a few more weeks, or in some cases, months. The clinical symptoms are milder than those caused by the other *Plasmodium* species of humans, and fevers above 40°C are rare. These characteristics made *P. ovale* rather unsuitable for malaria therapy, for which a high number of paroxysms with high fever were required to yield a better outcome for neurosyphilis. Relapses following clearance of a primary episode are uncommon, though they clearly occur in some patients or volunteers. It is unclear whether delayed primary episodes occur, as is observed with some *P. vivax* strains. Significant clinical and parasitological immunity is acquired against both homologous and heterologous strains following a single infection, a faster and more potent response than that observed for the other three species of human parasites. Furthermore, this significant immunity persists for many months, which probably explains the preponderance of *P. ovale* infections in children in endemic areas. Moreover, it might account for the paucity of relapses, which might remain asymptomatic and with low-level parasitaemias, thus escaping detection.

### Two distinct *P. ovale* types

The first hints that there was bivalent genotypic diversity within *P. ovale* came in 1995, when it was discovered that two isolates of *P. ovale*, one from the London School of Tropical Medicine and Hygiene (LS strain), and one from the Centres for Disease Control in Atlanta (Nigerian I/CDC strain),

### Glossary

**Belgian Congo:** former name of the Democratic Republic of the Congo from 1908 until 1960.

**Cryptic species:** morphologically indistinguishable species.

**Duffy negativity:** a condition of humans in which the Duffy Antigen Receptor for Cytokines (DARC) is absent from the surface of red blood cells, rendering the carrier resistant/less susceptible to blood-stage infection by *Plasmodium vivax*.

**Extrinsic period:** the time taken for the development of malaria parasites in mosquitoes, from ingestion of gametocytes to the presence of infectious sporozoites in salivary glands.

**Laverania:** a subgenus within the genus *Plasmodium*; it includes *P. falciparum* and related parasites of African great apes.

**Malaria therapy:** the treatment of neurosyphilis and other neurological disorders by induction of fever through controlled inoculation of malaria parasites and their subsequent clearance by drug treatment.

**Neurosyphilis:** a complication of syphilis (a sexually transmitted disease caused by *Treponema pallidum*) affecting the central nervous system.

**Ookinete:** motile mosquito midgut epithelium invasive forms of the malaria parasite that result from the fusion of male and female gametes.

**Pre-patent period:** the time taken for malaria parasites to become visible in thick blood smears following inoculation of sporozoites.

**Relapse:** infection of red blood cells by parasites originating from hypnozoites (the latent liver stage of some malaria parasites), occurring after the primary infection episode.

**Schüffner's dots:** stippling of infected red blood cell characteristic of *Plasmodium vivax* and *Plasmodium ovale*. Named after Wilhelm Paul Schüffner (1867–1949), who described them in 1899.

**Sporozoite:** the form of the malaria parasite inoculated into vertebrates via the bite of infected mosquitoes.

**Tertian (malaria) parasites:** those malaria parasite species with a periodicity of 48 h.

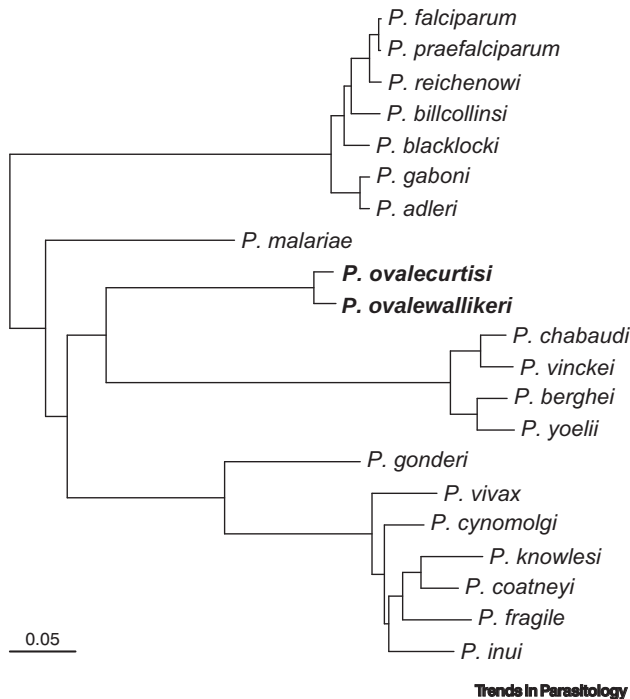
differed in their type A small subunit (SSU) rRNA gene sequences to a degree similar to that observed between pairs of species of bird or primate malaria parasites [18]. Similarly, two forms of type A SSU rRNA were found among *P. ovale* parasites collected from Vietnam [19]. Later it was found that *P. ovale* isolates with the two different rRNA sequences also differed in their **ookinete** surface proteins [20]. Further sequencing of other loci in 2004 led Win *et al.* to propose the existence of two types of *P. ovale* that they named 'classic' and 'variant', congruent with the Nigerian I/CDC strain, and the LS strain, respectively [21]. Both types were found in the same regions, in the same populations, and at the same times [19,22–24].

In 2010, Sutherland *et al.* presented the sequences of six independent loci for 55 *P. ovale* isolates from Africa and Asia. They found two distinct types at each locus, with no evidence of recombination, and so concluded that the 'classic' and 'variant' types were in fact two separate species [25]; they suggested the names *Plasmodium ovale curtisi* for the 'classic' type and *Plasmodium ovale wallikeri* for the 'variant' type, honouring David Walliker and Christopher Curtis in recognition of their significant contributions to the field of malaria. Subsequent comparisons of the genome sequences of these two *P. ovale* types have confirmed their genetic distinctiveness [26,27]. Of particular note, there are large differences between their *Plasmodium interspersed repeat (pir)* gene repertoires, as well as in other antigenic gene families such as the *surface-associated interspersed (surfin)* genes, supporting the genetic isolation of the two species [26].

The benchmark for interspecies divergence in *Plasmodium* has been the comparison between the human parasite *P. falciparum* and a close relative that infects chimpanzees, *Plasmodium reichenowi*. These two species from the subgenus **Laverania** are, like the two *P. ovale* species, morphologically indistinguishable, but clearly separate species. Initial comparisons, based on short mitochondrial DNA sequences, found that the extent of divergence between *P. ovale curtisi* and *P. ovale wallikeri* was about 43% of that between *P. falciparum* and *P. reichenowi* [25]. However, more recent analyses using data from nuclear genome sequences indicate that the *P. ovale* pair is much more divergent: comparisons of 186 protein sequences indicated the *P. ovale* pair to be nearly twice as divergent as the two *Laverania* species [28] (Figure 1).

Few population genetic studies have been conducted on the *P. ovale* species. However, a recent report analysing genes associated with drug resistance in 147 parasite isolates imported to China from Africa demonstrates dissimilarity between the two species in the mutations accumulated in the *dihydrofolate reductase (dhfr)* gene in response to antifolate drug pressure [29]. Not only were different *dhfr* mutant haplotypes observed in the two species, but the prevalence of mutant alleles also differed between them. This finding supports a lack of genetic exchange between the species and also suggests phenotypic and/or genotypic differences in their responses to drug selection pressure.

As well as being morphologically indistinguishable, the two *P. ovale* species also appear to be very similar in their phenotypes and clinical pathology. We entertained the hope that careful perusal of the few reports in the older literature might provide some robust feature(s) that would coincide with the genetic analyses above. However, the relative paucity and variable nature of these observations were of meagre help. Unique side-by-side comparisons of two strains, Donaldson and Liberian, from geographically distant origins (West Pacific and West Africa, respectively), failed to yield any discriminatory features, maybe because they were from the same species. A single tantalising potentially significant distinguishing feature concerns the sporozoite size, which was measured as 9 µm for the Belgian Congo strain [6] as compared to 13 µm for the Liberian strain [14], in both cases using the same mosquito species, *Anopheles*



**Figure 1. Evolutionary relationships among mammalian *Plasmodium* species.** Horizontal branch lengths are drawn to scale, indicating that *Plasmodium ovalecurtisi* and *Plasmodium ovalewallikeri* are more divergent than, for example, *Plasmodium gaboni* (a chimpanzee parasite) and *Plasmodium adleri* (a gorilla parasite), or *Plasmodium falciparum*/*Plasmodium praefalciparum* (from humans and gorillas, respectively), and *Plasmodium reichenowi* (from chimpanzees). The phylogeny was derived from analyses of more than 2700 proteins encoded by the nuclear genome. The scale bar indicates 0.05 amino acid replacements per site. Adapted from [50].

*maculipennis*. It is hoped that this may be confirmed as a distinguishing morphological trait by future comparative observations of mosquitoes infected with one or other *P. ovale* species.

There is some recent, though tentative, evidence to suggest that the latency period of the two species may differ, with several reports documenting a significantly longer latency period for *P. ovale curtisi* than *P. ovale wallikeri* [30–34], although others have found no differences [35]. A meta-analysis considering these and other reports suggested agreement with the former of these two possibilities [36]. Studies on *P. ovale* spp. infections imported into Spain suggested that there was an increased number of parasites per  $\mu\text{l}$  of blood in *P. ovale curtisi* infections compared to those of *P. ovale wallikeri*, and that this was associated with higher leucocyte and platelet counts in patients infected with the former species [30,31]. However, numerous other reports do not support this particular finding [37–42]. In terms of prevalence and distribution, the two species do not differ greatly, but it appears that *P. ovale curtisi* may be the more common of the two [36]. To date, nothing is known regarding potential differences between the species relating to their development in mosquitoes.

### Nomenclature

The names *P. ovale curtisi* and *P. ovale wallikeri* have been widely used by the malaria research community, despite the taxonomic discrepancy of trinomials, normally used for subspecies, being used for full species. Understandably, this has led to much confusion regarding the status of these parasites, with numerous reports referring to them as ‘subspecies’. We have compiled a list of more than 70 peer-reviewed papers published since 2010, in which the two species are referred to as subspecies (see Box S1 in the supplemental information online), as well as additional publications that include the correct species designation at one point but also refer to the two as subspecies elsewhere in the paper (see Box S2 in the supplemental information online).

The standard taxonomic practice after the realisation that a species in fact comprises two **cryptic species** would be for one species to retain the original name, and for the newly described species to be given a new designation. However, in the case of *P. ovale*, it is impossible to determine which of the two species was originally designated as such by Stephens in 1922 [3], rendering this approach problematic. Given this and the widespread adoption of the trinomial designations of *P. ovale curtisi* and *P. ovale wallikeri*, we hereby rename them '*Plasmodium ovalecurtisi* (Snounou *et al.* 2024)' and '*Plasmodium ovalewallikeri* (Snounou *et al.* 2024)', respectively, thus rendering their nomenclature binomial and consistent with their species status. The designation *P. ovale* (Stephens 1922) remains valid and the term '*Plasmodium ovale* complex' could be used for diagnostic purposes in situations where molecular analyses that distinguish between the two species have not been carried out. We propose that this solution provides the least disruptive manner for correcting the current taxonomic inconsistency, providing overt continuity with the names that have been used for the past 13 years.

### Concluding remarks: evolutionary speculations

It is intriguing that humans are infected by two distinct, yet closely related, parasite species that share similar geographic ranges and yet are not known to have different niches (see [Outstanding questions](#)). The other malaria parasites of humans, *P. falciparum*, *P. vivax*, and *P. malariae*, all have very close relatives infecting African apes in the wild [28,43]. In each case, the ape parasite species exhibits a much higher (by about an order of magnitude) level of genetic diversity. Ape samples have also yielded sequences closely related to the two *P. ovale* species, although as yet these are few in number [44–48].

Two possible alternative scenarios involving cross-species transmissions from apes have been suggested to explain the origin of the two related but divergent *P. ovale* species in humans. In the first, the two parasites in humans reflect successive transmissions from apes at time points sufficiently far apart that the species resulting from the first had diverged (in humans) to the extent that it could no longer recombine with the ape parasite by the time of the second transmission [49]. In the second, the divergence between the ancestors of *P. ovalecurtisi* and *P. ovalewallikeri* occurred because they infected different ape hosts, namely chimpanzees and bonobos on the one hand and gorillas on the other, before being independently transmitted to humans [43]. Under the first scenario, one of the human parasite species might be expected to have a much higher genetic diversity than the other, reflecting its longer history in the new host. It may be that *P. ovalewallikeri* has somewhat higher nucleotide diversity than *P. ovalecurtisi* [27], but neither of these species exhibits substantially more diversity than the other human malaria parasites. In fact, genetic diversity levels seem to be broadly similar across *P. falciparum*, *P. malariae*, *P. ovalecurtisi*, and *P. ovalewallikeri* [27,28,43]. It has been suggested that *P. falciparum* and *P. malariae* have similar levels of diversity because they started to diversify in humans at about the same time and that the common factor was that the origin of agriculture allowed stable and persistent transmission of these parasites among humans [28]. It may be that a similar explanation applies to the timescale of the origins of *P. ovalecurtisi* and *P. ovalewallikeri*. As yet, the actual evolutionary and epidemiological scenario under which these two species arose remains to be established, and elucidation may require more detail about the patterns of genetic variation among the human parasites, as well as more information about the related parasites in apes.

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### Declaration of interests

The authors declare no competing interests.

### Outstanding questions

What were the mechanisms involved in the initial speciation of *P. ovalewallikeri* and *P. ovalecurtisi*?

How can the two sympatric species coexist yet both survive? Do they occupy different niches?

Is hybridisation possible through laboratory manipulation?

What phenotypic/behavioural/pathological differences exist between the two?

Are both species equally infectious to the same mosquito vectors?

Are there morphological/phenotypic differences between the mosquito stages of the two species?

Do the relative prevalences of the two species differ geographically?

## Supplemental information

Supplemental information associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.pt.2023.11.004>.

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Supplementary file

### **The two parasite species formally known as *Plasmodium ovale***

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**Box S2. Papers referring to the two *Plasmodium ovale* species (but later to subspecies), or in doubt.**

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