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Plasmodium falciparum is not as lonely as previously considered

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Until very recently, only one species (*P. reichenowi*) was known to be a phylogenetic sister lineage of *P. falciparum*, the main malignant agent of human malaria. In 2009 and 2010, new studies have revealed the existence of several new phylogenetic species related to this deadly parasite and infecting chimpanzees and gorillas in Africa. These discoveries invite us to explore a whole set of new questions, which we briefly do in this article.

Key words: Plasmodium, Laverania, Great apes, diversity, origin, speciation, virulence

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The Plasmodium species infecting humans and non-human primates cluster into two distinct phylogenetic lineages (**Fig. 1**). One of these lineages (in yellow in **Fig. 1**) included, before 2009, almost all the known diversity of Plasmodium species infecting primates (more than 25 species[1]), with species infecting monkeys in Asia like *Plasmodium knowlesi*, in Africa like *P. gonderi* and in South America like *P. simium*, but also some species infecting humans like *P. ovale*, *P. malariae* and *P. vivax*. The other lineage (in red in **Fig. 1**), a deep evolutionary clade of its own, known as the Laverania subgenus,[1,2] included only two known species: *P. falciparum,* which infects humans and is responsible for the most acute and deadly form of human malaria and *P. reichenowi*, a sister species discovered in chimpanzees and gorillas at the beginning of the twentieth century[1] and for which only one chimpanzee-derived isolate was available for molecular analyses until 2009.[3] Other species of Plasmodium were described in great apes during the twentieth century (i.e., *P. rodhaini* and *P. schwetzi*) but they were finally considered to be respectively *P. malariae* and *P. vivax*[1] and were never subjected to molecular characterization.

The identification of Plasmodium species circulating in great apes in Africa was primarily done during the first half of the twentieth century, on the basis of morphological features.[1] This approach has several limitations.[4] First, phenotypic plasticity can lead to incorrect identifications. Second, morphological keys are often effective only for a particular life stage which cannot always be observed or is difficult to be. Finally, and perhaps most important, this approach overlooks morphologically cryptic taxa. These limitations, together with the difficulty to collect and manipulate great apes, were certainly, at least in part, responsible for the low known diversity of Plasmodium circulating in great apes in Africa.

The years 2009 and 2010 have witnessed a big bang in our knowledge of this diversity.[5-11] The use of molecular tools for species identification, combined with the use of non-invasive methods[9,11] to explore the diversity of Plasmodium species present in great apes, have revealed five new phylogenetic species in less than a year. **Figure 2** displays this diversity and the phylogenetic relationships between species. As shown, the Laverania lineage is now divided into two groups. Group A includes three different phylogenetic species: *P. GorA* infecting gorillas, *P. gaboni* and *P. billbrayi* infecting chimpanzees. Group B includes *P. reichenowi* and *P. falciparum*, as well as two new phylogenetic species: *P. GorB* that infects gorillas and which is located at the root of the entire group and *P. billcollinsi* from chimpanzees, which shares a common ancestor with *P. reichenowi* and *P. falciparum*.

It was also shown—in some cases, simply confirmed—that great apes could also

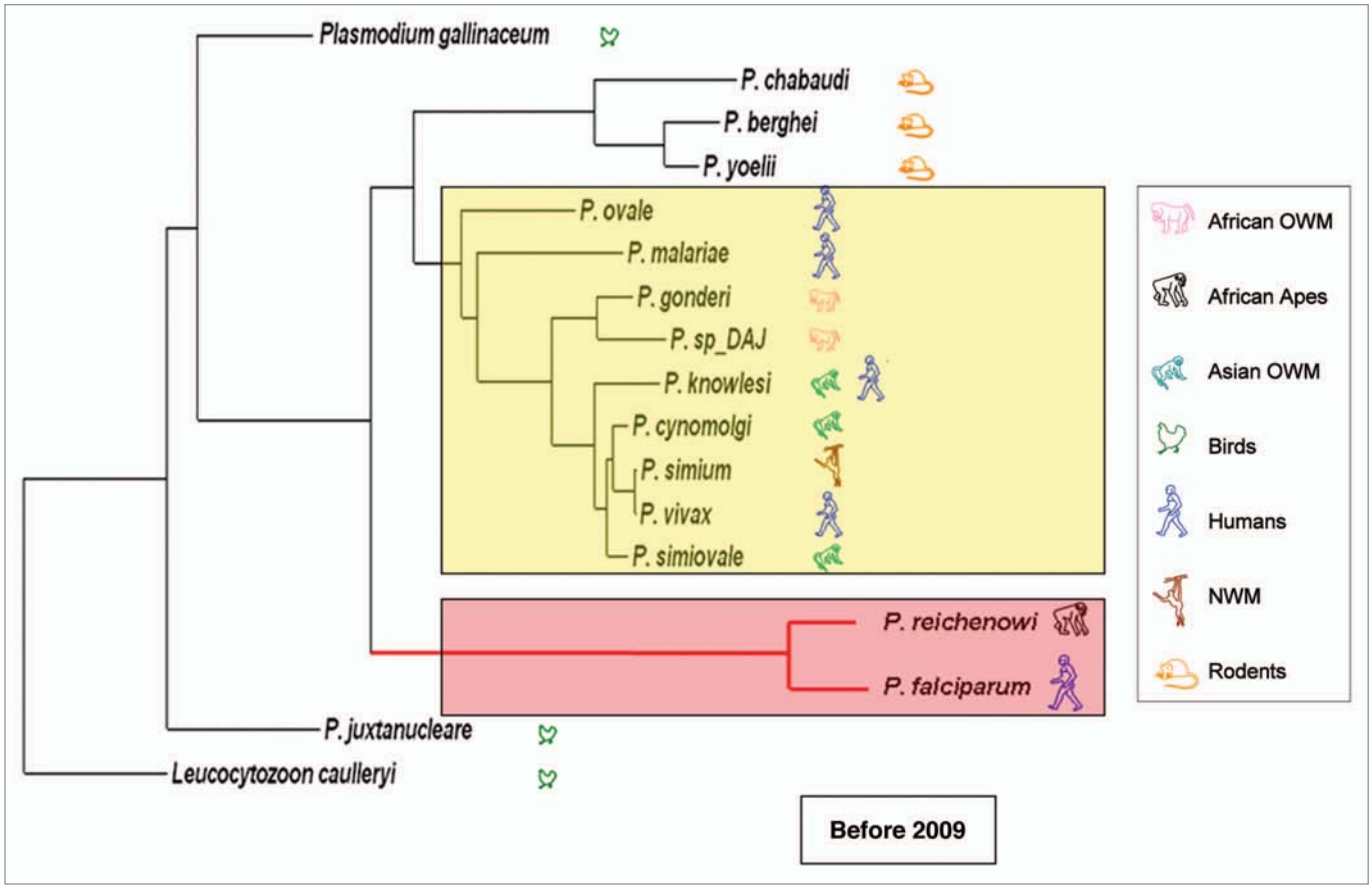

Figure 1. Simplified representation of the phylogeny of Plasmodium as it was known before 2009. The two boxes highlight the two main phylogenetic lineages of primate Plasmodium. Notice that not all known species of Plasmodium from primates are included in the lineage highlighted in yellow. OWM, Old World Monkeys; NWM, New World Monkeys.

host species such as *P. malariae*, *P. vivax*, *P. ovale* and *P. falciparum*.[5-9,11] This was not a complete surprise for *P. malariae* and *P. vivax*, since morphologically similar species had been previously reported in chimpanzees and gorillas.[1] This discovery was more surprising for *P. ovale* and even more for *P. falciparum*, which were, for a long time, considered as strictly human specific. For chimpanzees and bonobos, *P. falciparum* strains were discovered only in captive animals[5,8] while in gorillas, they were discovered in both captive and wild animals.[9,11] This latter observation, together with the fact that the diversity of gorilla *P. falciparum* is higher than the one observed in the human ones, has led Liu and colleagues[9] to propose that human *P. falciparum* may have originated from a transfer from gorillas.

The last two years have thus witnessed a turn in our knowledge of the most devastating human infectious disease in the world: (i) *P. falciparum* is not as unique or distinctive species as previously considered, but actually belongs to a diverse lineage of Plasmodium species from apes, the Laverania clade; (ii) the great apes are natural hosts to a large diversity of Plasmodium species, including some previously considered as human specific; (iii) *P. falciparum* originated in great apes, most likely in gorillas.

These recent discoveries invite us to explore new questions. First, have we now discovered the entire diversity of Plasmodium circulating in great apes or can we expect to discover more?

Ecologists know well that species richness estimates are strongly influenced by sampling size.[12] Estimates of community species richness are expected to be positively correlated with sampling size up to a plateau, beyond which additional sampling does not increase the observed richness.[12] **Figure 3A and B** represents the relationship between sampling size (the cumulative number of host individuals analyzed over all studies) and the number of Plasmodium species found to infect chimpanzees and gorillas, respectively. This graph manifests several important observations. First, over all studies, we notice that gorillas have received far less attention than chimpanzees—the total number of individuals sampled in gorillas is less than half than in chimpanzees. Second, in chimpanzees, the accumulation curve (**Fig. 3A**) approaches an asymptote as sampling increases, which suggests that the total diversity of Plasmodium species circulating in this host species may have now been discovered. For gorillas, however, it is not possible to conclude as the number of studies available is still too low to determine the asymptote of the accumulation curve (**Fig. 3B**). Nevertheless, it seems likely that the diversity of Plasmodium species

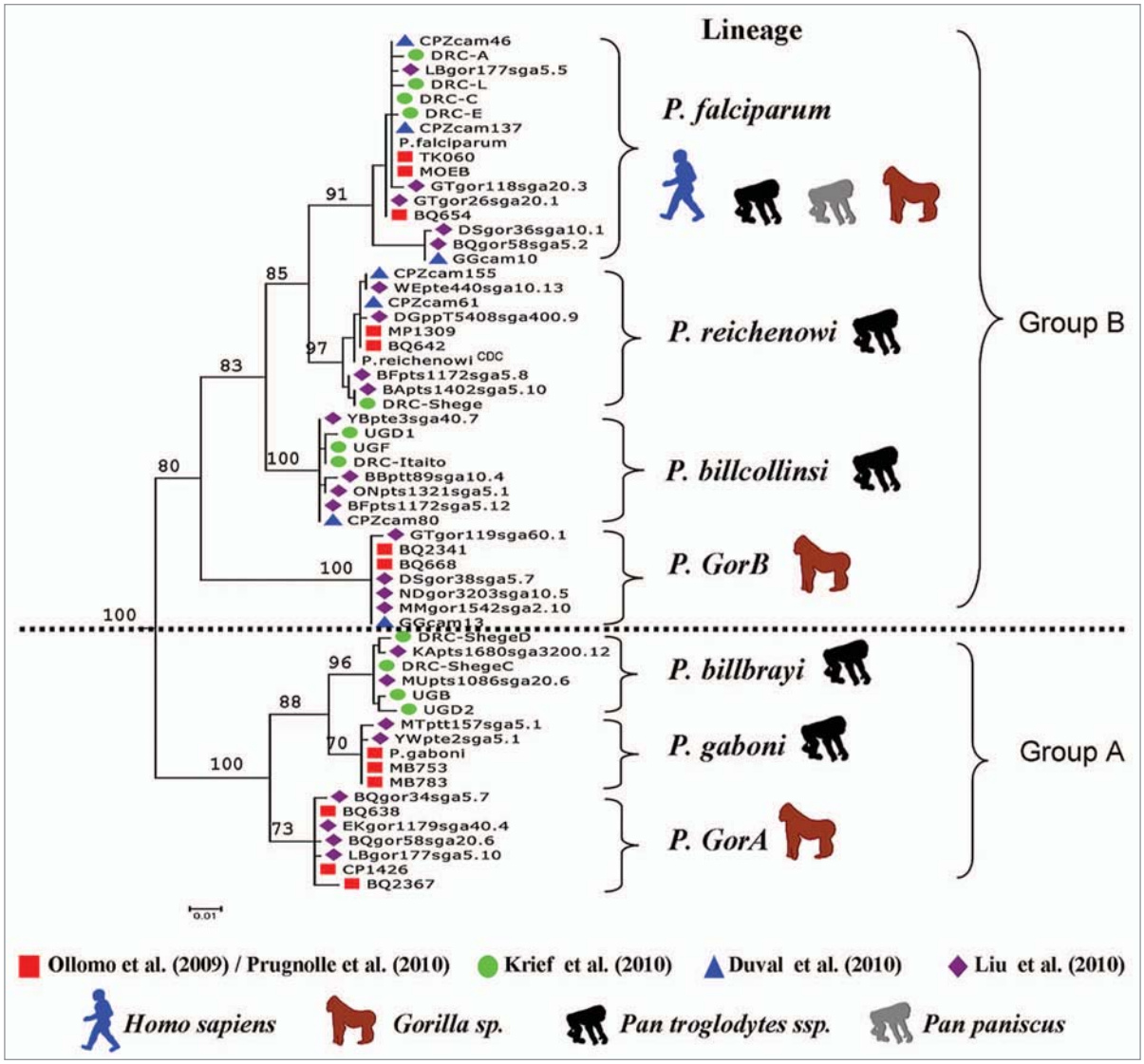

Figure 2. Phylogeny of the Laverania subgenus of Plasmodium based on partial Cytochrome b sequences (i.e., 593 nucleotides) and including strains isolated and characterized in Ollomo et al.,[10] Prugnolle et al.,[11] Duval et al.,[5] Krief et al.[8] and Liu et al.[9] The phylogeny was obtained using maximum likelihood methods, as detailed in Prugnolle et al.[11] Robustness was tested by means of 100 bootstraps.

that infect gorillas is lower than the diversity of Plasmodium species that infect chimpanzees.

What events in the history of the Laverania clade were responsible for the diversification of Plasmodium in the great apes?

Given that Plasmodium species are strictly dependent on their hosts, their diversification through evolutionary times can only occur through two main processes:[13] (i) subdivision of the parasite populations into discrete populations due to an isolation of their host populations (the cospeciation/codivergence scenario) or (ii) colonization of a new host (the host-switch scenario). Based on the phylogeny presented in **Figure 2** and the known phylogeny of the hominids (see **Fig. 4**), one possible scenario could be that lineages A and B (**Fig. 2**) were present in the African hominid ancestor, and then diverged along with their vertebrate hosts (**Fig. 4**). This hypothesis implies that: (1) Plasmodium *GorA* diverged from *Plasmodium gaboni/billbrayi* at the same time that P. GorB diverged from *P. billcollinsi/P. reichenowi/P. falciparum*; (2) This time of divergence should be congruent with the time of divergence of the gorilla lineage from the chimpanzee/human lineage, around 9 Myears.[14] If the cospeciation/codivergence hypothesis is extended to the divergence between *P. reichenowi* and *P. falciparum*, (3) these two species should have diverged congruently with the divergence between chimpanzees and humans, between 4 and 7 Myears.[14] The cospeciation/codivergence hypothesis cannot account (4) for the divergence (a) between *P. billcollinsi* and *P. reichenowi* and (b) between *P. gaboni* and *P. billbrayi*, since these four species are all parasites of chimpanzees. Similarly, this hypothesis cannot account for the divergence (c) between *P. falciparum* found in gorillas and *P. falciparum* infecting humans.

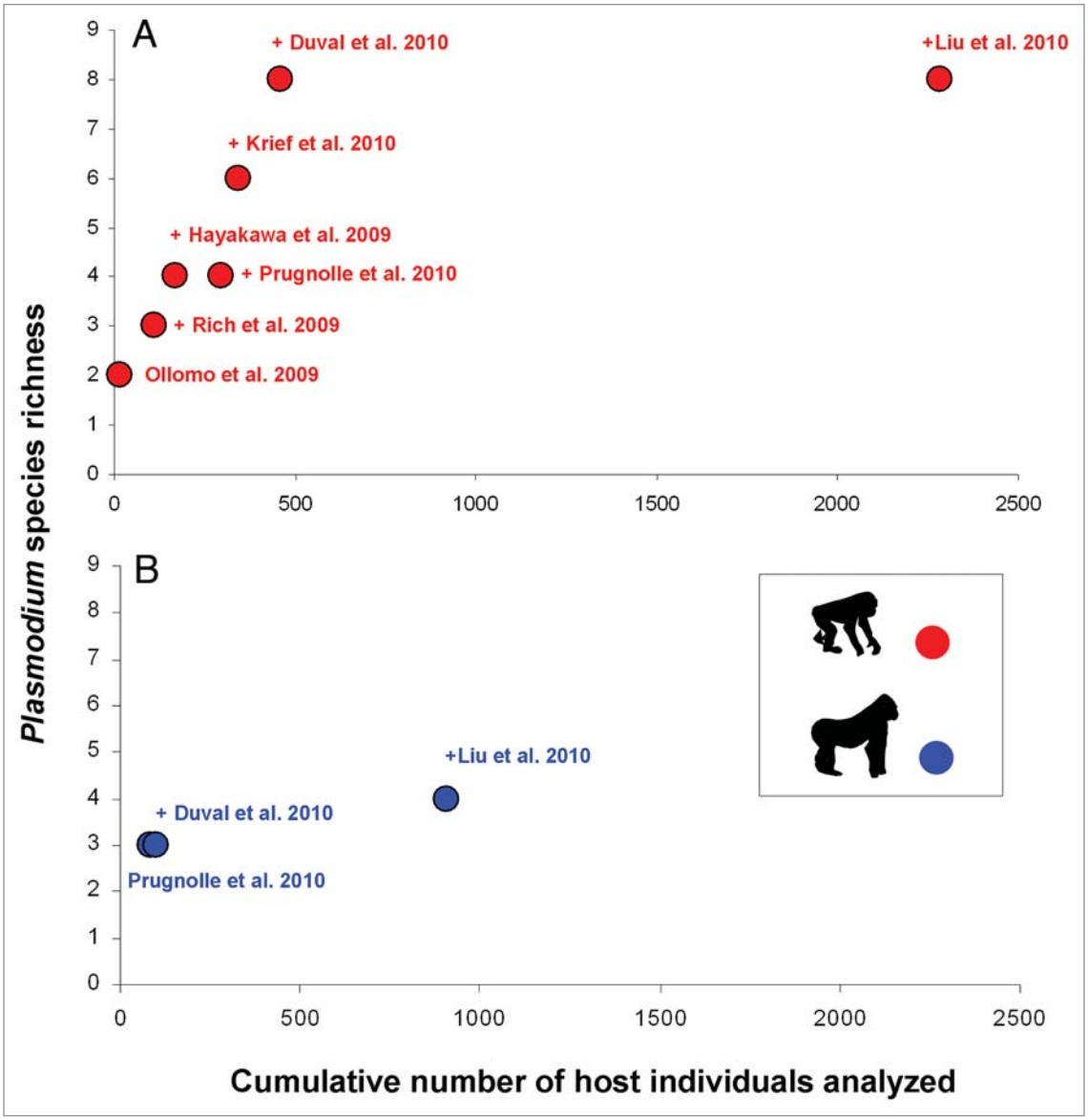

Figure 3. Relationship between Plasmodium species richness in great apes ((A) in chimpanzees, red circles; (B) in gorillas, blue circles) and the cumulative number of host individuals analyzed.

Concerning prediction (3), the hypothesis of a cospeciation between *P. falciparum/P. reichenowi* and humans/chimpanzees is still highly disputed. While certain authors have proposed that *P. reichenowi* is ancestral to *P. falciparum*[15] or that their divergence occurred later in the history (~2.5 MYears ago for Ricklefs and Outlaw in ref. 16), others have argued that *P. falciparum* and *P. reichenowi* indeed diverged at the same time as their hosts (reviewed in refs. 17 and 18). Concerning prediction (1), the data currently available, although limited, allows to evaluate it. Based on the phylogeny presented in **Figure 2**, prediction (1) is inconsistent with the genetic distances computed between the relevant parasite species. Thus the average genetic distance computed between P. GorB and *P. billcollinsi/P. reichenowi/P. falciparum* is 0.078 (standard error = 0.013; distances were estimated using program MEGA 4.0,[19]), about twice as large as the distance between P. GorA and *P. gaboni/P billbrayi* (d = 0.047 and standard error = 0.0089).

The large diversity of Plasmodium species observed in chimpanzees (i.e., four species) and in gorillas (i.e., two species) on the one hand, and their specificity for chimpanzees (i.e., *P. gaboni, P. billbrayi, P. billcollinsi* and *P. reichenowi*) and for gorillas (i.e., P. GorA and P. GorB, *P. falciparum*) on the other hand, call for information, now lacking, on mechanisms at the origin and evolution of these parasite species and their hosts. Paraphrasing the American physicist Robert Oppenheimer, "Let us not look at things as they are but as they could be." The patterns of Plasmodium diversity currently observed in great apes could thus be the result of several past events of speciation and extinction in both the host and parasite evolutionary lineages, or could involve

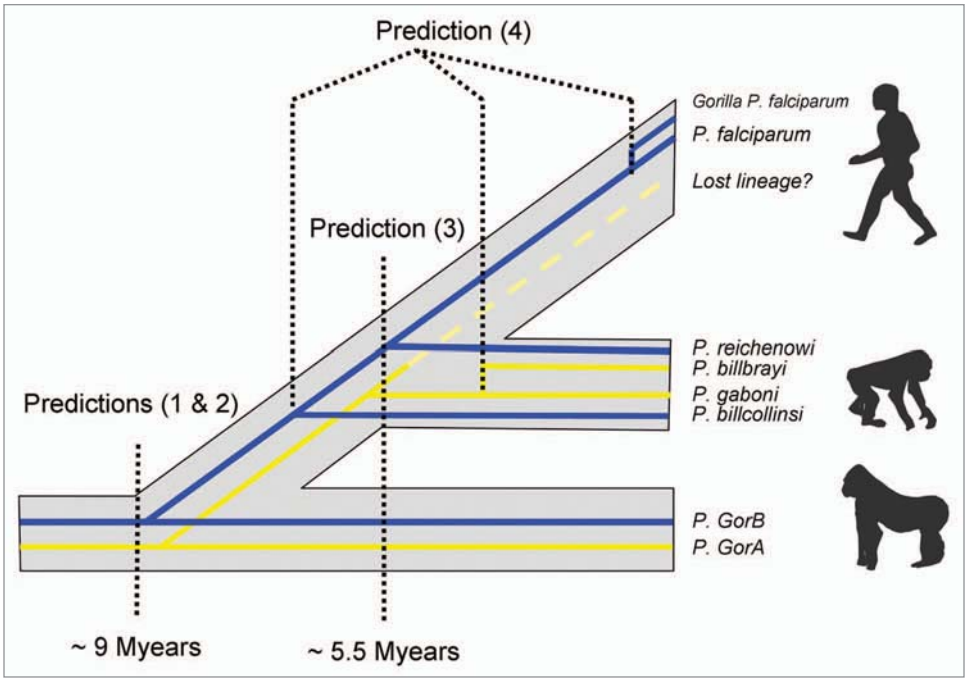

Figure 4. A putative cospeciation/codivergence scenario between Plasmodium and Hominids. For details about the four predictions, please refer to main text.

particular mutations as in the divergence between falciparum/human lineage versus reichenowi/chimpanzee lineage.[20,21] Much additional information will be needed, starting perhaps with reliable estimates of evolutionary rates within each lineage in order to date the events of divergence. Information about the vectors of these different species and on their evolutionary history would also be helpful.

Finally, one last question that deserves our attention is: how virulent are the other species of the Laverania subgenus compared to *P. falciparum*? Are they as deadly as *P. falciparum*?

P. falciparum is the most virulent species of Plasmodium infecting humans. It is responsible for more than one million deaths every year all over the world, especially in Africa. The origin of its extreme virulence has been debated for several decades. Some authors have attributed it to a recent host switch;[22] for others it is simply a direct consequence of the parasite's exploitation of the host during production of its transmission stages (reviewed in ref. 23). Ajit Varki and collaborators[20,21] have provided the best grounded account. *P. falciparum* expresses multiple binding proteins that recognize specific targets on the surface of erythrocytes (RBCs). The major targets are terminal sialic acids (Sias). A mutation, estimated to have occurred 2–3 Myears ago in human ancestors, eliminated biosynthesis of the common Sia n-glycolylneuraminic acid (Neu5Gc) causing the accumulation of its precursor N-acetylneuraminic acid (Neu5Ac). Chimpanzees and gorillas exhibit a mixture of Neu5Gc and Neu5Ac, while human RBCs exhibit abundant Neu5Ac. Martin et al.[20] demonstrated that the major merozoite RBC-binding protein EBA-175 of *P. reichenowi* preferentially binds to Neu5Gc, while that of *P. falciparum* binds to Neu5Ac. Rich et al.[15] have suggested that the final EBA-175 mutations responsible for the malignancy of *P. falciparum* may have occurred relatively recently (reviewed in ref. 21). In a series of in vitro experiments, Martin et al.[20] demonstrated that the effect of Pf EBA-175 is much more severe on human RBCs than on chimpanzee RBCs, but also much more severe than the Pr EBA-175 is on chimp RBC, which would explain the observation that human falciparum malaria is much more severe than chimpanzee malaria. This would also account for the observation by Krief et al.[8] that infection with *P. falciparum* did not seem to be noticeably harmful to bonobos.

Tarello[24] reported the death of a young chimpanzee (one-year-old) infected with *P. reichenowi*. The clinical outcomes observed before death were partially comparable to those produced by *P. falciparum* in human beings like anorexia, diarrhea and exhaustion, but a clear association between the presence of *P. reichenowi* and the death of the chimpanzee was not clearly made. Similarly, Dian Fossey reported, in her famous book *Gorillas in the Mist*,[25] the death of a young female mountain gorilla (Quince, 8 years, 3 months old) that was likely caused by malaria (see page 266 for a description of the post-mortem examination of the animal). The evidences regarding the infection by malaria were however only indirect. Again, it is difficult to make a direct link between the death of the gorilla and malaria in this case as well. More studies, and more systematic, are clearly needed if we are to obtain a fairly good picture of the virulence of the Laverania lineage species that infect great apes. The virulence of *P. falciparum* for chimpanzees and gorillas needs also to be investigated. In particular, do chimpanzees and gorillas transmit this parasite? Are they victims or reservoirs of this deadly human parasite? We have currently no answers to this question.

In conclusion, although the discovery of five new species in the subgenus Laverania represents a major advance towards the understanding of the evolution of *P. falciparum*, it also opens up numerous new questions, such as those we have briefly discussed in this article.

Acknowledgements

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