Evolutionary Relatedness of Some Primate Models of Plasmodium

Andrew P. Waters, * Desmond G. Higgins, † and Thomas F. McCutchan‡

*Laboratorium voor Parasitologie, Universiteit te Leiden; †European Molecular Biology Laboratory; and ‡Laboratory of Malaria Research, NIAID

Primate—and, specifically, monkey—malaria infections are commonly used for understanding the pathology of and immune response to the human disease because they are thought to resemble most closely the host-parasite relationship found in humans. *Plasmodium cynomolgi* is used extensively as a model for the human parasite, *P. vivax*, and *P. knowlesi* is used primarily as a model for the development of erythrocytic-stage vaccines. Both of these simian parasites can naturally infect man, resulting in mildly symptomatic episodes of the disease. The phylogenetic relationship between these two simian parasites and previously characterized *Plasmodium* species, including *P. vivax*, was examined by comparison of the asexually expressed small-subunit ribosomal RNA genes. Our analysis confirmed that *P. vivax* is most closely related to *P. cynomolgi* and that it remains an appropriate model of the human pathogen. Furthermore, with *P. knowlesi* and *P. fragile*, these two species form a group of closely related species, distant from other *Plasmodium* species. What is considered to be the most ancient of the human malaria pathogens, *P. malariae*, was also included in the analysis and does not group at all with other simian or human parasites.

Introduction

The assessment of relationships among pathogens has many ramifications for studying disease, including selection of appropriate animal models for investigating human infection. For example, malaria remains one of the chief causes of mortality and morbidity in the developing world. Animal models of malaria represent an essential component of attempts to understand the biology of the parasite and to develop vaccines against the disease. It is important to be able to assess the precise relevance of any chosen model system to the human condition. Relevance is a particularly important criterion for the choice of primate models of malaria, which is complicated by specific ethical and economic considerations. Morphological studies have been carried out in depth and have been combined with biological information, to estimate the phylogeny of the genus. The information of the time was synthesized into a working picture of the phylogeny of malaria parasites by Garnham (1966, pp. 60–84). He recognized four major evolutionary branches within the genus—namely, avian, simian, rodent, and saurian—and that distinctions could be made at the level of the subgenus.

We have also been able to provide estimates of the phylogeny of the genus, as a result of studies on the biology of the ribosomal RNA (rRNA) genes in *Plasmodium*.

1. Key words: 18S rRNA, malaria, *Plasmodium*, phylogeny.

Address for correspondence and reprints: Andrew P. Waters, Laboratorium voor Parasitologie, Universiteit te Leiden, Postbus 9605, 2300RC Leiden, The Netherlands.
and have suggested that the involvement of lateral transfer between subgenera complicates the traditional view of coevolution of host and parasite (Waters et al. 1991). The results of our analysis showed that *P. falciparum* was relatively more closely related to the avian parasites than to any other described species. Our interpretation of these data has been rightly questioned with regard to both direction and time of transfer (Ayala and Fitch 1992; Brooks and McLennan 1992), and in the present paper we address both of these objections. There appears to be further biogeographic evidence for other lateral transfers occurring within the genus. For instance, it has been suggested by Killick-Kendrick (1978, pp. 39–45) that the origin of murine infection by malaria may well have resulted through prolonged exposure to an extraordinary parasite.

It is against this background that we present a phylogenetic assessment of some primate malaria species naturally infective to macaques in Southeast Asia and superimpose this on previous analyses (Waters et al. 1991). *Plasmodium cynomolgi* has long been used as the species of choice for the study of a simian equivalent of *P. vivax*, and there is a large body of evidence supporting this choice [Coatney et al. (1971, pp. 1–98) present the evidence; also see Galinski et al. 1992]. However, given that apparent opportunistic transfer from host to host has occurred within the genus, a demonstration of the relationship between *P. vivax* and *P. cynomolgi* within the context of other simian malarias might be germane.

**Material and Methods**

*Plasmodium knowlesi* strain W1 was obtained from infection of rhesus monkeys, harvesting of parasites from infected blood, and drug cure of the animal. The material was a gift from Dr. G. H. Mitchell. *Plasmodium cynomolgi* (Vietnamese strain) was also obtained from infected rhesus blood, and these parasites were provided by D. Seeley, Jr.

DNA and RNA

DNA was prepared from infected material by phenol extraction (Wong-Staal et al. 1979). The small-subunit (SSU) rRNA genes were amplified by polymerase chain reactions (PCR) (Saiki et al. 1988) using phylogenetically conserved primers at the 5' and 3' ends of the structural sequence as described. Fragments were cloned into either PUC19 or pSPORI1 (Life Technologies, Gaithersburg, Md.). Sequencing was performed by using modified T7 polymerase (Sequenase; U.S. Biochemicals) in the manner of Sanger et al. (1977). Assignment of stage specificity was by hybridization of antisense oligonucleotides designed to bind to the distinct and specific regions of each SSU gene. Each oligonucleotide hybridized differentially to asexual RNA and sporozoite RNA from the appropriate species.

**Oligonucleotides**

The oligonucleotides used to amplify almost-full-length SSU genes from parasite DNA were 367R, 5' CGC GGC CGC TTC ACC TAC GGA AAC C 3' complementary to the 3' end of the SSU gene; and 368R, 5' CGC GGC CGC AAC CTG GTT GAT CTT GCC 3' complementary to the conserved 5' end of the SSU gene. Both oligonucleotides introduce a NotI site during amplification. The oligonucleotides used for the determination of stage-specific expression were 351R, 5' CAG TTA TGT GGA TTT ATA 3' (*P. knowlesi* “A” gene), and 354R, 5' ATG TGG ATT AAG CTA AAA 3' (*P. cynomolgi* “A” gene).
Data Analysis

Sequences were compiled by using the Staden Plus package (Amersham). Multiple alignments and trees were mainly generated by using the CLUSTALV program (Higgins et al. 1992). The SSU rRNA sequences of *P. cynomolgi* and *P. knowlesi* were automatically aligned with the asexually expressed sequences from *P. berghei* (Gunderson et al. 1986; GenBank/EMBL accession no. M14599), *P. falciparum* (McCutchan et al. 1988; M19172), *P. fragile* (Waters et al. 1991; M61722), *P. gallinaceum* (Waters et al. 1991; M61723), *P. vivax* (Waters and McCutchan 1989a; X13926), *P. lophurae* (Waters et al. 1989; X13706), and *P. malariae* (Goman et al. 1991; M54897). The multiple-alignment algorithm is that of Higgins and Sharp (1989). (The alignment is available by electronic mail from D.G.H.; to request it, please send a message to the address Higgins@EMBL-Heidelberg.DE.) Regions of the multiple alignment that were impossible to align unambiguously were removed, leaving 1,828 alignment positions for phylogenetic analysis. The fragments that were removed correspond to the following positions from the *P. berghei* sequence: 75-78, 133-134, 172-179, 191-204, 273-290, 499-501, 542-544, 656-721, 745-766, 817-827, 1109-1136, and 1704-1754. The two new sequences reported in the present article have been deposited in the GenBank/EMBL data bases, under the accession numbers LO7559 (*P. cynomolgi*) and LO7560 (*P. knowlesi*). Distances were calculated between every pair of sequences by using Kimura's (1980) two-parameter model to correct for multiple substitutions. For the distance calculations, any positions in the alignment where any sequence had a gap were removed, leaving 1,673 sites. Finally, phylogenetic trees were calculated by using the neighbor-joining method of Saitou and Nei (1987) and the DNAML and DNAMLK programs of the Phylip package (Felsenstein 1981). Confidence levels for each grouping in the tree were calculated by using a “bootstrap” procedure similar to that of Felsenstein (1985). The trees were produced by using the DRAWTREE program of the Phylip package (Felsenstein 1989).

Results

Sequence of the Asexually Expressed SSU rRNA Genes of *Plasmodium cynomolgi* and *P. knowlesi*

The amplified genes were cloned and sequenced by using both plasmid primers and a battery of 20 primers that map to phylogenetically conserved regions of the gene. All clones were sequenced on both strands. The PCR method truncates the genes at the 3' end, as a result of sequence ambiguity in the genes of *P. berghei* in that region (Gunderson et al. 1987). The 3' oligo is therefore designed to avoid that possible difficulty. Therefore, only 18 bp of each gene has not been cloned, and the expected length of each gene would be 2,065 bp for *P. cynomolgi* and 2,110 bp for *P. knowlesi*. The missing data are insignificant for the statistical assessment of phylogeny. Corrected distances between all nine species of *Plasmodium* are given in table 1. The genes appear typical for the genus, demonstrating the expected profile of complexity within the expansion regions of the molecule. The data contained within these aligned sequences can also serve as a basis for the construction of species-specific oligonucleotides, which in turn can be used for the direct detection of infection, via hybridization to the rRNA of the organism (Waters and McCutchan 1989b). Expansion of the data base for these purposes is also useful because that will allow the ready discrimination between possible zoonoses, transmission of infections to man from an animal reservoir, and infection by an authentic human pathogen. With such oligonucleotides, the pattern
Table 1
Distances between the SSU rRNA Sequences of Nine Plasmodium Species

<table>
<thead>
<tr>
<th></th>
<th>falciparum</th>
<th>gallinaceum</th>
<th>lophurae</th>
<th>berghei</th>
<th>malariae</th>
<th>vivax</th>
<th>knowlesi</th>
<th>cynomolgi</th>
<th>fragile</th>
</tr>
</thead>
<tbody>
<tr>
<td>falciparum</td>
<td>4.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gallinaceum</td>
<td>3.5</td>
<td>2.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lophurae</td>
<td>4.7</td>
<td>6.4</td>
<td>5.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>berghei</td>
<td>4.3</td>
<td>5.9</td>
<td>4.9</td>
<td>5.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>malariae</td>
<td>4.5</td>
<td>5.2</td>
<td>4.9</td>
<td>4.5</td>
<td>4.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vivax</td>
<td>4.0</td>
<td>5.0</td>
<td>4.4</td>
<td>4.1</td>
<td>3.7</td>
<td>0.8</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>knowlesi</td>
<td>4.4</td>
<td>5.2</td>
<td>4.7</td>
<td>4.4</td>
<td>3.9</td>
<td>0.8</td>
<td>0.5</td>
<td>1.1</td>
<td>0.8</td>
</tr>
<tr>
<td>cynomolgi</td>
<td>4.3</td>
<td>5.3</td>
<td>5.0</td>
<td>4.4</td>
<td>3.9</td>
<td>1.1</td>
<td>0.8</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>fragile</td>
<td>4.3</td>
<td>5.3</td>
<td>5.0</td>
<td>4.4</td>
<td>3.9</td>
<td>1.1</td>
<td>0.8</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

NOTE.—Distances are corrected by using Kimura’s (1980) two-parameter method and are the no. of substitutions/100 sites, based on 1,673 positions in the multiple alignment.

...of the expression of the two genes reported here was confirmed by their specific hybridization to RNA isolated from blood-stage parasites and not to that isolated from sporozoite-infected mosquitoes (data not shown).

Phylogenetic Analysis

An inferred phylogenetic tree, calculated from the distances in table 1 by using the neighbor-joining method (Saitou and Nei 1987), is shown in figure 1, along with confidence levels from the bootstrap procedure. A second tree, calculated by using the DNAML program, is also shown. The same topology was found by using the DNAML program, except that the relative positions of P. berghei and P. malariae were reversed, consistent with the low bootstrap figure for the branch between them. These are unrooted trees; the position of the root must be inferred on biological grounds, by inspection, or by assuming a constant rate of substitution and placing the root at the center of the tree. With the DNAMLK program, which calculates a maximum-likelihood tree by assuming a good “molecular clock,” the position of the root is estimated to lie along the branch connecting the “avian” group (P. lophurae, P. gallinaceum, and P. falciparum) with the rest, thus dividing the tree into two lineages: the “avian” species and the “mammalian” species.

Twenty bootstrap experiments were carried out to determine the stability of this position for the root and were analyzed with the DNAMLK program. In 12 of the 20 resulting trees, the root was found in the same position as described above. In the remaining eight cases, P. malariae was placed on the same side of the tree as was the “avian” group (P. lophurae, P. gallinaceum, and P. falciparum), with the rest of the species on the other side of the root. These two positions for the root are shown in figure 1. The implications of different positions for the root are discussed below.

Several features are immediately apparent from the tree. First, the simian species (P. vivax, P. cynomolgi, P. knowlesi, and P. fragile) form a group of closely related species, relatively distant from the rest of the tree. The bootstrap figure and the long branch leading to this group, compared with the short branches within the group, confirm this. Within the simian group, the bootstrap figures show that the exact branching order cannot be estimated very reliably. Nonetheless, the closest species to P. vivax is estimated to be P. cynomolgi; the bootstrap figures show that this grouping occurs >70% (709/1,000) of the time in the bootstrap sample trees. A more accurate estimation of the precise relationship of the four “simian/vivax” parasites was obtained
Fig. 1.—Phylogenetic trees of the SSU rRNA sequences of nine *Plasmodium* species, produced by (a) the neighbor-joining method and (b) the DNAMLK program. In the neighbor-joining tree, bootstrap confidence levels are shown underlined for each internal branch. The figures show the number of occurrences in 1,000 bootstrap sample trees. Branch lengths are shown for every branch, as number of substitutions/100 nucleotides. In the DNAMLK tree, the two possible positions for the root, found by the DNAMLK program, are indicated by arrows. Position 1 was found in 12/20 bootstrap replicate trees, while position 2 was found in 8/20. The vertebrate hosts for the various species are as follows: human—*P. falciparum*, *P. malariae*, and *P. vivax*; simian—*P. cynomolgi*, *P. fragile*, and *P. knowlesi*; murine—*P. berghei*; and avian—*P. gallinaceum* and *P. lophurae*. 

**Vertebrate Hosts**
- Human: *P. falciparum*, *P. malariae*, *P. vivax*
- Simian: *P. cynomolgi*, *P. fragile*, *P. knowlesi*
- Murine: *P. berghei*
- Avian: *P. gallinaceum*, *P. lophurae*
by aligning the entire SSU rRNA sequences of the four species and by using the SSU A gene of *P. berghei* as outgroup (data not shown). In this analysis, *P. vivax* and *P. cynomolgi* formed a monophyletic group in 997 of 1,000 bootstrap sample trees, demonstrating the close relationship of the two species.

The second feature is the isolated position of *P. malariae*, which does not group with either of the other two human parasites. The exact position of this species cannot be estimated accurately, as is revealed by the very low figure for the bootstrap confidence level of the branch connecting *P. malariae* and the “simian” species to the rest of the tree. *Plasmodium malariae* is just as likely to branch closer to the “avian” group; in fact, that is where it branches in the maximum-likelihood tree produced by the DNAML program. Finally, the previously established (Waters et al. 1991) grouping of *P. falciparum* and the bird parasites (*P. lophurae* and *P. gallinaceum*) is clear. Table 2 describes the host range of all the species of *Plasmodium* discussed in the present paper.

**Discussion**

The most important criterion used by Garnham (1966, p. 68) to classify the genus *Plasmodium* was the vertebrate host. This serves to subdivide the primate ma-

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Natural Host</th>
<th>Confirmed Laboratory Host</th>
<th>Infectious to Humans?</th>
</tr>
</thead>
<tbody>
<tr>
<td>vivax</td>
<td><em>Homo sapiens</em></td>
<td><em>Pan troglodytes</em></td>
<td>Yes</td>
</tr>
<tr>
<td>cynomolgi</td>
<td><em>Macaca irus</em></td>
<td><em>M. mulatta</em></td>
<td>Yes</td>
</tr>
<tr>
<td>knowlesi</td>
<td><em>M. irus</em></td>
<td><em>M. mulatta</em></td>
<td>No</td>
</tr>
<tr>
<td>fragile</td>
<td><em>M. radiata</em></td>
<td><em>M. mulatta</em></td>
<td>No</td>
</tr>
<tr>
<td>simium</td>
<td><em>Alouatta fasc, Brachyteles arachnoide</em></td>
<td><em>Saimiri sciureus</em></td>
<td>+/-</td>
</tr>
<tr>
<td>gonderi</td>
<td><em>Cercocebus sp.</em></td>
<td><em>M. mulatta</em></td>
<td>No</td>
</tr>
<tr>
<td>malariae</td>
<td><em>H. sapiens</em></td>
<td><em>Aotus trivirgatus</em></td>
<td>No</td>
</tr>
<tr>
<td>brasilianum</td>
<td><em>Brachyurus calvus</em>a,b*</td>
<td><em>Aotus trivirgatus</em></td>
<td>Yes</td>
</tr>
<tr>
<td>inui*a</td>
<td>*Macaca sp.*ad</td>
<td><em>M. mulatta</em></td>
<td>Yes</td>
</tr>
<tr>
<td>shortiti*c</td>
<td><em>M. radiata</em></td>
<td><em>M. mulatta</em></td>
<td>No</td>
</tr>
<tr>
<td>rhodaini*</td>
<td><em>P. stayrus verus</em></td>
<td><em>P. troglodytes</em></td>
<td>Yes</td>
</tr>
<tr>
<td>falciparum</td>
<td><em>H. sapiens</em></td>
<td><em>P. troglodytes</em>f</td>
<td>No</td>
</tr>
<tr>
<td>reichenowi</td>
<td><em>P. troglodytes</em></td>
<td><em>Rattus, Mus</em>a,a*</td>
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</tr>
<tr>
<td>berghei</td>
<td><em>Thammomys surdaster</em>a,g*</td>
<td><em>Rattus, Mus</em>a,a*</td>
<td>No</td>
</tr>
<tr>
<td>lophurae</td>
<td><em>Lophura igniti</em></td>
<td><em>Gallus sp.</em>,ab<em>Mus</em></td>
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</tr>
<tr>
<td>gallinaceum</td>
<td><em>Gallus sonneratii</em></td>
<td><em>G. gallus</em></td>
<td>No</td>
</tr>
</tbody>
</table>

*a* Example host species; the range is too large to catalogue.

*b* For a list, see Garnham (1966, p. 315).

* Some researchers consider these to be the same species (Coatney et al. 1971, p. 245) while others allow a distinction (Garnham 1966, pp. 300–308).

* For a list, see Coatney et al. (1971, p. 255).

* Thought to be identical to *P. malariae* adapted to the chimpanzee host (Coatney et al. 1971, p. 260).

* Asymptomatic infection.

* For a list, see Garnham (1966, pp. 446–451).

* For a list, see Garnham (1966, pp. 667–669).

* For a list, see Garnham (1966, pp. 609–612).
larias from the rest. Only *P. falciparum* and perhaps *P. reichenowi*, which were noted as exceptional, were placed outside the single subgenus, *P. plasmodium*, which encompasses all other species infectious to primates. In general the analyses provided here support that view, with the possible exception of *P. malariae*, whose exact position remains uncertain. *Plasmodium malariae* is the only human *Plasmodium* pathogen with quartan periodicity (72-h asexual cycle and consequent appearance of fever) and has been speculated to be an ancient parasite of man (Knowles et al. 1930), which may explain its diverged placement. The significance of the position of *P. malariae* will remain unclear until other relevant primate and avian malarias have been characterized.

The evolutionary history of primate malaria has long been a subject of interest, and one scenario may be favored. Coatney et al. (1971, p. 5) propose that in Asia *Plasmodium* coevolved with its host, there being a wide distribution of appropriate infections in a plethora of host species. The subsequent distribution of the primate malarias into Africa could then be explained by the migration of the peripatetic hominid precursors (Coatney et al. 1971, p. 5). The introduction of malaria into South America was more recent, possibly pre-Columbian (Bruce-Chwatt 1965) but also possibly during Western colonization (see Coatney et al. 1971, p. 6, and refs. therein). Three factors favor this interpretation: (1) Primate malaria has a curious distribution in the three continental landmasses in which it is found. The majority of simian malaria is represented by the Asian species, there being only one species, *P. gonderi*, known in Africa, and only two primate infections, *P. simium* and *P. brasilianum*, known in all of South America. (2) All four human malarias are found in each of these three continents (Coatney et al. 1971, pp. 43, 171, 209, 264). Support for the possibility that the simian malarias of Africa and South America are of human origin is found in the striking biological resemblance between *P. brasilianum* and the human parasite *P. malariae* and between *P. gonderi* and the human parasite *P. vivax*. Molecular analysis is consistent with this as well. For example, there is less sequence divergence between the circumsporozoite protein genes of *P. malariae* and *P. brasilianum* than that found between the same gene in isolates of both *P. falciparum* and *P. vivax* (Lal et al. 1988). (3) Host-parasite relationships are clearly favored but not absolutely adhered to. Parasites designated as pathogens of monkeys can occasionally infect man, and vice versa.

Understanding the interrelatedness of primate malarias has both practical and academic relevance. The three simian-infecting species now characterized are all Oriental, infect macaques, and, in some cases (*P. fragile* and *P. cynomolgi*), can give concomitant infections, so perhaps it is not surprising that they form a monophyletic branch. More surprising, *P. vivax* forms such a close association with *P. cynomolgi*. There are other similarities between the two, such as their promiscuous, oligoxenous range of infection of Anopheline vectors, their morphological similarity, and their immunological cross-reactivity (Coatney et al. 1971, pp. 43–98). This is confirmation that this simian parasite is a good, phylogenetically supported choice as a simian model for the human pathogen. Of the simian species now characterized by sequencing of the SSU rRNA genes, both *P. knowlesi* and *P. fragile* were regarded by Garnham as phylogenetically problematic (Garnham 1966, p. 110). The former was thought so because of its unique periodicity; the latter was thought so because of its ability to cytoadhere and the apparent morphological similarity of its asexual blood stages (especially the rings) to *P. falciparum*, despite the complete dissimilarity of its gametocytes (Garnham 1966, p. 110). Although this study resolves the specific affiliation of these
two species within the overall genus, their absolute position remains obscure and awaits characterization of further species within the complex.

The data here also clarify formerly unresolved issues regarding the relationship of the human malaria \textit{P. falciparum} to the avian subgenera. The exceptional case of \textit{P. falciparum} indicated that the current host distribution of these parasites did not occur through mutual long associations, and the thought that the human pathogen is a recent acquisition has been in the literature for >40 years (Boyd 1949). Previous work had shown that, on the basis of genome composition, rodent and avian parasites have a closer relationship to \textit{P. falciparum} than to other simian and human species (McCutchan et al. 1984). This was expanded in a study comparing the sequences of the SSU rRNA genes of six species of malaria parasite, which showed that \textit{P. falciparum} formed a significant and monophyletic grouping with two avian parasites (Waters et al. 1991). The study concluded that the phylogenetic relationships were incompatible with the pattern of host distribution unless lateral transfer events had occurred. The direction of transfer could not be stated with certainty on the basis of the tree, but the avian-to-human direction was favored after a review of associated biological evidence (Waters et al. 1991).

A consequence of the position of the root in that tree (Waters et al. 1991) called for a mammalian precursor for the \textit{Plasmodium} species characterized. The existence of such a precursor would require two transfers—from mammals to avians and back to mammals—to explain an avian source of \textit{P. falciparum}. Therefore, Brooks and McLennan (1992) pointed out that the simplest explanation for the observed topology and host-range data was that lateral transfer of parasites had taken place from human to bird hosts—and not as we suggested. The position of the root was inferred by using the SSU rRNA sequence of \textit{Acanthamoeba castellanii} (Gunderson and Sogin 1986) as an outgroup. \textit{Acanthamoeba} is too distantly related to \textit{Plasmodium} to allow the position of the root to be found reliably; sequences from other, equally distantly related organisms gave different positions for the root (data not shown). In most cases, the alternative root was to divide the species into “avian” and “mammalian” parasites, as found in our analysis above. Further, in our previous tree, we used all sites from a full-length automatic alignment of the sequences. Some parts of the sequences are variable and cannot be reliably aligned. When these regions are removed (as was done in the present analysis), the sequences behave in a more “clocklike” manner, and the position of the root can be estimated more easily. The most likely position for the root divides the sequences into two groups: the “avian” (including \textit{P. falciparum}) species and the “mammalian” species (the rest). This position for the root actually improves the case made in our previous work and removes the objections. The only way of conclusively resolving the position of the root will be to obtain data from suitable outgroup species.

It is appropriate to restate the conclusions of the previous analysis (Waters et al. 1991), which demonstrated a monophyletic group comprising the human parasite, \textit{P. falciparum}, and the avian parasites. That study concluded that the climactic effect of the establishment of agriculture would create the conditions where the sustained transfer of highly pathogenic infectious agents such as \textit{P. falciparum} would be tolerated and not lethally compromise the survival of the human host as a species. Date estimates based on the earlier analysis indicate that the divergence of the represented species was substantially earlier than such timing (Ayala and Fitch 1992). The ecological significance of agriculture is clear, but any resolution of the question regarding timing
of the introduction of *P. falciparum* into the human population will require the identification and characterization of the appropriate avian precursor.

The inferred branching pattern of the genus *Plasmodium*, presented here, reveals relatively deep branching roots, suggesting that the various lineages are quite distinct and not recently diverged. It is suggested that both the diverged position of *P. malariae* and the close grouping of *P. vivax* with the simian malarials are incompatible with the continued use of the subgenus *P. plasmodium* (Garnham 1966) and that *P. malariae* and its morphological and biological homologues be grouped in a separate subgenus, *P. quartans*. At present this would include *P. brasilianum* and *P. inui* [Also *P. rhodaini* and *P. shortti*, if these are accepted as species in their own right; for discussion, see Garnham (1966, pp. 300–308 for *P. shortti* and p. 273 for *P. rhodaini*); also see Coatney et al. (1970, p. 254 for *P. shortti* and pp. 259–260 for *P. rhodaini*)]. Therefore, it will be relevant to extend the analysis, to include other species infective to the different primate orders and to examine their interrelationships in comparison with the supposed relative evolution of their hosts. Such studies may also give more insight into the role of man—and his ancestors—in the global distribution of primate malaria. If this role and distribution prove to be significant, then the danger of zoonoses as a source for novel and potentially dangerous human pathogens would remain an area of health concern.

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**LITERATURE CITED**


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