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Primate malarias: Diversity, distribution and insights for zoonotic *Plasmodium*

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ABSTRACT

Protozoans within the genus *Plasmodium* are well-known as the causative agents of malaria in humans. Numerous *Plasmodium* species parasites also infect a wide range of non-human primate hosts in tropical and sub-tropical regions worldwide. Studying this diversity can provide critical insight into our understanding of human malarias, as several human malaria species are a result of host switches from non-human primates. Current spillover of a monkey malaria, *Plasmodium knowlesi*, in Southeast Asia highlights the permeability of species barriers in *Plasmodium*. Also recently, surveys of apes in Africa uncovered a previously undescribed diversity of *Plasmodium* in chimpanzees and gorillas. Therefore, we carried out a meta-analysis to quantify the global distribution, host range, and diversity of known non-human primate malaria species. We used published records of *Plasmodium* parasites found in non-human primates to estimate the total diversity of non-human primate malarias globally. We estimate that at least three undescribed primate malaria species exist in sampled primates, and many more likely exist in unstudied species. The diversity of malaria parasites is especially uncertain in regions of low sampling such as Madagascar, and taxonomic groups such as African Old World Monkeys and gibbons. Presence–absence data of malaria across primates enables us to highlight the close association of forested regions and non-human primate malarias. This distribution potentially reflects a long coevolution of primates, forest-adapted mosquitoes, and malaria parasites. The diversity and distribution of primate malaria are an essential prerequisite to understanding the mechanisms and circumstances that allow *Plasmodium* to jump species barriers, both in the evolution of malaria parasites and current cases of spillover into humans.

Introduction

Malaria is arguably the most important infectious disease in humans. Annually, it causes an estimated 584,000 deaths and leads to over 198 million cases globally [1]. *Plasmodium* parasites that cause malaria have also had a significant impact on the human evolution, as evidenced by hundreds of mutations that have arisen to high frequency due to selection by malaria [2,3].

While there are four *Plasmodium* species that primarily infect humans, there are over 250 *Plasmodium* species that infect other animals, including birds, lizards, snakes and mammals [4–6]. Studying these species can provide a critical insight into human malaria, especially malaria parasites of non-human primates (NHPs). Two of the most important human malaria parasites, *Plasmodium falciparum* and *Plasmodium vivax*, originated from a cross species transmission event of a NHP malaria to humans [7–10]. Additionally, zoonotic spillover of wild primate malaria is an emerging global public health concern [11–14]. This is especially important in Malaysian Borneo, where the majority of human malaria cases are caused by a monkey malaria, *Plasmodium knowlesi* [15–17]. Several authors have implicated changing land use in the region as the cause for the recent emergence of *P. knowlesi*, but the mechanisms underlying the recent rise in infections in humans remain unknown. Understanding the diversity and distribution of NHP malarias is an important first step to predict the potential zoonotic risk of NHP malarias.

The term 'malaria' has historically referred to disease caused by species from the Apicomplexan genera *Leucocytozoon*, *Haemosporidia*, *Plasmodium*, and *Hepatocystis*. Here, we will use just 'malaria' to describe disease caused by members of the genus *Plasmodium* and 'malaria-like' parasites to describe *Leucocytozoon*, *Haemosporidia*, and *Hepatocystis*[18]. All malaria and malaria-like parasites have a digenic (two-stage) life cycle that requires an intermediate vertebrate host and a definitive insect hosts. Insects in the order Diptera are typically utilized for sexual reproduction and transmission among vertebrate hosts. Although four species of *Plasmodium* utilize humans as intermediate hosts, at least twenty-six additional NHP parasites exist outside *Homo sapiens* hosts (Table A.1).

The first account of a NHP malaria parasite was recorded by Laveran (1905) in an orangutan, *Pongo pygmaeus*. The species *Plasmodium pitheci* was described a few years later, along with *Plasmodium inui*

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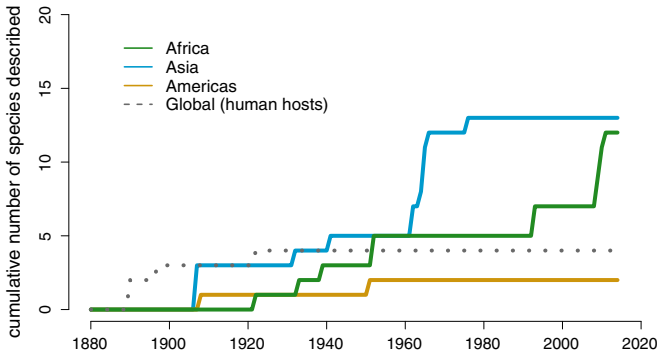

Fig. 1. Discovery of primate *Plasmodium* species. Colored lines are used to indicate geographic regions where the parasites are endemic. Aside from human malarias ('Global'), all primate malarias are restricted to the region in which they were described. We included parasites in Table A.1 that have been morphologically described by at least two groups or belong to the *Laverania* clade (see Methods for distinction of species). The last reviews of primate malarias were conducted in 1933 [58], 1941 [33], 1966 [4], and 1971 [21]. Fooden [75] also reviewed *Macaca* literature in 1994. The dataset gathered here nearly doubles the number of individuals examined in previous reviews and presents the most updated taxonomic distinctions.

and *Plasmodium cynomolgi*, that infect sympatric monkey species, *Macaca fascicularis* and *Macaca nemestrina*, in Borneo [19]. Discovery of primate malarias continued as parasitologists examined an increasing diversity of apes, monkeys, and lemurs from across the globe (Fig. 1, Table A.1). Sample sizes were often small and restricted to animals captured for zoos or killed for examination. The infection of a human with a monkey malaria in 1965 [20] initiated a resurgence of NHP malaria research focused on Southeast Asia, resulting in nine newly described malaria species in Asian apes and monkeys [21]. More recently, identification of a cluster of a monkey malaria, *P. knowlesi*, within humans in Southeast Asia [15], and discoveries of novel parasite species in apes [7–10,22–24] and monkeys [25–27] in Central Africa has reignited research interest in primate malarias. This is in part due to improved sampling techniques, leading to the identification of at least four novel clades in the *Laverania* subgenus of *Plasmodium* that are awaiting morphological description [28].

Malariologists have spent decades characterizing these parasite species, and over a century of surveys are available to inform our understanding of the distribution and host range of primate malarias. This presents the opportunity to revisit the global distribution and diversity of NHP malarias for the first time in over 30 years, this time using modern analytical techniques developed to understand patterns of biodiversity accumulation.

In this paper, we critically review all published surveys of primate malarias between 1905–2015 to present an updated global characterization of *Plasmodium* in primates. Whenever possible, we integrate historical literature with newer molecular work. Using species accumulation curves, we are able to predict expected species richness in primate taxa that have been sampled; and by quantifying sampling effort in each species, we highlight undersampled taxonomic and geographic areas that may harbor a hidden diversity of malaria. We also utilize primate host ranges and presence–absence parasite data to create a global map of non-human primate malarias, and draw attention to regions that may be at risk for zoonotic malaria.

Methods

Primate malaria database

To create a database of primate malarias, we used the search terms *Plasmodium* and/or malaria and all genera of primates in PubMed and Web of Science until January 1, 2015. We also used key reference books [4,21] and historical literature reviews to find obscure and non-English publications [31–33] to supplement online searches. We included recent ape *Plasmodium* species within the *Laverania* subgenus with the naming established by Rayner et al.[28]. These species have only been identified molecularly, and await morphological characterization to solidify their species status [34]. However we include these species because they have been isolated by several groups that have confirmed the genetic distinction as species and ensure their identity doesn't overlap with already named species. Other authors have reported novel malaria species in lemurs [35,36] and African Old World Monkeys [27,37] based on molecularly characterized samples. These species are listed in Table A.1 but we did not consider these as species in host species accumulation calculations, however they were included in group calculations few isolates or few groups have isolated the parasites, and it is unknown whether their identity overlaps with morphologically described species. For example, almost every lemur malaria parasite found has been named a unique *Plasmodium* species [35,36,38], but there is no corresponding molecular and morphological data from most of these parasites.

For each published account, the location, host species name (in the paper), subspecies (if applicable), number of hosts sampled, sampling method, identification method, *Plasmodium* species found, and any notes of interest were recorded (full data available in Table A.2). Because genus and species names have changed over the century the data was collected, we used locations and descriptions to update host names to the Mammals of the World 2005 nomenclature [39], which we cross-validated with species synonym files [40]. When possible, we contacted authors for missing information from publications to complete database entries.

A prerequisite for the data to be included in the geographic analysis was that a specific country of origin must be known. Occasionally authors utilized samples from zoos with unknown origin, or worked with samples that had been exported without a country of origin specified, so these were excluded from our presence/absence maps. For data to be included in the species accumulation analysis, the surveys they came from had to utilize methods that did not obviously bias species discovery. For example, if specific primers for a sub genus or particular *Plasmodium* species were used, then these were excluded from species accumulation analyses. These exclusions are identified for each entry in Table A.2.

The methods used to detect and identify malaria parasites in primates vary in their sensitivity and specificity. Historically, *Plasmodium* was identified by erythrocytic morphological characteristics using microscopy. Malaria parasites can also be identified in blood samples using molecular markers (i.e., a nested PCR protocol with species-specific primers). Microscopy has a limit of detection of approximately 40 parasites/μL, meaning well-trained malariologists must check at least 100 fields of thick blood films to detect parasites in an individual. Information on screening protocol was rarely given, so we are unable to compare sensitivities among microscopy studies. PCR using DNA isolated from blood is the most sensitive method for detection of active infections [41]. The last method is parasite detection from fecal samples. Although there are benefits to using non-invasively-collected samples, they degrade quickly in the field and range in their sensitivity (30–95%; [42]). Nevertheless, all of these data are informative for determining relative frequencies of infection, and predicting species richness in primates.

Predicting malaria species diversity

Datasets were separated into individual host species for analysis in R computing software version 3.0.2 [44]. To quantify sampling effort, we assumed that individual hosts belonging to the same species represented equivalent sampling units. Some surveys reported unknown *Plasmodium* species; these records were excluded from the analysis. From the vegan package (vs. 2.0), the functions "specaccum" and "specpool" were used to generate species accumulation curves and estimate total richness,