two pathogens may reduce the immunopathological effects of an overworked immune system and this may allow each pathogen to persist for longer in co-infected hosts. This could enhance either the transmission success of one or both pathogens or the colonization of a new host species by a novel pathogen.

Prior infection with one pathogen (the malaria parasite *Plasmodium)* may facilitate later infection with, and transmission of, another completely unrelated pathogen, the bacterium *M. gallisepticum*. A similar situation is the example of *Babesia microti* (a malaria-like parasite that infects red blood cells and is the cause of babesiosis) and *Borrelia burgdorferi*, a bacterium that causes Lyme disease [11]. Diuk-Wasser and colleagues showed how *Babesia* only successfully establishes in populations in which Lyme disease is already present. More subtly, these examples suggest that prior infections, such as malaria, or parasitic helminths, help facilitate the emergence of pathogens, such as Ebola, Nipah, Hendra, severe acute respiratory syndrome (SARS, or Zika virus. They also underline the previously underexplored potential role that co-infection interacting with host endocrine stress might have in mediating the emergence of novel pathogens. For example, pregnant mothers who are often immunologically stressed and have major upheavals in their endocrine system are more susceptible to mosquito bites [12]. Was this a significant factor in the emergence of Zika virus? To date, the role of endocrine and nutritional processes in the within-host dynamics of pathogens has been essentially ignored. However, it may prove to be an important missing link in many future studies of the emergence and subsequent coevolutionary dynamics of many host–parasite systems.

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Forum

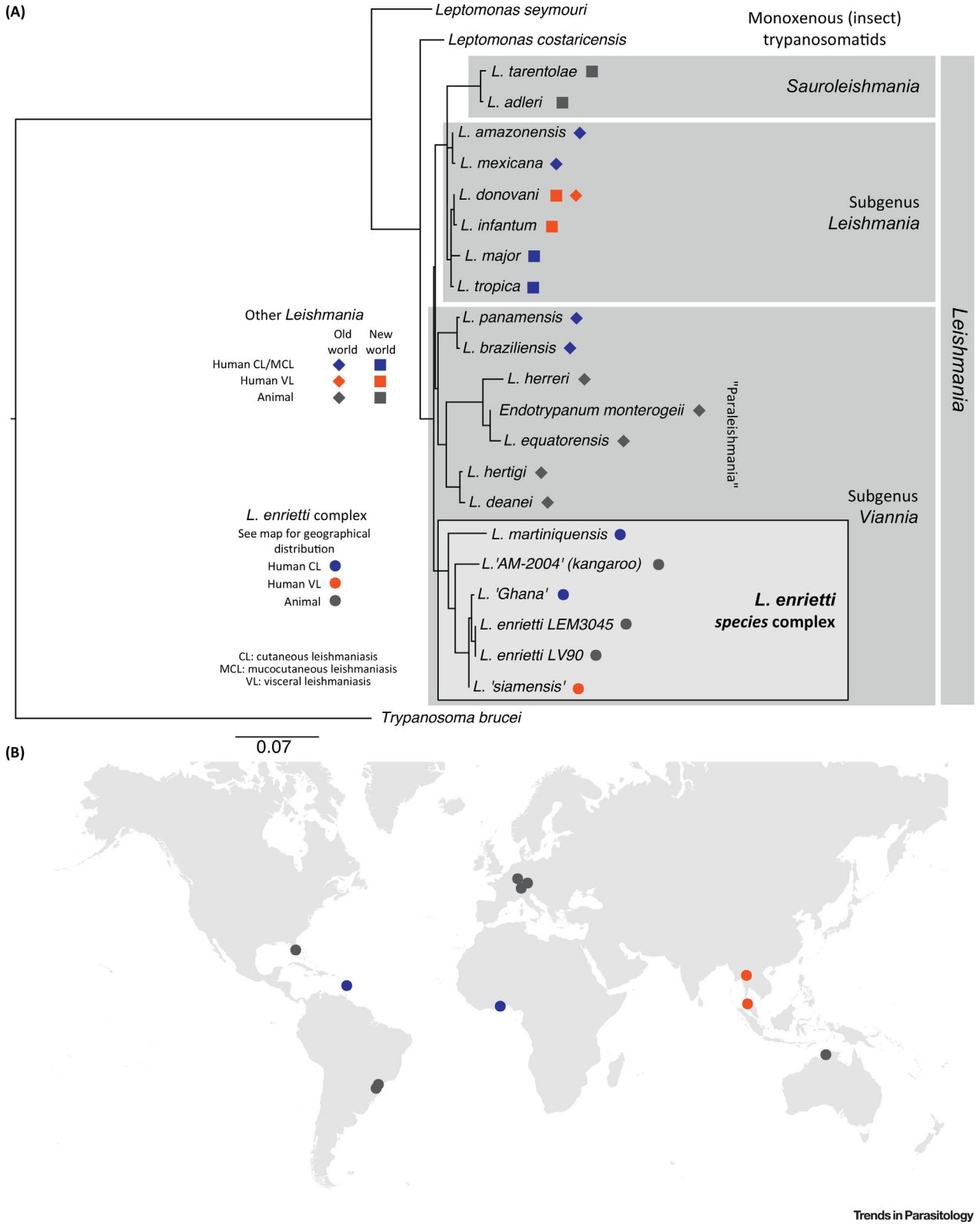
The Expanding World of Human Leishmaniasis

James A. Cotton[1,*,@]

New *Leishmania* isolates form a novel group of human parasites related to *Leishmania enrietti*, with cases in Ghana, Thailand, and Martinique; other relatives infect Australian and South American wildlife. These parasites apparently cause both cutaneous and visceral disease, and may have evolved a novel transmission mechanism exploiting blood-feeding midges.

Leishmania is a diverse and widespread genus of human pathogens that cause the neglected tropical disease leishmaniasis in South and Central America, Africa, Asia, and Europe. Their clinical diversity includes asymptomatic infections and symptoms ranging from localised ulcers to fatal infections. Cutaneous leishmaniasis (CL) appears when parasites remain in the skin, causing either localised symptoms at the site of insect vector bites, more widespread cutaneous disease (disseminated CL) or destructive mucocutaneous leishmaniasis (MCL). Systemic infections (visceral leishmaniasis; VL) occur when the parasites spread to other organs, in particular to the liver and spleen where they destroy immune cells. VL is generally fatal if not adequately treated.

One key to this clinical and geographic range is the biological diversity of *Leishmania*. Clinically important parasites are found in two clades – the new world subgenus *Viannia* and the subgenus *Leishmania* found in both the new and old worlds (Figure 1); the parasites largely responsible for VL are in *Leishmania,* while both groups contain parasites causing CL. The *Viannia* and *Leishmania* groups contain all the best known species, but only around 20 out of approximately 53 recognised species of *Leishmania* [1] are known to infect humans, and of these probably only 10 are of major public health importance. Other *Leishmania* species are much less well known: a basal group of *Leishmania* species known as *Paraleishmania* and a species called *Leishmania enrietti* infect wild mammals in South America. An unusual group (subgenus *Sauroleishmania*)



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has adapted to lizards in Africa and Asia, apparently a secondary adaptation of an originally mammalian parasite. Another nominal genus (*Endotrypanum*) is now widely accepted to be included within *Leishmania*.

Discovery of novel *Leishmania* species is not unusual – the most recent example being *L. waltoni* described in 2015 [2] from older isolates from patients with diffuse CL in the Dominican Republic during the 1980s; however, most of the recently described species are neotropical, and are often not known to infect humans. Most of these novel species are in fact closely related to known species (*L. waltoni* is closely related to *L. mexicana* and may represent a population isolated on the island of Hispaniola). However, in recent years, a number of *Leishmania* populations have been reported from some unexpected hosts, and in unexpected parts of the world, complicating this picture.

The first of these unexpected discoveries relates to a focus of cutaneous leishmaniasis in Martinique. While sporadic cases, apparently acquired on the island, had been reported since 1917, initial typing of the first parasites isolated from cases suggested that these were not *Leishmania* infections. Instead, they appeared to be caused by monoxenous trypanosomatids – parasites only distantly related to *Leishmania* but generally considered to have an insect-only lifecycle without the involvement of a vertebrate host. Infections of immunocompetent people (as in most of the Martinique cases) by monoxenous trypanosomatids are exceedingly rare and indeed, subsequent typing using molecular methods confirmed that the Martinique cases were in fact caused by *Leishmania*. This parasite has since been formally described as *L. martiniquensis* [3] and is related to *L. enrietti*, albeit rather distantly. *L. enrietti* is a well known but still slightly mysterious parasite: while it was historically used as a laboratory model for the genus, being particularly large and easy to grow in culture, it has only been isolated three times since its discovery in the 1940s [4]. Each time, it was found causing particularly large, spreading skin lesions in domesticated Guinea pigs (*Cavia porcellus*) kept in rural areas of North-East Brazil. No sandfly species have been found infected with this parasite, and it is not clear what the natural reservoir might be – attempts to infect wild species of *Cavia* apparently failed, so essentially nothing is known about the ecology of this species.

The second surprise came on the other side of the world, in Thailand, where a handful of reports of VL cases included patients with no history of travel to *Leishmania*-endemic regions. These were the first known autochthonous cases of leishmaniasis from South-East Asia. Molecular phylogenies show that these parasites are also related to, but distinct from, *L. enrietti*. Most of the Thai cases are from the south of the country, but recent analysis of an isolate from Northern Thailand has shown that parasites from this area may be a separate lineage to other isolates from the region. This Northern isolate was closely related to *L. enrietti* itself, while other Thai isolates are much more similar to *L. martiniquensis* [5]. Members of both of these lineages have been referred to as '*L. siamensis*'. As this species has not been formally described there is significant risk of confusion!

Most recently, parasites isolated from cutaneous lesions in the Volta region of Ghana have been shown to be related to *L. enrietti* and to parasites from Thailand [6]. These presumably represent the first new human-infective species of *Leishmania* to be isolated in Africa for over 40 years. Molecular phylogenetic evidence thus now links recently discovered parasites in three different foci of human leishmaniasis: causing CL in Ghana, VL in Thailand, and a different species causing CL and VL in Martinique and Thailand. This clade represents a new and widespread group of human-infective *Leishmania* species (Figure 1). The group is not restricted to human hosts: related parasites have also been identified in cows in Switzerland [7] and horses

Box 1. Outstanding Questions about the *Leishmania enrietti* Complex

- **Confirming the vector**. There is substantial evidence that midges may transmit these parasites, at least for the Australian parasite. A missing piece is evidence that midge bites transmit the disease to naive animals.
- **Unravelling the epidemiology.** Confirming the vectors involved in each focus should shed light on likely animal reservoirs, and on the likelihood of human cases in the foci of veterinary disease.
- **Understanding the pathogenesis of '*L. siamensis*' VL.** The Thailand outbreak is worrying, as these parasites cause VL symptoms in patients without known immunodeficiency or HIV coinfection [5]. Understanding these infections is clinically important, but also presents an opportunity to better understand VL, as these parasites have evolved to disseminate and survive in deep tissues independently of the *L. donovani* complex. Work in animal models could be possible, as *L. martiniquensis* can establish disseminated infection in mice [12].
- **Revealing the distribution and evolution.** The sporadic distribution of known parasites is striking and confusing. Surveys of under-studied regions, including those not endemic for human leishmaniasis, using molecular tools, and particularly in wildlife, could reveal how widespread parasites of this group are, and should help to understand the distribution and evolution of these parasites.

Figure 1. Phylogeny and Geographical Distribution of the *Leishmania enrietti* Species Complex. (A) Maximum-likelihood molecular phylogeny of selected *Leishmania* and related trypansomatid species based on previously published sequences of RNA polymerase II large subunit (RNAPolII) [5,6,11]. Main clinical presentations of species are indicated with symbol colors; note that many species can occasionally cause other symptoms and that many infections are asymptomatic. Geographical distribution and *Leishmania* species outside the *L. enrietti* complex are shown with the shape of symbols. (B) The global distribution of the *L. enrietti* species complex. For some foci no RNAPolII sequence is available, and so are missing from the phylogenetic tree, as, for example, the *L. martiniquensis*-like parasites from Southern Thailand.

in Switzerland, Germany, and the USA. A related species has been discovered in red kangaroos, and subsequently found in some other macropods in Australia's Northern Territory, the first endemic *Leishmania* species reported from Australia [8]. *L. enrietti* thus represents a novel group [8] within *Leishmania*, capable of causing both visceral and cutaneous disease and infecting a range of different mammals.

Given the wide geographical and host range of these parasites, perhaps the biggest mystery is their ecology, and ultimately, epidemiology. All *Leishmania* species are thought to share a single group of insect vectors – sandflies from the subfamily phlebotominae. While sandflies are present throughout the regions involved, and *L. siamensis* DNA has been detected in them, the vectors of this group of species are unknown and there is evidence that biting midges could be involved. Trapping of sandflies at sites of known transmission of the kangaroo-infective *Leishmania* in Australia failed to identify any flies containing *Leishmania* DNA, but parasite DNA was often found in a blood-feeding midge, *Forcipomyia* sp. Subsequent investigation showed that promastigotes of the kangaroo *Leishmania* species could be cultured from these midges [8]. While far from conclusively showing that these midges are responsible for transmitting the parasites, this was a major surprise, representing the first time that any vector other than a phlebotomine sandfly was implicated in the life cycle of *Leishmania*. Recent experimental work has confirmed that midges can support development of the kangaroo *Leishmania* and *L. enrietti*, and can be infected with the latter species by feeding on infected guinea pigs [9].

As they cause potentially fatal visceral disease and might be responsible for thousands of cases of CL in Ghana, these parasites are of public health importance, and research is needed to understand many aspects of their biology (Box 1). The availability of parasites in axenic culture will, as always, be key to further understanding of the biology of these parasites, and there are isolates from all of the human disease foci; other resources are becoming available, for example, with genome sequence data available for both *L. enrietti* and *L. martiniquensis*.

Perhaps the most strikingly unusual features of the *L. enrietti* complex is how geographically widespread the foci are, and how recently most were discovered. In an era of historically unprecedented climate change it is tempting to assume that the foci themselves are recent, but they may have been overlooked or, before widespread molecular typing, assumed to be imported cases of better-known *Leishmania* species. Molecular methods such as dating with the molecular clock should clarify how long the parasites have been in these regions – for example, to test whether the populations have been separate long enough for their distribution to be due to a shared Gondwanan origin, or are much more recent. In either case, other foci are likely to be uncovered – for example, no firm identification is available for the parasites recently found causing cutaneous leishmaniasis in East Timor [10]. Given the geographic position, it is plausible that this could be related to the Australian parasites.

More surprises undoubtedly await as more is learnt about the biology and epidemiology of this newly discovered group of human pathogens.

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