The Origin of Insect-Borne Human Diseases as Revealed in Amber

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Some of the greatest mysteries of medical entomology and pathology center around the origins of devastating human diseases such as malaria, leishmaniasis, and trypanosomiasis. Where did these pathogens come from, and when were they first vectored by blood-sucking insects? While scientists have used a plethora of methods attempting to unravel the seemingly complex origins of these diseases, it may be some time before the pathways are sorted out to everyone's satisfaction.

Due to the microscopic, fragile features of pathogenic microorganisms, paleontological evidence was never seriously considered for solving these problems. However, recent discoveries in amber of fossil vectors carrying vertebrate pathogens have provided valuable information on the minimum ages, origins, and early hosts of malaria, leishmaniasis, and trypanosomiasis. These discoveries attest to the remarkable qualities of amber in preserving microorganisms through millennia.

All three diseases discussed here are caused by members of the ubiquitous group known as protozoa or protists. Most protozoa are free-living, but others have formed commensal or symbiotic associations with insect vectors that cause millions of human deaths annually. Over 30 species of protozoa are known to infect humans, and if roughly the same number infects the other approximately 5,000 mammalian species on earth, it can be estimated that 150,000 species of parasitic protozoa reside in mammals alone (Manwell 1961).

Relationships between protozoa and insects were certainly established quite early in the evolution of both groups, and over time, these dual teams co-evolved various types of vector relationships with vertebrates. Investigations in amber show that the three very successful examples of co-evolution between insects and protozoa presented here have existed for at least 100 million years.

In deciphering the life cycles of these fossil pathogenic protozoa, I have relied on the principle of behavioral fixity, which dictates that the lifestyle of fossil organisms will be similar to that of their present-day descendants at the specific, generic, and often family levels (Boucot 1990; Boucot and Poinar 2011). Since their origin hundreds of millions of years ago, protozoa have undergone a multitude of changes and modifications as new species arose in response to environmental changes, the extinction and appearance of new hosts, and a continual genetic turnover as they modified their surface coats in response to vertebrate immune reactions.

Some issues in the etiology of vector-borne diseases are relevant here. During epidemics, some individuals die from a disease while others become infected but survive for a prolonged period, all the while carrying the pathogen inside their bodies. These living germ factories or reservoir hosts maintain the disease in the population for extended periods. Hematophagous arthropods feeding on reservoir hosts acquire pathogens, and under the right set of parameters, they transmit them to different vertebrate groups. Such bloodsuckers are known as bridge vectors, and the resulting host-switching is referred to as a lateral or horizontal transfer. Bridge vectors can move infections from birds to mammals, from lizards to birds, or just from one mammalian group to another. Reservoir hosts, bridge vectors, and lateral transfers play crucial roles in the co-speciation of vector-borne diseases.

Malaria

Malaria is a disease caused by parasitic protozoans that initiate infection in the vertebrate host with microscopic sporozoites. There are many types of malarial organisms with a wide range of arthropod vectors. The more primitive malarias infect reptiles and birds and

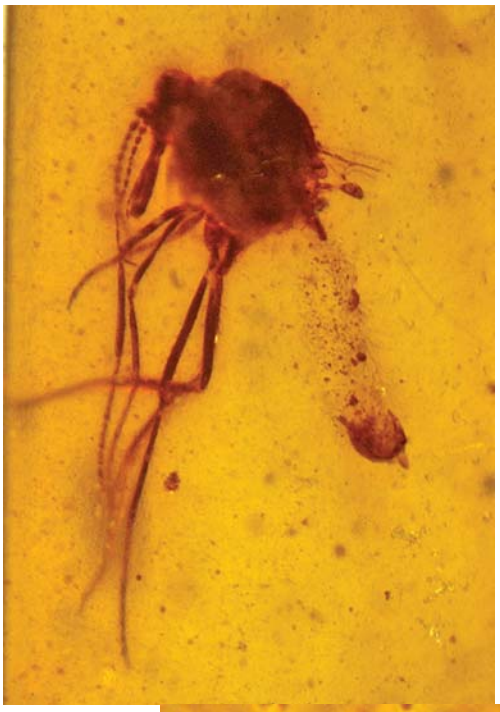

Fig. 1. A *Protoculicoides* sp. biting midge infected with the primitive malarial organism *Paleohaemoproteus burmacis* in Early Cretaceous Burmese amber.

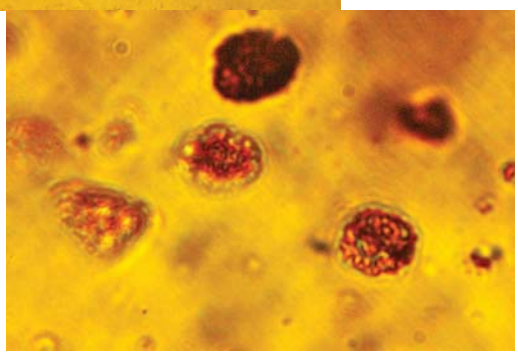

Fig. 2. Oocysts of *P. burmacis* developing in the body cavity of *Protoculicoides*.

are transmitted by biting midges, black flies, sand flies, and deerflies. One example of these is *Haemoproteus*, a group found in reptiles and birds and mostly vectored by biting midges of the genus *Culicoides*.

An examination of biting midges in Burmese amber revealed a specimen with a cleared abdomen that allowed an unobstructed view into its body cavity (Fig. 1). Inside were a number of developing oocysts (Fig. 2) and sporozoites (Fig. 3) of an archaic malarial parasite. This 100 million-year-old malaria, described as *Paleohaemoproteus burmanicus*, is an extinct species related to the extant *Haemoproteus* (Poinar and Telford 2005). The infected biting midge belonged to the extinct genus *Protoculicoides*, which is ancestral to the *Culicoides* that bite us today. Morphological features of *Protoculicoides* indicated that the vertebrate host was cold-blooded, and because this type of malaria commonly occurs in reptiles today, it is likely that the ancient hosts were reptiles. This remarkable find established a time, locality, and vector group for ancient malaria.

The ancestors of today's malaria-causing pathogens were probably free-living, amoeba-like organisms that at some point invaded the intestinal tract of insect larvae developing in the same habitat. Eventually, these symbiotic organisms entered the gut cells of their insect hosts, just as the protozoan parasite *Malameba* does in grasshoppers and crickets today (Ernst and Baker 1982). As the parasitic lifestyle co-evolved, the pathogens entered the body cavity of their insect hosts (Léger 1900). Somewhere along the evolutionary line, malaria-like forms appeared. Those species in blood-sucking insects

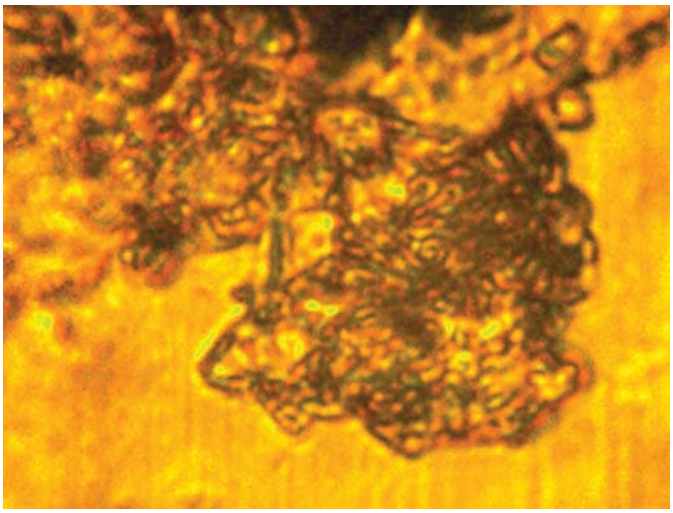

Fig. 3. Sporozoites of *P. burmacis* developing in the body cavity of *Protoculicoides*.

soon became dixenous (with two hosts in their life cycle) as their vertebrate hosts carried full-blown infections (Poinar and Telford 2005).

Plasmodium malaria

Probably the most advanced type of malaria is represented by members of the genus *Plasmodium*, where development of the oocyst occurs in the gut wall of mosquitoes and masses of sporozoites emerge from the oocyst to be transmitted back to vertebrates (Garnham 1966). The most notorious types of *Plasmodium* malaria are four species vectored by anopheline mosquitoes (*P. ovale, P. malariae, P. falciparum*, and *P. vivax*) that infect humans. Globally, 300–500 million new cases of human malaria occur each year, and over a million deaths result in Africa alone (Foster and Walker 2002). Before mosquito eradication procedures and antimalarial drugs, the disease was established in many temperate areas of the United States and was so severe in the 17th century that it hindered the colonization of the east coast from Massachusetts to Georgia. Even as late as 1934, outbreaks occurred in the U.S. that caused nearly 4,000 deaths that year and almost a million new cases the following year. By 1944, the death rate had dropped to about 600 (Hermes 1953), but because anopheline mosquitoes are widely distributed in North America, malaria has the potential to re-appear in the United States.

In *Plasmodium* malaria, the cycle begins when a mosquito acquires sexual stages, the gametocytes, from the victim's blood. The gametocytes fuse in the stomach of the mosquito, and the zygote develops into a motile worm-like body (ookinete) that penetrates through the insect's midgut and forms a minute cyst (oocyst) on the outer intestinal wall. The cyst grows and produces within it a number of microscopic spindle-shaped bodies called sporozoites. When the cyst bursts, the liberated sporozoites migrate through the vector's body cavity and collect in the salivary glands. At the next blood meal, the sporozoites are transferred in the mosquito's saliva into a new victim, where they initiate a number of asexual cycles and eventually end up in the blood, ready to be picked up by another mosquito (Garnham 1966).

The age, origin, and subsequent dispersal of *Plasmodium* malaria have been controversial topics, primarily due to the lack of fossil evidence. Various ages proposed for the origin of human malaria range from 15,000 to 8 million years (Pennisi 2001). Not all *Plasmodium*

malaria attacks humans or is carried by anopheline mosquitoes. When Ronald Ross deciphered the life cycle of malaria for the first time in 1901, he used a type of *Plasmodium* malaria carried by culicine mosquitoes that infected birds (Garnham 1966).

That type of *Plasmodium* malaria was discovered in a culicine mosquito (Fig. 4) in Dominican amber. The vector, *Culex malariager* (Poinar 2005a), contained gametes, an ookinete, oocysts (Fig. 5), and sporozoites (Fig. 6) of a malarial pathogen described as *Plasmodium dominicana* (Poinar 2005b). The large, pedunculated cysts of the fossil aligned it with a type of present-day bird malaria known as *Plasmodium juxtanucleare*. Aside from being the first fossil record of *Plasmodium* malaria, the discovery shows that the pathogen was established in the New World at least 15 million years ago, the minimum age suggested for Dominican amber. Others have provided evidence that some Dominican amber could be up 40 million years old (Schlee 1990).

Fig. 4. A culicine mosquito in Dominican amber. Extant culicines vector *Plasmodium* malaria to birds and reptiles.

Fig. 5. An oocyst of *Plasmodium dominicana* in the body cavity of its vector, *Culex malariager*, in Dominican amber.

This discovery raises the possibility that ancient bird malaria may have gradually evolved into a species that was laterally transferred to simians and eventually to humans when they entered the New World. Infected migratory birds could have spread the disease throughout Meso- and South America, as both culicine and anopheline mosquitoes were present in the mid-Tertiary to serve as vectors. The Dominican amber *Anopheles dominicanus* (Zavortink and Poinar 2000) (Fig.7) possibly acted as a bridge vector, transferring *P. dominicana* from birds to simians and humans. Culicine mosquitoes (Fig. 4) also had the potential to function as bridge vectors, as they presently transmit both bird and simian malaria (Hart and Williams 1933; Klein et al. 1988).

Support for such a theory is derived from molecular and laboratory studies that demonstrated a close relationship between bird and human malaria. One study showed that *P. falciparum* infecting humans had a recent avian progenitor and probably arose by lateral transfer from birds (Waters et al. 1991). In addition, avian malaria has been established in mammals (McGhee 1951) and can complete its development (forming mature sporozoites) in anopheline mosquitoes (Jeffrey 1944).

There is also an ongoing debate whether one or more species of human malaria originated in the Americas and was waiting to make the leap into *Homo sapiens* when they arrived, or whether all were introduced from the Old World (Africa, Asia, Europe) by Paleoamericans migrating across the Bering Strait 40,000 years ago, Spanish soldiers in the 16th century, or African slaves in the 17th century. Since all four species of human malaria are present worldwide today (Hart and Williams 1933), it is almost irresolvable when and where each species first appeared in human populations. However, while it is almost certain that some types of malaria arrived in the New World by the routes mentioned above, it is now apparent that a species of *Plasmodium* malaria was already established in the New World long before humans appeared.

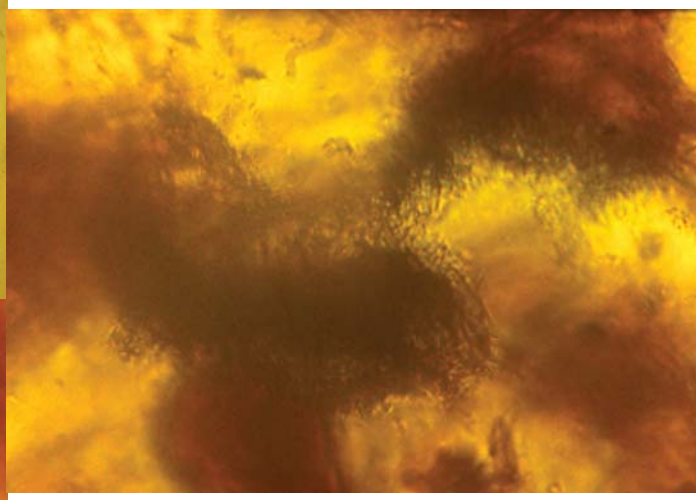

Fig. 6. Sporozoites of *Plasmodium dominicana* emerging from an oocyst in the body cavity of its vector, *Culex malariager,* in Dominican amber.

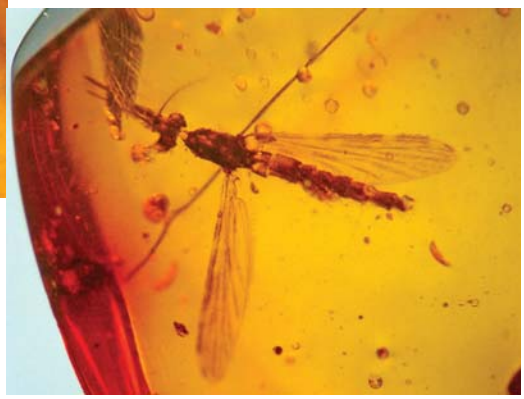

Fig. 7. The first New World fossil anopheline, *Anopheles dominicanus,* in Dominican amber. This species could have served as a bridge vector to transfer *Plasmodium dominicanium* from birds to primates.

When humans finally arrived in the New World, transfers of *P. dominicana* (or a close relative) from birds to simians probably had already occurred, and some of these new strains had subsequently mutated into the now widespread South American simian parasite *P.*

brasilianum. This species of simian malaria does not occur anywhere else in the world (Garnham 1966), and it likely evolved into the closely related human parasite *P. malariae*. Since either species can infect both simians and humans, it is doubtful that these two forms of malaria have been separate species for long (Garnham 1966).

There is historical evidence that malaria was established in the Americas before the Europeans arrived. When Spaniards first arrived in South America, they noticed that when Indians fell ill with fevers and chills, they drank infusions of cinchona bark to cure themselves (Garnham 1966). The natives had learned that some component (quinine) in the bark had anti-malarial properties. Some (see Rocco 2003) claim that other diseases could have caused these fevers, but Garnham (1966) pointed out that malaria is the only fever known to be susceptible to quinine. Others have presented the argument that the Indians did not know about *Cinchona* bark because malaria does not occur at heights where these trees grow (Rocco 2003). However, early botanical reports list *Cinchona* trees growing at elevations ranging from 2,300 to 9,000 feet (Fawcett 1906), which overlap with the altitudes at which malaria occurs. Herms (1953) reported malaria in Quito at 9,000 feet, in Mexico at 7,500 feet, and persistent malaria in California at about 5,500 feet.

Leishmaniasis

Most North Americans are unfamiliar with the human disease known as leishmaniasis, which is caused by several species of the protozoan *Leishmania* and transmitted by small biting flies known as sand flies or phlebotomine flies. The causal agent is a type of flagellated protozoan that shares its developmental cycle with two hosts (dixenous). Part of its development is in the gut of its insect vector, while the rest is completed in vertebrate blood cells. The malady is widely distributed throughout the world, with foci of infections in Asia, Southern Europe, Africa, the Middle East, and South America. It is estimated that in South America alone, some 15-20 million people are infected with leishmaniasis, with some 65 million at risk. Most of the victims are infected with cutaneous leishmaniasis that causes open sores on the skin. However, the infections can become internal and Kala Azar is a severe visceral form of leishmaniasis that enters the internal tissues. Kala Azar can reach epidemic proportions, and epizootics were responsible for 100,000 deaths in southern Sudan between 1989 and 1994 (Dedet 2002).

American military serving in Iraq and Afghanistan are well acquainted with sand fly-transmitted *Leishmania*. They have learned

Fig. 8. An extant sand fly (*Phlebotomus papatasi*) feeding on a human. This is one of the main vectors of *Leishmania major* in Africa. Photo by Dean Furman.

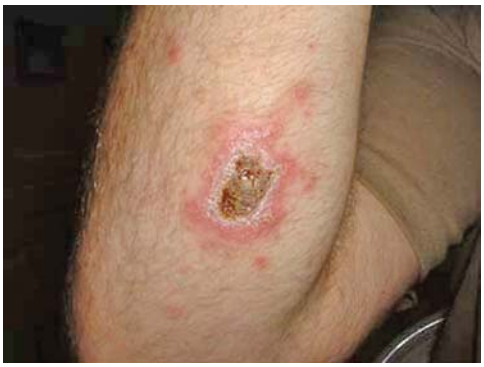

Fig. 9. Cutaneous leishmaniasis on a U.S. soldier at the Tallil Airbase in Iraq in 2003. Photo courtesy of Dr. Peter J. Weina, M.D., Walter Reed Army Institute of Research.

that the miniscule sand flies (Fig. 8) can pass through normal mosquito netting covering the tents. So far, over 750 U.S. military personnel have been infected in Iraq and subsequently hospitalized in the United States for diagnosis and treatment. Most were infected with the cutaneous form of the disease (Fig. 9), but a few acquired the potentially lethal visceral type (Berté 2005; Seppa 2004). *Leishmania* has become an even greater health risk since scientists discovered that it can work in concert with other diseases such as AIDS. The combined effect of human co-infections of *Leishmania* and HIV causes a serious new disease complex as the HIV virus weakens the immune system and allows the potentially lethal Kala Azar to enter the internal tissues (Dedet 2002).

Whether *Leishmania* arose in the Old World with reptiles as the original hosts or in the New World (the Americas) in rodents is a controversial and seemingly irresolvable topic (Kerr 1999; Noyes et al. 2000). However, a recent fossil has helped solve this riddle. An ancient form of *Leishmania* was discovered in a blood-laden sand fly

Fig. 10. The Early Cretaceous sand fly, *Palaeomyia burmitis*, vector of *Paleoleishmania proterus* in Burmese amber.

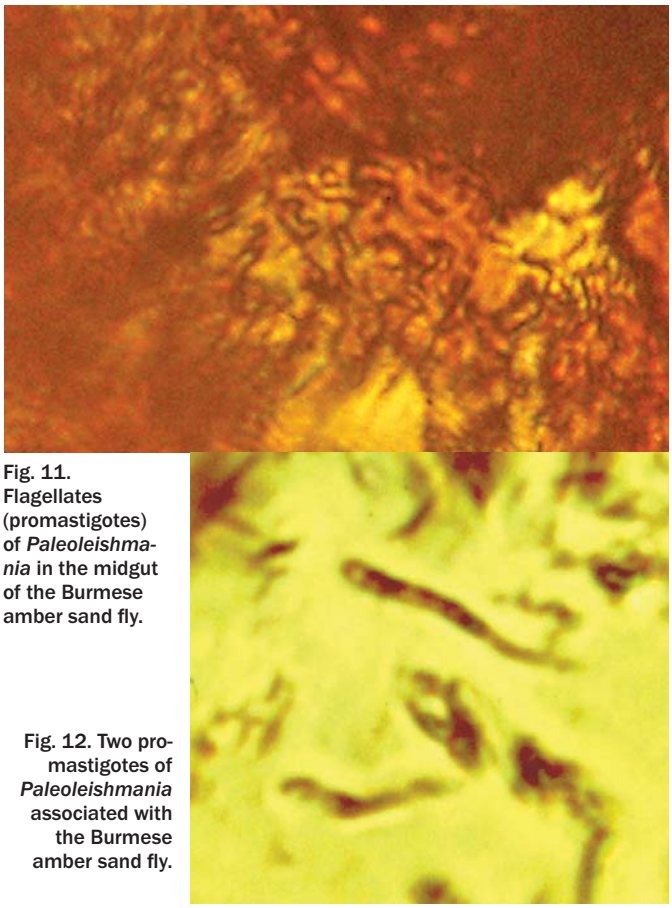

Fig. 11. Flagellates (promastigotes) of *Paleoleishmania* in the midgut of the Burmese amber sand fly.

Fig. 12. Two promastigotes of *Paleoleishmania* associated with the Burmese amber sand fly.

found in Cretaceous amber from Burma (Fig. 10). The mouthparts at the tip of the elongate proboscis of the sand fly *Palaeomyia burmitis* Poinar (2004) are similar to the piercing and sucking forms of present-day sand flies, showing that this 100 million-year-old specimen had the ability to imbibe blood from vertebrates (Poinar 2004). Comparison with the process of blood digestion in extant sand flies (Dolmatova 1942) indicated that *Palaeomyia* was in the early stages of this process when it became entrapped in tree resin.

Even more astonishingly, *Palaeomyia* carried the developmental stages of an ancient *Leishmania*, described as *Paleoleishmania proterus* Poinar and Poinar (2004a), in its alimentary tract. *Palaeomyia* contained minute round amastigotes in its proboscis, elongate flagellated forms known as promastigotes in its gut (Figs. 11,12), and procyclic stages (Fig. 13) in fluid that leaked out of its gut into the amber. Amastigotes are formed in vertebrate blood cells, showing that *Paleoleishmania* was dixenous, requiring both a sand fly and a vertebrate to complete its life cycle (Poinar and Poinar 2004a). The dual-host life cycle was further confirmed when *Leishmania*-infected reptilian blood cells (Fig. 14) were detected in the foregut of *Palaeomyia.* These infected blood cells were almost identical to lizard cells infected with *Leishmania* today (Poinar and Poinar 2004b). This spectacular fossil demonstrated that sand flies were transmitting leishmanial pathogens to reptiles 100 million years ago, lending support to the Old World–reptile hypothesis for leishmanial origins.

Exactly when *Leishmania* appeared in the New World is unknown, but the extinct sand fly *Lutzomyia adiketis* Poinar (2008a) (Fig. 15) was found in Dominican amber with promastigotes of *Paleoleishmania neotropicum* Poinar (2008a) in its foregut and midgut and amastigotes, promastigotes, and paramastigotes (Fig. 16) in its proboscis. The presence of amastigotes showed that *P. neotropicum* was digenetic, because in *Leishmania*, amastigotes are only formed in the vertebrate host.

It stands to reason that insect-transmitted diseases could not have arisen before the appearance of their respective vectors, but the first occurrence of vector groups is not always certain due to gaps in the fossil record. The earliest sand flies resembling modern forms occur in Lebanese amber ~130 million years ago (Poinar and Milki 2001), and that could be inferred as the earliest possible date for the origin of sand fly transmission of *Leishmania*.

How did sand flies originally acquire Leishmania? Some suggest that adult flies may have obtained flagellates while imbibing sap or nectar from plants infected with *Phytomonas*, a plant-parasitic trypanosome. (Sand flies are known to feed on plant sugars to increase their longevity.) Others (Baker 1965) suggest that vertebrates were the original hosts and became naturally infected when free-living flagellates invaded their guts and penetrated into their internal tissues. Sand flies would then have received their initial infections from parasitized vertebrates.

It is more likely that the free-living ancestors of *Leishmania* occurred in decaying organic matter and were ingested along with food items by developing sand fly larvae. The flagellates could have multiplied in the larval gut and then been carried through the pupal and into the adult stage. While many insects "void" their gut contents before entering the pupal stage, there are records of flagellates and bacteria being passed transtadially in sand flies and other insects.

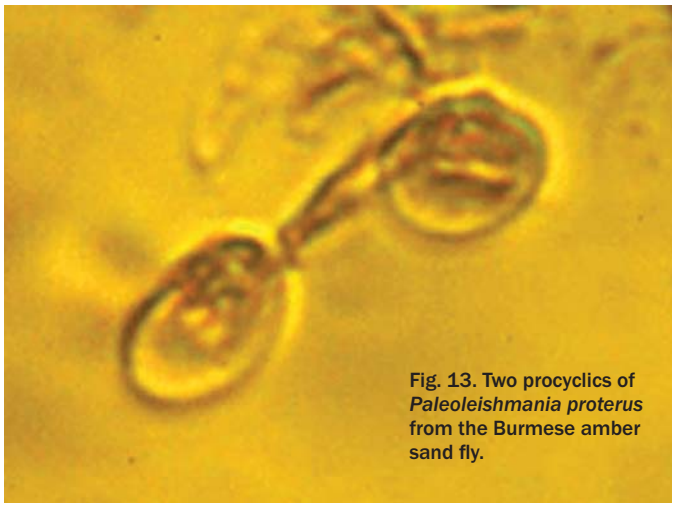

Fig. 13. Two procyclics of *Paleoleishmania proterus* from the Burmese amber sand fly.

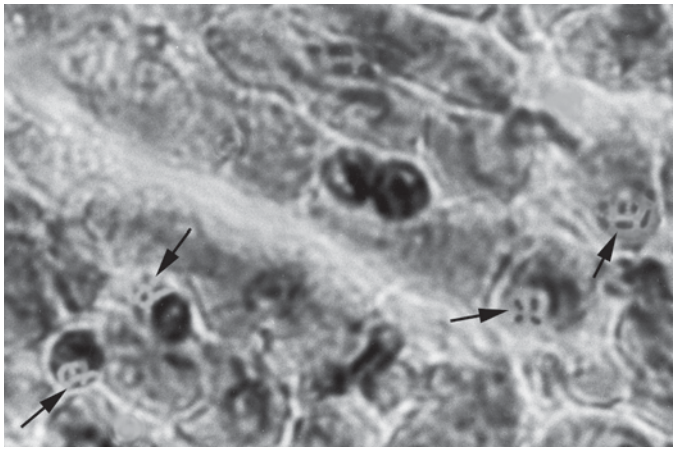

Fig. 14. Reptilian blood cells infected with *Paleoleishmania proterus* in the thoracic midgut of the Burmese amber sand fly. Arrows show amastigote stage of the parasite developing inside the vertebrate blood cells.

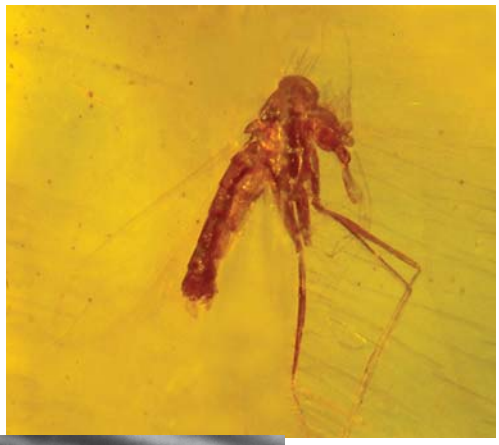

Fig. 15. The extinct sand fly *Lutzomyia adiketis*, a vector of *Paleoleishmania neotropicum*, in Dominican amber.

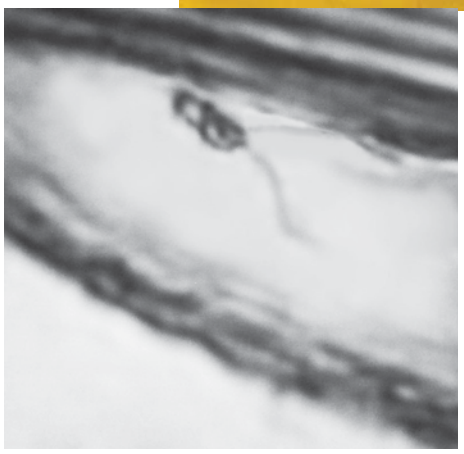

Fig. 16. Paramastigotes of *Paleoleishmania neotropicum* in the rostrum of *L. adiketis*.

Transtadial transmission of the trypanosomatid *Crithidia fasciculata* Leger was experimentally achieved in the mosquito *Culiseta incidens* (Thompson) (Clark et al. 1964), and bacteria were shown to be transmitted transtadially from larval to adult *Phlebotomus duboscqi* (Volf 2002). The trypanosomatid *Leptomonas ctenocephali* (Fantham) is carried through the pupal stage into the adult dog flea *Ctenocephalides canis* (Curtis), and when mealworm larvae were experimentally infected with *Leptomonas pyrrhocoris* L.& D., they carried the flagellates though the pupal and into the adult stage (Steinhaus 1949).

There is actual evidence for this hypothesis in Early Cretaceous amber. Two sand fly larvae in Burmese amber (Poinar et al. 2006) associated with the fruiting bodies of a coral mushroom in Burmese amber (Poinar and Brown 2003) had voided some of their intestinal contents, a behavior typical of mature sand fly larvae just before pupation (Lawyer and Perkins 2000) (Fig. 17). Associated with the fecal deposit and debris surrounding the larvae were numerous trypanosomatids. When a portion of the alimentary tract of one of the sand flies was exposed by further polishing of the amber, trypanosomatids were also found inside the gut of the sand fly larva (Poinar 2007) (Fig. 18). The great majority of flagellates resembled promastigotes, as they had kinetoplasts located in the anterior end. After establishing themselves in the gut of adult sand flies, the trypanosomatids would have been transferred to vertebrates during blood feeding. It probably took a period of time before trypanosomatids became established in vertebrates and were re-acquired by non-infected sand flies during feeding. An extraordinary degree of co-evolution seems to have occurred between the trypanosmatids and their insect and vertebrate hosts. The association between trypanosomatids and sand flies may have been established in even more primitive insect groups such as the Mesozoic scorpionflies (Mecoptera) from which the Diptera supposedly evolved, as supported by the trypanosomatids that occur in extant panorpid scorpionflies (Podlipaev et al. 2004a).

The establishment of the parasites in the vertebrate host and their subsequent re-infection of adult sand flies is undoubtedly a rare event and would only occur under ideal conditions. If the above scheme of flagellate–sand fly co-speciation is correct, different strains of trypanosomatids could have appeared at different localities and times over the past ~100 million years. The 100 million-year-old Burmese amber sand fly trypanosomatid *P. proterus* undoubtedly arose independently from *P. neotropicum*, which could well be the progenitor to one or more extant Neotropical *Leishmania* species.

American trypanosomiasis

There are other types of insects, aside from biting flies, capable of transmitting human diseases, and these include some assassin bugs. Assassin bugs comprise the family Reduviidae (Hemiptera), involving ~3,000 species of predatory insects, the great majority of which attack other invertebrates. However, a small fraction, placed in the subfamily Triatominae, exists on vertebrate blood (Lent and Wygodzinsky 1979) (Fig. 19). Within this triatomine group are vectors of the notorious Chagas' disease found in Central and South America. Chagas' disease is also known as American trypanosomiasis in order to separate this malady from the well-known tsetse fly-transmitted African trypanosomiasis that causes sleeping sickness.

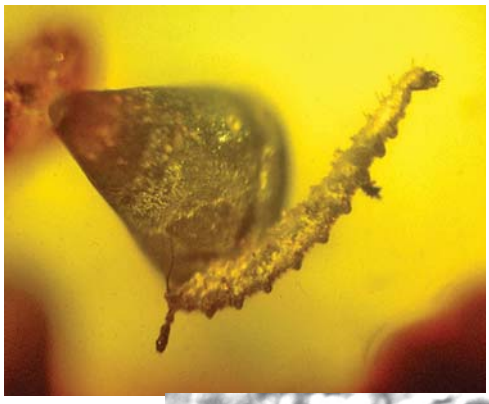

Fig. 17. A sand fly larva adjacent to its apparent food source, *Palaeoclavaria burmitis*, a coral mushroom, in Early Cretaceous Burmese amber.

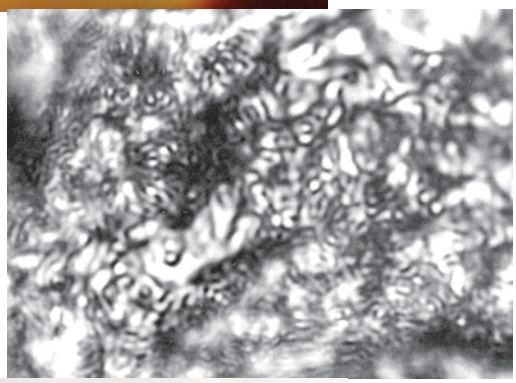

Fig. 18. Trypanosomes in the gut of the sand fly larva shown in Fig. 17.

Fig. 19. Feeding behavior of the triatomid bug, *Dipetalogaster* sp.

The large triatomine assassin bugs remain hidden in cracks or roof thatching during the day, but emerge at night to feed. They frequently bite the face of their victim near the eyes or mouth, giving rise to their common name, "kissing bugs." The bites themselves can result in severe reactions in sensitive individuals, causing nausea, rapid breathing, high pulse rate, and heart palpitations. Even more serious are what the bugs may be carrying in their bodies at the time of feeding: namely, flagellated protozoa known as *Trypanosoma cruzi*, the causal agent of Chagas' disease (Hoare 1972; Lent and Wygodzinsky 1979).

The transmission of Chagas' disease is different from that of most vector-borne pathogens. The infective stages occur in the insect's hindgut and reach the victim when they are defecated on the skin during and just after feeding. Each fecal droplet contains thousands of thrashing trypanosomes that are inoculated into the still-open wound when the victim responds to the irritation by scratching or rubbing. The flagellates can even enter through the eyes or mucous membranes lining the nose and mouth.

If not treated, the infection enters a chronic stage and the parasites invade the heart muscle, which may result in myocarditis, heart failure, and death. In his journals on the voyage of the *Beagle*, Charles Darwin (1860) recorded being bitten by these bugs. The famous Israeli parasitologist Saul Adler felt that Darwin's health problems later in life were the result of a chronic infection of Chagas' disease acquired during these insect attacks (Adler 1959).

The origin of kissing bug–trypanosome associations in the Americas is controversial, with one school suggesting that triatomine bugs evolved fairly recently from predatory ancestors, while others believe that their blood-sucking habits are much more ancient. There was little hope that fossils would solve this question, especially those in amber, since these bugs are quite large and large insects can usually escape from the sticky resin. While fossil triatomines do occur in amber, they are quite rare and I know of only two specimens: one undescribed species (Fig. 20) and *Triatoma dominicana* (Fig. 21), both in Dominican amber.

Fig. 20. An extremely rare adult female triatomine bug in Dominican amber.

Fig. 21. The extinct *Triatoma dominicana*, a vector of *Trypanosoma antiquus*, in Dominican amber.

The aforementioned debate was partly settled by the discovery of *Trypanosoma antiquus* (Fig. 22) in a fecal droplet that was adjacent to *Triatoma dominicana* in the amber (Poinar 2005c). The likely vertebrate host of this ancient vector–trypanosome duo was deduced from several bat hairs preserved adjacent to the bug.

Trypanosomes in the subgenus *Schizotrypanum,* to which both *T. antiquus* and the causal agent of Chagas' disease (*T. cruzi*) belong, have been reported from diverse genera of bats (Hoare 1972). In the Neotropics, triatomines are the suspected vectors of all bat trypanosomes (Marinkelle 1982; Ryckman 1986). Bat trypanosomes are morphologically indistinguishable from the human-infecting *T. cruzi*, and all invade their vertebrate hosts through fecal contamination (Lent and Wygodzinsky 1979), as was probably the case with *T. antiquus.*

The fossil not only shows that triatomine–trypanosome vector associations evolved millions of years ago, but also that bats were

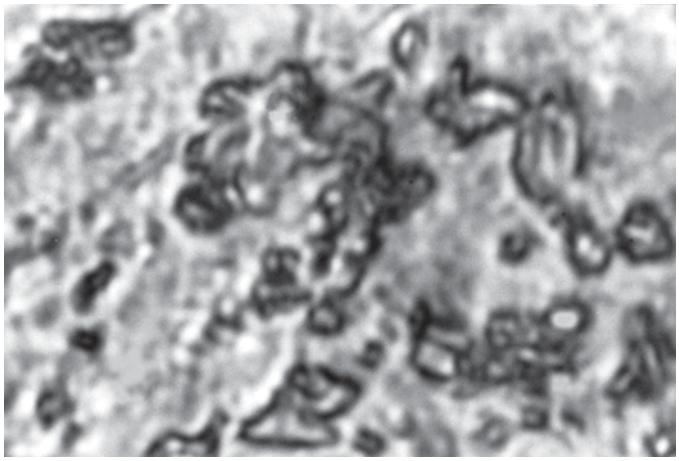

Fig. 22. Flagellates of *Trypanosoma antiquus* in a fecal droplet from *Triatoma dominicana* in Dominican amber.

ancient hosts for both kissing bugs and *Schizotrypanum* trypanosomes, including the human species that causes Chagas' disease.

Turning to the Old World, when was an insect vector–trypanosome association first established? While *Trypanosoma* in Africa is vectored by tsetse flies today (Fig. 23), a rare piece of amber shows that other insects vectored trypanosomes long before tsetse flies evolved. In a piece of Early Cretaceous amber from Burma, a biting midge, *Leptoconops nosopheris* Poinar (2008b), had taken a blood meal shortly before becoming entrapped in resin (Fig. 24). This female contained stages of the digenetic trypanosome *Paleotrypanosoma burmanicus* Poinar (2008b) in its gut and salivary glands. Some of the trypanosomatids had collected in a salivary secretion that had been released from the tip of the proboscis of the biting midge (Fig. 25). Today, females of the genus *Leptoconops* feed on reptiles, birds, and mammals (Auezova et al.1990; Mullens et al. 1997; Mullen 2002). The genus extends back ~130 million years, with species found in Lebanese amber (Poinar and Milki 2001). There are 134 extant and (including the present specimen) 14 fossil species, five of which occur in Burmese amber (Szadziewski and Poinar 2005). However, this is the first species of *Leptoconops*, extant or extinct, associated with trypanosomes. Both digenetic and monogenetic trypanosomes have been reported from other extant biting midges (Sharp 1928; Kremer et al. 1961; Baker 1976; Podlipaev et al. 2004b; Svobodová et al. 2007), and a monogenetic species is known from a biting midge in Burmese amber (Poinar and Poinar 2005). One extant trypanosome

Fig. 23. Blood-filled abdomen of the extant tsetse fly (*Glossina morsitans*), carrier of *Trypanosoma brucei*, the cause of human sleeping sickness in sub-Saharan Africa.

Fig. 24. Blood-filled Early Cretaceous biting midge, *Leptoconops nosopheris*, a vector of the trypanosome *Paleotrypanosoma burmanicus*.

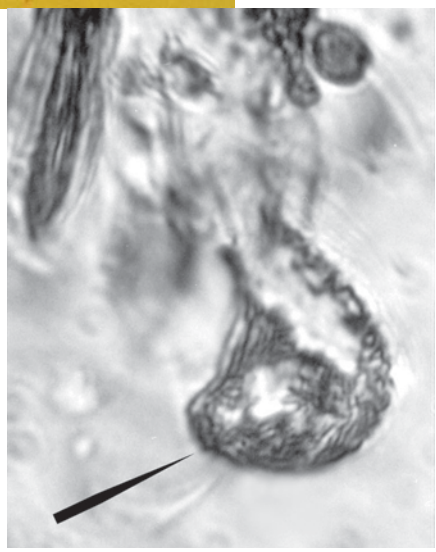

Fig. 25. Trypanosomatids of *Paleotrypanosoma burmanicus* in the salivary secretion (pointer) of *Leptoconops nosopheris* in Early Cretaceous Burmese amber.

isolated from a biting midge was closely related to a strain isolated from Egyptian rats and could have been transferred to the vertebrate by the insect during blood feeding (Podlipaev et al. 2004a).

It is possible that biting midges were the ancestral vectors of the Old World *Trypanosoma* lineage. Later, these flagellates could have been acquired from infected hosts by other biting Diptera, which acted as bridge vectors and transferred the parasites to new vertebrate hosts.

Conclusion

The previous cases show how insect vectors in amber can supply valuable information not only on the minimum dates when vertebrate pathogens were being transmitted, but also in what part of the world they occurred. Due to the fragility of microorganisms, unless they result in some structural deformity in vertebrates that can survive petrifaction, the actual existence of pathogenic forms is extremely difficult to ascertain with any type of fossilization process other than amber preservation. It takes time and patience to search through thousands of amber pieces to detect and identify these ancient organisms, and then to establish co-evolutionary relationships with their vectors. Nevertheless, at the end of the day, the excitement of discovering the remains of an ancient vertebrate pathogen in amber is the reward in itself.

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