

Review Article

Evolutionary History of Terrestrial Pathogens and Endoparasites as Revealed in Fossils and Subfossils

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Received 9 February 2014; Revised 29 April 2014; Accepted 2 May 2014; Published 12 June 2014

Academic Editor: Renfu Shao

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The present work uses fossils and subfossils to decipher the origin and evolution of terrestrial pathogens and endoparasites. Fossils, as interpreted by morphology or specific features of their hosts, furnish minimum dates for the origin of infectious agents, coevolution with hosts, and geographical locations. Subfossils, those that can be C^{14} dated (roughly under 50,000 years) and are identified by morphology as well as molecular and immunological techniques, provide time periods when humans became infected with various diseases. The pathogen groups surveyed include viruses, bacteria, protozoa, fungi, and select multicellular endoparasites including nematodes, trematodes, cestodes, and insect parasitoids in the terrestrial environment.

1. Introduction

The world is filled with pathogens that have been around for millions of years, yet we know very little about their origins and evolution. The present work uses fossils and subfossils to decipher the origin and evolution of terrestrial pathogens and endoparasites. Fossils furnish minimum dates for the origin of infectious agents, coevolution with hosts, and geographical locations. Subfossil remains, those that can be C^{14} dated (roughly under 50,000 years), provide time periods when humans became infected with various diseases. The detection of fossil infectious agents is based on morphology or specific features of their hosts while molecular and immunological techniques can be used as well in searching for subfossil pathogens and parasites.

Arthropods in amber that were vectoring vertebrate pathogens have provided unique information on the origin and evolution of human and animal diseases [1]. Such detailed preservation is due to the instant death of the vector, its rapid desiccation, and impregnation by natural fixatives. Dates for the various amber sites mentioned in the present work are Dominican, 20–15 mya based on foraminifera and 45–30 mya based on coccoliths; Mexican, 22–26 mya; Baltic, 40–50 mya; Myanmar (Burmese), 97–110 mya; and Lebanese, 130–135 mya.

The pathogen groups surveyed include viruses, bacteria, protozoa, and fungi in the terrestrial environment. Also included are multicellular endoparasites such as nematodes, trematodes, cestodes, and select insect parasitoids. Examples are included when both the pathogen/parasite and host occur together as determined by morphological, molecular, or immunological evidence. Reports of disease in the fossil record based on host signs have been summarized previously [2–4].

2. Pathogens

2.1. Viruses. Viruses are probably quite ancient and may have existed some 4 billion years ago with bacteria as hosts. The nearly 2000 species of viruses infecting plants and animals today are probably a conservative estimate of their diversity [5]. All estimates on the evolutionary history of viruses are based on molecular analyses since fossils of such small, fragile structures are almost nonexistent. Evidence of some viruses has been found in subfossil remains based on DNA analysis [4] but the only fossil evidence of infectious diseases caused by viruses associated with their hosts is insect polyhedrosis, polynida, and iridoviruses. The polyhedra of nuclear polyhedrosis viruses (Baculoviridae) and cytoplasmic polyhedrosis

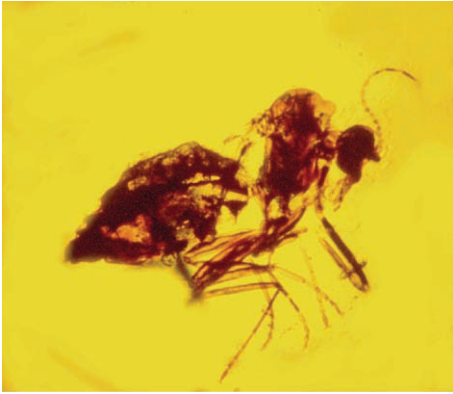


FIGURE 1: Early Cretaceous Myanmar amber biting midge (*Proto-culicoides* sp.; Diptera: Ceratopogonidae) that contains polyhedra of a cytoplasmic polyhedrosis virus in its midgut cells.

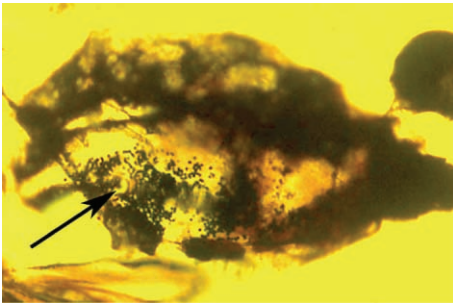


FIGURE 2: Group of polyhedra (arrow) of a cytoplasmic polyhedrosis virus in the midgut cells of the Early Cretaceous biting midge in Figure 1.

viruses (Reoviridae) are just large enough to be detected with the light microscope. Based on the size of the polyhedra, the particular organs in which they occur, and the type of insect hosts, it was possible to identify a cytoplasmic polyhedrosis virus infecting a biting midge (Figures 1, 2, and 3) and a nuclear polyhedrosis virus infection in a sand fly (Figures 4 and 5), both in Early Cretaceous amber from Myanmar [6]. These records provide a minimum date for the origin of the Baculoviridae and Reoviridae and suggest that some human arboviruses could have evolved first as pathogens of bloodsucking flies.

A specialized group of viruses that are carried by parasitic wasps of the families Braconidae and Ichneumonidae are the Polydnaviridae. Genomes of polydnaviruses are integrated into the wasp genome and virus replication occurs in the reproductive system of the female wasp. During oviposition, the virus is introduced along with the wasp eggs into the body of potential hosts. The virus replicates in the host and affects the host immune system by preventing it from encapsulating and destroying the wasp eggs. The transfer of these virus particles from wasp to host was first observed in 1975 with the ichneumonid, *Casinaria infesta*, and its pluteid moth host [7] and in 1976 with the braconid *Phanerotoma flavitestacea* and its pyralid moth host, *Paramyelois transitella* [8]. Since that time, the field has expanded and the polydnaviruses are

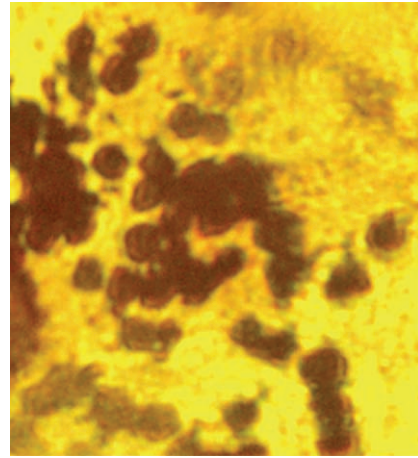


FIGURE 3: Detail of polyhedra of a cytoplasmic polyhedrosis virus in the midgut cells of an Early Cretaceous biting midge in Figure 1.

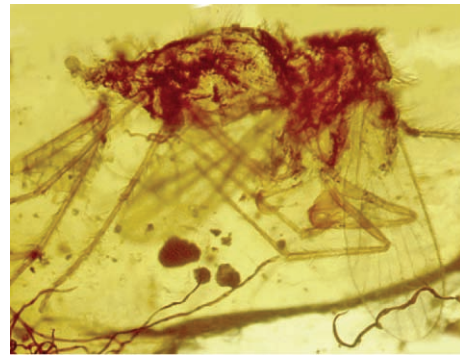


FIGURE 4: An Early Cretaceous Myanmar amber sand fly (Diptera: Psychodidae) infected with a nuclear polyhedrosis virus.

now included in the new discipline of Paleovirology, which infers viral evolution from host genome sequence data [9, 10].

Presently the family Polydnaviridae contains two genera. The genus *Bracovirus* occurs in the calyx cells and fluid of the female wasps of the family Braconidae and the genus *Ichnovirus* is associated with female wasps of the family Ichneumonidae. Because of the close association between the virus and members of these two families, one can assume that these viruses existed in ancient wasps of these lineages, such as the fossil braconid and ichneumonid wasps depicted in Dominican amber (Figures 6 and 7) and even in the primitive braconid wasp in 100 million year old amber from Myanmar (Figure 8).

A group of invertebrate pathogenic viruses whose presence often can be determined by color changes of the host are the iridoviruses. These double-stranded DNA viruses are icosahedral and hexagonal in cross section. The isopod iridescent virus infects terrestrial pillbugs and sowbugs and in the later stages of infection turns normal brown hosts to various shades of violet, blue, and purple [11]. In fact, an isopod thus colored can be assured of having an iridoviral infection. The bluish color is the result of paracrystalline arrays of viral particles (virions) filling the epidermal and

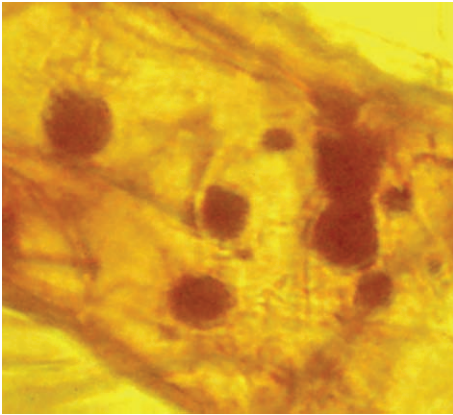


FIGURE 5: Detail of polyhedra of a nuclear polyhedrosis virus in the Early Cretaceous sand fly in Figure 4.



FIGURE 6: A female braconid wasp (Hymenoptera: Braconidae) in Dominican amber that is probably carrying the genome of a *Bracovirus* in its reproductive system.



FIGURE 7: A female ichneumonid wasp (Hymenoptera: Ichneumonidae) in Dominican amber that is probably carrying the genome of an *Ichnovirus* in its reproductive system.



FIGURE 8: A primitive braconid wasp (Hymenoptera: Braconidae) in Early Cretaceous Myanmar amber that could be carrying the genome of a polydnavirus in its reproductive system.



FIGURE 9: A bluish tinted terrestrial isopod in Early Cretaceous Myanmar amber that could be infected with the isopod iridescent virus.

other cells of the isopod that is eventually killed by the infection. An isopod in Cretaceous amber from Myanmar that has iridescent blue areas on its body is assumed to be infected with an ancient strain of the isopod iridescent virus (Figure 9). This is another example of identifying fossil viruses using indirect, but reliable evidence derived from our present knowledge.

2.2. Bacteria. Bacteria are ubiquitous and there are numerous reports of fossil associations between putative bacteria and various animals and plants [2, 3]. However, most of these reports refer to signs indicating a possible bacterial infection and actual bacteria are absent. One example is the dark areas on the body of the Burgess Shale onychophoran, *Aysheaia pedunculata*, that are thought to represent bacterial infections [12]. Other examples of indirect presence of bacteria are fossil fish with luminescent organs similar to those on their present day descendants that are operated by luminescent bacteria [3] or insects producing flatus in fossilized resin [13].

Filamentous cells occurring in the marine environment some 3.6 billion years ago represent some of the earliest known bacteria [14]. When terrestrial bacterial first appeared and formed associations with animals and plants is unknown since fossil bacteria are quite difficult to detect. Their small size and delicate structure (except for spores) are not prone to fossilization. Evidence of the early evolution of some specific



FIGURE 10: An Early Cretaceous Myanmar amber entomopathogenic nematode, *Proheterorhabditis burmanicus* (arrow), adjacent to its beetle host.

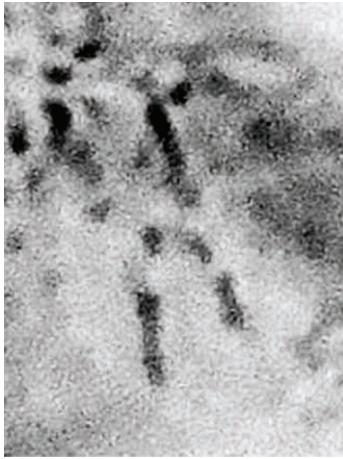


FIGURE 11: Bacterial cells of *Photorhabdus* inside the Early Cretaceous Myanmar amber beetle shown in Figure 10.

lineages of pathogenic bacteria has appeared in fossilized resin.

The oldest known definite fossil disease-producing bacteria are associated with an entomopathogenic nematode of the *Heterorhabditis* clade in Early Cretaceous amber [15]. Several infective stages of the insect pathogenic nematode, *Proheterorhabditis burmanicus*, were preserved as they emerged from their beetle host in Myanmar amber (Figure 10). Extant members of the *Heterorhabditis* clade carry and release spores of the luminescent bacterium, *Photorhabdus luminescens*, into the body cavity of insects. The bacteria multiply and kill the insects within 24 hours. The nematodes feed on the combined bacteria host breakdown tissues and the newly formed infective stage juveniles carry these bacterial cells into a new host. Bacterial cells in the fossil beetle (Figure 11) adjacent to the nematode resembled those of extant *Photorhabdus* spp. and are presumed to have been introduced into the beetle by *Proheterorhabditis burmanicus*.

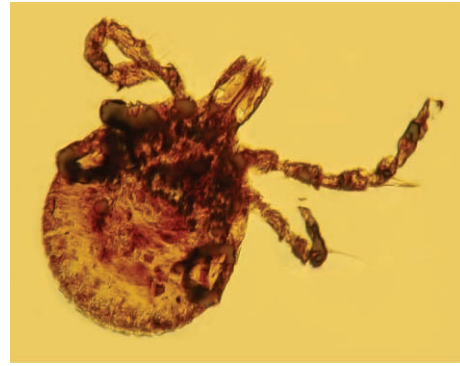


FIGURE 12: Larval *Amblyomma* hard tick in Dominican amber.

Ticks (Figure 12) carry a number of bacterial pathogens. One of special interest is the spirochete, *Borrelia burgdorferi*, the causal agent of Lyme disease. An ancient spirochete with characters of *Borrelia* was found in the midgut and hemocoel of a larval hard tick belonging to the genus *Amblyomma* (Arachnida: Ixodidae) in Dominican amber (Figures 13 and 14) [16]. The presence of spirochetes in the hemocoel indicated that the tick was a competent vector since spirochetes do not enter the hemocoel of incompetent vectors [17]. Vertebrate hosts of extant *Amblyomma* ticks are reptiles, birds, and mammals and remains of all three of these groups occur in Dominican amber [18]. This discovery shows how ancient some lineages of tick-vectored pathogens can be. It is likely that genetic material of this fossil spirochete contributed to the genome of extant *Borrelia* lineages, including those species that infect humans. The earliest record of Lyme disease was recently reported from the Tyrolean Iceman, a 5,300-year-old mummy discovered in 1991 in the Italian Alps. During a genomic analysis of the Iceman, sequences corresponding to ~60% of the genome of *Borrelia burgdorferi* indicated that the Iceman suffered from Lyme borreliosis [19].

The fossil record of hard ticks extends back to the Early Cretaceous [20, 21]. This sets a time period when these arachnids could have acquired spirochetes, followed by a period of coevolution that established the vertebrate cycle and increased vector competency. The origin of Borreliosis is a mystery. It is likely that *Borrelia* began as a free-living spirochete that could have made contact with tick eggs deposited in the soil. However, so little is known about interactions of *Borrelia* sp. with present day ticks that this scenario is difficult to prove. Yet if *Borrelia* began as a tick parasite, it could have been passed from mother to offspring by vertical or transovarial transmission (TOT). The spirochetes may have initially served as symbiotes, since TOT is normally how symbiotic microorganisms are passed in hosts. A later evolutionary step would be entering the salivary glands where they could be transferred to vertebrates.

An example of this evolutionary scenario is the establishment and transfer of rickettsial organisms in arthropods [22]. Rickettsias occur in many locations inside the alimentary tract and body tissues of a wide range of animals [23, 24]. Some symbiotic species, like *Wolbachia*, are beneficial and assist in the digestion of blood meals, while other rickettsias

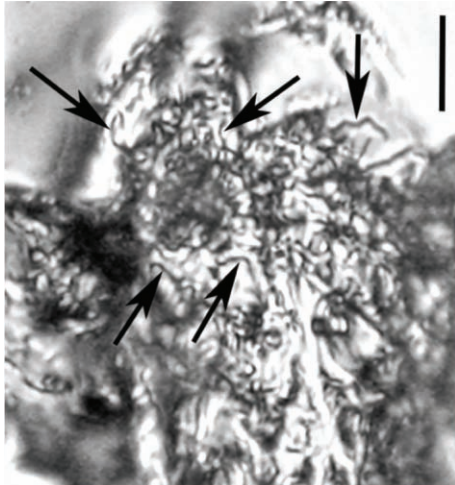


FIGURE 13: Spirochetes (arrows) within the cheliceral sheath and surrounding the hypostome of a larval *Amblyomma* tick in Dominican amber similar to the one shown in Figure 12. Scale bar = 16 μm .

are pathogenic to both arthropod vectors and their vertebrate hosts. Finding evidence of rickettsias in the fossil record is difficult but a recent study showed various stages of a rickettsial organism in the Early Cretaceous Myanmar amber hard tick, *Cornupalpatum burmanicum* (Figure 15). The rickettsial cells occurred in the remains of gastric ceca (Figure 16). Cocci, diplococci, and short, rod shaped cells borne singly or in short chains were dividing by binary fission. The rods ranged from 0.4 to 0.8 μm in width by 1.3–1.7 μm in length. In some areas, ovoid cells surrounded by an apparent mucoprotein, slime layer (haloes), were present. Such “halo” cells are known to occur in rickettsial populations and are considered to represent infective stages of the disease process [22, 23]. Since the cells were developing in the gastric cecal cells, the fossil could be related to any one of the many species in the families of Rickettsiaceae or Anaplasmataceae [17, 23]. Ticks are known to vector a range of rickettsial pathogens, including Q-fever, tick typhus, and Boutonneuse fever, some of which cause diseases in reptiles [24], which makes dinosaurs possible hosts [25].

Insects relying solely on blood meals throughout their entire development often harbor symbiotic microorganisms [26]. While they can be considered parasites since they develop on a combination of the blood meal and digestive secretions from the insect, they may supply essential elements to their host, since blood is considered a nutritionally inadequate diet deficient in B vitamins. Nocardiform bacteria such as *Rhodococcus* assimilate proteins and carbohydrates in the lumen of the alimentary tract of blood-sucking triatomine bugs [27]. Species of this genus occur in soil and water as well as in the lumen of insect alimentary tracts. Past evidence of this clade was discovered when cells of *Paleorhodococcus dominicanus* were found in a fecal droplet adjacent to the triatome bug, *Triatoma dominicana*, in Dominican amber [28, 29]. The fossil species consisted of numerous coccoid elements with associated mycelial fragments [29] (Figure 18).

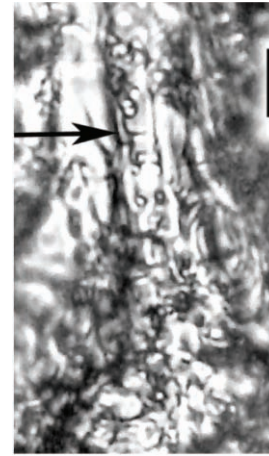


FIGURE 14: Spirochetes in the pharynx (arrow) of a larval *Amblyomma* tick in Dominican amber similar to the one shown in Figure 12. Scale bar = 13 μm .

While *P. dominicanus* could have been beneficial if it was assisting in the breakdown of blood components, it could also have been infectious since some species of *Rhodococcus* are pathogens, such as *R. equi* that infects horses.

A *Bacillus* sporangium inside of the body of the mycetophagous nematode, *Oligaphelenchoides atrebora* in Mexican amber (Figure 19), could indicate the presence of an infectious disease in the entire nematode population [30]. *Bacillus* sp. are known to infect extant nematodes [31] as well as insects and humans. However, fossil evidence of such infections is rare, even though the ancient presence of *B. sphaericus*, *B. subtilis*, and *B. circulans* was found in amber, sedimentary rocks, and salt deposits [32].

One of the most studied bacterial diseases is the plague, which has killed countless humans throughout history. Plague is caused by a small, pleomorphic bacterium known as *Yersinia pestis* that is transmitted by fleas. This pathogenic strain probably evolved from one of the free-living species of *Yersinia* that occur in soil, fresh water, foods, fish, and various animals [33]. The chances of finding fossil cells of *Y. pestis* are extremely slim. Some bacterial cells attached to the proboscis of a fossil *Rhopalopsyllus* flea in Dominican amber (Figure 20) have the morphological features of *Yersinia* (Figure 21) although it is not possible to confirm their identity. However, the extant *Rhopalopsyllus cavicola* is one of 125 species of fleas capable of transmitting *Y. pestis*, in this case to cavy rodents in the American tropics [34, 35].

Sophisticated molecular and immunological methods can now be used not only to identify pathogens, but to compare various strains that appeared throughout history. One of the best sources of ancient DNA for such studies is roots and crowns of teeth from the victims of various epidemics (Figure 22). These were used to characterize strains of *Y. pestis* that were responsible for the Black Death that struck Europe in the 14th to 17th centuries [36].

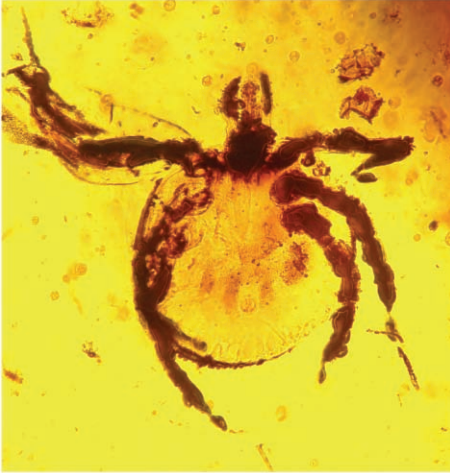


FIGURE 15: Early Cretaceous Myanmar amber larval hard tick, *Cornupalpatum burmanicum* that contains rickettsial organisms.

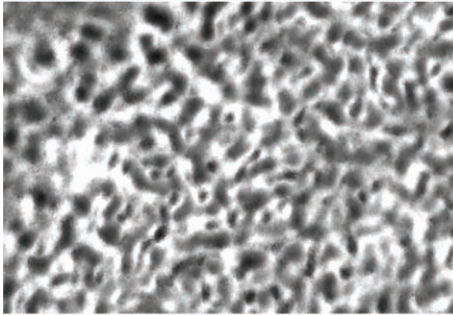


FIGURE 16: Short rods and cocci of a rickettsial organism in a gastric ceca of the Early Cretaceous larval hard tick shown in Figure 15.

Past cholera epidemics also resulted in high mortality rates (Figure 23). Using a preserved intestinal portion from a victim of the 1849 cholera outbreak in Philadelphia, molecular techniques were used to reconstruct the genome of the causative organism, *Vibrio cholerae* [37].

Teeth are also a good source of decay-causing bacteria. One report characterizes filamentous actinomycetes in a tooth from an Eocene rabbit in Montana [38], thus documenting the earliest record of tooth infection in placental mammals.

2.3. Protozoa. Protozoa, with some 120,000 described species, are an ancient group with a fossil record extending back to the Archean Eon, some 2 billion years ago. Protozoa probably originated in the sea and soon afterwards formed symbiotic associations with terrestrial life forms. Eventually, they entered the lumen of the alimentary tract of invertebrates and developed on partially digested food. Some of these became permanent residents in the guts of various insects such as termites, where the protozoa served a mutualistic role of digesting cellulose. Other protozoans invaded the internal organs of their hosts and became parasites. During their evolution, some became

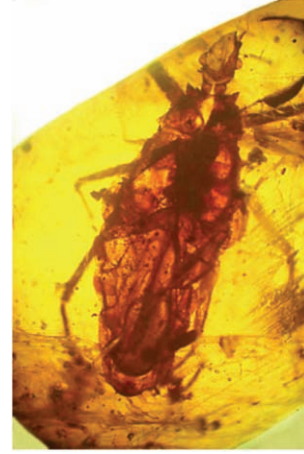


FIGURE 17: A Kissing bug, *Triatoma dominicana*, in Dominican amber with an actinobacteria infection.

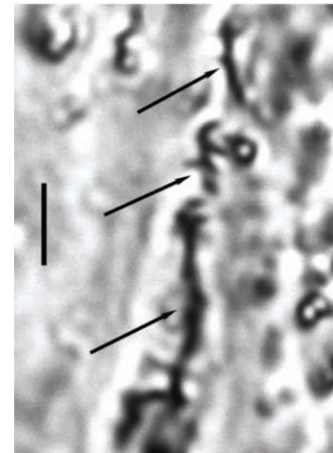


FIGURE 18: Filaments of the actinobacteria, *Paleorhodococcus dominicana* (arrows) in an anal deposit of the Dominican amber Kissing bug shown in Figure 17. Bar = 7 μm .

closely associated with blood-sucking arthropods, which transported them into vertebrates where they became established as true parasites with the potential of causing disease. A few invaded the tissues of plants but the present diversity of plant parasitic protozoa is quite low.

Today a number of arthropods vector parasitic protozoa and early lineages of some of these became entrapped in amber, thus providing clues to the evolution of human diseases. Two basic types of development evolved between arthropod vectors and their protozoan parasites. The first is where the protozoans only develop in the gut lumen of the vector. Examples are members of the trypanosome lineage that include *Leishmania* vectored by sand flies. The second is where the protozoans penetrate the gut wall and undergo development in the body cavity of the vector. Examples are the various malarial pathogens vectored by several arthropods, especially mosquitoes.

The first fossil protozoan pathogen to be discovered was one of the former types that were in the gut lumen

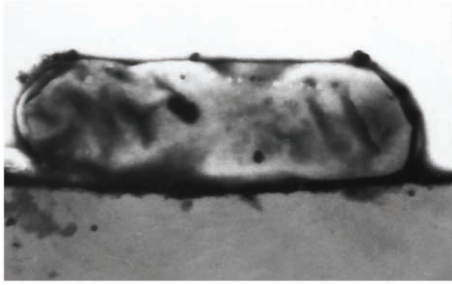


FIGURE 19: A *Bacillus* sporangium developing inside the body cavity of the mycetophagous nematode, *Oligaphelenchoides atrebora*, in Mexican amber.



FIGURE 20: A flea (*Rhopalopsyllus* sp.) in Dominican amber.

of a phlebotomine sand fly entrapped in Early Cretaceous Myanmar amber (Figure 24) [39]. In the midgut lumen of the sand fly, *Palaomyia burmitis*, were various stages of the leishmanial parasite, *Paleoleishmania proterus* [40] (Figure 25). Aside from normal promastigotes, elongate nectomonad promastigotes (Figure 26) and short procyclic promastigotes (Figure 27) were also present. Vertebrate infective amastigotes occurred in the proboscis of the fossil sand fly (Figure 28) and a view into the thoracic lumen of the fossil sand fly revealed nucleated vertebrate blood cells. Some of these cells contained developing amastigotes (Figure 29), obviously acquired during a recent blood meal. Based on morphological characteristics of the blood cells, the vertebrate host was considered to be a reptile and *Paleoleishmania proterus* was thought to be an ancestor of the genus *Sauroleishmania* that infects lizards and snakes today [41].

The life cycle of *Paleoleishmania proterus* was probably similar to that of leishmanial parasites vectored today by sand flies [42]. Amastigotes were acquired from the blood of a vertebrate during feeding. These developed into short ovoid procyclic forms that then formed elongate nectomonad stages in the lumen of the alimentary tract of the sand fly. Metacyclic (paramastigote) forms were transferred back into a vertebrate during blood-feeding.

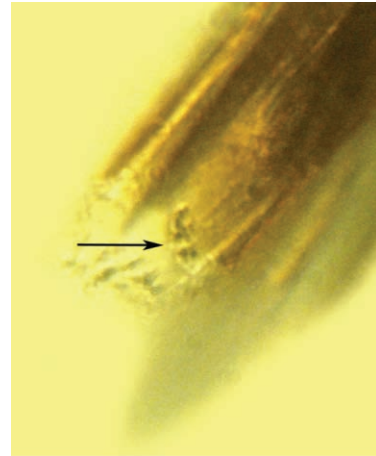


FIGURE 21: Bacterial cells (arrow) attached to the tip of the proboscis of the Dominican amber flea shown in Figure 20.



FIGURE 22: Human teeth from a victim of the plague discovered in a 16th century burial pit in Tuscany. Such teeth are the “treasure trove” for archaeologists wishing to obtain DNA from ancient remains (photo courtesy of Debi Poinar).



FIGURE 23: DNA analysis showed that this individual from a burial pit in Tuscany died from cholera around 1850 (photo courtesy of Debi Poinar).

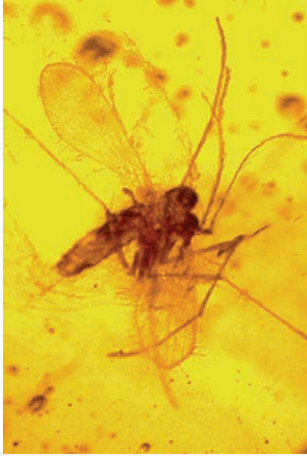


FIGURE 24: The Early Cretaceous Myanmar amber sand fly, *Palaeomyia burmitis*, with stages of the trypanosomatid, *Paleolishmania proterus*.



FIGURE 27: Short procyclic promastigotes of *Paleolishmania proterus* developing in the midgut of the Early Cretaceous sand fly shown in Figure 24.

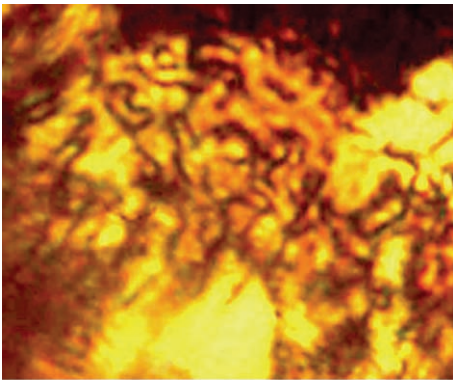


FIGURE 25: Promastigotes of the trypanosomatid, *Paleolishmania proterus*, developing in the midgut of the Early Cretaceous sand fly shown in Figure 24.

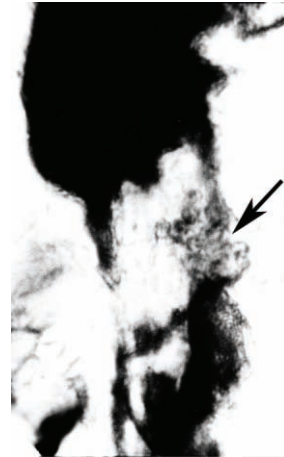


FIGURE 28: Amastigotes (arrow) of *Paleolishmania proterus* acquired from a vertebrate host in the proboscis of the Early Cretaceous sand fly shown in Figure 24.



FIGURE 26: Elongate nectomonad promastigotes of *Paleolishmania proterus* developing in the midgut of the Early Cretaceous sand fly shown in Figure 24.

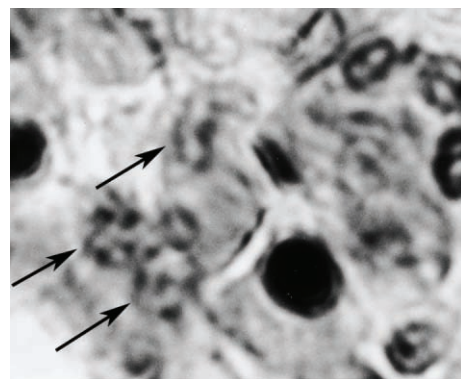


FIGURE 29: Amastigotes (small dark bodies [arrows]) of *Paleolishmania proterus* developing inside vertebrate blood cells in the thoracic lumen of the Early Cretaceous sand fly shown in Figure 24.

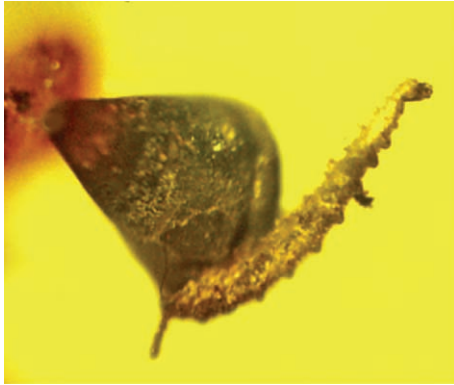


FIGURE 30: One of two Early Cretaceous Myanmar amber sand fly larvae that contained flagellates inside the lumen of their alimentary tracts. Similar flagellates were associated with the adjacent club fungus.

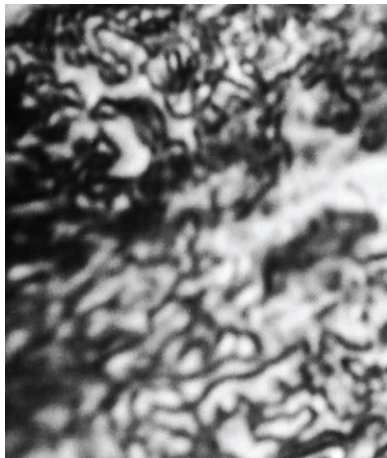


FIGURE 31: Flagellates inside the lumen of the alimentary tract of the Early Cretaceous sand fly larva shown in Figure 30.

How, when, and where protozoan vector associations originated are difficult to determine, especially when every association could have evolved slightly differently. Also past evidence of vector associations is extremely rare since most protozoans are too small and fragile to be recognized [43]. However, in some instances, clues to possible mechanisms exist. In the case of *Leishmania* and sand flies, the discovery of trypanosomes associated with sand fly larvae originating from the same Early Cretaceous Myanmar amber deposits provided a possible scenario for how these flagellates became established in sand flies. The two fossil sand fly larvae were adjacent to and probably feeding on a fruiting body of a coral mushroom, *Palaeoclavaria burmitis* (Figure 30) [44–46]. Associated with some expelled fecal material from both larvae were numerous flagellates. Similar flagellates surrounded the bases of the fungal fruiting bodies and were associated with debris attached to the cuticle of both sand flies. After polishing away a portion of the alimentary tract of one of the amber sand flies, numerous flagellates similar

to those at the bases of the fungal fruiting bodies were also discovered in the lumen of its midgut (Figure 31).

Based on these findings, the following scenario for the acquisition of *Leishmania* by sand flies is presented. Free-living trypanosomes developing in the same microhabitat as the sand fly larvae and club fungi were ingested by the larvae. They may have served as food at first, but eventually the trypanosomes began to multiply inside the gut of the sand fly and were carried transtadially from the larva through the pupa and into the adult. Once established in the gut of the adult sand fly, the flagellates were eventually transferred to vertebrates during blood feeding and a vector association was established. Molecular studies showing that insects were the first hosts of trypanosomatids [47] support the above scenario on the origin of parasitic *Leishmania* through larval acquisition of free-living trypanosomatids.

Flagellate-insect associations, some of which could evolve into vector relationships, occur today as shown by free-living trypanosomatids in the alimentary tract lumen of various insects. Species of the aquatic flagellate *Crithidia* can be found inside the gut lumen of mosquito larvae and *Herpetomonas* spp. occur in the larval habitats, as well as inside the gut lumen of fruit flies (Drosophilidae), scuttle flies (Phoridae), and muscid flies (Muscidae) [48]. Transtadial transmission of *Crithidia fasciculata* was experimentally achieved in the mosquito, *Culiseta incidens* [49]. Not only protozoa but also bacteria are known to be transmitted transtadially from larvae into adult *Phlebotomus duboscqi* [50]. The trypanosomatid, *Leptomonas ctenocephali*, is carried through the pupal stage into the adult dog flea, *Ctenocephalides canis*, and when mealworm larvae were experimentally infected with *Leptomonas pyrrocoris*, they carried the flagellates through the pupal and into the adult stage [51].

The scenario of insect larvae acquiring free-living flagellates that are eventually carried into the adult stage may be how many insect vectors originally acquired pathogenic microorganisms. When such microbes were first transmitted into vertebrates, they would have had to deal with immune responses, which was probably achieved by entering specific tissues like the liver or blood cells. How many attempts it took before free-living trypanosomatids were established in sand flies and a vector relationship was established will never be known. However, it is obvious that a combination of confluent events resulted in successful host-vector associations.

So when did sand flies begin to transmit *Leishmania*? A minimum date of 100 million years can be established from actual fossils in Early Cretaceous Myanmar amber. However, the fossil record of sand flies extends back to 125 million year Early Cretaceous Lebanese amber [52], although some suspect that sand flies existed in the Jurassic [53] or even in the Triassic [54].

As pathogens coevolve with their vectors, it is only natural that they would coevolve with their vertebrate hosts. Thus, successful establishment of *Leishmania* in mammals may have been predisposed by first infecting reptiles, especially since *Paleoleishmania proterus* infected reptiles in the Early Cretaceous [41]. Reptiles were probably the original hosts of *Leishmania* [55, 56]. This scenario is supported by molecular

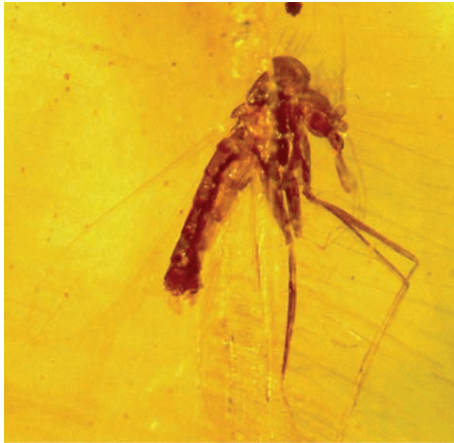


FIGURE 32: *Lutzomyia adiketis*, the Dominican amber sand fly vector of *Paleoleishmania neotropicum*.

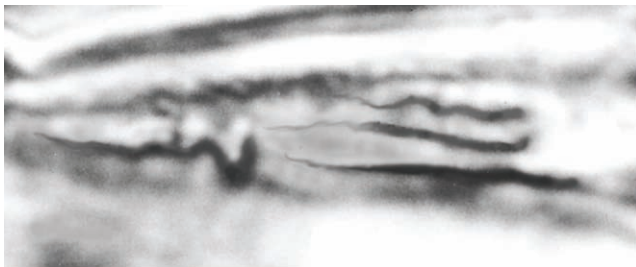


FIGURE 33: Four promastigotes of *Paleoleishmania neotropicum* in the proboscis of the Dominican amber sand fly shown in Figure 32.

data [57] and numerous reptilian trypanosome infections transmitted by sand flies today [58–61].

Did several foci of sand fly-*Leishmania* evolve over time in different geographical areas or was this vector association a single event? Two theories supporting the latter scenario were proposed on the place of origin of *Leishmania*. One proposes a Palearctic origin with reptiles as the first vertebrate hosts. The pathogen was later carried across Beringia into the Nearctic and then into the Neotropical region via the Panamanian land bridge [62]. The second theory placed the origin of *Leishmania* in the Neotropical region with sloths as the first vertebrate hosts. Infected porcupines and other mammals carried the infections across the Panamanian land bridge into North America and eventually across Beringia into Eurasia [63]. The presence of *Paleoleishmania* in Early Cretaceous Myanmar amber sand flies supports the former of these two theories.

If leishmaniasis did evolve in the Old World, just when it arrived in the New World is a mystery. However, a Dominican amber fossil provides a minimum date of some 20–30 million years. The evidence was in a *Lutzomyia* sand fly that was vectoring *Paleoleishmania neotropicum* [64] (Figure 32). In the proboscis of this Dominican amber sand fly were promastigotes (Figure 33), paramastigotes (Figure 34), and a group of amastigotes (Figure 35). Amastigotes are only formed in the vertebrate host and are normally ingested with

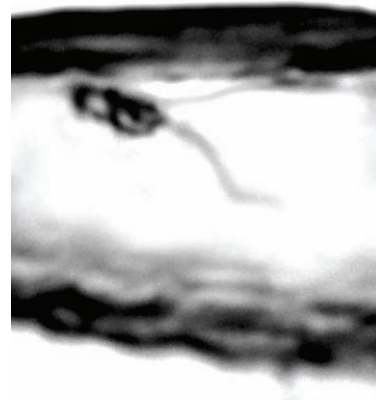


FIGURE 34: Two paramastigotes of *Paleoleishmania neotropicum* in the proboscis of the Dominican amber sand fly shown in Figure 32.

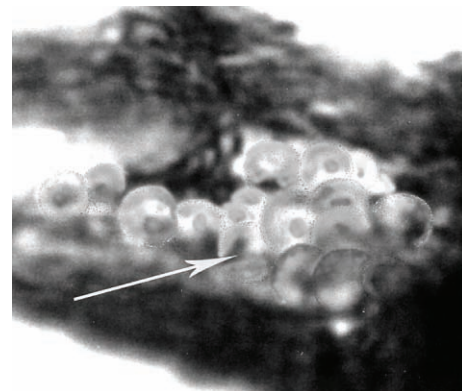


FIGURE 35: A cluster of amastigotes (arrow) of *Paleoleishmania neotropicum* in the proboscis of the Dominican amber sand fly shown in Figure 32.

the blood meal. The flagellates may have been multiplying in the proboscis since in *Leishmania chagasi*, amastigotes may divide during their transformation into promastigotes [65–67]. The promastigotes and paramastigotes (metacyclic stages) are produced inside the gut of the sand fly and indicate that there were at least two blood meals taken. Whether the origin and evolution of the New World sand fly *Leishmania* were the same as those of the Old World cannot be determined but they could have evolved independently of one another.

Biting midges are also vectors of trypanosomes, especially those infecting birds. Some idea of the antiquity of biting midges transmitting trypanosomes was the discovery of the Early Cretaceous Myanmar amber *Leptoconops nosopheris*. The body of the fossil biting midge had partially cleared, revealing trypanosomes of *Paleotrypanosoma burmanicus* in its midgut (Figure 36). Trypanosomes also occurred in the head, salivary glands, and salivary ducts of *L. nosopheris* and some flagellates were in a salivary droplet that collected on the tip of the proboscis (Figure 37) [68]. Extant

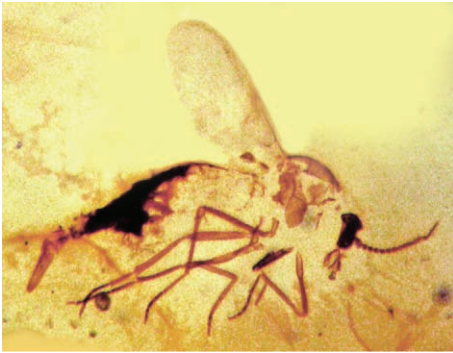


FIGURE 36: The Early Cretaceous Myanmar amber biting midge, *Leptoconops nosopheris*, a vector of the trypanosomatid, *Paleotrypanosoma burmanicus*.



FIGURE 37: Stages of *Paleotrypanosoma burmanicus* in a salivary secretion protruding from the proboscis of the Early Cretaceous biting midge shown in Figure 36.

species of *Leptoconops* feed on reptiles, birds, and mammals; however, based on its age and characters resembling those found on extant reptile feeders, the vertebrate host of *Paleotrypanosoma burmanicus* was suspected of being a reptile [69, 70]. Molecular techniques indicated that vertebrate parasitism by trypanosomes evolved multiple times in the past [71]. It is possible that biting midges were the original ancestral vectors of the *Trypanosoma* lineage, since *Leptoconops* clades extend back at least 120 million years [72].

Flies are not the only insect vectors of trypanosomes. Kissing bugs (Hemiptera: Reduviidae) are well known for their bloodsucking habits (Figure 38) and they transmit the devastating Chagas disease (*Trypanosoma cruzi*) in the American tropics. Two fecal droplets containing metatrypanosomes of *Trypanosoma antiquus* (Figure 39) were adjacent to a fifth instar nymph of *Triatoma dominicana* in Dominican amber (Figure 17). The fossil metatrypanosomes resemble those of *T. cruzi*. Analysis of mammalian hairs adjacent to the fecal droplets indicates that the vertebrate host for both the triatomine and trypanosomatid probably was a bat. Extant triatomines not only feed on bats but also are the



FIGURE 38: Kissing bugs (Hemiptera: Reduviidae), such as this extant *Dipetalogaster* sp., transmit *Trypanosoma cruzi* to humans in the American tropics.

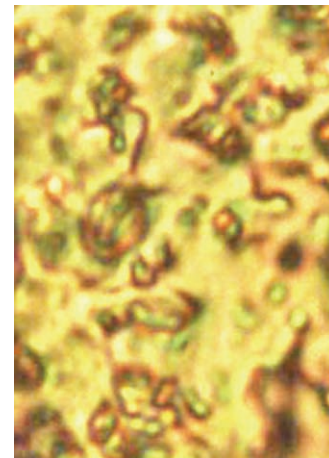


FIGURE 39: Metatrypanosomes of *Trypanosoma antiquus* in a fecal droplet of the Dominican amber *Triatoma dominicana* shown in Figure 17.

suspected vectors of all New World bat trypanosomes [73–75]. It is possible that some lineages of trypanosomes evolved in bats and that these mammals were the original vertebrate hosts in Central and South America. Additional evidence of a long association between bats and trypanosomes is the absence of disease symptoms in these mammals [73]. Records of Chagas disease in humans date back to 4,000-year-old mummies in the Atacama Desert [76].

Other types of flagellates have been discovered in triatomines. Epimastigotes of a species of *Blastocrithidia* were detected in an anal droplet of *Panstrongylus hispaniolae* in Dominican amber (Figures 40 and 41) [77]. The fossil flagellates are similar in size and shape to the epimastigotes of *Blastocrithidia triatomae* that were described from the intestine of *Triatoma infestans* [78]. *Blastocrithidia* can be pathogenic in triatomines when populations built up in the midgut [79].

One of the most notorious protozoan diseases of humans is malaria and aside from episodic epidemics of plague and cholera, malaria continues to cause human suffering in many parts of the world today. That may be because malarial organisms are the ultimate pathogens. Few other pathogens



FIGURE 40: A Kissing bug, *Panstrongylus hispaniolae*, in Dominican amber.



FIGURE 41: Epimastigotes (arrows) of a *Blastocrithidia* sp. protozoan in the anal droplet of *Panstrongylus hispaniolae* depicted in Figure 40. Bar = 77 μm .

have the ability to change their surface coat so readily as modern day *Plasmodium*.

A separate lineage of ancient malarial organisms is vectored by biting midges that date back 100 million years. These primitive malaria-biting midge associations still survive today in various parts of the world. The earliest evidence of malaria was obtained from a partially cleared female biting midge (*Protoculicoides* sp.) in Early Cretaceous Myanmar amber [80] (Figure 42). A number of developing oocysts and sporozoites of the malarial parasite, *Paleohaemoproteus burmaces*, were inside the abdomen of the biting midge (Figure 43). Morphological features of the vector were used to determine that the host was large and cold-blooded [81]. Likely hosts in Early Cretaceous Myanmar were lizards, crocodylians, and dinosaurs [25].

The development of *Paleohaemoproteus burmaces* was probably very similar to malarial parasites vectored by biting midges today. During blood feeding, the female vector acquires vertebrate blood cells infected with gametocytes. After their release from the vertebrate blood cells in the stomach of the biting midge, the gametocytes unite and initiate the zygote or sporogonic stage of the life cycle. The

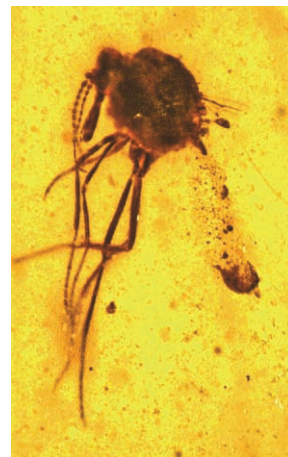


FIGURE 42: The Early Cretaceous Myanmar amber biting midge (*Protoculicoides* sp.) with its malarial pathogen, *Paleohaemoproteus burmaces*.

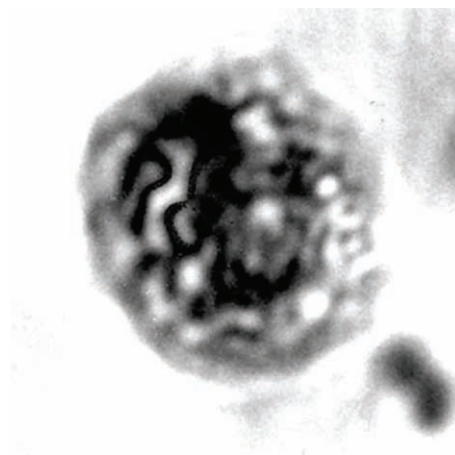


FIGURE 43: An oocyst of *Paleohaemoproteus burmaces* developing in the body cavity of the Early Cretaceous biting midge depicted in Figure 42. Sporozoites are developing inside the oocyst.

zygote differentiates into a motile ookinete, which penetrates the epithelial cells of the insect's midgut and forms an oocyst. Vertebrate-infective sporozoites develop within the oocysts (Figure 43) and when released, they migrate to the salivary glands. They are introduced into a vertebrate during subsequent blood meals.

Paleohaemoproteus burmaces has many characteristics of the extant genus *Haemoproteus* that are vectored by biting midges to birds and reptiles [82–87]. Today haemoproteid malaria caused by *Haemoproteus* spp. is commonly transmitted by *Culicoides* biting midges [82, 88].

Discovering *Plasmodium* malaria in the Dominican amber mosquito, *Culex malariager*, showed that not all lineages of human malaria evolved in the Old World [89, 90] (Figure 44) and were later introduced into the New World. Now we know that at least one lineage of *Plasmodium* malaria was being vectored by mosquitoes in the Americas some 20–30 mya [90]. Inside the fossil *Culex malariager* were various

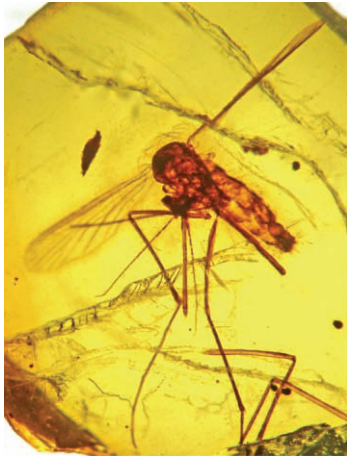


FIGURE 44: The Dominican amber mosquito, *Culex malariager*, a vector of the malarial pathogen, *Plasmodium dominicana*.

stages (oocysts, sporozoites, ookinete, and microgametocyte) of *Plasmodium dominicana*. Two oocysts were entire (Figure 45); however, two others had ruptured and groups of sporozoites were moving into the hemocoel (Figure 46). Some of the released sporozoites had managed to reach the salivary glands before the mosquito became entrapped.

The large, pedunculated oocysts aligned *Plasmodium dominicana* with the extant bird parasite, *Plasmodium juxtanucleare*, which is transmitted by *Culex* spp. [83, 91]. This latter species had been assigned a basal position among avian-infecting *Plasmodium* species [91], suggesting that the genus *Plasmodium* could have evolved with birds in the Mesozoic with Cretaceous culicine mosquitoes as vectors [92].

The *Plasmodium dominicana* lineage could easily have been transferred from birds to mammals by culicine mosquitoes since such lateral transfers of malaria from birds to mammals occur naturally and have been demonstrated experimentally [83, 93, 94]. Once established in mammals, anopheline mosquitoes, such as the Dominican amber *Anopheles dominicanus*, could have transmitted *Plasmodium* from simians to humans [95]. Another possible pathway is that anopheline mosquitoes could have acquired infections directly from birds and passed them on to mammals, including simians. So the evolution of human malaria in the New World could have begun in birds and then shifted to mammals and finally to humans. Human malaria in the Old World may have evolved by a completely different pathway but probably also existed millions of years ago. The discovery of *Plasmodium falciparum* malaria in Egyptian mummies using antigen immunoassay techniques establishes the disease in humans at a minimum date of 3200 B.C. [96, 97].

Fossils provide no clues of how *Plasmodium* evolved from free-living ancestors; however, I believe that the original host in a vector association is the one in which the sexual stages of the parasite occur, which in the case of malaria would be the mosquito. A possible scenario is that the free-living aquatic

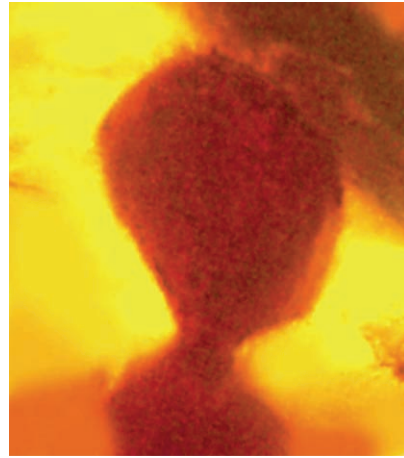


FIGURE 45: An oocyst of *Plasmodium dominicana* in the hemocoel of the Dominican amber mosquito shown in Figure 44.

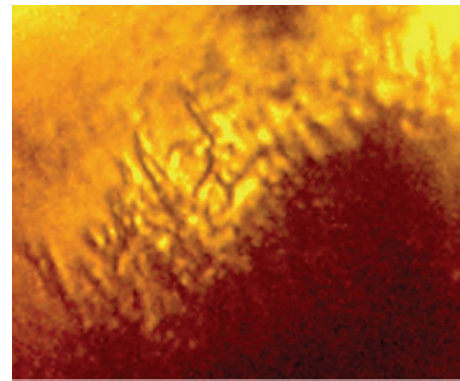


FIGURE 46: Sporozoites emerging from an oocyst of *Plasmodium dominicana* in the Dominican amber mosquito shown in Figure 44.

ancestors of *Plasmodium* were first acquired by mosquito larvae. The parasites were then carried through the pupa stage into the next generation of adults and this single host cycle was established. Eventually, some sporozoites entered the salivary glands and were transferred into vertebrates. Their establishment in the liver and blood cells served to protect them from host defense responses.

An opposing but less likely evolutionary scenario is that *Plasmodium* was originally a blood parasite of vertebrates that reproduced by merozoite division. Mosquitoes ingested vertebrate blood cells infected with merozoites and over time the released merozoites began to diversify and eventually a sexual stage was established in mosquitoes. This resulted in the establishment of a sporogonic stage with the production of vertebrate-infective sporozoites.

There are numerous types of malaria in a wide range of vertebrates; however, very few have left a fossil record. A rare malarial pathogen was discovered in a Dominican amber streblid bat fly, *Enischnomyia stegosoma* (Figure 47) [98, 99]. Two ovoid oocysts of *Vetufefrus ovatus* attached to the midgut wall of the bat fly contained short, stubby sporozoites (Figure 48) and similar sporozoites in the salivary

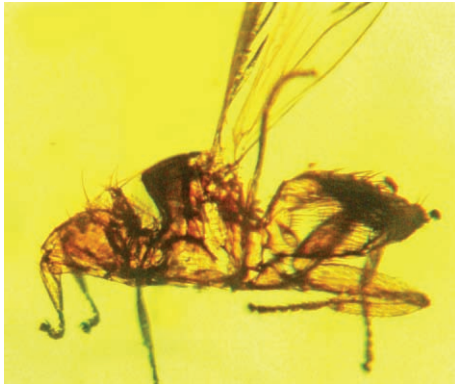


FIGURE 47: A Dominican amber bat fly, *Enischnomyia stegosoma*, vector of the malarial pathogen, *Vetufebus ovatus*.

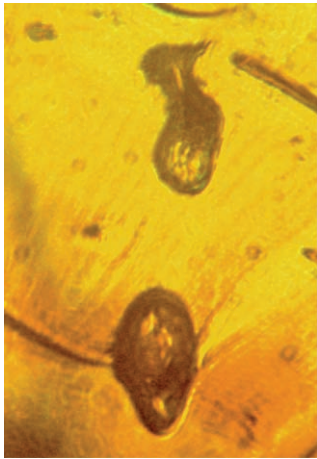


FIGURE 48: Two oocysts with developing sporozoites of *Vetufebus ovatus* in the Dominican amber bat fly shown in Figure 47.

glands and ducts indicated that representatives of Neotropical Hippoboscoidea were vectoring malaria to bats in the Cenozoic.

Protozoans cause a number of alimentary tract infections in all vertebrates; however, the origins of such infections are difficult to determine. They probably date back to the early evolution of vertebrates since the passage of microbes into the lumen of the alimentary tract would have occurred from eating infested food. The establishment of some of these microbes, at least for short periods, would have occurred multiple times. Reptiles were one of the early hosts of intestinal protozoan diseases [100] as shown by the discovery of mature cysts of *Entamoebites antiquus* in a dinosaur coprolite from the Early Cretaceous Bernissart Iguanodon shaft in Belgium [101] (Figure 49). The fossil cysts resembled species in the widespread modern genus *Entamoeba* that infect amphibians, reptiles, birds, and mammals. The extant *E. invadens* destroys leucocytes and causes disease in reptiles when it invades the liver and causes hepatitis and nephritis. Common symptoms of gastrointestinal infections in lizards



FIGURE 49: An Early Cretaceous cyst of *Entamoebites antiquus* recovered from a dinosaur coprolite at the Bernissart Iguanodon shaft in Belgium.

and snakes are vomiting and severe diarrhea [102–104]. *Entamoebites antiquus* provides a minimum date when pathogenic protozoa were established in the gut of vertebrates.

There is widespread subfossil evidence of intestinal pathogens based on samples from coprolites, latrines, and cesspits. Using an immunoassay test for determining the presence of adhesion antigens, the pathogenic *Entamoeba histolytica* could be detected in human samples from Brazil 6000 to 5000 BP [105]. A review of human intestinal parasites from around the world based on the microscopic examination of treated samples has been recently summarized [106].

Gregarines are a very complex group of protozoans that evolved as monoxenous parasites of invertebrates [107, 108]. Their origins are enigmatic since there are no closely related free-living forms; however, since they parasitize a wide variety of marine and terrestrial invertebrates, gregarines are certainly an ancient group. Gregarines parasitize a wide range of insects today, including a number of cockroaches, which may have been one of the original host groups [109]. Discovering trophozoites and oocysts of *Primigregarina burmanica* from an Early Cretaceous Myanmar amber cockroach showed that the basic developmental stages of extant gregarines were firmly established some 100 million years ago [110, 111] (Figures 50 and 51).

2.4. Fungi. Fungal pathogens are ubiquitous and parasitize a wide range of animals and plants; however, actual evidence of parasitism is rare. Most fossil examples of animal-parasitic fungi occur on arthropods in amber and in many cases, the fungi can usually be placed in extant families and genera. The oldest evidence of animal parasitism by fungi is the insect pathogen, *Paleophiocordyceps coccophagus*, parasitizing a scale insect in Early Cretaceous Myanmar amber [112]. Two synnemata covered with a palisade of elongated cylindrical phialides bearing conidia at their tips had emerged from the insect (Figures 52 and 53). Evidence indicated that the hypocrealean ancestor of *P. coccophagus* evolved in the Early



FIGURE 50: An Early Cretaceous Myanmar amber cockroach adjacent to a gametocyst (arrow) of the gregarine protozoan parasite, *Primigregarina burmanica*.

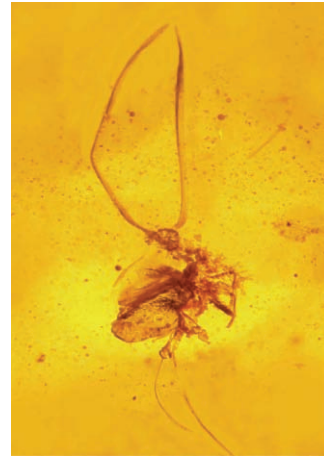


FIGURE 52: Two extended synnemata of the Early Cretaceous Myanmar amber fungus, *Paleoophiocordyceps coccophagus*, emerging from the top of a scale insect (Albicoccidae).

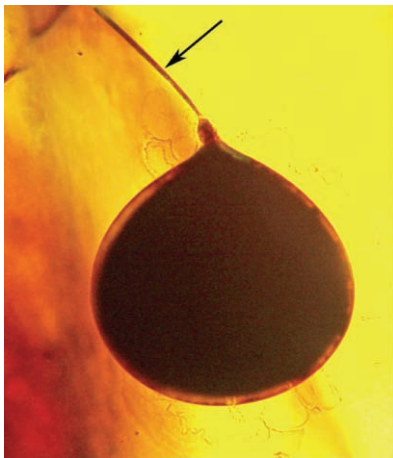


FIGURE 51: Detail of the gametocyst of the gregarine parasite, *Primigregarina burmanica* that was released from the gut of the Early Cretaceous cockroach shown in Figure 50. Arrow shows a spore-filled sporoduct.

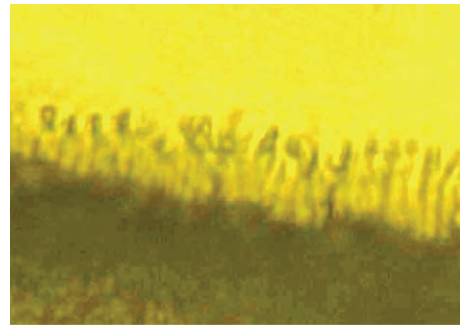


FIGURE 53: A layer of conidiogenous cells and conidia on the surface of one of the synnemata of the Early Cretaceous fungus shown in Figure 52.

Jurassic (193 mya) as a plant parasite that later shifted to animal hosts [112].

Additional examples of fungal pathogens of insects in amber include a member of the Entomophthorales infecting a winged termite in Dominican amber [113]. The termite was covered with a white mat of mycelium that had produced budding conidia, characteristic of extant members of the genus *Entomophthora* (Figure 54). Another Entomophthorales infection was found on a fungus gnat in Dominican amber. The fungus formed a felt-like hymenium over the back of the fly (Figure 55), which is very similar to the growth pattern of *Entomophthora* infections on extant fungus gnats [6].

A Dominican amber *Azteca* ant heavily infected with a species of the extant genus *Beauveria* (Figure 56) establishes

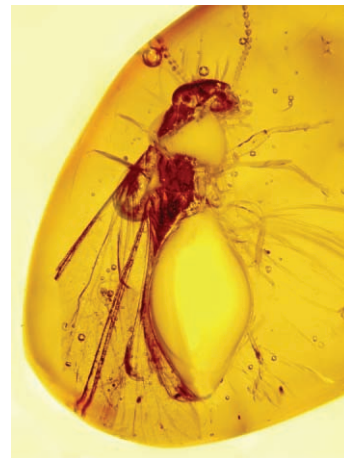


FIGURE 54: A Dominican amber termite bearing white colonies of an Entomophthorales fungus.



FIGURE 55: An entomophthorales infection of a fungus gnat in Dominican amber. The fungus formed a felt-like hymenium (arrow) on the fly.



FIGURE 56: Dominican amber *Azteca* worker ant infected with a *Beauveria* fungus.

a minimum date for the appearance of that clade. Conidiophores and conidia of the fungus covered portions of the ant's body [114] (Figure 57).

The Trichomycetes comprise a group of cosmopolitan, microscopic fungi that parasitize a wide range of arthropods. A member of this order found on a *Culex* mosquito in Dominican amber establishes a minimum date for this group. Several types of thalli had emerged from between the junctions of the abdominal tergites on the back of the mosquito [6] (Figure 58). Some were slender, elongate forms that were producing trichospores while others were short and swollen (Figure 59).

Some additional examples of fungal pathogens in Dominican amber are a synnemata produced by a *Hirsutella*-like fungus on a *Troctopsocopsis* bark louse (Figure 60) and an undescribed fungus emerging from a winged ant (Figure 61).

Evidence of pathogenic fungi attacking nematodes was found among a population of the mycetophagous nematode, *Oligaphelenchoides atrebora*, in Mexican amber [30]. The inner tissues of infected nematodes had been replaced by fungal mycelia (Figure 62), some of which were sporulating (Figure 63) [115].

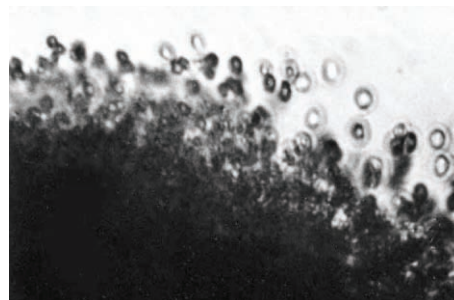


FIGURE 57: Conidiophores and conidia of the *Beauveria* fungus growing on the Dominican amber *Azteca* ant depicted in Figure 56.



FIGURE 58: Group of thalli (arrow) of a trichomycete fungus emerging between the abdominal tergites of a *Culex* mosquito in Dominican amber.

Ancient fungal pathogens of vertebrates are quite rare; however, a case of coccidioidomycosis was reported in two Holocene bison dated at 8500 BP [116]. Spheres and endospores of the pathogen were found in the mandibles and adjoining bones of the bison. The subfossil stages were similar to those of extant species of *Coccidioides*, a genus that infects a wide range of vertebrates, including humans. This discovery sets a locality and minimum time for the occurrence of vertebrate parasitic Ascomycetes. It is interesting that humans are considered one of the vectors of this disease, dispersing it as they migrated between North and South America [116]. Skeletal pathology in a Native American dated at 1,000 to 1,400 A.D. was attributed to coccidioidomycosis when a microscopic examination revealed both spherules and endospores of *Coccidioides immitis* [117].

There are numerous reports of fossil fungi on plants dating from the Palaeozoic onwards. However, few of these show the actual pathogen in association with their plant host. Many of these reports have been summarized previously [3, 118]. The fossil record of fungi begins in the Ordovician, some 460 mya [119], although molecular estimates of fungal phylogeny place the origin of terrestrial fungi (Ascomycota, Basidiomycota, and Glomales) in the Proterozoic [120].

The origins of plant pathogenic fungi may have begun with simple hosts such as algae and fungi. Fungal parasitism of Early Devonian algae (400 mya) probably represents one of the first host parasitic associations with fungi [121]. The same is true for mycoparasitism where fungi parasitize other fungi.

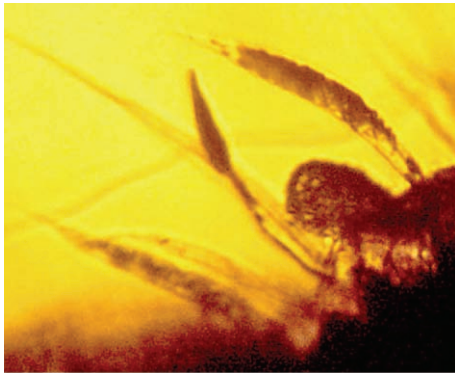


FIGURE 59: Slender, narrow thalli, and a short, swollen thallus of the trichomycete fungus parasitizing the Dominican amber *Culex* mosquito depicted in Figure 58.



FIGURE 63: Developing spore of a fungal pathogen inside the body of the nematode, *Oligaphelenchoides atrebora*, in Mexican amber.



FIGURE 60: Synnematum of a *Hirsutella*-like fungus parasitizing a bark louse in Dominican amber.

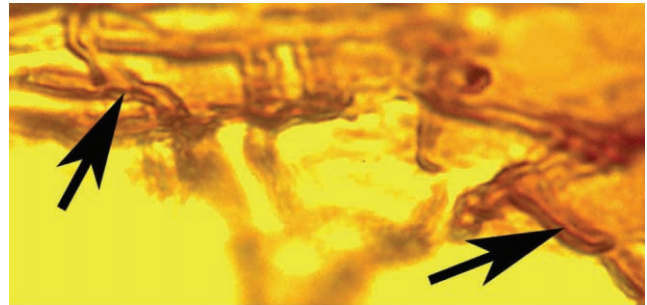


FIGURE 64: Parasitic and hyperparasitic fungi (arrows) developing on an agaric mushroom in Early Cretaceous Myanmar amber.

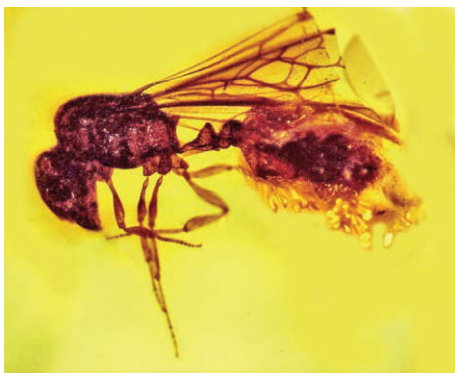


FIGURE 61: A fungus sporulating on the abdomen of a winged ant in Dominican amber.



FIGURE 62: Fungal hyphae inside the body of the nematode, *Oligaphelenchoides atrebora*, in Mexican amber.

Records of such associations have been found in Devonian deposits [122] and in Early Cretaceous amber, where an agaric mushroom was parasitized by a fungus that in turn was parasitized by a second hyperparasitic fungus [123] (Figure 64). Fungi were probably the first parasites of vascular plants. An early record of pathogenic fungi associated with a vascular plant is the 400 mya Devonian *Paleopyrenomycites devonicus* that was developing in the cortex of aerial stems and rhizomes of the primitive *Asteroxylon* (Figure 65) [124]. Rust and smut fungi are also probably very primitive although thus far, only a Tertiary record of a smut fungus infecting an Eocene angiosperm in British Columbia is known [125].

Spot fungi are an interesting heterogeneous group that evolved various methods of either penetrating surfaces of leaves and petals or entering through natural openings (stomata). Fossil records of leaf spot fungi have been reviewed and date back to the Late Carboniferous [3, 126]. Some fossil spot fungi occurred on a petal in Mexican amber and on a dicot and monocot leaf in Dominican amber [127]. Several pycnidia of *Leptothyrites dominicanus* were present on the monocot leaf (Figure 66) and one pustule contained conidiophores and conidia (Figure 67).

Most groups of parasitic fungi probably evolved from saprophytic species developing on dead arthropods and plants. Physiological adaptations allowing them to attack

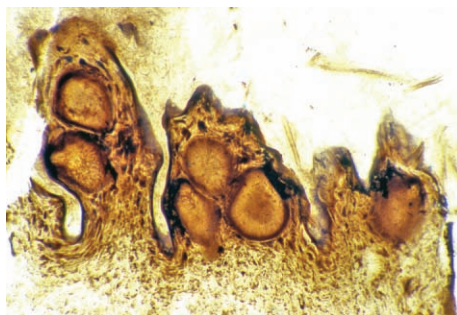


FIGURE 65: Perithecia of the Devonian ascomycetous fungus, *Paleopyrenomycites devonicus*, developing in the vascular plant, *Asteroxylon*, at the Early Devonian Rhyne chert site (Photo courtesy of T. N. Taylor).

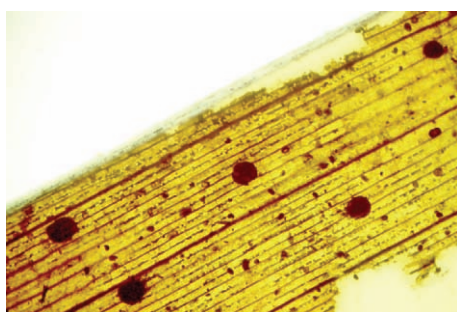


FIGURE 66: Five spherical, dark pycnidia of the coelomycete, *Lep-tothyrites dominicanus*, parasitizing a monocot leaf in Dominican amber.

dying or weakened animals and plants could have been further modified for entering and obtaining nourishment from healthy organisms.

3. Multicellular Endoparasites

3.1. Nematomorpha. Hairworms are an ancient group of arthropod parasites that live inside invertebrates and usually kill the latter when their development is completed. A specimen in Early Cretaceous Myanmar amber provides a minimum date of 100 my for their existence [128]. Evidence that they parasitized cockroaches in the Tertiary is shown by the hairworm, *Paleochordodes protus*, emerging from a blattid in Dominican amber [129] (Figure 68). Since this group of parasites is not closely related to any existing free-living or parasite group, it is difficult to determine their origins and evolutionary development. Hairworms appear to be an ancient group that arose independently and successfully maintained themselves to the present, in spite of ongoing environmental disruptions that are destroying their host's habitats.

3.2. Nematodes. Nematodes are another primitive group that has no close affinity to any extant invertebrate group and the lineage from which they evolved is probably extinct. Thus attempting to trace their origins is highly speculative,

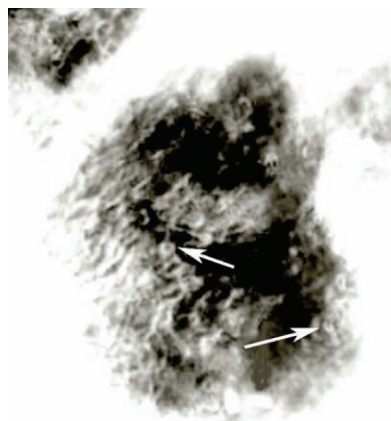


FIGURE 67: Conidia (arrows) inside one of the pycnidia of *Lep-tothyrites dominicanus* shown in Figure 66.

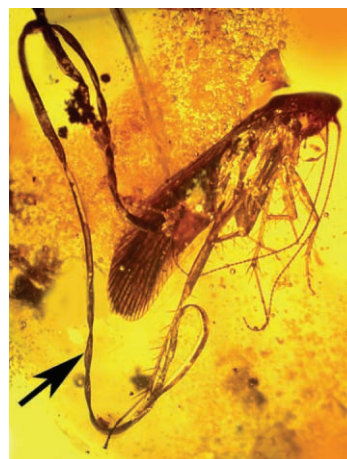


FIGURE 68: The hairworm, *Paleochordodes protus* (arrow), emerging from a cockroach in Dominican amber.

even with molecular techniques. It is possible that nematodes originated in the sea and first parasitized marine invertebrates and vertebrates in the Cambrian. The most complete fossil record of any particular nematode group is of the Mermithidae, a group well represented in amber and easily detected as they are leaving or have just emerged from their arthropod hosts.

Mermithid nematodes superficially resemble hairworms in size and development. They parasitize a wide range of terrestrial invertebrates and probably evolved from free-living dorylaimids or mononchs. A recent study reported free-living dorylaimids that had entered the body cavity of some terrestrial insects, thus showing a possible first step in an evolutionary scenario indicating that mermithids evolved from free-living Dorylaimida [130].

Many mermithids parasitize small flying insects, which is why the most complete record of their evolutionary history is in amber. Evidence in Early Cretaceous Lebanese amber suggests that chironomid midges or their ancestors were the original insect hosts of mermithids (Figure 69) [131].

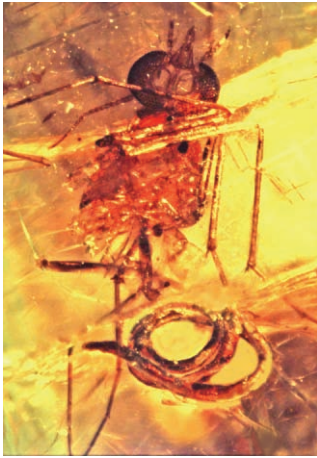


FIGURE 69: Parasitism of an Early Cretaceous Lebanese amber chironomid midge by the mermithid nematode, *Cretacimermis libani*. The nematode is coiled in the abdomen of its host.



FIGURE 70: Parasitism of a Baltic amber ant by the mermithid nematode, *Heydenius formicarius*.

However based on the earliest fossil record of all their host groups, an estimated date for the origin of mermithids would be in the Permian [15]. Evidence that mermithids had diversified by the mid-Tertiary is provided by a wide range of parasitized hosts, including ants (Figure 70), hemipterans (Figure 71), trichopteran (Figure 72), and arachnids in Baltic and Dominican amber [15, 132].

Several clades of the nematode group Tylenchomorpha also parasitize insects and among these the acugutturids, iontonchids, allantonematids, and sphaerulariids have a fossil record. Those with the greatest selection of hosts are the Sphaerularidae and Allantonematidae. In contrast to mermithids that only mature but do not multiply in their hosts, the small size of these tylenchomorphs allows them to undergo one or even two generations inside their host. Often the juveniles can be observed within the body cavity or emerging from various parts of insects in amber. Fossil records of sphaerulariids and allantonematids include species parasitizing beetles, ants, and flies in Tertiary amber [15].

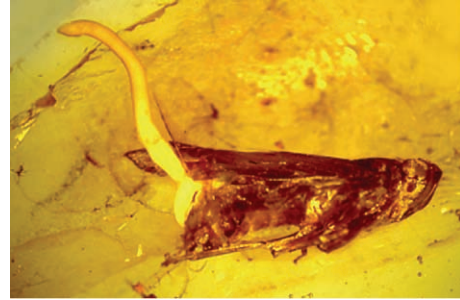


FIGURE 71: The mermithid nematode, *Heydenius brownii*, emerging from a planthopper in Baltic amber.

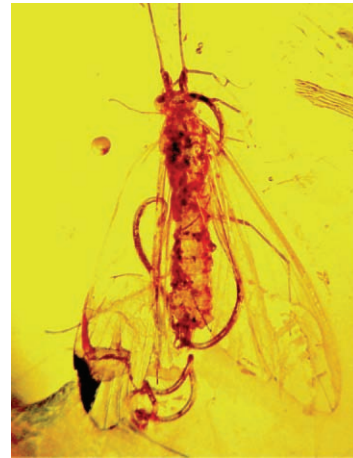


FIGURE 72: Two mermithid nematodes, *Heydenius trichorosus*, adjacent to their caddis fly host (*Triaenodes balticus*) in Baltic amber.

The earliest fossil record of sphaerulariids is *Tripius balticus* that was parasitizing a sciarid fly in Baltic amber. However, based on the earliest fossil record of their host group, an estimated date for the origin of sphaerulariids would be in the Jurassic [15].

The Dominican amber *Palaeoparasitylenchus dominicanus* establishes a location and minimum time when drosophilid flies were victimized by allantonematids. However, based on the earliest fossil record of their host group, an estimated date for the origin of allantonematids would be in the Permian [15]. Using the same criterion, an estimated date for the origin of the iontonchids would be in the Cretaceous [15]. However, the oldest fossil record of an iontonchid is *Paleoiontonchium dominicanum* in a parasitized fungus gnat in Dominican amber. The iontonchids are especially interesting since they provide evidence that insect parasitic tylenchomorphs evolved from mycetophagous clades. While the life cycle of the fossil *Paleoiontonchium dominicanum* remains unknown, it is probably similar to that of its recent descendant, *Iotonchium californicum*, that was studied in California [133]. This latter species has two alternating life cycles, a fungal feeding cycle with the nematodes developing on mushrooms and an insect-parasitic cycle where development only occurs inside the body of a fungus gnat that feeds on the mushroom. *Iotonchium* is a nematode that

has the ability to utilize both fungi and insects as hosts. It appears to be at a stage in its evolution that is ready to sever the ties with the more primitive fungal portion of its life cycle.

A representative of the acugutturid moth parasites, *Setonema protera*, also occurs in Dominican amber; however, based on the earliest fossil record of its host group, an estimated date for the origin of this group would be in the Carboniferous [15].

Representatives of the nematode suborder Rhabditina are mainly microbotrophic free-living nematodes; however, some lineages developed amazing specialized patterns of parasitism. One of these lineages is a member of the family Diplogasteridae that evolved the ability to parasitize fig wasps. Diplogasterids are common inhabitants of soil and decaying organic matter and their associations with arthropods are mainly phoretic in nature. Yet some of these nematodes became parasites of fig wasps. Fig wasps are very tiny hymenopterans that develop in fig synconia within the flower clusters. Their entire life cycle takes place in the fig with female flight patterns related to entering and exiting the fig flowers as they pollinate the new flowers and deposit their eggs. The *Parasitodiplogaster* lineage of diplogasterids is carried by the adult fig wasps to the fig flowers and then parasitizes the developing fig wasp larvae. Originally, the nematodes were probably strictly phoretic and developed on pollen and bacteria in the fig flowers, using the wasps only for transport. At some point, juvenile nematodes emerging from eggs deposited in the fig flowers entered the fig wasp larvae and adults. That this association has continued for 20–30 million years is evident from a parasitized fig wasp surrounded by released stages of the diplogasterid, *Syconema dominicana*, in Dominican amber [15]. However, based on the earliest fossil record of their host group, an estimated date for the origin of this nematode lineage would be in the Eocene [15].

Another unique mode of parasitism originated independently in two genera of rhabditine nematodes, *Steinernema* and *Heterorhabditis*. These two nematode genera evolved from separate lineages of free-living, bacterial-feeding, soil nematodes but developed a very similar pattern of insect parasitism. Each lineage became associated with its own specific insect-pathogenic bacteria that were originally acquired from the immediate environment. These bacteria are retained in the gut of the infective stage juveniles and released when the latter penetrate into a living insect. The released bacteria kill the insect within 24 hours and the nematodes feed on the tissues of the corpse that have been modified by the specific bacteria. These so-called entomopathogenic nematodes are sold commercially as biological control agents. The discovery of infective juveniles of one of these clades in Early Cretaceous Myanmar amber shows that entomopathogenic nematodes were present some 100 mya (Figure 10) [15]. The fossil infective stage *Proheterorhabditis burmanicus* was adjacent to a beetle that contained bacteria in its tissues similar to those carried by extant heterorhabditids (Figure 11) [134]. Based on the earliest fossil record of their host group, an estimated date for the origin of both the heterorhabditids and steinernematids would be in the Permian [15].

Helminthiasis refers to diseases of humans and animals caused by parasitic worms such as nematodes, trematodes (flukes), and cestodes (tapeworms). Past remains of vertebrate parasitic nematodes are mainly found in coprolites and corpses. Two common animal parasites are ascarids or stomach worms and oxyurids or pinworms. The oxyurids evolved from microbial soil inhabitants and it was not that large a step to shift from feeding on microbes in the environment to those in the gut lumen of invertebrates and vertebrates. The earliest record of an oxyurid parasitizing invertebrates is *Paleothelastoma tipulae* from an Early Cretaceous crane fly in Myanmar amber [15]. Based on the earliest fossil record of their host group, an estimated date for the origin of the invertebrate parasitic oxyurids would be in the Silurian [15].

Oxyurids found in subfossil deposits show the wide range of vertebrate hosts of these parasites. *Syphacia* was found in the remains of a 10,000–12,000-year-old ground squirrel in Eastern Russia [135], eggs of *Parapharyngodon sceleratus* were recovered from 9,000-year-old coprolites of an iguanid in Brazil [136], and juveniles of *Agamofilaria oxyura* were recovered from a 10,000 ± 180-year-old Shasta Ground Sloth bolus [137]. Based on the earliest fossil record of their host group, an estimated date for the origin of the vertebrate parasitic oxyurids would be in the Carboniferous [15].

It is difficult to determine when humans were first infected by the oxyurid pinworm, *Enterobius vermicularis*. In spite of the 10,000-year-old record from North America and molecular traces of *E. vermicularis* in 27 pre-Columbian coprolites ranging from 1,100 to 6,110 years in North and South America [138, 139], the first human infections were probably in the Old World, even though such records from archaeological sites are rare [106]. The oldest Old World records are from a 2100-year-old Chinese mummy [140] and surprisingly, the only report of pinworms in Egyptian mummies was from a site in Roman-occupied Egypt that dates from 30 to 395 AD [141]. However, the discovery of *E. vermicularis* in a chimpanzee [142] provides an avenue for early human infection in the Old World and peoples migrating across the Bering Strait could have brought this nematode to the New World [143].

Ascarids or stomach worms are probably the most common nematode parasites of vertebrates. The complicated life cycle of many ascarids, including the giant roundworm that infects humans, makes determining their early evolution difficult. Why it is necessary for the immature worms to traverse the wall of the intestine, migrate through the tissues of the host, and then return to small intestine to continue their development is perplexing. The oldest fossil evidence of this group is Early Cretaceous eggs of *Ascarites* spp. from a dinosaur coprolite in Belgium [101]. However, based on the earliest fossil record of their host group, an estimated date for the origin of ascarids would be in the Permian [15]. Ascarids radiated in numerous lines of vertebrates and invertebrates and have been recovered from more archaeological sites than any other human parasite. A selection of Pleistocene and Holocene sites containing the human ascarid, *Ascaris*

TABLE 1: Records of the human stomach worm, *Ascaris lumbricoides*, from global archaeological sites.

Country	Site	Date	Reference
France	Clairvaux, Jura	3600 BC	[149]
	Chalain, Jura	2700–2440 BC	[149]
	Bobigny	2nd century AD	[175]
	Paris	14th–15th century AD	[176]
	Marly-le-Roi, Yveline	17th–18th century AD	[177]
Germany	Drobintz girl	600 BC	[178]
	Bremerhaven	100 BC–500 AD	[179]
	Lübeck and other cities	15th century AD	[180]
England	Somerset	4100–2600 BP	[181]
	Winchester	Roman period	[182]
	Lindow Man	2nd century AD	[183]
	York	2nd–3rd century AD	[184]
	York	9th–10th century AD	[185]
	Winchester	1000 years	[186]
	Winchester	1100 AD	[187]
	Southampton	13th–14th century AD	[188]
	York	14th–16th century AD	[189]
	Worcester	15th century	[190]
N. America	Kentucky Salts Cave	1125–290 BC	[191]
	Tennessee Big Bone Cave	2177 ± 145 BP	[192]
	Arizona Elden Pueblo	1070–1250 AD	[193]
	Adak Island, Alaska	840 ± 40 BP	[194]
	Williamsburg, Virginia	1720 AD	[195]
Africa	Kruger Cave, Rustenburg	10,000–7,000 BP	[196]
Middle East	Egypt PUM mummy	200 BC	[197]
	Acre, Israel	13 century AD	[198]
S. America	Huarmey Valley, Peru	2277 BC ± 181	[199]
	Gentio Cave, Brazil	3490 ± 120 BP	[106]
	Brazil sites	8800–7000 BP	[200]
New Zealand	Wellington	150–200 years	[201]

lumbricoides, extending back 30,000 years [144] is presented in Table 1 and shows the geographical range of this parasite over various time periods. It has been suggested that humans acquired *A. lumbricoides* from pigs, since they harbour the closely related species, *A. suum*. However, *A. lumbricoides* has been recovered from Old World chimpanzees, gorillas, and bonobos as well as from New World howler monkeys [145–147] and the original infections in humans were probably acquired from apes and simians.

Hookworms are another clade of nematode intestinal parasites recovered from archaeological finds and reports of these parasites have been recently summarized [106]. The two species attacking humans, *Ancylostoma duodenale* and *Necator americanus*, are usually lumped together since the stages normally recovered (eggs and first stage juveniles) are so similar that it is impossible to distinguish them by morphological methods. New World records date back to 7230 BP [148], while Old World records are slightly younger (3600 BP) [149]. Hookworms probably evolved from free-living microbotrophic soil nematodes. Based on the earliest fossil record of their host group, an estimated date for the

origin of hookworms would be in the Palaeocene [15]. Again, there is a good possibility that humans originally acquired infections from apes and simians since *N. americanus* has been found in chimpanzees [150] and *A. duodenale* infects Bonobos [151] and Howler monkeys [146].

Another intestinal parasite that has been traced in archaeological remains is the human whipworm, *Trichuris trichiura*. Most findings are from the Old World, with the oldest record of 10,000–7,000 years BP coming from Africa [152], although eggs of this genus from rodent coprolites date back to 30,000 [153]. Reports of these have been recently summarized [106]. Based on the earliest fossil record of their host group, an estimated date for the origin of whipworms would be in the Carboniferous [15]. Whipworms infect a range of vertebrates, including dogs, cats, mice, pigs, and sheep. The presence of the human whipworm in Old World simians and primates [145, 147, 154–157] provides an avenue for human infections.

While parasites in the lumen of the alimentary track of vertebrates can be discovered in mummies and coprolites, the remains of insect vectored nematodes are usually not available in archaeological deposits unless molecular and

immunological methods are used. Filarial nematodes adjacent to their insect vectors in Dominican amber establish the presence of filariids in the Cenozoic along with their blackfly and mosquito insect vectors [15]. Based on the earliest fossil record of their host group, an estimated date for the origin of filariids would be in the Carboniferous [15].

Fossil evidence of phytoparasitic nematodes is quite rare. The earliest record of plant parasitism by nematodes is the 400 mya Devonian parasite, *Palaeonema phyticum*, that was developing in the stomatal chambers of the Early Devonian (396 mya) land plant, *Aglaophyton major*. This species was an epistrate feeder that mechanically disrupted cortical cells and ingested the contents [158]. *Palaeonema* probably evolved from free-living microbotrophic clades that entered the natural opening of *Aglaophyton* to escape predators and explore a new food source. Lineages with this type of behavior are rare today since most plant parasites have evolved a stylet that is used to penetrate plant tissue and withdraw cell sap. Such stylet bearing nematodes are quite rare as fossils of higher plants and thus far are only represented by a population of *Oligaphelenchoides maximis* inside plant tissue in Dominican amber [15]. Their early lineages were probably free-living fungal feeders.

3.3. Trematodes. Trematodes or flukes can also be found in ancient human remains and reports of liver flukes, *Fasciola hepatica*, and schistosomes, *Schistosoma* spp., in archaeological sites have been recently summarized [106]. It is interesting that *F. hepatica* has only been recovered in Old World coprolites, in spite of its global occurrence today in cattle and sheep. Subfossils of both genera date back some 5,000 years [159, 160]. It has been suggested that humans acquired schistosomes from ruminant and rodent definitive hosts sometime in the Miocene [161]. This is quite possible since the oldest fossil evidence of this group is an egg of the trematode, *Digenites proterus*, recovered from a dinosaur coprolite in the Early Cretaceous Bernissart Iguanodon shaft in Belgium [101].

3.4. Cestodes. Cestodes or tapeworms are another parasite group that occurs in coprolites. The oldest fossil records of this group are 270 million-year-old tapeworm eggs in a shark coprolite [162], which suggests that terrestrial tapeworms might have originated in a marine habitat. Archaeological finds usually reveal the three tapeworm species, *Taenia saginata*, *T. asiatica*, and *T. solium* that infect humans through cattle and pigs. There is evidence that these and probably other tapeworms were originally acquired when humans devoured wild bovids and swine before these animals were domesticated [163]. Early records of *Taenia* eggs in human mummies date back to 3200 BP [164].

4. Parasitic Arthropods

While there are many examples of parasitic wasps in the fossil record, there are very few examples of actual parasitism. Some samples occur in amber and several of these show evidence

of dryinid wasp parasitism of planthoppers [3]. Evidence of an early stage ichneumonid wasp larva that was dislodged from its feeding position on the abdomen of a spider appeared in Dominican amber [165]. Such larvae first feed on spider hemolymph from the outside and then eventually consume the organs of the spider. Other evidence of spider parasitism by wasps in Dominican amber is an ichneumonid wasp larva that devoured the eggs of a spider and then pupated adjacent to the adult spider [166].

Worker *Formica* ants are parasitized by small braconid wasps that deposit their eggs directly into the body cavity of the host. The parasitic larvae develop in the ant's hemocoel and when mature, emerge between the abdominal segments and form a cocoon for pupation in the soil [167]. Evidence that the ancestors of extant *Elasmosoma* wasps have been around for some 40 million years came from a braconid wasp larva emerging from a worker ant in Baltic amber [168].

Strepsiptera, a unique order of insects, have another method of parasitizing insects. They possess active first instar larvae called triungulins that search the environment for planthopper hosts. After finding and entering the body of a planthopper, the active triungulin molts into a sedentary larva that develops to an adult inside the host. The winged adult males emerge and search for females that remain in their planthopper hosts with part of their body protruding for mating purposes. After fertilization the developing eggs hatch into a new generation of triungulins that swarm out of the female's brood pouch prepared to search for another victim. The oldest records of actual parasitism by this group are puparia inside an Eocene ant [169] and a female inside a Baltic amber ant [170]. A group of triungulins leaving their delphacid planthopper host was captured in Dominican amber when the infected planthopper landed in some resin [171]. An empty puparium in a halictid bee shows another host group for Tertiary strepsiptera [171].

A larval hymenopteran emerging from an adult trichopteran was found in Baltic amber [172]. This association is presently unknown, since today only the larval and pupal stages of trichopterans are parasitized by hymenopterans. Some tachinid fly parasitoids deposit eggs directly on their insect hosts and this behavior was noted in Dominican amber where the leaf beetle, *Stenaspidiotus microptilus*, had a tachinid egg attached to its pronotum [173].

Some interesting arthropod parasites were recently described from dog mummies in Egypt [174]. Numerous specimens of the dog tick, *Rhipicephalus sanguineus*, and a specimen of the louse fly, *Hippobosca longipennis*, were found still attached to the remains of dogs in tombs at the El Deir site in Egypt. While only dating to Roman times, they do show vector associations and suggest possible disease transmission scenarios.

5. Discussion and Conclusions

Tracing the history of diseases is similar to climbing the evolutionary scale, with free-living organisms at the base and a series of mutated parasitic stages appearing along the ascent until ultimately, human pathogens appear at the peak. The

origins and coevolution of infectious agents are reflected in their present life histories and associations with their hosts. Whether the infection is latent or virulent, includes single or multiple hosts, is permanently associated with the host or has a resistant free-living stage, has both an intermediate as well as a definitive host, and is vector spread or a contagion are reflected in the evolutionary history of the infectious agent, which often extends back millions of years.

The evolutionary history of host-pathogen associations may also determine how long the relationships will continue under future fluctuating environmental and climatic conditions. It is often the least specialized associations that survive such changes. An example is the polydnaviruses that occur only in certain groups of parasitic wasps and probably date back to the early lineages of these wasps. While very successful now, their host specificity makes polydnaviruses especially vulnerable to extinction if conditions change. If a particular wasp lineage becomes extinct because its host is eradicated, the virus also disappears. In contrast, the iridoviruses infecting the sow bug have a wide host range, crossing phyla, and even infecting vertebrates. The origin of iridoviruses probably extends back to early invertebrate lineages and these viruses have a much better chance of survival since if one host group disappears, the infection continues in other hosts. The polyhedral viruses fall between polydnaviruses and iridoviruses in being restricted to more than one genus of insects but rarely crossing families and never crossing phyla. These viral groups survived the long period of coevolution with their hosts although others certainly became extinct along the way. Unfortunately very little is known about the evolution of pathogenic viruses in reservoir hosts and how long it took before the infection became latent or if it ever was virulent. Undetectable amounts of viruses certainly exist as latent infections in all vertebrates (reservoir hosts) and some of these eventually enter humans as highly virulent pathogens.

With pathogenic bacteria, a similar situation occurs between the infectious agent and the host. Several species of the genus *Bacillus* coevolved with insects and became overt pathogens. At least one, *B. anthracis*, became a vertebrate pathogen that also infects humans. The apparent absence of reservoir hosts and the persistence of the pathogen in the environment in the form of resting spores separate this group from most pathogenic forms such as plague bacteria and Lyme disease spirochetes. These traits suggest that *Bacillus* is in an early evolutionary stage with its hosts.

Fossil evidence of protozoan infections indicates that lineages of *Leishmania* evolved in their insect vectors long before they infected vertebrates. The pathway of infection was initiated through sand fly larvae feeding on flagellates in the environment. The presence of gametogenesis and syngamy of *Plasmodium* in a mosquito in Dominican amber indicates the vector as the original host. Another character used to determine the original host in arthropod-vector associations is the amount of damage done to both arthropod and vertebrate hosts. The host showing the least amount of damage is considered the primary host.

Parasitic helminthes obviously evolved from free-living ancestors but their origins are a mystery since no early

progenitors have been found. An estimate of the date for their origins can be made from the earliest fossil record of their host group. As with the above pathogen groups, some lineages are associated with specific hosts and others with multiple hosts. An example of the former is nematodes of the genus *Parasitodiplogaster* that coevolved with fig wasps and are at risk of extinction if their host lineage perishes. In contrast, vertebrate parasitic nematodes of the genus *Ascaris* coevolved with a wide range of hosts and their existence is much more secure.

Based on our present knowledge and evidence from the past, it is obvious that both contagion and vector-borne pathogens are quite ancient. There is no definite subfossil evidence that any of these pathogens originated in humans. Humans acquired them from reservoir hosts (zoonosis) that had asymptomatic infections after a precarious balance was established with the pathogen [174]. Such a balance is absent when the pathogens are introduced into humans and then they become overt and highly virulent.

Unfortunately, we know very little about the development of human pathogens in reservoir hosts and arthropod vectors or why some vectors are more efficient carriers than others. All of these traits are connected with the coevolution of pathogens with their hosts that occurred millions of years ago.

The host-parasite associations around us today represent various stages of coevolution. Some may be at the end of their evolutionary line while others may be in the middle or just beginning. Most of these associations will be unsuccessful for one reason or another while others will survive and fall into one or more of the following recognized stages of symbiotic relationships: inquilinism (two organisms living in the same habitat), commensalism (one of the two organisms benefits), mutualism (both organisms benefit), or parasitism (one organism takes nourishment from the other, which suffers). Determining the intricacies of these evolutionary stages presents a challenge to future generations of scientists.

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The author thanks Roberta Poinar for discussions and comments on earlier drafts of this paper, T. N. Taylor for supplying Figure 61, Debi Poinar for supplying Figures 18 and 19, and Raymond Jacobson for supplying references and comments.

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