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Spirochete-like cells in a Dominican amber *Ambylomma* tick (Arachnida: Ixodidae)

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Abstract

Amber preserves microscopic, soft-bodies organisms and is a good medium in which to trace the evolution of pathogen-vector associations. Spirochetes-like cells (Spirochaetales: Spirochaetaceae) in the hemocoel and lumen of the alimentary tract of a larva tick (*Amblyomma* sp. Arachnida: Ixodidae) in Dominican amber are described in the collective fossil genus and species, *Palaeoborrelia dominicana* n. gen., n. sp. The size and shape of the fossil spirochetes closely resemble those of present day *Borellia* species. This discovery represents the first record of spirochetes associated with fossil ticks.

Keywords: Ixodidae; Dominican amber; Tertiary hard tick

Introduction

Bacteria are an ancient group that date back some 3.6 billion years, when filamentous types appeared in the marine environment (Awramik et al. 1983). When terrestrial bacterial first formed associations with invertebrates is unknown since fossil terrestrial bacteria are quite rare. Their small size and delicate structure (except for spores) are not prone to fossilization. Fortunately, amber is one medium that preserves such small, intricate organisms and previous inclusions in fossilized resin have provided direct evidence of free-living, symbiotic and insect-pathogenic bacteria (Blunck 1929, Katinas 1983, Poinar 1992, 2010, 2011b, Waggoner 1996). Four *Amblyomma* tick larvae in Dominican amber were examined for the presence of internal microbes. One specimen contained large numbers of spirochete-like cells in the hemocoel and lumen of the alimentary tract. The present study characterizes these cells and shows their resemblance to extant tick-borne *Borrelia* species.

Materials and Methods

A series of 4 unengorged tick larvae in Dominican amber were examined in this study.

They were all well preserved and complete (Fig. 1). While the hemocoel and lumen of the alimentary tract of three of the ticks were free of inclusions, numerous spirochete-like cells were noted in the fourth larva. The ventral surface of this larva was polished away to better view the internal tissues (Fig. 2). This revealed an exceptionally large population of spirochete-like cells in the hemocoel and lumen of the alimentary tract. These cells were often several layers deep and had been fixed and dehydrated from natural compounds in the original plant resin. They appeared black under bright field lighting, were orientated haphazardly in three dimensions and had dried in a variety of shapes. Unfortunately fine details of small microscopic structures are rarely preserved in amber thus fibers and flagella could not be distinguished. Since it is not possible to section such small delicate material in amber without putting the specimen in jeopardy, identification of the cells was based on their physical features and location in the body of a hard tick. Observations and photographs were made with a Nikon Optiphot compound microscope (with magnifications up to 1000x).

The amber containing the fossils originated from amber mines in the northern mountain range (Cordillera Septentrional) of the Dominican Republic between Puerto Plata and Santiago. Dating of Dominican amber based on foraminifera is 20-15 mya (Iturralde-Vincent and MacPhee 1996). The specimen is deposited in the Poinar amber collection (accession # A-10-270) maintained at Oregon State University, Corvallis, Oregon, USA.

Systematic Palaeontology

The putative fossil spirochetes occur throughout the hemocoel and lumen of the alimentary tract of the fossil tick (Figs. 3-8). Viewing the fossil cells is similar to examining a dry mount of modern forms with sub-stage illumination. Because they overlap each other, entire cells were difficult to photograph. However, by changing the fine focal adjustment on the microscope, select cells could be distinguished and outlined. These are presented separately adjacent to the original photographs and demonstrate the range in cell shapes (Figs 6-8). Some of the cells were surrounded by light borders that possibly represent outer sheaths (Fig. 9).

Description

Phylum: Spirochaetes Garrity and Holt 2001

Class Spirochaetia Paster 2010

Order Spirochaetales Buchanan 1917

Family Spirochaetaceae Swellengrebel 1907

Of the three families recognized within the Order Spirochaetes, the fossil cells fall within the family Spirochaetaceae. Currently, four genera are recognized in this family (Paster 2010). Members of the genus *Spirocheta* Ehrenberg 1835 are free-living in aquatic environments. Species in the genus *Cristispira* Gross 1910 range from 30 µm – 180 µm in length and occur in the digestive tract of freshwater and marine mollusks. Representatives of the genus *Treponema* Schaudinn 1905 have regularly and rigidly coiled cells and are obligate anaerobes that occur in the mouth, gut and genital tract of animals. Members of the genus *Borrelia* Swellengrebel 1907 have loose, coarse, irregular coils, range from 3 µm -30 µm in length and most are vectored by ticks (Paster 2010; Kelly 1984). The fossil spirochetes would fall into the genus *Borrelia*, however since millions of years separate the fossils from the extant species of *Borrelia*, the fossil spirochetes are described in a collective fossil genus. The description is based on the large population of spirochetes in the hemocoel and lumen of the alimentary tract of the fossil larval *Ambylomma* tick.

Palaeoborrelia Poinar, n. gen.

This taxon is established as a collective fossil genus for spirochetes in the hemocoel and/or lumen of the alimentary tract body of fossil ticks (Ixodida).

Diagnosis: Cells elongate, narrow, with rounded ends, some elongate and curved; others with irregular 3-8 loose coils; occurring in hemocoel and/or lumen of alimentary tract of fossil ticks (Ixodida).

Palaeoborrelia dominicana Poinar, n. sp. (Figs. 3-8)

Description. Cells elongate, narrow, with rounded ends and various shapes, most of which range from elongate curved forms to those with coarse, irregular 3-8 loose coils; length cells (N=20): 9 µm (7 µm -13 µm); width cells (N=20): 0.4 µm (0.3 µm -0.5 µm).

Diagnosis: The species is erected for spirochetes occurring in the hemocoel and/or lumen of the alimentary tract of fossil ticks in Dominican amber.

Specimen. Deposited in the Poinar amber collection (accession # A-10-270) maintained at Oregon State University, Corvallis, Oregon, USA.

Locality. Amber mine in the Cordillera Septentrional of the Dominican Republic.

Etymology. The generic name is from the Greek "palaeo" = old and the name of the extant genus *Borrelia*. The specific epithet indicates the geographical origin of the fossil.

Vector tick. The larval tick has elongate palps, 11 festoons and eyes, which places it in the genus *Amblyomma*. The length (excluding capitulum) is 0.53 mm and the width 0.57 mm, which falls within the range of unengorged larval *Ambylomma* ticks previously reported in Dominican amber (Keirans et al. 2002). An adult *Ambylomma* tick was earlier characterized from the same amber source (Lane and Poinar 1986).

Remarks. The spirochete-like cells can be separated from other possible bacteria carried by extant ticks by size alone, since the latter are all under 2 µm in length (Paster 2010; Kelly 1984). While helical bacteria are fairly common in the environment, members of the genus *Borrelia* are the only representatives of this group that occur in ticks (Paster 2010; Kelly 1984). Consideration was given to the possibility that the fossil cells represent some type of extracellular symbiont, similar to nodiocardiform organisms that occur in the alimentary tract of triatome bugs (Poinar 2011). Besides, all known symbionts of extant ticks are intracellular, thread-like forms with chains composed of small rods and granules that reside in the Malpighian tubules and developing eggs (Buchner 1965; Steinhaus 1946). Besides, if they were some type of extinct extracellular symbiont, they would likely be present in the body cavities of the other 3 larval *Ambylomma* ticks examined in the present study. There was no sign of these structures in the other larval ticks.

The apparent absence of a nucleus and kinetoplast in the fossil cells eliminates the possibility that they are promastigotes of a trypanosome. Also trypanosome cells have pointed ends, which is not the case with the fossil cells (Burgdorfer et al. 1973). The possibility that the fossil cells represent fibrin strands or matrix proteins can be dismissed because such structures have never been observed in ticks (Bain 2005; Stein 1986). The shape of the fossil cells were similar to those of extant desiccated spirochetes (Fig.10)(Lane and Burgdorfer 1987).

Discussion

The fossil cells described in the present study closely resemble in size and shape tick-borne spirochetes of the extant genus *Borrelia*. Their presence in the body of a hard tick is also characteristic of *Borrelia* spirochetes. The fact that these fossil cells occur both in the body cavity and gut lumen supports the contention that they are not artifacts acquired from a host or intracellular products that formed after the tick became entombed in amber. The time of death of organisms in resin occurs immediately after entombment and tissue preservation begins instantly. If these cells suddenly spontaneously developed they should be present in the other ticks examined as well as in additional organisms entrapped in amber. Such is not the case.

It is interesting that the fossil spirochete-like cells are in the hemocoel as well as in the alimentary tract of the fossil tick. Their acquisition could have been by transovarial transmission (TOT) or by feeding on an infected host. The extant *Borrelia miyamotoi* has up to a 90% - 97% TOT rate in larvae of the western black-legged tick (Lane and Burgdorfer 1987; Barbour et al. 2009). Even though there is no evidence of blood in the fossil tick, there could have been some initial host contact since ixodid ticks can attach, initiate feeding and then become dislodged from the host (Sonenshine 1993; Sonenshine et al. 2002). Thus it is conceivable that the tick could have acquired at least some of the fossil cells while attached to an infected host, but then became dislodged before any significant amount of blood was consumed

The fossil record of hard ticks extends back to the Early Cretaceous some 100 mya (Poinar and Brown 2003; Poinar and Buckley 2008). This sets a minimum time period when these arachnids could have acquired spirochetes, followed by a period of co-evolution that established the vertebrate cycle and increased vector competency.

Fossil parasites associated with their vectors provide us with minimum time and place records of various extant diseases. Such records are valuable for determining the evolutionary history of specific pathogens. The present study establishes a minimum time period of 20-15 mya when hard ticks were infected with *Borrelia*-type spirochetes.

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Figures

1. One of four unengorged larval *Amblyomma* ticks in Dominican amber that was investigated in this study. In this specimen, as well as in 2 others, no inclusions were present in the hemocoel or lumen of the alimentary tract. Scale bar = 220 µm.

2. A larval *Ambylomma* tick in Dominican amber containing spirochete-like bodies. The integument is partially removed to better view the cells. Scale bar = 200 µm.

3. *Palaeoborrelia* cells (arrows) within the chelicerial sheath and surrounding the hypostome of a larval *Ambylomma* tick in Dominican amber. Scale bar = 16 µm.

4. *Palaeoborrelia* cells (arrows) around the anus of a larval *Ambylomma* tick in Dominican amber. Scale bar = 11 µm.

5. *Palaeoborrelia* cells in the pharynx (arrow) of a larval *Ambylomma* tick in Dominican amber. Scale bar = 13 µm.

6. A. *Palaeoborrelia* cells in the lumen of the alimentary tract of a larval *Ambylomma* sp. tick in Dominican amber. Scale bar = 10 µm. B. Outlines of 5 cells from Fig. 6A examined at various focal levels with background removed. Numbers correspond to those in Fig. 6A. Scale bar = 10 µm.

7. A. *Palaeoborrelia* cells in the hemocoel of a larval *Ambylomma* sp. tick in Dominican amber. Scale bar = 13 µm. B. Outlines of 10 cells from Fig. 7A examined at various focal levels with background removed. Numbers correspond to those in Fig. 7A. Scale bar = 13 µm.

8. A. *Palaeoborrelia* cells in the hemocoel of a larval *Ambylomma* sp. tick in Dominican amber. Scale bar = 5 µm. B. Outlines of 6 cells from Fig. 8A examined at various focal levels with background removed. Numbers correspond to those in Fig. 8A. Scale bar = 5 µm.

9. Two *Palaeoborrelia* cells showing adjacent clear zones that could represent periplasmic spaces between the protoplasmic cylinders and their surrounding sheathes. Scale bar = 2.5 µm.

10. Photo from the web (inverted in black and white by the present author) showing a dry mount of *Borrelia burgdorferi* cells with configurations similar to those of the fossil cells. Scale bar = 6 µm. Photo by Claudia R. Molins with permission.