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**Rickettsial-like cells in the Cretaceous tick,
Cornupalpatum burmanicum (Ixodida: Ixodidae)**

George Poinar, Jr.

Department of Integrative Biology, Oregon State University, Corvallis, OR 97331, USA

Phone: 541 752 0917

Fax: 541 737 0501

Email: poinarg@science.oregonstate.edu

Abstract

Rickettsial-like cells are reported from the body cavity of the Myanmar amber larval tick, *Cornupalpatum burmanicum* (Ixodida: Ixodidae). These cells are characterized and described in a new collective fossil genus erected for putative rickettsial in fossil ticks. The size and shape of the fossil cells resemble those of present day members of the Rickettsiaceae, many of which occur in the body cavity of present day ticks.

Key words: rickettsial-like cells, Cretaceous tick, Ixodidae

Introduction

Bacteria are an ancient group that date back some 3.6 billion years BP, 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 inclusions in fossilized resin have provided direct evidence of not only free-living, but symbiotic and insect-pathogenic bacteria (Blunck, 1929; Poinar, 2011; Schmidt and Schäfer, 2005; Waggoner, 1996).

In re-examining the paratype of the Early Cretaceous hard tick larva, *Cornupalpatum burmanicum* Poinar and Brown (2003) in Myanmar amber, rickettsial-like cells were observed in the body cavity. The present study describes these cells and shows their resemblance to extant members of the Rickettsiales.

Materials and methods

The Myanmar amber piece containing the paratype of *Cornupalpatum burmanicum* (accession number A-10-261) is rectangular in outline, measuring 22 mm in greatest length, 15 mm in greatest width and 4 mm in greatest depth. This specimen shares with the holotype of *Cornupalpatum burmanicum* Poinar and Brown (2003) the unique feature of palpal claws, which distinguishes the genus from all extant Ixodidae. The length of the paratype larva (idiosoma and capitulum) is 490 μm . 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 presence in the body of

a hard tick. Observations and photographs were made with a Nikon Optiphot compound microscope (with magnifications up to 1000x).

The tick specimen originated from the Noiye Bum 2001 Summit Site in the Hukawng Valley, southwest of Maingkhwan in the state of Kachin, Myanmar. Amber-bearing strata in this mine were assigned to the Upper Albian (97-110 Ma) of the Early Cretaceous on the basis of paleontological evidence (Cruickshank & Ko, 2003).

Results

The resemblance of the rickettsial-like cells in the fossil tick to extant rickettsiae is based on size, developmental types, presence of “halos” on some individuals, multiplication by binary fission and occurrence in the body cavity of a tick. The fossil cells occur throughout the body cavity of the fossil tick but are especially concentrated in four groups extending posteriorly from the main portion of the tick’s body (Fig. 1). In his study of the internal anatomy of *Dermacentor andersoni* Stiles, Douglas (1943) noted that the midgut is composed of some 14 diverticula that ramify throughout the body cavity. Two pairs of caudal diverticula extend from the midgut proper (stomach) to the caudal margin of *D. andersoni*. These four caudal diverticula in *D. andersoni* correspond to the four groups of fossil rickettsial-like cells extending from the midgut area to the caudal margin of the fossil tick.

Description

? Order Rickettsiales Gieszczykiewicz 1939 (Dumler and Walker, 2005)

Based on the morphological characters of the fossil cells, they are tentatively placed in the Order Rickettsiales.

Palaeorickettsia Poinar, n. gen.

This taxon is established as a collective genus for rickettsial-like bodies in the body cavity of fossil ticks (Ixodida). It is recognized that collective genera have no taxonomic standing.

Diagnosis: Rickettsial-like cells occurring in the body cavity of fossil ticks (Ixodidae).

Type species: *Palaeorickettsia protera* Poinar n. gen., n. sp.

Palaeorickettsia protera Poinar n. sp. (Figs 1-5)

Description. Coccoid, diplococcoid and short, rod-shaped cells dividing by binary fission; individual rods from 0.4-0.8 μm in width and 1.3-1.7 μm in length; coccoid forms, including those surrounded by halos, 0.4- 0.7 μm in diameter.

Diagnosis: This species is erected for rickettsial-like bodies occurring in the body cavity of fossil ticks in Myanmar amber. Aside from the above morphological features, it is also characterized by its presence in the extinct, Early Cretaceous tick, *Cornupalpatum burmanicum*. *Palaeorickettsia protera* can be distinguished from other groups of tick-borne bacteria, such as spirochetes, by its small size (cells under 2 μm in length) and its apparent intracellular association with the midgut diverticula.

Specimen. Deposited in the Poinar amber collection (accession # A-10-261) maintained at Oregon State University, Corvallis, Oregon, USA.

Locality. Amber mine in the Hukawng Valley, southwest of Maingkhwan in the state of Kachin, Myanmar (26°20'N, 96°36'E).

Etymology. The generic name is from the Greek “palaeo” = old and the name of the extant genus *Rickettsia*. The specific epithet is from the Greek “proteros” = earlier.

Remarks: The four groups of fossil cells corresponding to midgut diverticula of *Cornupalpatum burmanicum* measured 73 µm, 90 µm, 80 µm and 85 µm in length, respectively (Fig. 1). The cells in the center of the groups were mostly small rods, many of which were dividing (Figs 2-5). In shape and size, the fossil rods closely resemble those of the extant *Rickettsia rickettsii* Brumpt, 1922 (Fig. 6). The coccoid cells surrounded by “halos” often formed along the outer margin of a central group of dividing rods (Figs. 2, 3). In some areas, small clusters of these “halo” cells were arranged within larger groups (Fig. 4).

Discussion

The fossil cells described above closely resemble in size and shape those of extant rickettsia. Due to their small size, age and presence in amber, it is not possible to stain them to see if they possess a gram-negative cell wall. It is difficult to determine the location of the fossil cells in the living tick but since they cover the entire area of the proposed diverticula, they could have been intracellular.

In extant ticks, rickettsiae can occur in various tissues throughout the body cavity, especially in the midgut epithelium (Sonenshine, 1993). While cells of *Palaeorickettsia proteran* are present throughout the body cavity of the tick, most are concentrated in the four cell groups corresponding with the midgut diverticula (Fig. 1).

The “halo” is considered to represent the slime layer that appears as a translucent zone around extant intracellular rickettsial cells and is thought to attach rickettsiae to potential host cells (Weiss and Moulder, 1984; Austin and Winkler, 1988; Sonenshine, 1993; Yu and Walker, 2005). Similar halos have been noted around coccoid cells of insect parasitic rickettsiae (Poinar and Thomas, 1984).

Since the tick is unengorged, it is possible that the rickettsial-like cells were acquired by transovarian transmission, which is the normal manner of vertical transfer for many pathogenic tick-borne rickettsiae (Sonenshine, 1998; Yu and Walker, 2005).

Fossil parasites associated with their vectors provide minimum time and place records for understanding the evolutionary history of extant lineages. The present study, which is the first fossil record of rickettsial-like cells, establishes a minimum time period of 97-110 mya when hard ticks were associated with these organisms.

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Legends to illustrations

Fig. 1. Paratype of *Cornupalpatum burmanicum*, an unengorged ixodid tick larva in Early Cretaceous Myanmar amber. Arrows show 4 groups of *Palaeorickettsia protera* that were probably associated with midgut diverticula. Bar = 130 μm .

Fig. 2. Developing stages of *Palaeorickettsia protera* in the first group of cells in *Cornupalpatum burmanicum* in Early Cretaceous Myanmar amber. Groups of coccoid “halo” stages (C) occur on the outer margins of a central group of dividing cells. Bar = 6 μm .

Fig. 3. Various stages of *Palaeorickettsia protera* in the second group of cells in *Cornupalpatum burmanicum* in Early Cretaceous Myanmar amber.

D= diplococci; C= coccoid “halo” stages; P = pleomorphic stage; A = tick anus. Bar = 7 μm .

Fig. 4. Stages of *Palaeorickettsia protera* in the third group of cells in *Cornupalpatum burmanicum* in Early Cretaceous Myanmar amber. Arrows show clusters of coccoid “halo” forms. Bar = 7 μm .

Fig. 5. Detail of a group of *Palaeorickettsia protera* in the fourth group of cells in *Cornupalpatum burmanicum* in Early Cretaceous Myanmar amber. Bar = 2.0 μm .

Fig. 6. Cells of the extant *Rickettsia rickettsii* in the cytoplasm of a hemocyte of *Dermacentor andersoni*. Note similarity of these cells to the fossil cells in Figure 5. Photo courtesy of W. Burgdorfer. Bar = 1.5 μm .