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ORIGINAL RESEARCH

Spiroplasma burmanica sp. nov. (Spiroplasmataceae: Mollicutes) from a Fossil Plant Louse (Psylloidea: Sternorrhyncha) in mid-Cretaceous Burmese Amber

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ABSTRACT
A new species of spiroplasmid, *Spiroplasma burmanica* sp. nov. (Mollicutes: Entomoplasmatales: Spiroplasmataceae) is described from the body cavity of a fossil plant louse (Psylloidea: Sternorrhyncha) in Burmese amber. The new species is pleomorphic with body shapes varying from oval to helical. The majority of the helical cells occur in the head, thorax (including leg cavities) and abdomen of the fossil psyllid. The association between *S. burmanica* and the psyllid is considered to be a case of symbiosis, similar to extant relationships. This discovery of the first fossil spiroplasmid shows that psyllids carried these microorganisms some 100 million years ago.

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Introduction

Insects have associations with many different microorganisms. These associations can be mutually beneficial and asymptomatic (symbiotic), parasitic or pathogenic. Most insect symbiotes are specific, occur in a single or related host, and are vertically transmitted (Bressan, 2014).

Members of the family Spiroplasmataceae are enigmatic, parasitic microorganisms characterized by the absence of cell walls, flagella, and other organelles of locomotion. They possess a wide range of cell shapes (from spherical to helical) and are found in both insects and plants. Spiroplasmas in herbivorous insects may be vectored to and established in the insect's host plant (Fletcher et al., 2006; Gasparich 2010). Spiroplasmas also may cause various structural malformations in insects (Williamson et al., 1999). The present study describes a spiroplasmid in the body cavity of a fossil psyllid (Psylloidea: Sternorrhyncha) in Burmese amber, showing that the family Spiroplastmataceae extends back at least to the mid-Cretaceous.

Materials and methods

The amber specimen originated from the Noije Bum Summit Site mine in the Hukawng Valley, located southwest of Maingkhwan in Kachin State (26º20´N, 96º36´E) in northern Myanmar. Based on paleontological evidence the site was dated to the late Albian of the Early Cretaceous (Cruickshank & Ko 2003), placing the age at 97–110 Mya. A recent Zircon U-Pb and trace element analyses of amber from different locations in Myanmar confirmed an age of around 100 Ma for amber from the Hukawng Valley as well as an age range of 72 Ma to 110 Ma for amber from other sites in northern Myanmar (Xing & Qui, 2020).

The amber was polished close enough to the insect's abdomen to observe the spiroplasmas under oil immersion with a Nikon Optiphot compound microscope. Helicon Focus Pro X64 was used to stack photos for better overall clarity and depth of field. Images of microorganisms in amber are difficult to photograph since photos must be taken through the amber matrix as well as through the insect body wall, which explains why the resulting images can never be as clear as with extant forms.

Results

A wide range of cell shapes (from spherical to helical) were observed in the body cavity of the fossil psyllid. Tightly appressed cells of various shapes (from spherical to short helical) occurred in several extracellular locations in the abdominal hemocoel (Figs.1,2). The remainder of the abdomen, as well as the thorax, head and leg segments of the fossil psyllid were filled with helical cells in various positions, indicating that they were in motion when preserved.

Placement of the microbes in the genus *Spiroplasma*, which is the only genus in the family Spiroplasmataceae, is based on the helical (spiral) shape of the great majority of cells and the absence of cell walls, flagella, and other organelles of locomotion (Whitcomb & Tully,1982, 1984). It was not possible to confirm the presence of spiroplasmas in the alimentary tract since it was opaque.

Phylum: Firmicutes Gibbons and Murray 1978

Division: Tenericutes Rasin & Freundt 1984
Class: Mollicutes Edward & Freundt 1967
Order: Entomoplasmatales Tully et al. 1893
Family: Spiroplasmataceae Skripal 1983
Genus: Spiroplasma Skripal 1974

Spiroplasma burmanica sp. nov. (Figs.1-7)

This taxon is established for spiroplasmas in the body cavity of fossil plant lice (Hemiptera: Sternorrhyncha: Psylloidea) in Burmese amber.

Diagnosis: Species pleomorphic, with both spherical and helical cells lacking cell walls, flagella, and other organelles of locomotion. Helical cells occur in the hemocoel of the abdomen, thorax, head, and legs. Spherical cells occur in clusters in the abdominal cavity.

Description: Species polymorphic, lacking cell walls; with clusters of spherical cells, ranging from 0.06 to 0.10 µm in diameter, in the abdomen. Helical cells, presumed to be motile based on their various shapes, ranging from 0.3 to 0.5 µm in length and from .03 to .05 µm in diameter, occurring in the abdomen, thorax, head and leg segments. Genetic and growth features unobtainable.

Specimen. Deposited in the Poinar amber collection (accession # B-He- 4-37) maintained at Oregon State University.

Type locality: Myanmar (Burma), state of Kachin, Noije Bum Summit Site amber mine in the Hukawng Valley, SW of Maingkhwan (26º20´N, 96º36´E).

Etymology: The specific epithet indicates the geographical origin of the fossil.

Host insect: *Alloeopterus anomeotarsus* (Hemiptera: Sternorrhyncha: Psylloidea: Dinglidae).

Figure 1. Dorsal view of *Alloeopterus anomeotarsus* gen. et sp. nov. in Burmese amber. Scale bar = 400 µm.

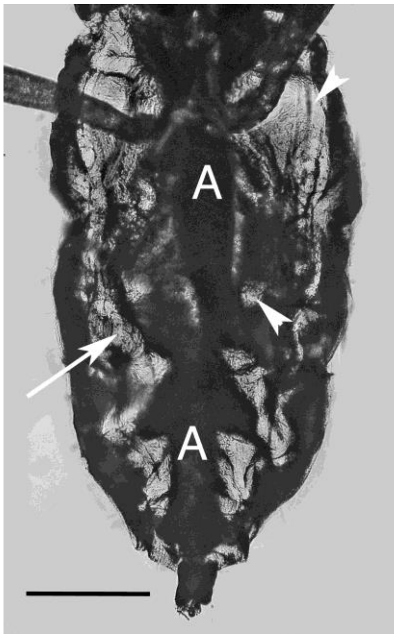

Figure 2. Dorsal view of abdomen of *Alloeopterus anomeotarsus* in Burmese amber. A= alimentary tract. Arrow shows location of spherical and short-helical stages of *Spiroplasma burmanica* sp. nov. shown in Fig. 3. Lower Arrowhead shows area of helical stages of *S. burmanica* sp. nov. shown in Fig. 4. Top arrowhead shows area of helical stages show in Fig. 5. Scale bar = 160 µm.

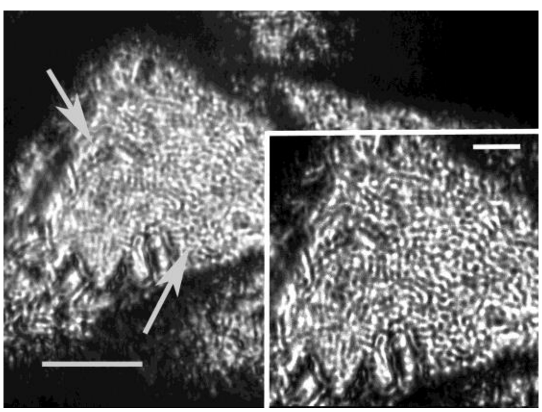

Figure 3. Spherical and short-helical cells of *Spiroplasma burmanica* sp. nov. in the abdominal hemocoel of *Alloeopterus anomeotarsus* in Burmese amber. Scale bar = 0.7 µm. Insert. Detail of same area. Scale bar = 0.3 µm.

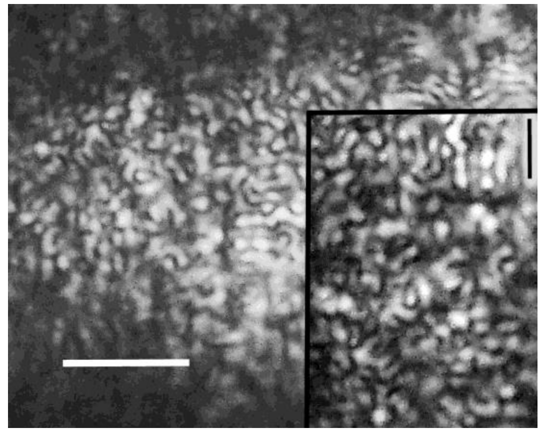

Figure 4. A. Helical cells of *Spiroplasma burmanica* sp. nov. in the hemocoel of the abdomen of *Alloeopterus anomeotarsus* in Burmese amber. Scale bar = 0.4 µm. B. Same as 4A except under different optical conditions. Scale bar = 0.2 µm.

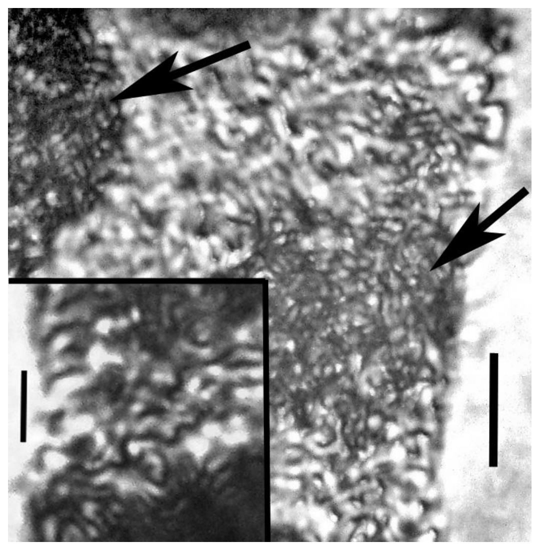

Figure 5. Clusters of helical cells (arrows) of *Spiroplasma burmanica* sp. nov. along the edge of the abdominal cavity of *Alloeopterus anomeotarsus* in Burmese amber. Scale bar = 0.7 µm. Insert. Detail of helical cells in same area. Scale bar = 0.2 µm.

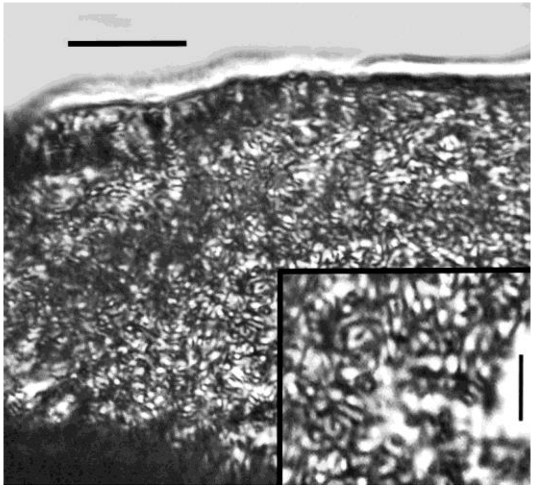

Figure 6. Helical cells of *Spiroplasma burmanica* sp. nov. in the lumen of the mesofemur of *Alloeopterus anomeotarsus* in Burmese amber. Scale bar = 1.5 µm. Insert. Detail of helical cells in same area. Scale bar = 0.4 µm.

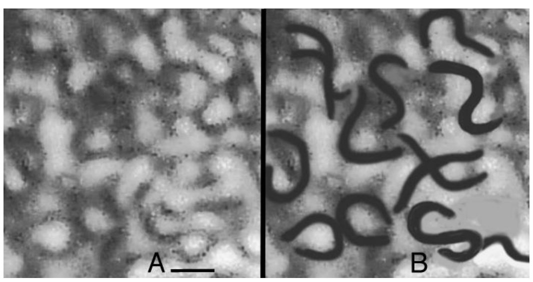

Figure 7. Helical cells of *Spiroplasma burmanica* sp. nov. in the abdominal cavity of *Alloeopterus anomeotarsus* in Burmese amber. A. Enlarged view of several helical cells. Scale bar = 0.02 µm. B. Same photo as A but with select cells blackened. Scale same as in A.

Discussion

Spiroplasma burmanica sp. nov. can be separated from many other insect microorganisms by the presence of its minute helical cells. This feature distinguishes it from the spherical to short, straight to slightly curved, rod-shaped mycetocytes found in extracellular, spherical mycetomes of extant psyllids (Profft, 1936; Chang & Musgrave,1969 or 64).

Another widespread group of microorganisms found in a wide range of invertebrates, including psyllids (Nakabachi et al., 2020), are rickettsia (Weiss & Moulder, 1984). Due to their medical importance, the rickettsias of blood-sucking insects, especially those of tsetse flies, have been most studied. Tsetse rickettsia, as well as those of psyllids (Nakabachi et al., 2020), are straight rods approximately 1.90 µm in length and 0.56 µm in width (Pinnock & Hess, 1974; Dale & Maudin, 1999). Their intracellular location and shape distinguish them from the ovoid and helical cells of *Spiroplasma burmanica* sp. nov. (Roubaud,1919; Wigglesworth, 1929). While the coccoid cells of *Spiroplasma burmanica* sp. nov. superficially resemble those of the bacterial genera *Staphylococcus* Rosenbach and *Micrococcus* Cohn that are parasites of numerous insects (Poinar & Thomas, 1984), the presence of cell walls and the chain-like growth pattern of the cells in the latter two genera distinguish them from the spherical cells of *Spiroplasma burmanica* sp. nov.

The small size, absence of cell walls and flagella and helical cells distinguishes *Spiroplasma burmanica* sp. nov. from the much larger, rod-shaped insect pathogenic bacteria, such as the ubiquitous *Pseudomonas aeruginosa* (Schroeter) and *Serratia marcescens* Bizio (Poinar & Thomas, 1984).

The helical cells of *Spiroplasma burmanica* sp. nov. exhibit a similar shape to some protozoan flagellates that occur in the body cavity of arthropods, especially blood-suckering insects. However, trypanosomatids are normally restricted to the gut and salivary glands of arthropods, possess flagella, undulating membranes and are much larger (over 5 µm in length) than the helical cells of *Spiroplasma burmanica* sp. nov. (Marinkelle, 1982; Schaub, 1994; Wallace, 1966; Kudo, 1966).

The polymorphic feature of spiroplasmas, with cell shapes ranging from spherical to helical (Fig. 3) is shared with mycoplasmas (Razin & Freundt, 1984; Whitcomb & Tully, 1982, 1984). Both groups have cells that are spherical, pear shaped, filamentous or helical. However, the cells of mycoplasmas are usually not motile or at most have a gliding movement and are parasites of birds and mammals (Razin & Freundt, 1984; Meloni et al., 1980).

It is not possible to determine the plant host of the fossil psyllid, since the host range of extant psyllids includes at least 45 plant families (Hodkinson, 2009). However, psyllids mainly feed on perennial dicotyledonous angiosperms and are usually host specific (Hodkinson, 2009). Their restriction to a single (or closely related) host plant makes psyllids excellent vectors of plant parasitic microorganisms. In Africa and Asia, psyllids vector phloem-limited bacteria such as *Candidatus* Liberibacter africanum and *Candidatus* L. asiaticum (Rhizobiaceae) that cause "citrus greening", a disease resulting in the dieback of shoots and leaves and the deterioration of fruit quality (Gullan & Martin, 2003; Nadarasah & Stavrinides, 2011). The minute rod-shaped bacteria of *Candidatus* range from 0.2-0.4 µm in length (Puttamuk et al., 2014). No helical cells are associated with cultures of *Candidatus* (Nadarasah & Stavrinides, 2011).

There are no known examples of psyllids vectoring spiroplasmas to their host plants and it is very likely that *S. burmanica* has a symbiotic association with the psyllid, involving either commensalism (one organ benefits and the other organism is not harmed), mutualism (both organisms benefit, and neither is harmed) and is carried transovarially from one generation to the next. A symbiotic association is strengthened by the discovery of helical cells of *Spiroplasma burmanica* in the body cavity of another Burmese amber psylloid, *Mirala burmanica* Burckhardt & Poinar (2020). The present study extends the distribution of the family Spiroplastmataceae back at least to the mid-Cretaceous and shows psyllida as being early hosts of this association.

Conflict of Interest statement

The author claims that there is no conflict of interest regarding this paper.

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