Paleorhodococcus dominicanus gen. nov., sp. nov., a fossil Actinobacteria in a fecal droplet of *Triatoma dominicana* (Hemiptera: Reduviidae: Triatominae) in Dominican amber.

George Poinar, Jr.

Department of Zoology, Oregon State University, Corvallis, OR 97331

Correspondence

poinarg@science.oregonstate.edu

Paleorhodococcus dominicanus n. gen., n sp. (Actinobacteria) is described from a fecal droplet of *Triatoma dominicana* (Hemiptera: Reduviidae: Triatominae) in Dominican amber. The fossil can be distinguished from most extant species of the closely related extant genus *Rhodococcus* Zopf, 1891 by its spherical cocci forming substrate filaments with elementary branching, the clustering of coccoidal elements , the short filaments bearing reduced side branches and its occurrence in a fecal droplet of the extinct triatomine bug, *P. dominicanus*. This is the first fossil record of an Actinobacter and shows that these organisms formed symbiotic associations with insects by the mid-Tertiary.

Introduction

The "nocardioform" Actinobacteria comprise several genera of microorganisms that form fugacious mycelia that break up into rod-shaped or coccoid nonmotile elements (Lechevalier, 1984; Garrity et al., 2004). *Rhodococcus* is a genus in this group that forms aerobic, nonsporulating, nonmotile, Gram-positive coccoidal elements with associated aerial or substrate mycelium. Species of this genus occur in a variety of environments, including soil, water, and insect alimentary tracts (Lechevalier, 1984; Garrity et al., 2004). Several species are symbionts in the intestine of bloodsucking triatomine bugs (Hemiptera) (Brecher & Wigglesworth, 1944).

In a fecal drop (Fig. 1) of a previously described fossil bug, *Triatoma dominicana* Poinar (2005) in Dominican amber, were numerous coccoid elements with associated mycelial fragments (Figs.2-3). These structures, which are described below, are considered to represent spores and mycelial elements of a "nocardioform" Actinobacteria (Lechevalier, 1984; Garrity et al., 2004).

Methods

Amber location.The piece of amber containing the fossil triatomine with the fecal droplets originated from La Toca mine, between the cities of Puerto Plata and Santiago, in the Cordillera Septentrional mountain range in the northern portion of the

Dominican Republic. Dating of Dominican amber is still controversial with the latest proposed age of 20-15 mya based on foraminifera (Iturralde-Vinent & MacPhee, 1996) and the earliest of 45-30 mya based on coccoliths (Cêpek in Schlee, 1999). What makes dating the amber difficult is that it is secondarily deposited in turbiditic sandstones of the Upper Eocene to Lower Miocene Mamey Group (Draper et al., 1994). The plant species that formed the amber is a member of the legume family (*Hymenaea protera* Poinar, 1991) and the original environment was similar to a present day moist tropical forest (Poinar & Poinar, 1999).

Amber piece.The triangular amber piece containing the fecal droplet was polished through the drop in order to better view the contents, which included both metatrypanosomes and actinobacterial organisms. The final piece measured 9 mm x 8 mm x 8 mm and the oval fecal droplet (Fig. 1) was 3.3 mm in length and 1.5 mm in width. The fecal droplet was from the fossil triatomine bug, *Triatoma dominicana* Poinar (2005). Observations and photographs were made with a Nikon stereoscopic microscope SMZ-10 R and Nikon Optiphot TM at magnifications up to 1000X.

Results and Discussion

Description of fossil. Since various cultural, physiological and biochemical characters used to classify Actinobacteria are not available for fossils, characters of the organism described as the new collective genus *Paleorhodococcus* are based on morphology and host. This genus is established for Actinobacteria found in the alimentary tract of fossil insects. Systematic hierarchy is taken from Garrity et al., (2004).

Phylum Actinobacteria

Class Actinobacteria

Subclass Actinobacteridae

Order Actinomycetales

Suborder Corynebacterineae

Family Nocardiaceae

Description of *Paleorhodococcus* gen. nov.

Established for Actinobacteria found in the alimentary tract of fossil insects.

Generic characters same for those of species.

Type species: *Paleorhodococcus dominicanus* Poinar

Description of *Paleorhodococcus dominicanus* sp. nov. (Figs. 2-3)

Paleorhodococcus from "paleo", Greek for old and extant genus *Rhodococcus*.

"dominicanus" refers to the Dominican Republic, the place of origin of the fossil.

Numerous small, spherical to subspherical coccoid elements ranging from 1.3 µm - 2 µm in greatest diameter; coccoid elements solitary or in clumps of 2-5; mycelial (hyphal) fragments arising from cocci thin, with elementary branching (side projections); from 0.5 µm to 20 µm in length and 0.8 µm to 1.5 µm in diameter; conidia and endospores absent.

Type specimen: In fecal droplet of *Triatoma dominicana* in amber from the Dominican Republic: deposited in the Poinar amber collection maintained at Oregon State University (accession number P-3-3).

Type locality: La Toca amber mine in the Dominican Republic.

Diagnosis: While the composition of the cell wall is presently the main basis for separating species of this genus, morphological and cultural differences were used in the past. Some species produce aerial hyphae or serial synnemata, while others are amycelial or produce well-branched substrate mycelia. Also cocci may generate into short rods, form filaments with side projections, show elementary branching or have fragmentation of the filaments. The closest extant genus to *Paleorhodococcus* based on morphology and host is *Rhodococcus* Zopf, 1891(Lechevalier, 1984; Garrity et al., 2004). There are some 30 extant species of *Rhodococcus* recognized today (Garrity et al., 2004).

The fossil is characterized by its spherical-subspherical cocci forming substrate filaments with elemental branching. The extant species of *Rhodococcus* associated with Hemiptera only exhibit elementary branching (Lechevalier, 1984). The dimensions of the coccoid elements and hyphal lengths and diameters are similar to some extant species of *Rhodococcus* (Rowbotham & Cross, 1977; Lechevalier, 1984), however the clustering of coccoidal elements and the short filaments bearing reduced side branches are uncommon features (Lechevalier, 1984). Its occurrence in a fecal droplet of an extinct species of triatomine bug also distinguish *P. dominicanus* from extant members of the genus.

Comment: The fecal droplet is adjacent to the fossil triatomid, *T. dominicana*, in the amber and it is assumed that the bug voided the droplet as it was being covered with resin. In the same fecal droplet with *P. dominicanus* are numerous metatrypanosomes of the fossil trypanosomatid, *Trypanosoma antiquus* Poinar (2005). Based on

mammalian hairs in the amber, it was concluded that the host of both *T. dominicana* and *T. antiquus* was a bat (Poinar, 2005).

Biology

Insects (such as triatomines) relying solely on blood meals throughout their entire development harbor symbiotic micro-organisms (Lehane, 1991). They probably supply essential elements, since blood is considered a nutritionally inadequate diet deficient in B vitamins (Marshall, 1987). Nodiocardiform organisms are well known for their catabolic potential and their ability to assimilate proteins and carbohydrates and these are the most common symbionts reported from the alimentary tract of Triatomine bugs. *Rhodococcus rhodnii* Goodfellow & Alderson (1977) occurs in the alimentary tract of the reduviid bug *Rhodnius prolixus* and *Rhodococcus rhodochrous* (Overbeck) was described from the alimentary tract of *Triatoma protracta* (Uhler)(Marchette & Hatie, 1965). Brecher & Wigglesworth (1944) reported similar nocardioform organisms in the alimentary tracts of *Triatoma rubrofasciata* (DeGeer), *T. infestans* (Klug) and *T. flavida* (Neiva), mentioning that they also have been reported in 9 other triatomine species.

Evidence of their role in the life cycle of these hemipterans was demonstrated by Brecher & Wigglesworth (1944). When *R. rhodnii* was absent from the alimentary tract of *Rhodnius prolixus*, few bugs reached the adult stage and those that did were incapable of reproduction. These authors also showed that *R. rhodnii* was transferred into hatchling bugs when they fed on fecal droplets from older infested stages. It is

obvious that nocardioform organisms are of common occurrence in triatome bugs and explains their occurrence in *T. dominicana*.

This is the first fossil record of nocardioform organisms and shows that these Prokaryotes formed symbiotic associations with insects by the mid-Tertiary.

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FIGURES

Fig.1. Fecal droplet of *Triatoma dominicana* containing *Paleorhodococcus dominicanus* in Dominican amber. Bar = 500 µm.

Fig. 2. Clusters of coccoidal elements (arrows) of *Paleorhodococcus dominicanus* in Dominican amber. Bar = 5 µm.

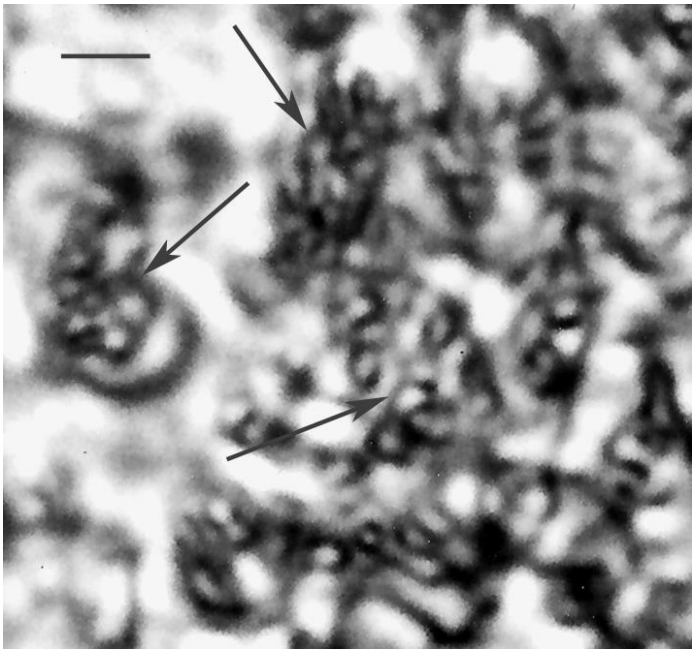

Fig. 3. Filaments of *Paleorhodococcus dominicanus* with side projections (arrows) in Dominican amber. Top arrow shows a filament arising from a germinating coccus. Lower 3 arrows shows filaments with elementary branching. Bar = 7 µm.