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Exquisite preservation of a widespread filamentous microorganism in French Cretaceous ambers: Crucial for revising a controversial fossil

Préservation exceptionnelle d'un microorganisme filamenteux dans les ambres crétacés de France : une clé pour la compréhension d'un fossile controversé

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ABSTRACT

Cretaceous ambers from different localities often contain abundant filamentous microorganisms that extend from the surface of the lumps of amber towards their center. These microfossils have been interpreted in the past as sheathed bacteria, cyanobacteria, and fungal hyphae, respectively. Here, we applied various techniques such as optical microscopy, confocal microscopy, and SEM to constrain the actual nature of these microorganisms. We evaluate published views and new evidence and conclude that the observed morphological and ultrastructural features correspond to sheathed bacteria. We propose a scenario explaining the observed differential preservations as various stages of the sheath construction around the bacterial filaments growing in the resin and the consequences of the transformation of the resin to amber. We suggest an abundant occurrence of at least one extinct resinicolous *Leptothrix*-like taxon in the Cretaceous Period.

Mots clés :
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RÉSUMÉ

L'ambre du Crétacé de différentes provenances contient souvent d'abondants microorganismes filamenteux, qui se développent depuis la surface vers le centre des pièces d'ambre. Ces microfossiles ont été identifiés comme des champignons, des cyanobactéries ou des bactéries. Grâce à l'utilisation de diverses techniques d'observation (microscopie optique, microscopie confocale, MEB), il est possible de mieux comprendre la nature de ces microorganismes. L'évaluation des travaux publiés et les nouvelles données obtenues permettent de conclure que les caractéristiques morphologiques et ultrastructurales correspondent à une bactérie à gaine de type *Leptothrix*. Les préservations différentielles observées sont rapportées aux différentes étapes de la construction de la gaine de filaments bactériens se développant dans la résine et les conséquences de la transformation de la résine en ambre. Ce type de microorganisme semble avoir disparu de la niche résinicole après le Crétacé.

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1. Introduction

Amber acts as a particularly favourable medium for the exceptional preservation of organic inclusions. Arthropods trapped in amber have been the subject of countless works. In contrast, the world of microorganisms was only discussed in detail later. Preservation of microorganism communities in amber is known from the Carboniferous onwards in many deposits the world over (Girard, 2010). Nevertheless, difficulties of approach and inaccuracies result from observational limits imposed by the size of the objects and the identifications based on morphological characters, which are often unclear when only classical optical microscopy is used.

This micro-world entrapped in amber comprises many filamentous microorganisms such as bacteria (including actinomycetes), cyanobacteria and fungi (see review in Saint Martin et al., 2012). Some microorganisms can be considered as resinicolous colonizers (Beimforde and Schmidt, 2011; Breton, 2012; Breton et al., 2013; Girard, 2010; Saint Martin et al., 2012, 2013; Speranza et al., 2015), presumably using the resin as a nutrient substrate functioning like a culture medium (Breton, 2011). Among these, a particular type of inclusion consists of networks of regular tubular filaments, slightly sinuous, exhibiting a distinct central lumen under the optical microscope. The diameter of filaments ranges between 6 to 10 μm while the lumen diameter is approximately 1 μm. The network develops mostly at the periphery of amber pebbles and more rarely invades the nodule from the outer to the inner part of the fossil resin. This filamentous network was first observed in Albian–Cenomanian amber from Kansas and assigned to the extant sheathed bacterium *Leptothrix* (Waggoner, 1996). Later, the fossil species *Leptotrichites resinatus* Schmidt 2005 was defined by Schmidt and Schäfer (2005) as a sheathed filamentous prokaryotic inclusion abundant in the Cretaceous amber of Schliersee (Germany) and considered to be close to the extant genus *Leptothrix*. Micro-inclusions resembling and/or attributed to *L. resinatus* were also identified in some Cretaceous ambers from France (Breton, 2012; Girard et al., 2009a; Girard, 2010; Girard et al., 2013a; Néraudeau et al., 2016; Saint Martin et al., 2012, 2013), England (Brasier et al., 2009), Spain and Japan (Beimforde and Schmidt, 2011) and were considered to be prokaryotic remains. In other cases, similar sheathed filamentous inclusions were tentatively assigned to a cyanobacterium and designated as the new fossil species *Paleocolteronema cenomanensis* Breton and Tostain, 2005 (Breton, 2007; Breton and Tostain, 2005; Breton et al., 2013; Girard, 2010; Girard et al., 2009a, 2013b). However, the same kind of micro-inclusions described in Cretaceous amber from Spain were instead considered to represent fungal hyphae (Ascaso et al., 2003, 2005; Martín-González et al., 2009; Speranza et al., 2010, 2015). The cylindrical structures around the lumen are therefore differently interpreted: as the sheaths of two types of filamentous prokaryotes (bacteria and/or cyanobacteria) or as the cell wall of eukaryotic mycelia (fungi). For reliable identification physical and chemical approaches were performed based on the putative presence of remains of chitin and EPS (Speranza et al., 2015) or phycocyanin pigment (Girard et al., 2009a), the authors recommending the systematic use of their own methods as discriminating. However, such methods are difficult to implement for each amber sample and assume the same degree of preservation of the organic substances despite quite variable taphonomic and diagenetic histories of the original resin.

To overcome the uncertainties about this controversial filamentous organism and to allow a better understanding of the morphology of microorganisms and their mode of preservation, we examined numerous French Cretaceous ambers from various sites and of diverse ages. We paid particularly attention to the finest structures and to the original organic compounds more or less preserved. Following the methodology of Ascaso et al. (2003), we applied various methods of investigations such as classical optical microscopy, Scanning Electron Microscope (SEM) and Confocal Laser Scanning Microscope (CLSM). Thus, the objective of this study is:

- to update the knowledge with complementary observations and data, providing extensive illustrations;
- to account for the mode and the quality of preservation;
- to underline the need to implement various investigative techniques on the same amber samples for better observation and identification of microorganisms, in order to avoid the problem of preservation bias.

2. Material and methods

2.1. Provenance of studied samples

Cretaceous ambers are widely distributed in France. Lacroix (1910) carried out a broad survey of the amber-bearing sites, which was recently updated (Nel et al., 2004; Perrichot and Néraudeau, 2014; Perrichot et al., 2007; Ragazzi et al., 2009), many deposits not being currently accessible. Our observations are based on samples collected from various amber-bearing deposits all over France, ranging in age from Albian to Campanian (Fig. 1, Table 1). According to Nohra et al. (2015), chemical characterization indicates that French Cretaceous ambers are produced by conifers belonging to diverse families.

In most cases, we collected *in situ* amber samples. We paid particular attention to samples from sites in Charente-Maritime (Archingeay, Cadeuil, Puy-Puy) (Girard, 2010) remarkable for the exceptional preservation of filamentous microstructures.

For comparison, extant samples of *Leptothrix* were collected from a natural water trickle.

2.2. Microscopic analyses

For optical and confocal microscopy, petrographic thin sections of approximately 30 microns standard thickness, covered with a cover-slip, were made.

About 60 thin sections were examined with an optical microscope (Zeiss Axioscope 40 equipped with a photographic device) provided with ×40, ×63, and ×100 immersion objectives. For the images taken at several focus levels, a specific image processing software (Helicon Focus®) was used.

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