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Spider web in Late Cretaceous French amber (Vendée): The contribution of 3D image microscopy

Les toiles d'araignée préservées dans l'ambre du Crétacé supérieur de France (Vendée) : apport de l'imagerie 3D

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ABSTRACT

Late Cretaceous amber from La Garnache (France, Vendée) contains filamentous networks that have all the characteristics of spider webs. Using methods of classic and confocal microscopy, the web architecture and the thread structure are described. The geometry of the webs preserved in amber is presented in 3D imaging. Unlike most of the spider webs identified in amber, there are no regular radiating webs growing in two dimensions characteristic of the well-known orb web. A number of the characters would correspond to the apparently irregular organization of cob-type web or sheet web. By using confocal microscopy, we can highlight the preserved autofluorescence of spider silk proteins and new characters are proposed to identify the spider webs or fragments of webs in amber.

RÉSUMÉ

L'ambre du Crétacé supérieur de La Garnache (France, Vendée) renferme des réseaux filamenteux présentant toutes les caractéristiques de toiles d'araignée. Des méthodes de microscopie conventionnelle et confocale sont utilisées pour décrire l'architecture des toiles et la structure des fils. La géométrie des toiles est présentée en imagerie 3D. Contrairement à la plupart des toiles d'araignée recensées dans l'ambre, il ne s'agit pas de toiles régulières rayonnantes qui se développent en deux dimensions, du type orbiculaire le plus connu. Un certain nombre de caractères les rapporteraient à des toiles à l'organisation plus irrégulière (toiles en tube, en nappes, en dômes, diffuses...). Grâce à la microscopie confocale, on peut mettre en évidence l'autofluorescence préservée des protéines des fils de soie ; de nouveaux caractères sont proposés pour identifier les toiles ou fragments de toiles d'araignée dans l'ambre.

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1. Introduction

Amber is known as a medium particularly favorable to the exceptional preservation of organic inclusions. Amber is increasingly, but controversially, considered a good Lagersttäte paradigm (Allison, 1988; Breton, 2007; Durán-Ruiz et al., 2013; Knight et al., 2010; Nudds and Selden, 2008a,b; Saupe and Selden, 2011; Savrda, 2010; Savrda et al., 2009; Selden and Nudds, 2012; Selden and Penney, 2010), especially as a "preservational trap". Amber can also preserve remarkably varied manifestations of organic activities, such as predatory strategies, rarely or never preserved during common fossilization processes and poorly investigated (Labandeira, 2002). These activities include the production of silky structures organized in networks, such as the cocoons woven by various insects, and spider webs (Arillo, 2007; Poinar, 1998, 2010; Zschokke, 2004). According to Arillo (2007), the spider web reflects predation behaviour, resulting in interspecific interactions revealed by the mode of preservation in amber.

In the fossil record, the presence of spider webs was reported in amber of various ages and provenances (Table 1), the oldest known occurrence being those of the Early Cretaceous of England (140 Ma) (Brasier et al., 2009). In France, only a possible occurrence of spider web remains has been reported by Girard (2010) in the mid-Cretaceous amber of Cadeuil (SW France).

In favourable cases, the bodies of victims have been found in the web, supporting the hypothesis of predation activity (Peñalver et al., 2006; Poinar and Buckley, 2012; Poinar and Poinar, 1999). Controversial ethological inferences have been drawn from the joint presence in a preserved web of male and female spiders and presumed prey (Poinar and Buckley, 2102, 2014; Penney, 2014).

From optical microscopy observations of Early Cretaceous amber, Brasier et al. (2009) established criteria for discriminating spider webs included in amber versus fungal and prokaryotic filaments also putatively present in the same samples, including: marked variations in thickness along the length, thread branching and junctions accompanied by marked variations in filament thickness, threads occurring in twisted pairs with parallel orientations and similar twists and kinks, common tension kinks, and the presence of fluid droplets. Considering these criteria, here, we identify spider webs in French amber of Cretaceous age. Moreover, using various microscopy techniques, such as traditional optical microscope, CLSM (confocal laser scanning microscope) and SEM, we provide additional data for describing the different structures constituting these spider webs, including: autofluorescence characteristics, comprehensive analysis of the web architecture and the fine structure of the threads, and distinctive elements from other filamentous inclusions.

Application of confocal laser scanning microscope (CLSM) technology to amber provides various advantages complementing traditional optical microscopy (Ascaso et al., 2003). An autofluorescence signal can be emitted by organic molecules preserved in amber, revealing poorly distinguishable features. The examination under CLSM of specific planes of depth allows the elimination of any

Table 1
Fossil spider web occurrences in amber of various ages and provenances and their main characteristics according to literature.
Tableau 1
Toiles d'araignée fossiles d'âges et de provenances variés, recensées dans l'ambre et principales caractéristiques, selon la littérature.

Age	Provenance	References	Description comments
Miocene	Dominica	Penney (2008) Poinar (1998, 2010) Poinar and Poinar (1999) Zschokke (2004)	Silk with droplets Araneoid spiders
Eocene	Baltic	Weitschat and Wichard (1998) Bachofen-Echt (1934) Gerhard and Rietschel (1968) Krzemihska and Krzemihski (1992) Zschokke (2004)	Spider constructing his silk Silk with droplets Araneoid spiders
Lower Eocene	India	Alimohammadian et al. (2005)	Silk with droplets Araneoid spiders
Upper Cretaceous (Santonian)	USA	Knight et al. (2010)	Spider with web Silk with droplets Araneoid spiders
Early Cretaceous	Lebanon	Zschokke (2003) Zschokke (2004) Poinar and Buckley (2012,2014)	Silk with droplets Web with prey Araneoid spiders
Lower Cretaceous	UK	Jarzembowski et al. (2008)	
Early Cretaceous	UK	Brasier et al. (2009)	Silk with droplets Helical twists Araneoid spiders
Early Cretaceous (Early Albian)	Spain El Soplao	Najarro et al. (2009)	Web with prey Araneoid spiders
Early Cretaceous	Spain San Just amber	Peñalver et al. (2006) Peñalver et al. (2008)	Strands with droplets Hanks (pelotes) Web with prey Araneoid spiders
Early Cretaceous	Burma	Zschokke (2004)	Silk with droplets Araneoid spiders

refractive optical illusion. CLSM is also especially interesting because three-dimensional (3D) information may be obtained by acquisition of stacks of closely spaced sections. Until now, CLSM observations in amber were only devoted to the study of microorganisms, such as protists or fungi (Ascaso et al., 2003, 2005; Martin-Gonzáles et al., 2009; Saint Martin et al., 2013, Speranza et al., 2010). Here, for the first time, the CLSM technique is used for organic but non-living structures and allows us to present fossil spider webs in 3D.

2. Material and methods

The amber under study comes from an outcrop temporarily exposed between 2003 and 2006 near La Garnache (Vendée, France), north of Challans, during the construction of a road. Considering the regional geological map (Ters and Viaud, 1983), and the few available palynological data (Legrand et al., 2006), the exact age of the amber deposit is still debatable but a Cenomanian–Santonian interval is finally considered (Néraudeau et al., in preparation).

The sampled amber pieces do not generally exceed a diameter of one centimetre. Of sixty pieces observed, about 3% show in some parts translucent amber networks (Fig. 1) or alignments of structures resembling spider webs. Several preparations were undertaken in relation to the different modes of observation and analysis. For optical microscopy, observation slides were prepared by including fragments or fine splinters of amber in resin Eukitt® mounting medium, with or without a coverslip, depending on the size of the fragments. Larger fragments were simply observed by optical microscopy after recovery by oil specific for microscopy. Petrographic thin sections of standard thickness of 30 μm, covered with a coverslip, were used for both optical and confocal observations.

For optical microscopy images taken at several focus levels, a specific image processing software (Helicon Focus®) was used.

The CLSM observations were performed on a Leica TCS SP5 microscope (upright and inverted) at the University Pierre-et-Marie-Curie and a Zeiss LSM700 microscope at the Aix-Marseille University. Samples were observed through 40 × 1,25–0,7 NA and 63 × 1.40 NA oil-immersion objectives. For spectral analysis, the excitation wavelength was 405 nm and the emission was monitored in the 415 to 765-nm range, with a bandwidth of 10 nm. The images obtained from confocal microscopy allow the viewing of autofluorescent objects in false colours. Thus, for a more consistent rendering, the images were converted to grayscale and then treated with the open source software *Image J* (Rasband, 1997–2013), permitting optimization of the 3D data.

For comparison, slides of modern spider webs of geometric and irregular architectures from diverse habitats (garden and house) were prepared by including fragments of web in the Eukitt® mounting medium and observed under the same experimental conditions as the amber pieces.

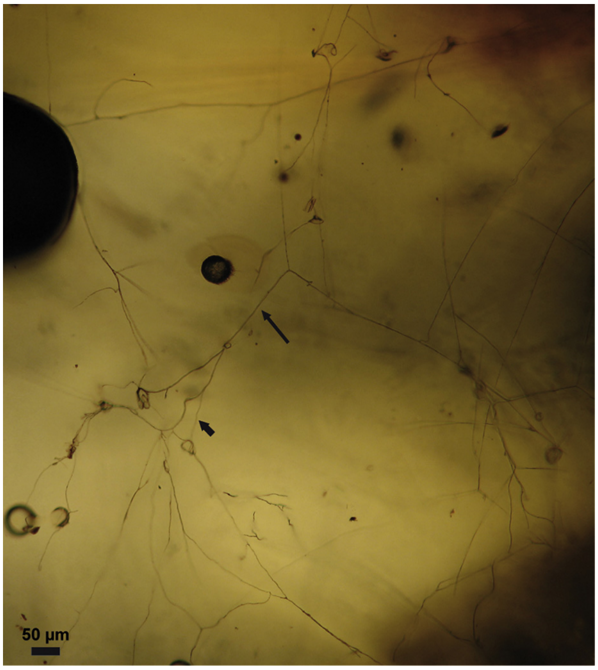

Fig. 1. (Color online). General view of spider web, consisting of major (large arrow) and secondary threads (short arrow) in an amber grain from La Garnache under optical microscope (image processes by Helicon Focus®).

Fig. 1. (Couleur en ligne). Vue d'ensemble d'une toile d'araignée, constituée de fils principaux (flèche longue) et secondaires (flèche courte) dans un grain d'ambre de La Garnache en microscopie optique (traitement de l'image par Helicon Focus®).

3. Spider web characteristics

3.1. CLSM results

The CLSM observations of spider web threads revealed a homogenous autofluorescence easily used for the 3D reconstructions. The fluorescence is limited to the range of blue/green wavelength, generally between 450 and 630 nm. It is important to note that the same signal is given by the threads included in amber and by the silk of the extant spider webs.

Spectral analysis of the threads reveals a similar spectral signature with an emission peak for amber-included spider webs at 469 nm, and an emission peak of 505 nm for garden spider webs (Fig. 2).

3.2. Web architecture

Amber pieces from La Garnache contain in abundance filamentous structures presented in two more or less complementary forms: web-like structures and isolated or sets of threads. As distinguishable from optical observations, the web structures can occupy space without a privileged direction, with a loose network extending over several centimetres in length (Fig. 1). The 3D reconstructions performed by the CLSM provide similar features for the web organization of La Garnache (Fig. 3A) and present-day samples (Fig. 3B). Clusters of threads are fairly common, but despite the high density of some of these clusters, they do not really affect the transparency of the amber (Fig. 3C). In

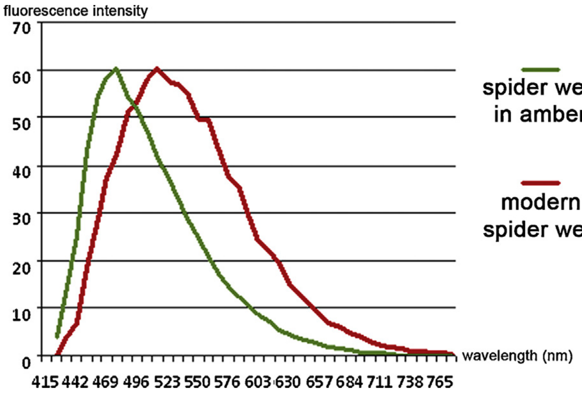

Fig. 2. (Color online). Spectral analysis of spider web threads from Cretaceous amber and current spider web.

Fig. 2. (Couleur en ligne). Analyse spectrale de fils de toiles d'araignée de l'ambre du Crétacé et de toile d'araignée actuelle.

some cases, the alignment of threads can be observed in the direction of stretching of the casting resin (Fig. 3D,E).

The web is generally composed of threads of different thickness (1 μm to 20 μm in diameter) delimiting principal and secondary elements of the web construction (Figs. 1 and 4 A and B). The architecture of the web is characterized by junction points of threads, often marked by thickening. The junction point may involve more than two threads and as many as four. From these branching points, threads fall in different directions and thus in several optical planes (Figs. 1 and 3). Triangular and trapezoidal figures resulting of branching and junctions are often visible delimiting an irregular frame (Fig. 3A). The threads can form loops that close on themselves (Fig. 4A,B), as also visible on present-day webs (Fig. 4C). Threads may be more intertwined, forming twisted braids (Figs.4D and 5A), similar to present-day features (Fig. 4E). Very often, the threads are associated as strongly intertwined pairs with parallel

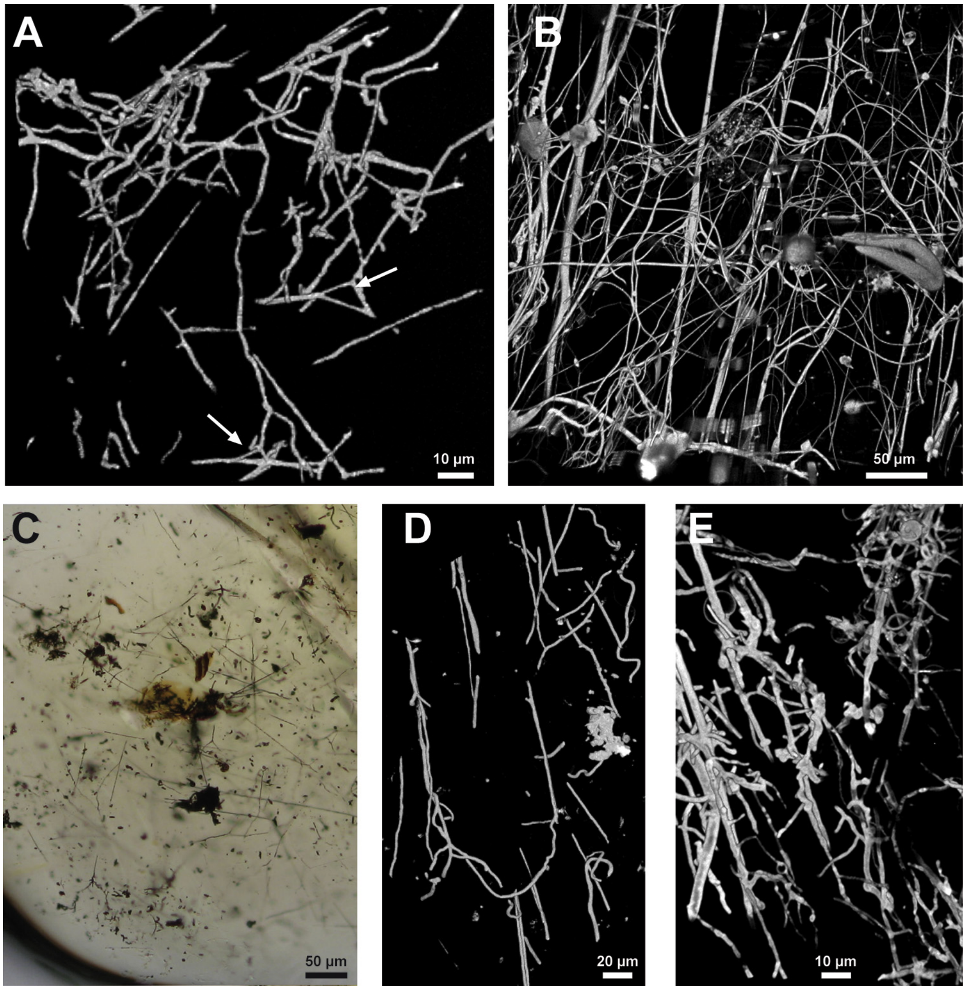

Fig. 3. (Color online). A. General view of CLSM 3D reconstitution of the network of spider web preserved in amber. B. CLSM 3D view of current spider web embedded in Eukitt®. C. Optical view of amber grain showing isolated threads and clusters of threads aligned in the same direction. D. CLSM 3D view of aligned threads. E. CLSM 3D view of aligned clusters of threads. Note the thickness variation of the various fibers of the web.

Fig. 3. (Couleur en ligne). A. Vue générale d'une Image 3D réalisée au microscope confocal du réseau d'une toile d'araignée, préservée dans l'ambre. B. Image 3D en microscopie confocale d'une toile d'araignée actuelle montée avec Eukitt®. C. Vue en microscopie optique montrant des fils isolés et des ensembles de fils alignés dans la même direction. D. Image 3D en microscopie confocale de fils alignés. E. Images 3D en microscopie confocale d'ensembles de fils alignés. Noter les variations d'épaisseur des diverses fibres de la toile.

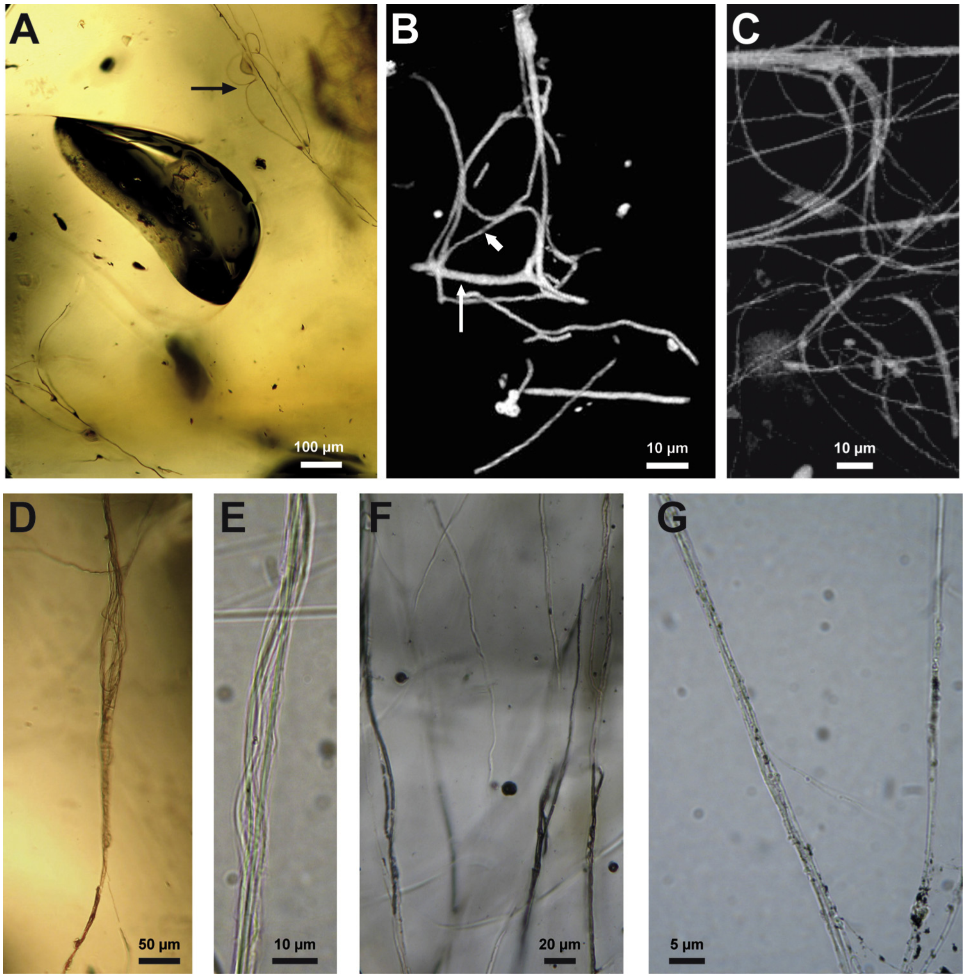

Fig. 4. (Color online). A. Network of spider silk forming loops closed on themselves (arrow) in amber under optical microscope (image processes by Helicon Focus®). B. CLSM 3D view of secondary thread (short arrow) forming loops attached to a major thread (large arrow) in amber. C. CLSM 3D view of thread network with loops in current spider web. D. Twisted braids of intertwined threads in amber under optical microscope. E. Twisted braids of intertwined threads in current spider web embedded in Eukitt® under optical microscope. F. Intertwined pairs of threads with parallel orientations and similar twists and kinks in amber under optical microscope. G. Intertwined pairs of threads in current spider web embedded in Eukitt® under optical microscope.

Fig. 4. (Couleur en ligne). A. Réseau de soies d'araignée dans l'ambre, formant des boucles enroulées sur elles-mêmes en microscopie optique (traitement de l'image par Helicon Focus®). B. Image 3D en microscopie confocale de fils secondaires (flèche courte) en boucles, se rattachant à des fils principaux (flèche longue) dans l'ambre. C. Image 3D en microscopie confocale d'un réseau de fils avec des boucles dans une toile d'araignée actuelle. D. Tresses torsadées de fils entremêlés dans l'ambre en microscopie optique. E. Tresses torsadées de fils entremêlés dans une toile d'araignée actuelle, préparée avec Eukitt® en microscopie optique. F. Paires de fils entrelacés dans l'ambre avec des orientations parallèles er les mêmes torsades et nœuds en microscopie optique. G. Paires de fils entrelacés dans une toile d'araignée actuelle préparée avec Eukitt® en microscopie optique.

orientations and similar twists and kinks (Fig. 4F), a setting again common in current webs (Fig. 4G).

A number of the threads show more or less loose kinks and spirals (Fig. 5B–E), frequent in current spider webs (Fig. 5F). As Brasier et al. (2009) emphasized concerning the spider web preserved in the Cretaceous amber of Hastings, twisting of the threads may reflect tension, and kinks result from the relaxation of initial tension. In some cases, the winding of the threads is accomplished by the formation of a short circular spiral circular (Fig. 5G–I). Such geometry was reported by Benjamin et al. (2002) in the construction of some current spider webs.

Infrequent droplets may fall irregularly along the threads (Fig. 6A–B). Although some spherical droplets are visible, they are more or less elongated in the direction of the thread.

3.3. Thread structure

Under classical microscopy, the threads included in amber, like extant spider web threads, appear as “full” homogeneous filaments that may be irregularly twisted, sometimes imparting a rosary appearance which might suggest cells of filamentous microorganisms such as bacteria or microfungi. The CLSM observations highlight this character more precisely and allow us to see that the thickness of the threads may vary; irregularly distributed thickenings are often observed (Fig. 6 C).

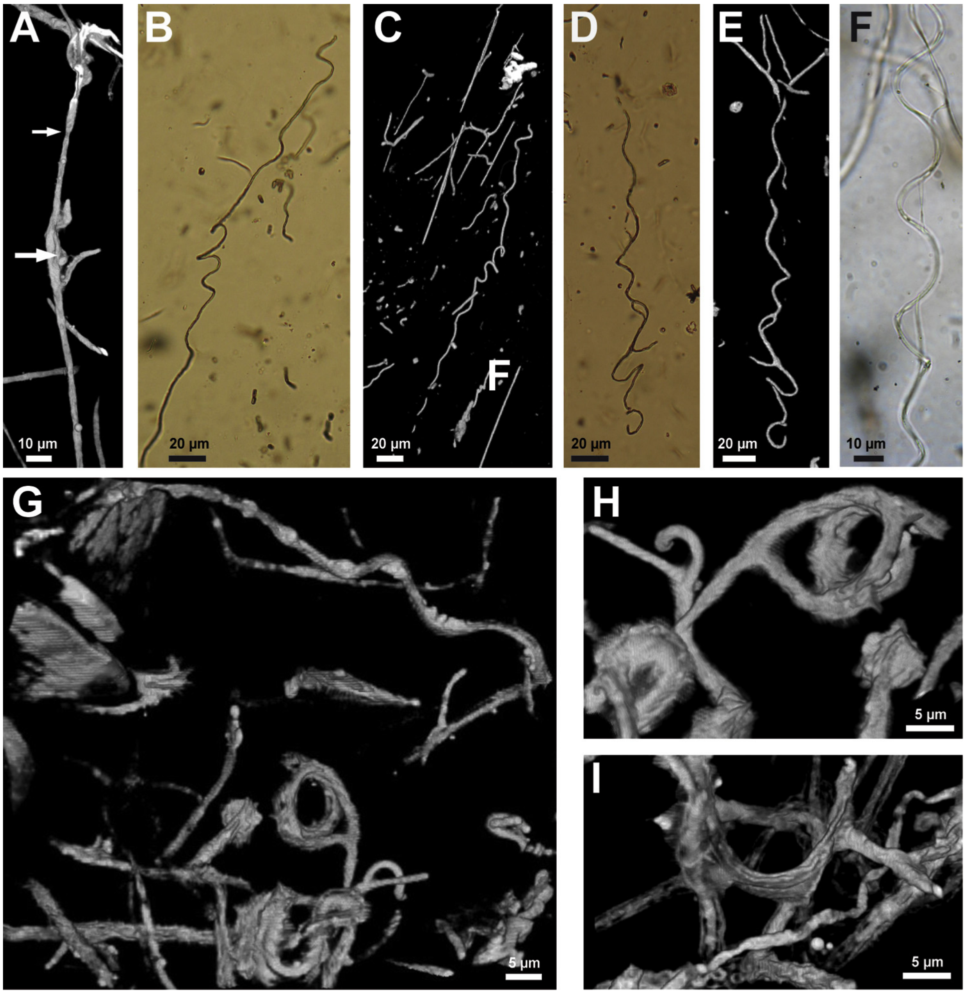

Fig. 5. (Color online). A. CLSM 3D view of intertwined threads (big arrow) showing thickness variations (little arrow). B. Loose spiral of thread under optical microscope. C. Same thread as in A in 3D view under CLSM. D. A. Loose spiral of thread under optical microscope. E. Same image as in C in 3D view under CLSM. F. Spiral thread of current spider web embedded in Eukitt® under optical microscope. G. CLSM 3D view of short spirals, circular in shape. H–I. Details of CLSM 3D view of short spiral, circular in shape.

Fig. 5. (Couleur en ligne). A. Image 3D de fils entremêlés (grande flèche) montrant des variations d'épaisseur (petite flèche). B. Spirale lâche de fil en microscopie optique. C. Image 3D en microscopie confocale du même fil qu'en B. D. Spirale lâche de fil en microscopie optique. E. Image 3D en microscopie confocale du même fil qu'en C. F. Fil spiralé de toile d'araignée actuelle montée avec Eukitt®. G. Image 3D en microscopie confocale de courtes spirales de contour circulaire. H–I. Détails de fils spiralés, de contour spiralé, en imagerie 3D confocale.

4. Discussion

4.1. *Identification of spider webs in amber*

When we deal with filamentous networks in amber, we can question their real nature: filamentous microorganisms or spider web?

Brasier et al. (2009) pointed out that the spider threads identified in amber invariably lack any evidence for cellular organization, such as regular cell wall divisions or reproductive bodies. On the contrary, Ascaso et al. (2003) remarked that mycelia present in amber show a clear differentiation evidenced by CLSM, including the mode of preservation. Our observations under optical microscopy and CLSM confirm that the filamentous network of the supposed spider web from La Garnache amber exhibits no concentric differentiation, no walls, no central lumen, no partitioning, and no constrictions, unlike many filamentous organisms observed in the same samples (Fig. 6D), and, more generally, in other Cretaceous amber. These observations allow us to provide supplementary criteria for the discrimination of the spider web in amber using CLSM. The spider webs do not alter the transparency of the amber, whereas dense networks of filamentous mycelia give a milky or opaque appearance to the amber (Saint Martin et al., 2012, 2013). Moreover, the autofluorescent ability of spider silk and their spectral characteristics could also be a criterion of discrimination. Provocatively, we can hypothesize that some misidentified filaments observed in amber and considered mycelia of microorganisms could be in fact fragments or threads of spider webs. The examination of the material under CLSM then proves a good means of investigation and discrimination.

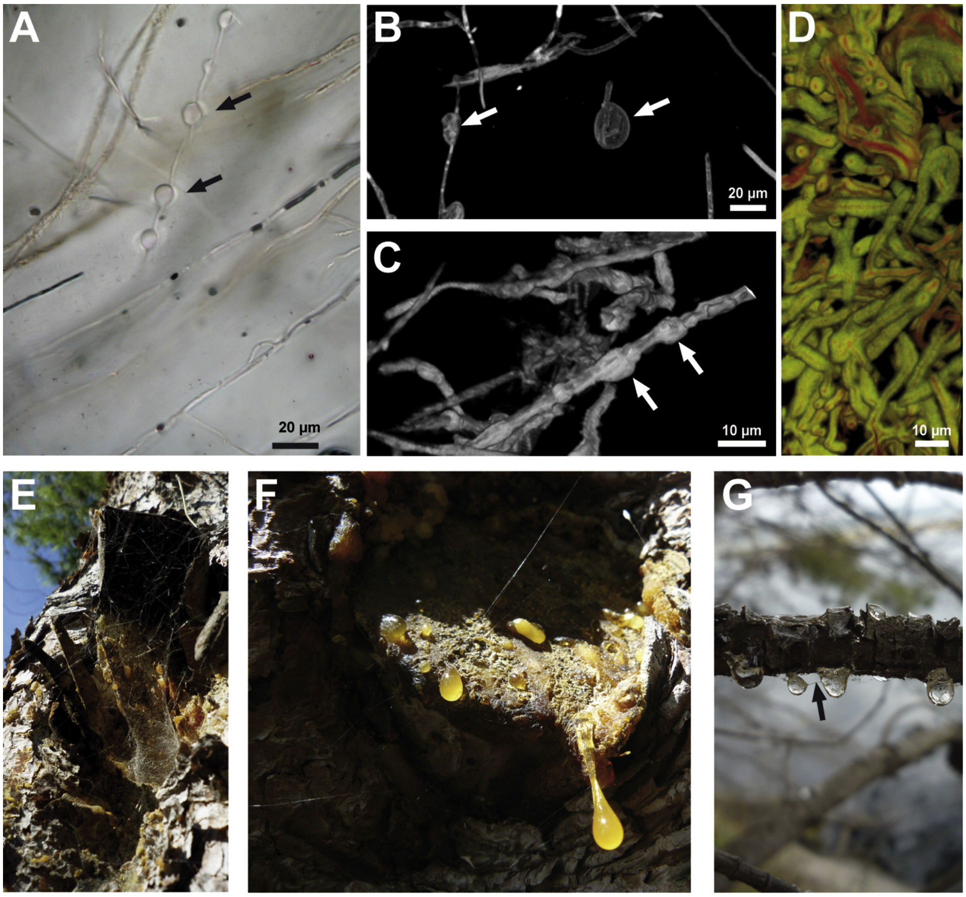

Fig. 6. (Color online). A. Droplets (arrows) along one thread under optical microscope. B. CLSM 3D view of droplets (arrows) attached to isolated threads. C. Thickenings (arrows) along a silk thread. D. CLSM 3D view of filamentous microorganisms showing a complex organization (central core and sheath). E–G. Current spider web and close resin flows.

Fig. 6. (Couleur en ligne). A. Gouttelettes (flèches) disposées le long d'un fil en microscopie optique. B. Image 3D en microscopie confocale de gouttelettes adhérant à des fils isolés. C. Épaississements (flèches) le long d'un fil. D. Microorganismes filamenteux montrant une structure composée (filament central entouré d'une gaine) en microscopie confocale. E–G. Toiles d'araignée actuelles dans la proximité de coulées de résine.

4.2. *Spider silk autofluorescence*

The characteristics of the webs evidenced in the La Garnache amber are highlighted by the autofluorescence exhibited by the threads. In extant organic silks, the fibre is mainly composed of proteins called fibroins and more especially spidroin for the spider silks. Recent studies (Hayashi and Lewis, 2001; Lefèvre et al., 2011; Motriuk-Smith et al., 2005) provide important insights into fibroin-spidroin structure, showing that these proteins are high molecular-weight biopolymers composed of an alternating amino acid rich blocks between non-repetitive *N*- and *C*-terminal domains. The fibroin contains three aromatic amino acid residues (tryptophan, tyrosine, and phenylalanine), which may contribute to their intrinsic fluorescence (Georgakoudi et al., 2007). Tryptophan is the most used to assess protein structure, since it has the highest quantum yield and extinction coefficient of the three aromatic amino acids (Georgakoudi et al., 2007).

The spectral analysis performed on the La Garnache amber revealed very similar emission spectra with the spectra reported by Rice et al. (2008) using two-photon microscopy of silkworm silk fibroin-based biomaterials. These authors observed peaks around 465 nm for dehydrated biomaterial and around 500 nm for hydrated biomaterial. The blue shift of the emission spectra of dry silk fibroin has been explained by Rice et al. (2008) as being a response to a more polar environment for the dry fibroin. Furthermore, fluorescence of amino acids, such as tryptophan has long been known to be sensitive to the polarity of their local environment (Gaines et al., 2010, Vivian and Callis, 2001, Weber, 1960). This is consistent with our data where amber spider webs, representing the dehydrated form show a blue-shifted spectrum in comparison to garden spider webs, which were mounted in Eukitt® mounting medium prior to observation, thus, representing the hydrated form.

The autofluorescence signal observed and its spectral characteristics therefore suggest that amber preserved, at least in part, the original character of the organic material that constitutes the spider web.

4.3. Spider web type and origin

Spiders produce several types of webs generally associated with different families, including spiral orb webs, tangle webs, cobwebs, sheet webs, and funnel webs. Orb webs represent the best known type of spider web and are woven by numerous families of spiders, especially the Araneidae.

In the fossil record, it is mostly the droplets associated with spider web threads that have attracted attention (Alimohammadian et al., 2005; Brasier et al., 2009; Knight et al., 2010; Peñalver et al., 2006; Zschokke, 2003). However, Peñalver et al. (2006) clearly described in Cretaceous amber from Spain an evident radial architecture organised in a single plane characteristic of orb webs. More generally, the webs are effectively rather recognized as orb webs and the araneid spiders often invoked as the weavers (Peñalver et al., 2006; Poinar and Buckley, 2012, 2014; Zschokke, 2003).

According to Blackledge et al. (2011), the orb webs are distinguished from other types of spider webs in their suspension in the air column upon discrete networks of frame threads and their two-dimensional (2D) capture surfaces. One of the main characteristics is the radial aspect of the construction. The webs observed in amber from La Garnache do not present specific characteristics typifying the orb webs as follows: the network of threads is three-dimensional and their organization appears quite confused, the threads have loops and multiple connection points are possible, no radial element is distinguishable, and the droplets are infrequent. Thus, the webs of La Garnache may be more likely cobweb or sheet web types.

It was considered that a cobweb is a highly complex, disordered, three-dimensional meshwork (Moore and Tran, 1999). More recently, Benjamin and Zschokke (2002, 2003) stated that cobweb spiders, as the Theridiidae, spin three-dimensional webs apparently organized chaotically but actually constructed using stereotyped behaviours that result in some structural elements. Features of the webs of La Garnache resemble this type of three-dimensional construction that seems to show no specific organization (see Fig. 3). Other aspects, such as the connections at short spirals could also evoke the characters of sheet webs described by Benjamin et al. (2002). Nevertheless, it seems very difficult to give a more precise assignment in the absence of identifiable anchorage dispositive and of the overall web geometry defining the weaving strategies (Benjamin and Zschokke, 2003; Zevenbergen et al., 2008). However, some features of observed webs can reveal construction processes and behaviour of the responsible weaver spider. For instance, the thickness variations along the same thread (as seen in Fig. 6C) may be explained by the fact that usually a cobweb reinforces original guy lines (Moore and Tran, 1999). The general architecture of the web trapped in amber exhibits two types of areas regarding the density of threads as observed in Figs. 1 and 3A: areas with spaced lines and areas with more interconnected lines. This kind of organisation was observed in modern cobweb and explained by two different specific functions: widely spaced lines are devoted to prevent collapse, while interconnected lines serve as a meshwork for catching prey (Moore and Tran, 1999). The general architecture of webs may also reflect the physiological state of the spider. The studies of modern spider web by Zevenbergen et al. (2008) showed that "spiders in poor body condition spin webs that are more effective at capturing prey than the webs spun by spiders in good body condition, providing a functional benefit to behavioural plasticity in these spiders". Effectively the authors observed that spiders in poor body condition spin cobwebs presenting many threads coated with glue (gum-footed threads) at their base, while spiders in good condition spin webs that lack gum-footed threads. From this point of view, concerning the architecture of the webs found in La Garnache amber, we may hypothesize that the web was woven by an almost starved spider because there are only a few glue droplets observed.

According to Breton (2012), spider webs encompassed in modern resin exuded along pine trunks would be cobwebs, which confirms our own observations (Figs 6E–G).

4.4. Taphonomy

The abundance of spider web remains, and sometimes their density, in the amber of La Garnache, allows us to assume that weaver spider activity was important in the production environment of the original resin. Current observations of coniferous trees show the pervasiveness of more or less complete spider webs, or at least the threads of disorganized webs, near or in contact with resin in production (Fig. 6E–G). Some spider webs have been encompassed in resin flows near their location. Deriving thread clusters have also been included within resin and aligned along the flow of the resin.

The silk of spider webs is reputed to be fragile and degraded very quickly. The inclusion in resin is probably the only way to preserve them in geologic time. However, even preserved in amber, silk has certainly undergone physicochemical changes that altered its original nature. The differences in the spectral analysis of the silk from the amber of La Garnache and an extant web could result from these changes. More comprehensive analysis of the spider webs preserved in amber and various types of current spider webs are needed to discriminate what amounts to the effects of preservation or the specificity of silks depending on kinds of spider.

5. Conclusion

For the first time, the presence of spider webs is shown in the Late Cretaceous amber from France. Using CLSM technology, a fossil spider web can be analysed with 3D imaging. Key features are:

- the threads, of variable thickness, are often arranged in pairs;
- the structure of the web is characterized by many junction points, loops, spirals and a construction in several directions;

- glue droplets, although present, are infrequent;
- the silk threads show autofluorescence clearly expressed as the threads of current spider silk;
- spectral analysis of silk threads is very similar to their current equivalent.

All these observations and the method we used have allowed us to provide new elements of discrimination when comparing these structures with other types of filaments, especially filaments of fungal or bacterial mycelia whose general appearance may be similar. Finally, fossil amber may contain more remains of spider web than generally reported. Moreover, here, we demonstrate for the first time that amber can preserve the proteins that constitute the spider web silk and cause the autofluorescence.

The spider webs described here differ from 2D geometric webs commonly known as orb web. So, this is the first identification of webs to be placed in categories as cobweb/sheet web/funnel web. More comparative research between former and current material would probably be needed to characterize more precisely the silk, the type of web construction and, thus, the weaving spider.

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