

Palaeoecology of the Cenomanian amber forest of Sarthe (western France)

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ABSTRACT

Cretaceous ambers have been discovered in France since the beginning of the 18th century. The best known are those from south-western France which are Late Albian-Early Cenomanian in age, but there are other important amber deposits in other regions. Here, we summarise the data on one of these other Cretaceous amber regions, the Sarthe Department. These deposits have been mentioned in the literature since the end of the 18th century, but they have remained relatively unknown. The material, that has been studied during the 1970's and 1980's, yielded a well-diversified arthropod fauna (72 arthropod specimens, including arachnids, cockroaches, bugs, beetles, flies, wasps...) dated to late Early-Middle Cenomanian. In the last decade, 4 types of bacteria, a possible testate amoeba and fungal remains were also found. A re-examination of the historical collections of the Sarthe amber, housed in the "Musée Vert" (Le Mans, France), allows to estimate the geographical extent of the amber deposits in the Sarthe Department. The study of the microfossils of these samples provides new data on their palaeoenvironment.

KEYWORDS | Amber. Arthropods. Microorganisms. Palaeoecology. Cenomanian.

INTRODUCTION

The Cenomanian stratotype has been defined in the region of Le Mans, in the western border of the Paris Basin (western France). The Cenomanian was one of the warmest periods of the Phanerozoic and corresponds to one of the most important transgressive episodes of the geological record. In France, Cenomanian deposits are mostly of marine origin, but sediments rich in terrestrial fossils were accumulated along the coast (Néraudeau,

2005). These deposits are particularly rich in plant debris (including amber), allowing a better understanding of the mid Cretaceous terrestrial biotopes of France.

The Late Albian-Early Cenomanian amber deposits from the northern part of the Aquitaine Basin are well known (Néraudeau *et al.*, 2002; Perrichot, 2005; Girard, 2010; Adl *et al.*, 2011), but works are very few (mainly by Th. Schlüter in the 1980's) concerned with the amber deposits of Sarthe (late Early-Middle Cenomanian). The

study of the amber collections housed in the “Musée Vert” in Le Mans provides new data on the ecology of Cenomanian terrestrial biotopes of the eastern part of the Armorican Massif.

MATERIAL AND METHODS

Stratigraphy and sedimentary environments of the Cenomanian stratotype

The main lignitic deposits with plant debris and amber come from the Sables du Maine Formation that is

considered to be late Early- Middle Cenomanian in age (Fig. 1). For more details about the regional stratigraphy, see Juignet and Médus (1971), Juignet (1974), Juignet *et al.* (1978) and Lasseur (2008).

The depositional environment of the lower-middle Cenomanian deposits of Sarthe was deltaic or coastal, as shown by the presence of numerous ripples and megaripples (Juignet, 1974; Lasseur, 2008). Several transgressive pulses are reflected in the sedimentary section by different shifts from flooding facies to tidal ones (Juignet and Louail, 1987). The presence of five levels with plant debris shows that the marine palaeoenvironment

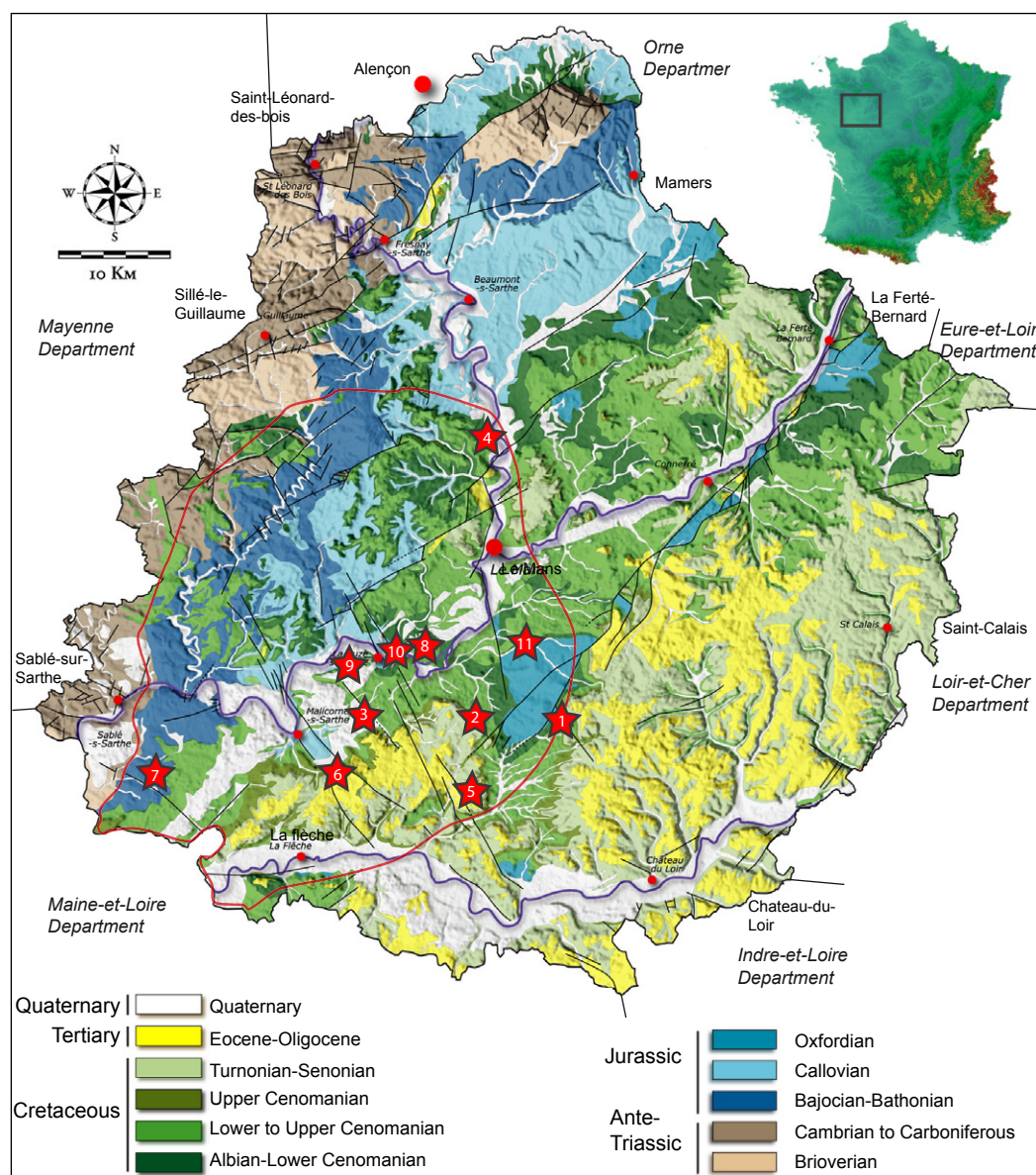


FIGURE 1 | Simplified geological map of the Sarthe Department (France) after Cattaneo and Mourgues (2009). The stars represent the different Cenomanian amber localities in the Sarthe Department. The area limited by the red line represents the supposed extension of the Cenomanian. 1: Écommoy; 2: Fessard; 3: Mézeray; 4: La Bazoge; 5: Mansigné; 6: La Brousse; 7: Précigné; 8: Roëzé-sur-Sarthe; 9: Saint-Jean-du-Bois; 10: La-Suze-sur-Sarthe; 11: Moncé-en-Belin.

was repeatedly disrupted by continental inputs (Juignet *et al.*, 1983). Unfortunately, it is now impossible to access the quarry, in Écommoy, in which these deposits were observed in the 1970's (Juignet and Médus, 1971), as it has been transformed into a fishing reserve. The black clay has also been located, by drilling, near the towns of La Flèche and Mayet (Juignet and Médus, 1971). Juignet and Médus (1971) interpreted the black clay as continental deposits.

Amber of the Cenomanian stratotype

Amber has been reported near Roëzé-sur-Loire (Maulny, 1799), and near the localities of Précigné and La-Suze-sur-Sarthe (Lacroix, 1910). Delaunay (1934), in a work on the rocks and soils of Sarthe, also indicated the presence of amber near the localities of Fessard, Mansigné, Précigné and Roëzé-sur-Sarthe. More recently, amber has been reported in the quarry of Bezonnais at Écommoy (Juignet and Médus, 1971). This amber is still the only one from Sarthe that has provided fossils, including arthropods (Schlüter, 1978; Perrichot *et al.*, 2007b) and microorganisms (Breton and Tostain, 2005; Breton, 2007). Galippe (1920) reported the cultivation of *Microzymas* (*i.e.* bacteria) from a piece of amber found near Précigné. However, it seems that Galippe's bacteria were recent contaminants as the surface of the samples were not sterilized.

The Sarthe amber is homogeneous, opaque and generally orange (when fresh) to reddish (when oxidised) (Fig. 2).

Apart from the samples collected by Pierre Juignet during the 1970's in the quarry of Bezonnais at Écommoy, the amber collection of the "Musée Vert" consists of material from the following localities: La Bazoge, Mansigné and Roëzé-sur-Sarthe. This material and amber reprints in the available literature show that Cenomanian amber deposits were well developed in the south of the Sarthe Department (Fig. 1). These deposits extend toward the south-west, in the Maine-et-Loire Department (Schlüter 1978; Perrichot *et al.*, 2007b). It was probably also the case northwesternly as Durand and Louail (1971) mentioned amber near the area of Neau in the Mayenne Department.

Concerning the depositional environment of the Sarthe amber, Juignet and Médus (1971) and Breton and Tostain (2005) indicated that the Écommoy amber was deposited in a swamp, more or less isolated from marine environments. This conclusion was supported by the presence of green grains of glauconite in the Level 3 of the black clay (Juignet and Médus, 1971). We confirm it as we found an amber lump from the "Musée Vert" collection encrusted by fossil oysters suggesting that the resin was produced on the land and then transported and deposited into marine or brackish water.

Sample preparation

The studied amber samples were decontaminated using a three-step protocol (Girard *et al.*, 2009b): i) ultrasonic cleaning, ii) oxidation with 9-10% H₂O₂, and iii) acid attack with 5% HF. Then small fragments of amber were detached with a scalpel and mounted in Canada balsam on a slide. Preparations were then observed under a Leica DMLP microscope. The Helicon Focus software was used to combine photos of an inclusion at different foci which facilitates better illustration.

Specimens shown in Breton and Tostain (2005), by Breton (2007) and Girard (2010), are housed in the "Muséum du Havre" (Coll. Juignet) under the reference MHPJ 0800. The other specimens are housed in the "Musée Vert" (Le Mans, France) and referenced under the inventory numbers MHNLM2009.3.1 (Roëzé-sur-Sarthe), MHNLM2009.3.2 (Mansigné), MHNLM2009.3.3, MHNLM2009.3.5 and MHNLM2009.3.6 (Écommoy) and MHNLM2009.3.4 (La Bazoge).

RESULTS

The fossil organisms from the Sarthe amber are mostly bacteria. They were found in all amber localities, and are more abundant in the Écommoy amber. Only a few unicellular eukaryotes have been identified. Kühne *et al.* (1973) and Schlüter (1978) also described a well-diversified entomofauna, partly revised by Matile (1981) and Perrichot *et al.* (2007b).

With regard to the systematics of the eukaryotic inclusions, the classification given by Adl *et al.* (2005) was followed. We adopted a similar hierarchical system, *i.e.* without formal rank designations. Single dots (•) indicate the super-groups as defined by Adl *et al.* (2005), double (••), triple dots (•••) and four dots (••••) correspond to first, second and third ranks, respectively. A similar system has been applied for the bacteria, with rod-shaped bacteria, actinomycetes and cyanobacteria considered as super-groups.

Bacteria

- Rod-shaped bacteria (Fig. 3A). Many colonies of rod-shaped bacteria have been observed (Fig. 3A, G). The spherical shape of the colonies suggests that these bacteria possibly grew in the resin after their trapping. Their diameter, from a few micrometres to more than 100µm, suggests that the colonies grew in the semifluid-non-solidified resin during 2-4 days, based on the observation of colonies of modern bacteria.

- **Actinomycetes.** Three different kinds of actinomycetes have been identified based on the diameter of the filaments, their branching and preservation. These organisms differ from sheathed bacteria by the absence of a sheath. The actinomycetes are also different from fungal filaments. As prokaryote organisms, they are devoid of vacuole, so they look homogeneous, and no membrane or cell wall is visible.

The first type (Type A) corresponds to filaments (0.6–1.0 µm in diameter) that regularly branch dichotomously (Fig. 3B). They form spherical colonies (diameter ranging from 50 to 100 µm), indicating that these actinomycetes possibly also grew in the resin during 2–4 days (based on observation of modern colonies of actinomycetes). The second type (Type B) corresponds to unbranched filaments (0.6–0.9 µm in diameter) (Fig. 3C). These actinomycetes are found on particular surfaces within the amber nodules corresponding to old surface resin flows. They developed on semifluid resin before being covered by another resin flow. The last type of actinomycetes (Type C; Fig. 3D) was described by Schlüter (1978) as ascomycetes developed on arthropod bodies. However, the illustrations given by Schlüter (1978) show that

they are actinomycetes. We found similar filaments developed on the unique specimen of arthropod from the Sarthe amber housed in the National Museum of Natural History, Paris (Fig. 3B). These filaments have a diameter of about 1 µm and their dichotomic branching indicates an actinomycetal origin. The lack of reproductive structures does not allow a more precise determination. They were decomposers that developed on cadavers.

- **Cyanobacteria.** Two different cyanobacteria have been found. *Palaeocolteronema cenomanensis* Breton and Tostain 2005 (Fig. 3E) was described from the Écommoy amber (Breton and Tostain, 2005) and is present in all the Sarthe ambers. *P. cenomanensis* was an important microorganism of the mid Cretaceous forest of France and has been found in all the Cenomanian amber localities of south-western France (Girard *et al.*, 2009a). This organism has recently been found in a Cenomanian amber lump from Saint-Lons-Les Mines (Landes, France) housed in the collection of the “Musée Vert” (Girard, pers. obs.). *P. cenomanensis* forms crusts (0.2–0.6 cm thick) at the periphery of some amber lumps. Its filaments are always centripetally orientated, indicating that *P. cenomanensis* grew in the resin during some days before its solidification. The presence of bubbles

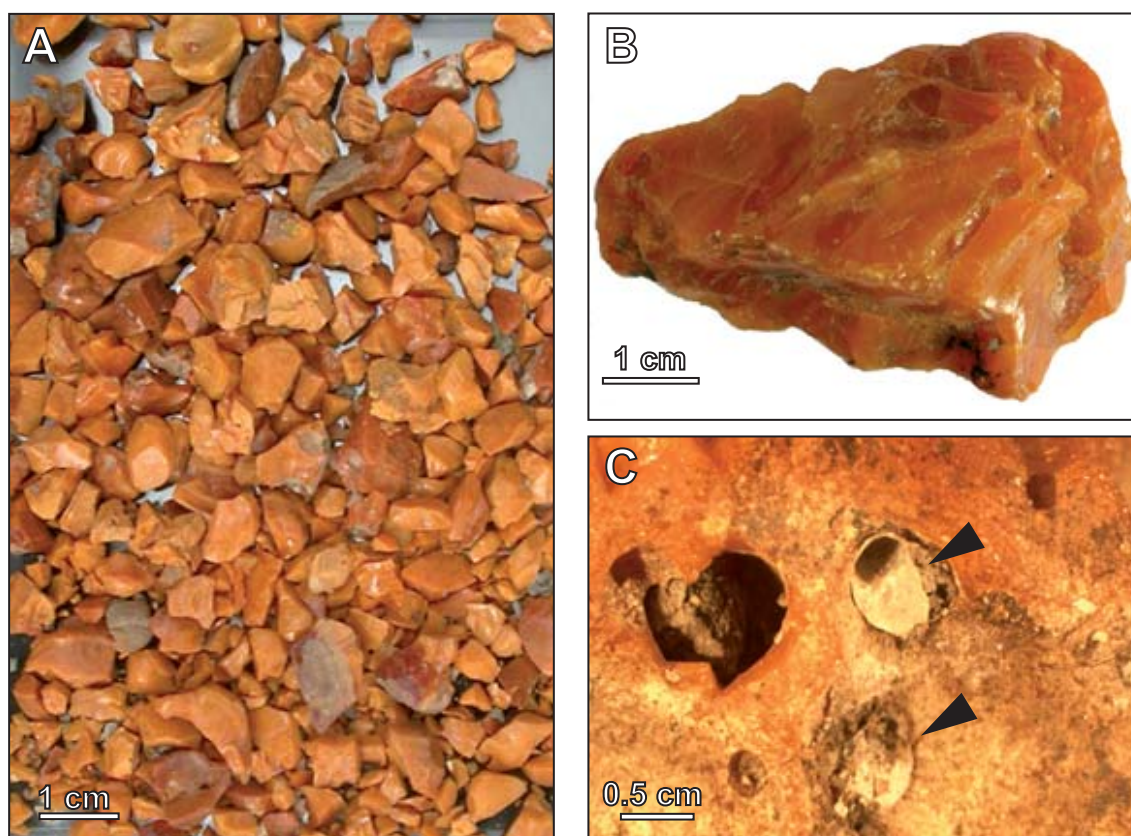


FIGURE 2 | Sarthe amber. A) Amber lumps from Mansigné. This material shows the typical characteristics of the Sarthe amber, *i.e.* its orange colour and its opacity. B) Amber lumps from Écommoy. C) Detail of the surface of an amber lump from Mansigné. Note fossil oysters (arrows) attached to the surface, indicating that the Sarthe amber was deposited into a marine or brackish environment.

on its filaments (“budding filaments” *sensu* Waggoner (1994)) shows that *P. cenomanensis* grew in freshwater. The presence of water probably delayed the solidification of the resin, allowing *P. cenomanensis* to develop such important crusts.

A second kind of cyanobacteria has been identified (Fig. 3F). Morphologically it is very similar to *P. cenomanensis*, but it has a greater diameter (about 50 µm, *i.e.* five times larger than *P. cenomanensis*). The association of these two cyanobacteria in the same amber piece indicates

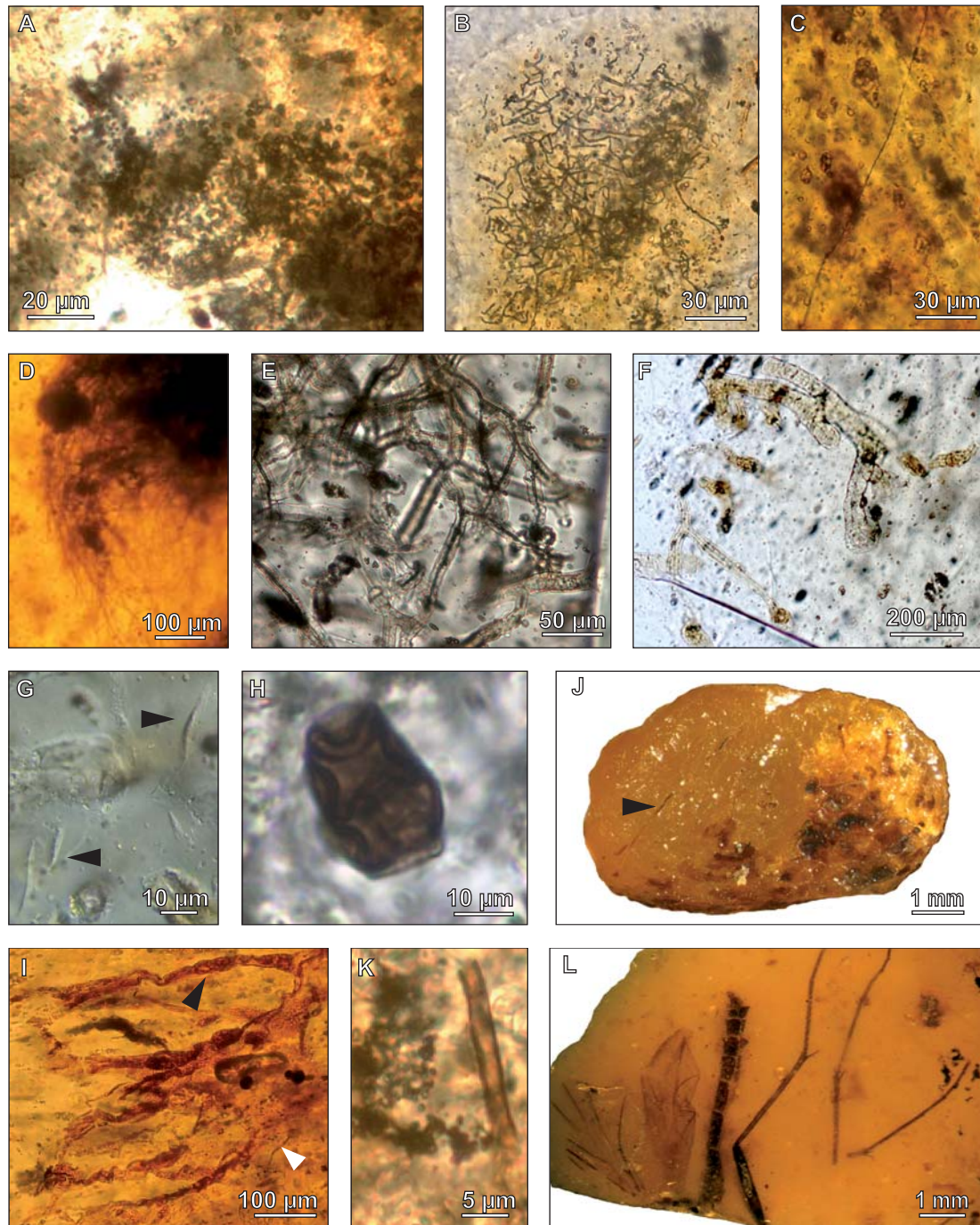


FIGURE 3 | Examples of microorganisms from the Sarthe amber. A) Colony of rod-shaped bacteria. B) Colony of actinomycetes Type A. C) Isolated filament of Type B actinomycetes. D) Filaments of Type C actinomycetes developed on the insect fragment illustrated in K. E- Net of filaments of *Palaeocolteronema cenomanensis* Breton and Tostain, 2005. F) *P. cenomanensis* (bottom) and a bigger cyanobacterium (top). G) Possible euglenids (arrows). H) Possible testate amoebae of the clade Arcellinida. I) Filaments of the green alga cf. *Trentepohlia* sp. J) Wood fibres (arrow). K) Small fragment of hyphae of an undetermined higher fungi (right) and a colony of rod-shaped bacteria (left). L) Holotype of *Schlueterimyia cenomanica* Matile, 1981 housed in the collection of the Muséum National d'Histoire Naturelle, Paris.

that they prospered in the same palaeoenvironment but probably occupied different ecological niches.

Eukaryotes

• Excavata

- Euglenozoa
- Euglenida

Questionable specimens of euglenids (Fig. 3G) have been found in the Écommoy amber (Breton and Tostain, 2005; Breton, 2007; Girard, 2010). Their state of presentation does not allow a more precise identification. Foissner *et al.* (1999) demonstrated that some microorganisms are not well preserved when they are trapped in conifer resins. Infrared spectroscopy showed that the Écommoy amber is derived from an araucarian resin (Schlüter, 1983). This could explain why the specimens of euglenids are poorly preserved. But, a non-microbial origin of these inclusions cannot be totally excluded, they may be pseudofossils as defined by Girard *et al.* (2011).

• Chromalveolata

- Alveolata
- Ciliophora

A few ciliates have been found in the Écommoy amber (Breton and Tostain, 2005; Breton, 2007; Girard, 2010). Their preservation state does not allow a precise determination. As for the possible euglenids, a non-microbial origin cannot be totally excluded for these inclusions too and they also may be pseudofossils as defined by Girard *et al.* (2011).

• Amoebozoa

- Tubulinea
- Testacealobosia
- Arcellinida

Breton and Tostain (2005) and Breton (2007) described naked amoebae. However, re-investigation of these inclusions did not confirm its microbial origin. Actually, it is difficult to be certain about the systematics of amber microorganisms with well-defined shapes, and it becomes extremely complicated with microorganisms not characterised by a specific shape. The interpretation of Breton and Tostain (2005) and Breton (2005) cannot be totally excluded. A non-biological origin (pseudofossil *sensu* Girard *et al.* (2011)) cannot be excluded.

A badly preserved testate amoeba has been found, in the Écommoy amber (Fig. 3H), with its shell laterally compressed at its posterior end. For this reason, its identification is difficult. Its brown colour and the apparent lack of xenosomes indicate that it is an Arcellinida. Indeed, species such as *Arcella mitrata* Leidy, 1876 and *A. vulgaris* Ehrenberg, 1830 have similar colour and surface aspect to those of the amber specimen.

• Archaeplastida

- Chloroplastida
- Chlorophyta
- Chlorophyceae

A few remains of green algae were found. Breton and Tostain (2005) mentioned a specimen of *cf. Trentepohlia* sp. (Trentepohliales; Fig. 3I) but, it is poorly preserved. Its filaments are red and are interlaced with filaments of actinomycetes. The diameter of the algae cells is about 15–20 µm and their length varies from 35 to 45 µm. The filaments of the algae are regularly dichotomously branched. For more details about the determination, see Breton and Tostain (2005) and Breton (2007).

• Opisthokonta

- Fungi
- Higher Fungi

A few remains of higher fungi have been observed (Fig. 3K). They mostly consist of unidentified fragments of mycelium. No reproductive structures have been observed. The filaments have a diameter varying from 1.0 to 2.5 µm. Some septae have been observed in a few fragments of mycelia (Fig. 3K). These septae and the diameter of the filaments indicate that they belong to higher fungi, but their precise taxonomic identity remains unclear.

• Metazoa

- Animalia

During the 1970's and 1980's, an important entomofauna from the Sarthe amber was described. For example, Schlüter (1983) mentioned 72 arthropod specimens in only 36 amber lumps. However, it has been impossible to find the majority of this material and only one specimen could be observed in the collection of the Palaeoentomology Laboratory of the National Museum of Natural History, Paris. Based exclusively from bibliographic references, a list of the arthropods found in the Sarthe amber, mainly from Écommoy, is given in Perrichot *et al.* (2007b).

••• Arachnida

Schlüter (1978) described one species of harvestmen, two species of mites and two species of spiders from the Écommoy amber.

••• Insecta

Insects are more diversified than arachnids in the Sarthe amber. Schlüter (1978) identified specimens belonging to 9 different orders, whose determinations have been partly updated (Matile, 1981; Perrichot *et al.*, 2007b). Cockroaches, bugs and barklice are represented by only one unidentified species. Isopterans (including termites) are well diversified with at least seven unidentified species (none of them have been placed in known families of isopterans).

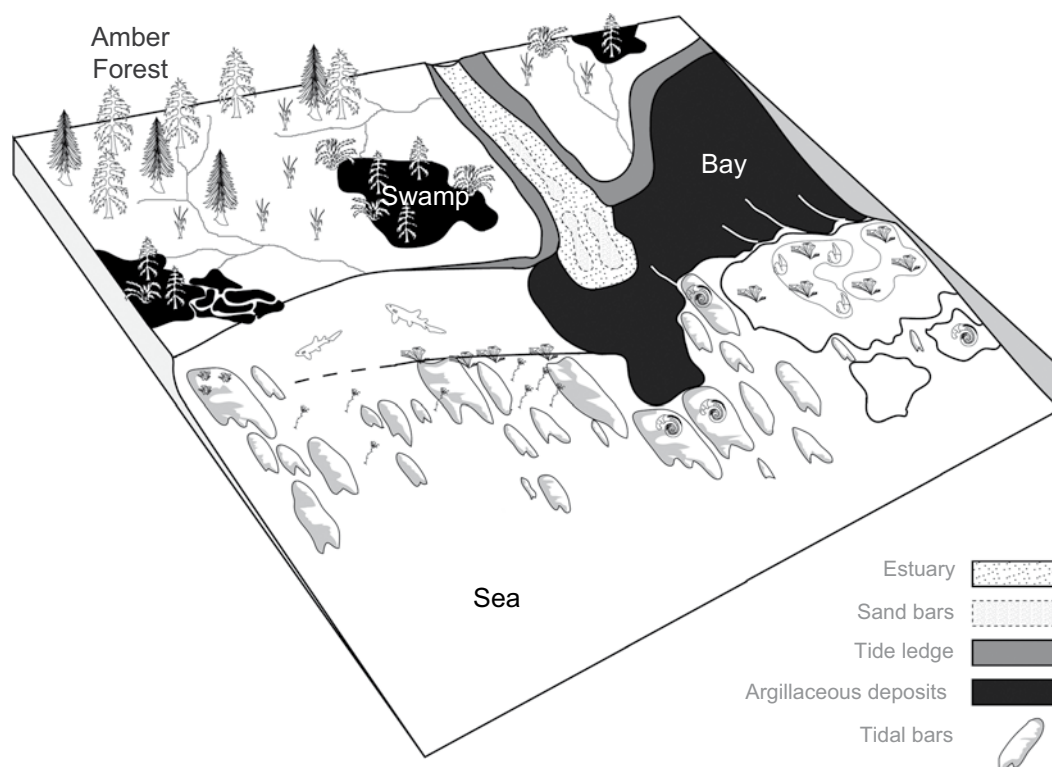


FIGURE 4 | The situation of the amber forest in the Cenomanian landscape of Sarthe. During the Cenomanian, Sarthe was mostly under marine influence. On its western rim, only part of Sarthe Department experienced continental conditions, and a resiniferous forest that produced Sarthe amber developed here. Swamps probably existed in this forest, in which resin flows were produced allowing the preservation of freshwater organisms such as *Palaeocolteronema cenomanensis*. Solidified resin was then transported (by runoff and small channels) into the depositional basin, a protected area under paralic influence that allowed the sedimentation of the black clay (as observed in the quarry of Bezonnais at Écommoy (Juignet and Médus, 1971)) and the encrusting of oysters on some amber pieces such as those of Mansigné.

Lacewings are represented by one species of Rachiberothidae (“Berothidae” in Schlüter, 1978) and moths by one species of Micropterygidae (Kühne *et al.*, 1973). Two species of Staphylinidae (beetles) have been identified. The first one belongs to the sub-family Staphilininae and the other to the Steninae. The hymenopterans are the most diverse group and are represented by five different families (Perrichot *et al.*, 2007b): one species of Mymarommatidae (“Mymaridae” in Schlüter (1978)), one incomplete and doubtful specimen of Diapriidae, four species of Platygastriidae (“Scelionidae” in Schlüter, 1978), three species of Sphecidae and one possible, incomplete, Bethyidae (“Falsiformicidae” in Schlüter, 1978). The dipterans (Fig. 3L) are the second most important order. Five families have been identified: Ceratopogonidae, Cecidomyiidae, Keroplatidae (Matile, 1981; “Fungivoridae” in Schlüter (1978)), Empididae and an incomplete specimen of intermediate Tipuloida (Perrichot *et al.*, 2007a; Perrichot, 2010). For more details about the arthropod fauna of the Sarthe amber, see also Schlüter and Stürmer (1982), Schlüter (1989) and Szadziewski and Schlüter (1992).

Non-organic inclusions

Long and translucent micro-crystals with parallel extinction are abundant in the Sarthe amber (especially in Mansigné amber). Pyrite is rare in the Sarthe amber.

DISCUSSION

Pollen and fossil leaf assemblages have already been used to reconstruct the mid-Cretaceous forest of Sarthe (Crié, 1884; Juignet and Médus, 1971; Azéma *et al.*, 1972; Laing, 1975). This forest was dominated by Polypodiales ferns (60% of the pollen assemblage), which constituted the undergrowth of the forest. Coniferales (Araucariaceae, Cheirolepidiaceae, Pinaceae and Taxodiaceae) and Ginkgoales were the main trees of this palaeoecosystem and represent about 30% of the plant remains found in Cenomanian layers in Sarthe. Angiosperms (including both monocots and dicots) were less common (only 10%) and probably restricted to specific environments (riparian vegetation?). Rare remains of Bennetitales and Cycadales completed the fossil assemblage. This plant assemblage of

Sarthe shows similarities with the Cenomanian floras of Charentes (Perrichot, 2005) and probably grew on coastal environments under a warm climate.

In contrast to the amber-bearing layers, only a few plant remains have been found in the Sarthe amber itself. Schlüter (1978) mentioned the presence of trichomes, which he attributed to angiosperms. However, we cannot confirm this attribution. Indeed, many plants can produce this kind of structures. Ferns, that formed an important part of the undergrowth of Cretaceous forests, could also have yielded the trichomes found in the Sarthe amber. In fact, the scarcity of angiosperms in the mid Cretaceous forests suggests that they could hardly have been the source of the trichomes. Wood fibres of gymnosperms (Fig. 3J) have also been observed, but their poor preservation did not allow a precise identification.

Compared to other contemporaneous French ambers (Perrichot, 2005), only a few inclusions have been found in the Sarthe amber, but were important to characterise the biotope in which the resin was secreted. The majority of the identified microfossils correspond to freshwater microorganisms. The distribution of the microfossils within the amber lumps indicates that the resin had a complex history. “Aerial” taxa (such as fungi) are found in the core of the amber lumps, suggesting an early entrapment, whilst freshwater taxa (such as *P. cenomanensis*) are always preserved at the edges of the amber lumps. The resin was probably aerially secreted (allowing the preservation of the tree bark microorganisms), and subsequently fell off the flooded soil, which allowed the preservation of the freshwater microorganisms.

The Sarthe amber was probably secreted in a freshwater swamp (Fig. 4), similar to the modern freshwater *Taxodium* swamps of Florida (USA). In this environment, resin flows can be secreted, on the trunk or branches and on immersed roots (Schmidt and Dilcher, 2007). Observations made on the Sarthe amber are corroborated by those of Schmidt and Dilcher (2007) as we observed both freshwater and continental taxa in Sarthe amber. In Florida Swamps (Schmidt and Dilcher, 2007), several freshwater organisms were trapped at the periphery of the resin flow whilst filamentous organisms (bacteria and fungi) colonised the resin and developed filament networks into the resin. In Sarthe amber, the average length of filaments of *P. cenomanensis* has been estimated to be 1 millimetre. Compared with the data of Schmidt and Schäfer (2005) on sheathed bacteria, we can estimate the growth of *P. cenomanensis* at five or six days before complete solidification of the resin.

The arthropod fauna from the Sarthe amber, described by Kühne *et al.* (1973) and Schlüter (1978) and partly

revised by Matile (1981) and Perrichot *et al.* (2007b), also provides some data about the amber forest of Sarthe. Arthropods mostly consist of flying organisms (flies, wasps, moths, etc.). These organisms probably flew over the forest soils, seeking for food, for swarming... The rest of the entomofauna is typical of a forest litter. Acari are abundant in modern forest litter where they eat diverse microarthropods, protists and fungi. The Specimens from the Sarthe amber probably had the same ecology. Unfortunately, they are quite rare in the fossil assemblage (taphonomic bias or missing data?), their precise ecology remains obscure. The coleopterans of the family Staphylinidae live mostly in well-developed litter. The fossil specimens from the Sarthe amber could have been active inhabitants of the forest litter. The Ceratopogonidae are also typical of litter forest. Their larvae are mostly burrowers in humid soils, in particular coastal environments (Downes and Wirth, 1981). Their occurrence in the Sarthe amber confirms that the amber forest grew close to the sea-shore (Szadziwski, 2004; Pérez de la Fuente *et al.*, 2011).

We can conclude that bacteria and fungi were the main decomposers of the Sarthe forest, but termites probably played an important role in the decomposition of dead wood. Mites and other small invertebrates fed on unicellular organisms (such as amoebae and ciliates) and fungi. Termites may have been the prey of the larvae of lacewings (as it is the case of neuropterans in modern forests). We can not compare the trophic structure of the Sarthe forest with that of the Charentes forest. However, it is clear that these two forests developed in similar environments, close to the sea, under marine influences (at least temporarily) as suggested by the presence of marine organisms in the Charentes amber (Girard *et al.*, 2008) and Ceratopogonidae in the Sarthe amber.

CONCLUSIONS

The analysis of historical collections from the “Musée Vert” (Le Mans, France) allows us to characterise the development of the amber-bearing layers in Sarthe. The Écommoy amber is the most well-known from Sarthe, but many other outcrops provided samples. The distribution of these outcrops indicates that an important littoral environment developed in the south-western part of the Sarthe Department during the Cenomanian (Fig. 1). This environment extended to the south, in the Maine-et-Loire Department, as suggested by the presence of amber in this area (Schlüter, 1978; Perrichot *et al.*, 2007b). Its northern extension remains unclear, but probably it was also well developed to the north as shown by the presence of amber in the localities of La Bazoge (Sarthe) and Neu (Mayenne) (Durand and Louail, 1971).

Microfossils have been found in all Sarthe localities. These microfossils indicate that the Sarthe amber was probably produced in a freshwater environment similar to the modern freshwater *Taxodium* swamps of Florida. The hypothesis that the Écommoy amber was secreted in a littoral environment, made by Breton and Tostain (2005) and Breton (2007) based on diatoms (*cf. Nitzschia* sp.), is not taken into account here, as a reinvestigation showed that the inclusions are probably pseudofossils (*sensu* Girard *et al.* (2011)), no diatoms. In contrast to the Charentes amber (Girard *et al.*, 2008), no clear influence of brackish to marine environments has been highlighted by fossils found inside the Sarthe amber. The links between the amber-producing (freshwater swamps) and the amber-depositional environments are still difficult to determine. Amber could have either been produced in coastal freshwater swamps or been transported from a distant forest.

We were unable to revise the arthropods studied by Schlüter (1978) as this material, supposedly housed in the palaeontology collections of the Freie Universität (Berlin, Germany), is missing. Thus, the links between the amber microcoenosis and the entomofauna from the Sarthe amber layers remains unclear.

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