Evidence of Scenedesmaceae (Chlorophyta) from 100 million-year-old amber

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ABSTRACT

Mid-Cretaceous ambers from Aix Island and Cadeuil (Charente-Maritime, southwestern France) have preserved a rich microorganism assemblage of cyanobacteria, testate amoebae, and algae. The assemblage contains the first fossil record of the modern green algae genus *Enallax* Pascher, 1943 (Chlorococcales, Scenedesmaceae) and a new species, *Enallax napoleoni* n. sp., is described. This discovery pushes back the origin of the genus *Enallax* to the Cretaceous. *Enallax napoleoni* n. sp. probably grew in freshwater ponds of the mid-Cretaceous amber forests of southwestern France under a warm climate, associated with the cyanobacterium *Palaeocolteronema cenomanensis* Breton & Tostain, 2005.

KEY WORDS
Algae,
Scenedesmaceae,
Enallax,
amber,
mid-Cretaceous,
France,
new species.

RÉSUMÉ

Présence de Scenedesmaceae (Chlorophytes) dans un ambre vieux de 100 millions d'années.

Les ambres médio-crétacés de l'île d'Aix et de Cadeuil (Charente-Maritime, sud-ouest de la France) ont préservé un riche assemblage de microorganismes composé de cyanobactéries, d'amibes testées et d'algues. Le premier fossile du genre actuel d'algues vertes *Enallax* Pascher, 1943 (Chlorococcales, Scenedesmaceaee) est décrit dans cette étude, comme une nouvelle espèce, *Enallax napoleoni* n. sp. Cette découverte repousse l'origine du genre *Enallax* jusqu'au Crétacé. *Enallax napoleoni* n. sp. se développait probablement dans des mares d'eau douce des forêts médio-crétacées à ambre du sud-ouest de la France, associée à la cyanobactérie *Palaeocolteronema cenomanensis* Breton & Tostain, 2005.

MOTS CLÉS
Algues,
Scenedesmaceae,
Enallax,
ambre,
Crétacé moyen,
France,
espèce nouvelle.

INTRODUCTION

Chlorophyceae – or green algae – are a widely diversified group of aquatic plants comprising more than 7800 modern species distributed in 520 genera. Bourrelly (1966) divided them into 14 different orders according to morphological and physiological features.

Their fossil record extends back to the Cambrian, 500 to 600 million years ago, mainly with fossils of Dasycladales (Knoll 2003). However some authors consider they originated much earlier, around 2 Ga (Teyssèdre 2006), and then probably gave rise to multicellular plants. Because of their poor potential of fossilization (most of them are soft-bodied), only a few fossils of green algae have been recorded thus far. Most of them correspond to a few families of calcareous green algae, i.e. green algae that edify a sort of calcareous skeleton. Among these, Botryococcaceae have been reported from diverse Eocene localities, e.g., from the oil shales of Messel (Hofmann *et al.* 2005); Cordiaceae have been found from calcareous rocks, the oldest ones being Silurian in age (Lemosquet & Poncet 1974); and the most recorded are the Dasycladaceae, with fossils ranging from the Cambrian to very recent times (Bassoulet *et al.* 1977). During the last couple of years, it has been demonstrated that amber can improve significantly the fossil record of rarely preserved organisms. It is also true for green algae as Schönborn *et al.* (1999) described *Chlamydomonas* Ehrenberg, 1833 (Volvocales) and *Chloromonas* Gobi, 1899 (Volvocales) from the Cenomanian amber of Schliersee, Germany, which was considered to be Triassic in age at that time (see Schmidt *et al.* 2001 for re-dating).

Since 1999, several Cretaceous amber localities of Albian and Cenomanian ages have been found in France. The ones of Aix Island and Cadeuil (Charente-Maritime, southwestern France) exhibit lignitic mid-Cretaceous layers containing amber pieces in which fossil insects (Perrichot *et al.* 2008; Néraudeau *et al.* 2008, 2009 [this volume]) and diverse microfauna and microflora were found. This includes a large proportion (95%) of the sheathed cyanobacterium *Palaeocolteronema cenomanensis* (Girard *et al.* in press), but also rare microorganisms such as the Scenedesmacean specimens (Chlorococcales, Scenedesmaceae) described here. These specimens belong to one of the oldest known Scenedesmaceae and provide new evidence for the evolutionary history of the group as well as further data about the mid-Cretaceous palaeoenvironments of Aix Island and Cadeuil.

GEOLOGICAL SETTING

The amber pieces containing fossils described herein come from two different localities (Fig. 1).

In the quarry of Cadeuil, the uppermost Albian to Lower Cenomanian deposits represent transgressive deposits which have eroded the underlying strata (Néraudeau *et al.* 2008). These deposits are mainly composed of fluviatile and paralic sand. They contain several clayey intercalations with local concentrations of fossil plant cuticles. These alternating sand and clay beds belong to the lithological unit A, subdivided into two sub-units (Néraudeau *et al.* 2008 and references herein). A1 (latestmost Albian in age) corresponds to sand of various grain sizes, arranged in large cross beddings, bearing abundant lignite and amber accumulations. A2 (earlymost Cenomanian in age) corresponds to fine sand arranged in horizontal beds, bearing rare wood remains. The unit A is overlain by a falun *sensu* Vullo *et al.* (2003) rich in orbitolines and oysters, corresponding to the base of the lithological unit B (sub-unit B1). In the Cadeuil area, the main amber deposit is located at the top of the sub-unit A1 (Néraudeau *et al.* 2008).

The tidal flat of Aix Island provides sandstone, clay and limestone of Lower Cenomanian age. Two lignitic intercalations contain amber (Perrichot 2005) and vertebrate remains (Vullo 2007). The oldest one corresponds to the lithological sub-unit B1b as described by Néraudeau *et al.* (1997, 2009 [this volume]). It is composed of a grey limestone rich in centimetric to plurodecimetric wood fragments and containing a few amber droplets. The younger sub-unit B2 (Néraudeau *et al.* 1997, 2009 [this volume]) is the more developed paralic facies of Aix Island and the richest in amber. It is divided into two parts, B2a which is a clay poor in plant debris and devoid of amber, and B2b which is a lignitic clay sometimes rich in amber. This facies ends with a glauconitic carbonaceous sandstone (B2c) which is rich in the oyster *Rhynchostreon suborbiculatum* Lamarck, 1801, and contains lignite, large amber pieces (sometimes more than 10 cm in diameter), and lenses of sand and gravel rich in lignitic debris and vertebrate remains.

MATERIAL AND METHODS

The specimens described in this study are preserved in a 1 cm piece of amber labelled Aix15c from Aix Island and in a 3-4 cm piece of amber labelled CDL26c from Cadeuil. They are of red colour and are surrounded by a white to grey cortex of sheathed filaments related to *Palaecolteronema cenomanensis* (Girard *et al.* in press). These amber pieces come from the few kilograms of Aix Island and Cadeuil amber conserved in the collections of the Geosciences Rennes laboratory (University of Rennes I).

Because of their outcropping conditions, Aix Island and Cadeuil ambers have been subjected to a great surface and cracks contamination by recent microorganisms such as green algae or diatoms. The amber pieces considered herein have been treated to eliminate contamination using the protocol described by Girard *et al.* (2009). It was cleaned by ultrasounds and then washed in 9-10% H_2O_2 during 5 hours to eliminate contaminant organic matter (such as recent bacteria or mycelia) and finally washed in 5% HF during 5 minutes to eliminate contaminant inorganic matter (such as diatom frustules). Then very thin fragments of the piece were mounted on slide with Canada balsam and observed under a Leica DLMP microscope. Immersion oil has been used to observe more details.

SYSTEMATIC PALAEONTOLOGY

Division CHLOROPHYTA Pascher, 1914
Class CHLOROPHYCEAE Kützing, 1843
Order CHLOROCOCCALES Pascher, 1915
Family SCENEDESMACEAE Oltmanns, 1904
Subfamily SCENEDESMOIDEAE
Hegewald & Hanagata, 2000

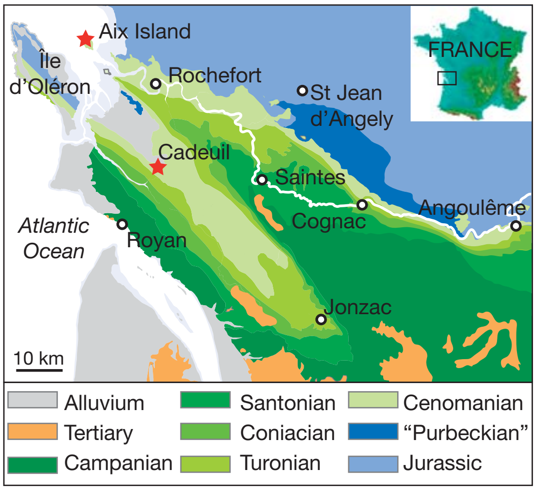

FIG. 1. — Simplified geological map of Charente-Maritime, south-western France. The stars indicate the outcrops of Aix Island and Cadeuil in which the amber pieces containing specimens of *Enallax napoleoni* n. sp. were found.

Genus *Enallax* Pascher, 1943

TYPE SPECIES. — *Enallax alpinus* Pascher, 1943.

DIAGNOSIS. — Coenobia with (2)-4-8 more or less parallel, three-dimensional arranged elongate cells, which are often shifted against each other. Cells fusiform to ellipsoid, rarely slightly asymmetric. Pressing of the cells to each other results in a convex outer side and a concave inner side of the single cells. Cell wall thick with meridional arranged ribs or ridges. A single chloroplast, parietal (attached to the wall), with some swellings, with a single pyrenoid. Reproduction by 2-4-8 autospores already arranged as a coenobium inside the mother cell. The autospores develop wrinkle-like structures (i.e. ribs or ridges) at the wall and emerge when the mother cell breaks. Resting stages show thick walls and red oil bodies inside the protoplast.

Enallax napoleoni n. sp.
(Fig. 2)

TYPE MATERIAL. — Holotype specimen Aix(15'-1)c-6, paratype specimen Aix(15'-1)c-7, preserved in the same piece of amber (Aix15c) with the holotype. Paratype specimens CDL26cc-7, CDL26cd-1 and CDL26cd-1 preserved in a single piece amber labelled CDL26c. Deposited in the amber collection of Géosciences, Université Rennes I, Rennes, France.

Etymology. — In reference to the history of Aix Island. The emperor Napoléon I, after he was defeated in Waterloo, stayed for a time on Aix Island before to be exiled and to die on Sainte Hélène Island.

Type locality. — Bois-Joly on Aix Island, Charente-Maritime, France.

Stratigraphic horizon. — Lower Cenomanian, lithological sub-unit B2b *sensu* Néraudeau *et al.* (2009 [this volume]).

Diagnosis. — *Enallax napoleoni* n. sp. is distinguished from all other species of *Enallax* by its larger size, and from *E. acutiformis* by its larger number of longitudinal ribs.

Description

Cell fusiform, nearly twice longer than large, with six strong longitudinal ribs forming a 6-order symmetry (when great axis of cell as axis of symmetry). One specimen slightly asymmetric, with one apex more pointed than other (Fig. 2B – possibly resulting from preservation). No mineralized test; intracellular structures not visible by preservation. Coenobia are unknown. Absence of coenobia does not indicate that *E. napoleoni* n. sp. was not able to form it. It probably reflects the fact that a possible coenobium might be destroyed during the embedment in the resin. Proximity of the two specimens described here (no more than 80-100 µm) could reflect this. The first specimen does not allow to see any structure into the cell; in the second it is possible to distinguish a central ellipsoidal structure interpreted as a chloroplast (Fig. 2B).

Measurements

First specimen from Aix Island about 49 µm long and 22 µm wide; second specimen 61 µm long and 28 µm wide. Specimens from Cadeuil about 65-70 µm long and 40-45 µm wide.

Discussion

These fossils can be attributed to the family Scenedesmaceae within Chlorococcales algae based on their fusiform shape, strong costulation, and dimension. In his inventory of the Scenedesmaceae, Bourrelly (1966) noticed that the shape of their coenobia, i.e. their colonies, is the main characteristic to classify these algae. Amber fossils have been found isolated. Although Aix Island specimens occur in a single piece of amber, they are indeed not connected to each other but separated by 100 µm.

Among the Scenedesmaceae, the new fossils fit in the genus *Enallax*, described by Bourrelly (1966) as a fusiform alga showing a strong longitudinal costulation and a slight asymmetry. *Scenedesmus acutiformis* Schröder, 1897 was also characterised as a large and straight unicellular alga with longitudinal ribs, but it was later transferred into *Enallax* by Hindák (1990).

The new species can be distinguished from the two modern species of *Enallax* as follows: *E. alpinus* is smaller (30 µm long and 14 µm wide according to Bourrelly [1966: pl. 36 fig. 19; Fig. 2C] instead of 50-60 µm long and 22-28 µm large for *E. napoleoni* n. sp.); and *E. acutiformis* has only four longitudinal ribs instead of six and is also smaller (20 µm long and 14 µm wide).

Species of the genus *Scotiella* Fritsch, 1912 (Chlorococcales, Oocystaceae) also exhibit morphological features similar to those of *Enallax* species. Cells of *Scotiella* have a central or parietal chloroplast with a median H-shaped plate. The presence of an elliptical chloroplast on the second specimen of *E. napoleoni* n. sp. from Aix Island allows to differentiate amber fossils from the genus *Scotiella*. The genus *Scotiellopsis* Vinatzer, 1975 (Chlorococcales, Scenedesmaceae) is also closely similar to amber fossils, but can be distinguished by the symmetry of *Scotiellopsis* cells and their weak costulation (Noguerol-Seoane & Rifón-Lastra 2000).

Specimens from Aix Island amber show peculiar obtuse cell pole (Fig. 2A, B) in comparison with the acute cell poles of *E. alpinus* and the specimens of Cadeuil amber (Fig. 2C). This difference is probably due the taphonomical processes that allowed the preservation of these two fossils. One should be surprised to found floating algal cell in amber because of the hydrophobic properties of the later, however Schmidt & Dilcher (2007) already proved that resin flows of *Taxodium* are able to engulf floating algal cells. The amber flows that have provided the specimens of *Enallax* should have been produced directly in water such as the one of *Taxodium*.

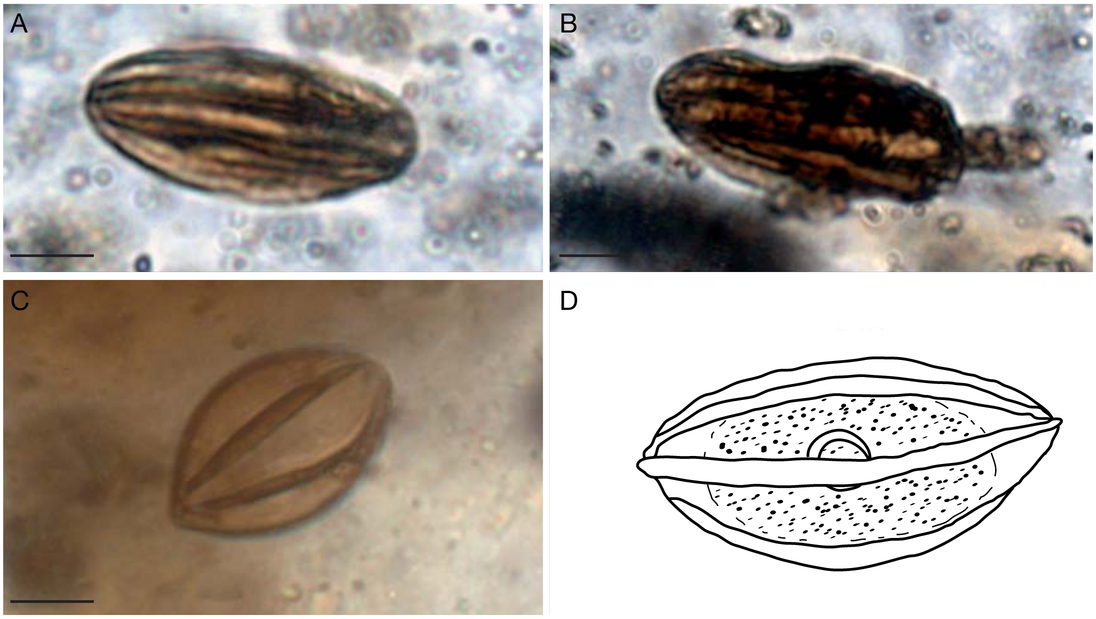

FIG. 2. — **A**-**C**, *Enallax napoleoni* n. sp.; **A**, **B**, from Cenomanian Aix Island amber; **A**, holotype specimen Aix(15'-1)c-6; **B**, paratype specimen Aix(15'-1)c-7, stipple lines highlight the ellipsoidal chloroplast of the specimen; **C**, paratype specimen CDL26cc-7 from Cadeuil; **D**, *E. alpinus* Pascher, 1943 redrawn from Bourrelly (1966). Scale bars: 20 µm.

PALAEOECOLOGY

Modern species of *Enallax* mainly live in cold environments. *Enallax alpinus* is mentioned by Bourrelly (1966) as a typical alga of alpine environments, especially of acid water such as those of peat bogs. *Enallax acutiformis* is also reported by Hegewald (1989) as a component of cold environments but he specified that it can also grow at 30°C. Da *et al.* (1997) mentioned its presence in Ivory Coast, confirming that *Enallax* can grow in cold and warm environments.

Specimens of *E. napoleoni* n. sp. are preserved as syninclusions with sheathed filaments of the cyanobacterium *P. cenomanensis* (Girard *et al.* in press). This cyanobacterium was first described from the Cenomanian amber of Ecommoy (western France) and mentioned in the Albian and Cenomanian amber of Archingeay-Les Nouillers and Cadeuil (southwestern France) (Breton & Tostain 2005). It supposedly grew into freshwater ponds of the amber forest (Breton & Tostain 2005; Girard *et al.* in press). Other amber samples of red amber from Aix Island and Cadeuil preserved freshwater testate amoebae (including *Arcella* Ehrenberg, 1832 specimens), actinomycetes, rod-shaped and diverse mycelium filaments. Sedimentological and palaeontological data indicate that this microorganism assemblage occurred in freshwater microenvironments such as ponds, among a coastal forest and under a warm climate (Néraudeau *et al.* 2009 [this volume]).

Therefore, the new fossils provide evidence that some early representatives of *Enallax* grew in warm, tropical conditions. But a more complex palaeoecology similar to that exhibited by modern species remains possible. The absence of an extensive fossil record at high latitude and the supposed mid-Cretaceous slight relief are the main limitation for additional discovery of early *Enallax* fossils.

CONCLUSION

As soft-bodied organisms, green algae have a scarce fossil record. The discovery of several specimens of the genus *Enallax* in Aix Island and Cadeuil amber contributes to the fossil record of the Scenedesmaceae, hitherto poorly documented. This family had already been described from the Albian Mattagami Formation, Ontario (Zippi 1998). Only three other occurrences of such algae have been recorded: one from Early Cretaceous of English Wealden (Batten & Lister 1988a, b), one from Palaeocene of Colorado and New Mexico (Fleming 1986, 1989) and one from mid-Miocene amber of Amazonia (Antoine *et al.* 2006). The present finding also provides a new opportunity to increase our knowledge of this important group of algae, indicating that the genus *Enallax* was already present in warm environments during the mid-Cretaceous. Whether *Enallax* already existed in cold environments (mountains, high latitude) has still to be evidenced.

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