

Review of the El Soplao Amber Outcrop, Early Cretaceous of Cantabria, Spain

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Abstract: El Soplao outcrop, an Early Cretaceous amber deposit recently discovered in northern Spain (Cantabria), has been shown to be the largest site of amber with arthropod inclusions that has been found in Spain so far. Relevant data provided herein for biogeochemistry of the amber, palynology, taphonomy and arthropod bioinclusions complement those previously published. This set of data suggests at least two botanical sources for the amber of El Soplao deposit. The first (type A amber) strongly supports a source related to Cheirolepidiaceae, and the second (type B amber) shows non-specific conifer biomarkers. Comparison of molecular composition of type A amber with *Frenelopsis* leaves (Cheirolepidiaceae) strongly suggests a biochemical affinity and a common botanical origin. A preliminary palynological study indicates a regional high taxonomical diversity, mainly of pteridophyte spores and gymnosperm pollen grains. According to the preliminary palynological data, the region was inhabited by conifer forests adapted to a dry season under a subtropical climate. The abundant charcoalfied wood associated with the amber in the same beds is evidence of paleofires that most likely promoted both the resin production and an intensive erosion of the litter, and subsequent great accumulation of amber plus plant cuticles. In addition, for the first time in the fossil record, charcoalfied plant fibers as bioinclusions in amber are reported. Other relevant taphonomic data are the exceptional presence of serpulids and bryozoans on the surfaces of some amber pieces indicating both a long exposure on marine or brackish-water and a mixed assemblage of amber. Lastly, new findings of insect bioinclusions, some of them uncommon in the fossil record or showing remarkable adaptations, are reported. In conclusion, a documented scenario for the origin of the El Soplao amber outcrop is provided.

Key words: fossil resin, chemotaxonomy, paleobotany, charcoal, arthropod bioinclusions, taphonomy, Early Albian

1 Introduction

Amber preserves delicate organic fossils, including microorganisms, cells and tissues, but the most abundant record is constituted by insects, sometimes showing interactions between them, such as mating interaction, commensalism and parasitism (e.g. Grimaldi, 1996; Martínez-Delclòs et al., 2004; Grimaldi and Engel, 2005). Early Cretaceous amber is remarkably important, because it was during this period that

there occurred explosive radiations of the flowering plants and many modern families of insects (Grimaldi and Engel, 2005). During the last decade several new deposits of Cretaceous amber have been discovered in Spain and France (e.g. Delclòs et al., 2007; Perrichot and Néraudeau, 2009), and researches on various aspects of Cretaceous amber have increased greatly. However, most of the researches made concern the taxonomical descriptions of arthropods and microorganisms embedded in amber. Only a few studies have been conducted on the origin of both the amber and the deposits that contain it. One of the most

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important aspects concerning Cretaceous amber is the plant source, if there was only one, but this aspect has been shown to be very difficult to ascertain with confidence.

The first detailed description of Spanish amber was published by Casal (1762). Approximately 150 years later Boscá (1910) first indicated the possible presence of insects preserved in Spanish amber. After the discovery of two remarkable amber deposits rich in bioinclusions, Peñacerrada I in the north and San Just in the north-east of Spain, a third major amber deposit was discovered in 2008 in Cantabria, named El Soplao. Thus it was not included in the most recent review of Spanish amber outcrops by Delclòs et al. (2007). Najarro et al. (2009) described in detail the regional geology, the features of the amber pieces, including infrared spectroscopy analyses, and a first attempt of the bioinclusions and the plant cuticles associated with the amber. Subsequent papers deal with biogeochemistry of amber and descriptions of new arthropod taxa preserved as bioinclusions. Thus, Menor-Salván et al. (2009a, 2009b) investigated biomarkers from amber pieces and *Frenelopsis* leaves from the El Soplao outcrop, concluding that they share the same origin; in addition, they identified the chemical compound form that produces an intensive fluorescent blue glow when this amber is under normal sunlight. More recently, Menor-Salván et al. (2010) report some paleochemotaxonomical aspects of the biological diterpenes preserved in El Soplao amber. On the other hand, Pérez-de la Fuente et al. (2010) and Nel et al. (2010) described the new insect taxa *Cantabroraphidia marcanoi* (Raphidioptera: Mesoraphidiidae) and *Tethysthrips hispanicus* (Thysanoptera: Thripidae), respectively. Lastly, Ortega-Blanco et al. (2010a) report the fauna of false fairy wasps (Mymarommatoidea) that has been recorded in Spanish Cretaceous amber, including a paratype specimen from El Soplao amber.

We report relevant new data about El Soplao amber deposit, such as new detail on stratigraphy of the amber-bearing levels, new geochemical and biogeochemical information of the amber and fossil leaves, palynological data, the presence of a great abundance of charcoal associated with the amber, the presence of marine invertebrates on the surface of some amber pieces and new discoveries about the bioinclusions. These new contributions permit us to portray a more documented scenario for the origin of the El Soplao amber outcrop.

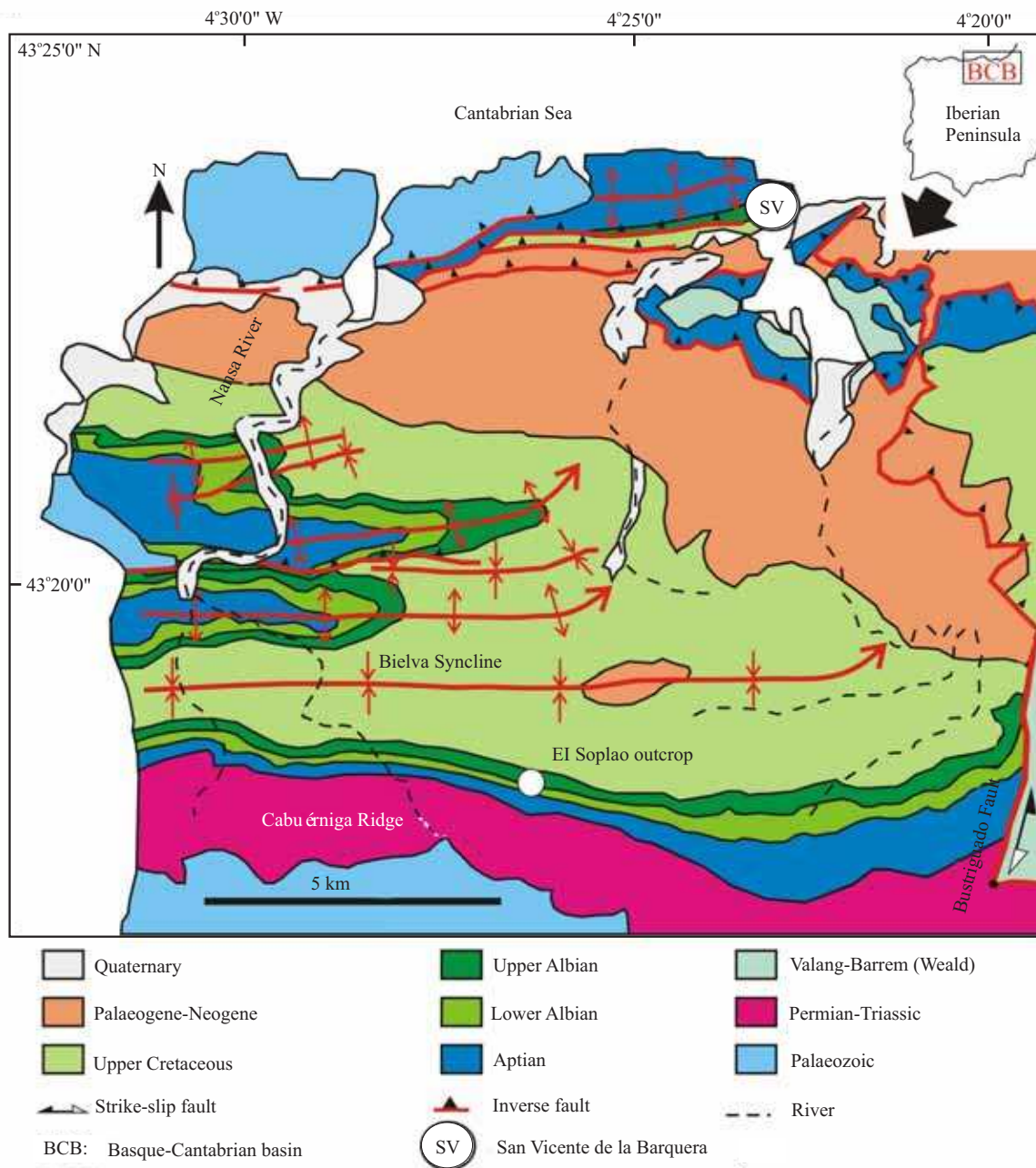
2 Geology

The El Soplao outcrop belongs to the Cretaceous succession of the north-western margin of the Basque-Cantabrian Basin. During the Cretaceous, this basin was affected by extensional tectonics, and perhaps strike-slip, associated with the opening of the North Atlantic Ocean and the Bay of Biscay (e.g. Le Pichon and Sibuet, 1971; Rat, 1988; García-Mondéjar et al., 1996; Soto et al., 2007). During the Late Jurassic–Early Cretaceous a major rift phase developed that led to the formation of several narrow sub-basin controlled by E–W, NW–SE and SW–NW trending faults, in which variable thicknesses of continental to marine sediments accumulated (García-Mondéjar et al., 1996; Soto et al., 2007). The

El Soplao area lies in the North Cantabrian sub-basin located immediately to the north of the Cabuérniga Ridge (Wilmsen, 2005), which is an E–W late-Variscan structure reactivated first as a paleo-high bounded by normal faults during the Early Cretaceous, and later as a reversal fault during widespread Tertiary (Pyrenean) compression (Fig. 1). The Lower Cretaceous (Aptian–Albian) deposits of the El Soplao area lie unconformably on lower Triassic (Buntsandstein facies) basement. They constitute a relatively thin (~300 m) sedimentary wedge weakly deformed and affected only by gently folding. The El Soplao amber outcrop is located in the southern flank of the Bielva syncline (Fig. 1), where the average strike of the succession is E–W and the dip about 40° N.

Thermal maturity of the area based on vitrinite reflectance values (% *Ro*) performed on vitrinite macerals from plant fragments have yield relatively low reflectance values that range between 0.50% and 0.61% (mean 0.56%) (Menor-Salván et al., 2010). These values suggest that the organic matter associated with the amber is only early mature, and the estimated temperatures from these indices of thermal maturation of organic matter are in the range of 60–70°C (e.g. Sweeney & Burnham, 1989), which are the burial maximum temperatures suffered by the amber deposit. These low maturity levels may be responsible for the good conservation of the molecular composition of the amber and its biological inclusions.

A synthesis of the stratigraphy of the Soplao area is represented in Fig. 2 (after Najarro et al., 2009, 2010). The amber-bearing deposit of El Soplao is included within the Las Peñasas Formation (Fig. 2), which is a Lower Albian unit (~112–110 Ma) of continental to transitional marine siliciclastic deposits interbedded in a succession of shallow marine, rudist and coral carbonate platform deposits. General sedimentary descriptions, depositional environments and fossil content of the Las Peñasas Formation have been already discussed in Najarro et al. (2009). In the El Soplao outcrop, the amber-rich beds occur in the lower-middle part of the Las Peñasas Formation. There, this unit rests disconformably above a thin (1–2 m) bed of continental red clay with root traces, deposited at the top of shallow marine limestones of the Reocín Formation (Fig. 2). The El Soplao amber deposit is characterized by dark, carbonaceous, pyritiferous shales with subordinated siltstones and sandstones laminae and cross-laminated centimetric sandstone layers, forming wavy and lenticular bedding. They contain remarkable accumulations of plant remains and amber pieces of different sizes and forms, as well as some remains and small shells of marine gastropods and bivalves. The principal amber-bearing shale bed of the El Soplao outcrop forms a lenticular body with maximum thickness of 1.5–2.0 m and a width of at least 10 m in N–S cross-section. In the strike of the bed, the amber-bearing shale extends more than 75 m. The base of this lens-shaped shale bed is erosional and truncates highly bioturbated, medium to coarse-grained sandstones with cross-bedding. At its top, the amber-bearing shale bed is overlain by heterolithic carbonaceous sandstones and muddy siltstones to sandstones with flaser bedding, which are also relatively rich in amber pieces. All of these deposits accumulated in a proximal estuarine bay system with small bayhead deltas (Najarro et al.,



2009) and represent the transgressive inundation of a continental fluvial plain and an incised valley fill. Based on geometry and facies, we interpret the amber-bearing shale of the El Soplao outcrop to have accumulated in a restricted tidal channel with low circulation and anoxic bottom-water, as suggested by widespread early pyritization.

3 Methods

3.1 Paleontological excavations

Three paleontological excavations have been carried out in El Soplao amber outcrop. During the first one (October 2008) different extraction methods were applied to obtain amber pieces. Amber was obtained manually with small tools. In addition a large prospect hole, approximately $7 \times 2.5 \times 2$ m in

size, was dug in one of the amber-rich areas of the outcrop using a bulldozer. Several tons of amber-bearing sediment from the large prospect hole were transported to a washing area located in the same outcrop, where a cement mixer and a sieve were used to obtain all ranges of amber sizes as described in Corral et al. (1999) and Alonso et al. (2000). This permitted to obtain a sampling without taphonomic biases introduced by the extraction methods towards the large and medium sizes. During the last excavation (July 2009) additional small prospect holes were dug close to the limits of the outcrop to find lateral extensions of the amber deposit. This task showed a high abundance of amber and revealed that the amber bed is at least 75 m long laterally, supporting the assertion of Najarro et al. (2009), which is that the El Soplao is the largest site of amber with arthropod inclusions that has ever been found in Spain so far.

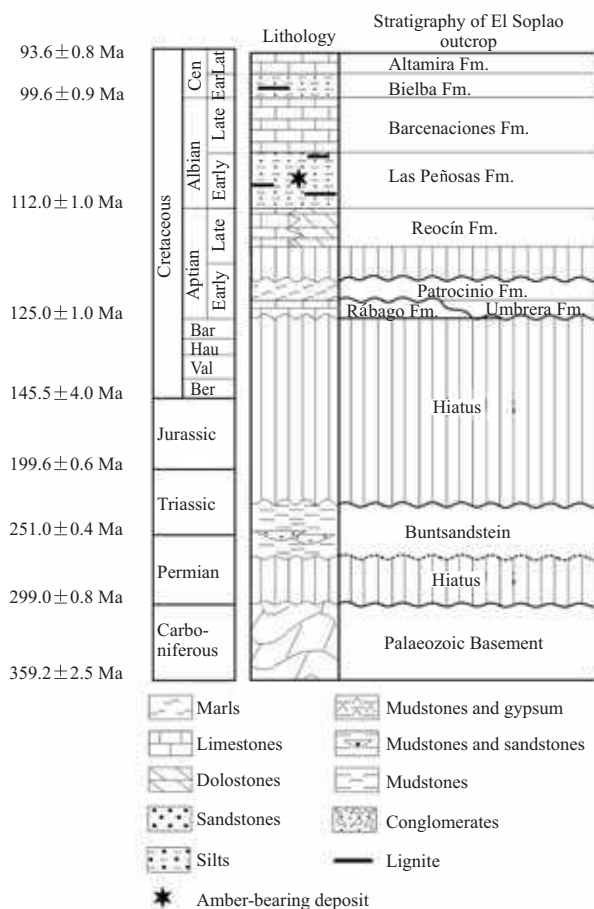


Fig. 2. Chrono- and lithostratigraphy of the El Soplao area. Modified from Hines (1985) and Najarro et al. (2010). Chronostratigraphy after Gradstein and Ogg (2009).

During the second paleontological excavation (March 2009) a new method was used to obtain abundant amber material increasing the collection of bioinclusions. The technique involves the use of high pressure water (Fig. 3.1 and 3.2) to extract entire large amber pieces that would be too fragile to resist conventional methods (Fig. 3.3). The water at high pressure disintegrated the sediment of the prospect hole and exposed the amber pieces, which were manually extracted to avoid fracturing. On the other hand, the small and medium pieces were retained using a large sieve where water and mud flowed (Fig. 3.2). The pieces from the mixture of mud, plant cuticles, coal and amber retained by the large sieve were separated in the washing area described above. This high pressure water method used for the first time to extract amber is already used in mining to fragment mineral seams, and to extract the useful parts from the waste rock. In conclusion, the new method was useful in obtaining numerous large amber pieces in a short period of time.

3.2 Biogeochemical analyses

For the biogeochemical analysis amber pieces, fossil wood, and sediments rich in plant cuticles were collected from the El

Soplao deposit during the first excavation on October 2008. Two types of amber pieces were collected: type A, characterized by a strong blue purple color under natural light (Fig. 3.3 and 3.4), purple-reddish under artificial light, and type B, less abundant, yellow under artificial light and with a bluish tinge under natural light. Plant cuticles were obtained from claystones by rinsing the plant-rich sediment in an ultrasonic bath of distilled water to remove all of the clay and silt sediment. The organic residue was air-dried. Plant fragments and leaves were distinguished and separated under a stereomicroscope. In addition, several resin and leaf samples from extant conifers of the families Cupressaceae and Araucariaceae (*Cupressus arizonica*, *Agathis australis* and *Araucaria angustifolia*) were collected from living trees at the Royal Botanic Garden of Madrid and in the kauri forests of New Zealand, in order to compare their compounds with those of the amber and fossil leaves and also to determine potential affinities of the amber with fossil taxa.

For the analytical characterization, several representative pieces of amber of the types A and B of about 50 g each, with the highest transparency available and free of major inclusions, crusts and debris, were selected from the El Soplao deposit. Following standard techniques, each piece was crushed and extracted for 4 h with dichloromethane:methanol (2:1) using a Büchi model B-811 automatic extractor. One aliquot of extract was injected directly into the injection port of the gas chromatograph. The bulk extract was concentrated to a volume of 20 mL and fractionated by use of flash chromatography on silica gel. The elution was carried out using hexane, dichloromethane, dichloromethane:methanol (1:1), and methanol, and subsequently 25 fractions were collected. Each fraction was concentrated by evaporation of the solvent under N_2 and analyzed by gas chromatography/mass spectrometry (GC/MS). The fractions with similar compositions were combined. The polar fraction (eluted with methanol) and the fractions containing ferruginol were recombined, further separated using a glass column (20 cm) filled with chromatographic-grade silica gel, and eluted sequentially with n-hexane:dichloromethane (1:1), pure dichloromethane, dichloromethane:methanol (1:1), and methanol. Four fractions were collected, designated A through D. All fractions were dried and the alcohols and acids converted to trimethylsilyl derivatives by reaction with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) at 65°C for a period of 3 h. Finally, the derived fractions were diluted with n-hexane and injected into the port of the gas chromatograph.

To study the molecular composition of fossil *Frenelopsis* and *Arctopitys* leaves (genus name *Mirovia* changed to *Arctopitys*; see Nosova and Weislo-Luraniec, 2007), 5 g of leaves were extracted for 4 h with dichloromethane:methanol (2:1) using a Büchi model B-811 automated extractor. The bulk extract was filtered and analyzed directly by gas chromatography-mass spectrometry. After, extract was fractionated using silica gel chromatography in two fractions by elution with hexane:dichloromethane (3:1) and dichloromethane:methanol (4:1). The polar fraction was dried and derived using the method described above. For comparison, the chemotaxonomy of extant Cupressaceae and Araucariaceae was

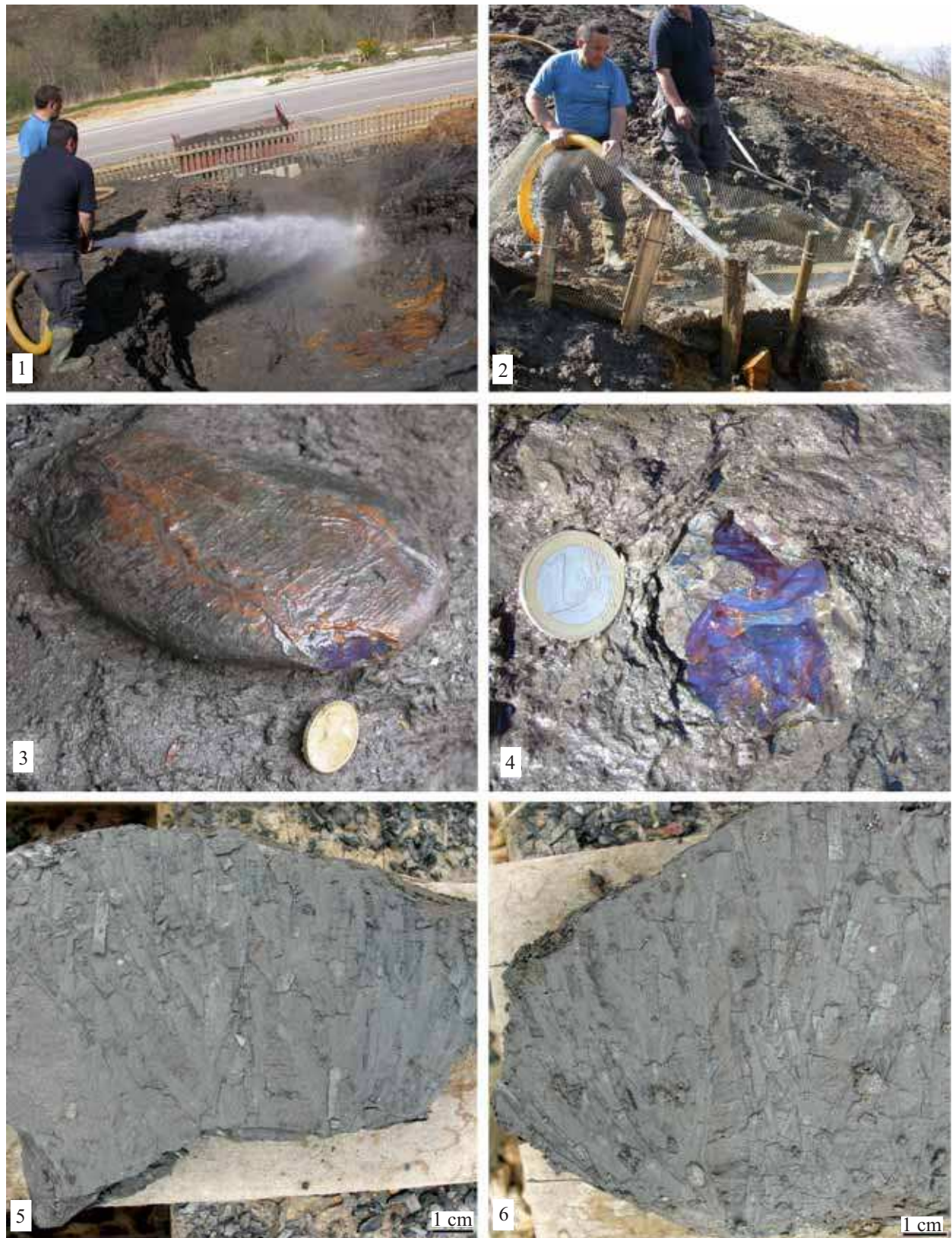


Fig. 3. Paleontological excavation in El Soplao amber outcrop (March 2009) with a new extractive method and some of the pieces obtained *in situ*.

1: High pressure water directed to the prospect hole; 2: large sieve to which water and mud flowed; 3: flattened amber piece virtually complete exposed *in situ* by high pressure water showing intensive fluorescent blue glow on its fracture (coin diameter 24 mm); 4: fragment of amber *in situ* showing intensive fluorescent blue glow (coin diameter 23 mm); 5–6: part and counterpart of four-time branched shoots of *Frenelopsis* sp. collected during the last excavation carried out (July 2009).

investigated using resin samples from *Cupressus arizonica*, *Agathis australis*, and *Araucaria angustifolia*. The resin was dissolved in dichloromethane:methanol (1:1) and fractionated by chromatography on silica gel in two fractions: the less-polar fraction being eluted with n-hexane:dichloromethane (1:4), and the second polar fraction being eluted with methanol. The polar fraction was dried under a nitrogen stream, yielding a white powder composed mainly of resin acids and highly polar compounds. The polar components were converted into trimethylsilyl derivatives by BSTFA and analyzed using GC-MS as described previously (Menor-Salván et al., 2010).

3.3 Palynological method

Two samples, Sop-Peñosas (this from the amber outcrop) and Peñosas-Cóbreces, both from Las Peñosas Formation, were prepared for palynological studies in the laboratory of ALICONTROL (Madrid, Spain). The rock samples were treated following the standard palynological preparation technique (Batten, 1999), which consists of an acid attack with HCl, HF and HNO₃ at high temperature. The residue was concentrated and sieved throughout sieves of different grid sizes (500, 250, 75, 50 and 12 µm). Then, the samples were mounted in glycerin jelly on glass slides for light microscopy. The samples were studied with an Olympus BX51 optical microscope. Both samples yielded representative and well-preserved assemblages: Sop-Peñosas yielded 488 miospores and Peñosas-Cóbreces 681 miospores.

3.4 Microscopic photography

Scanning electron micrographs of the charcoal were taken using a HITACHI model S-2500 of the University of Valencia. Optical photography used both a digital camera attached to a microscope Olympus BX51 and a digital camera Leica DFC420 attached to a stereomicroscope Leica MS5.

4 Biogeochemistry of the Amber

The overall aim of this part of the research was to identify the bioterpenoids preserved in the fossil resin and to determine their possible botanical source. Due to exceptional preservation, the amber-bearing deposit at El Soplao offers a unique opportunity to compare the molecular composition of the amber with that of the plant remains that appear in the same deposit.

The analysis of the polar terpenoids of the amber from El Soplao indicates that most likely at least two resin producers contributed to the amber record (Fig. 4). The main parent resin (type A; Fig. 4.1) originally contained phenolic abietanes (dominated by ferruginol), totarol, dehydroabietane and pimarane/isopimarane acids. The dominant resin acids found were 13-dihydroagathic and bisnordehydroabietic acids, as well as various other alteration products and minor quantities of callitrisic acid and hinokiol. The second parent resin (type B; Fig. 4.2) shares some general compounds characteristic of conifers with type A amber, but shows remarkable differences in specific biomarkers. It contained pimarane/isopimarane acids as the only identifiable biological precursors preserved and shows

absence of phenolic terpenoids (ferruginol, totarol, hinokiol) and other specific biomarkers that are present in type A amber (e.g. dehydroabietane, callitrisic acid).

The direct diagenetic products of the pimarane/abietane and labdane class terpenoids constitute the main geoterpenoids extractable from both types of El Soplao amber. The moderate degree of burial diagenesis of the studied material deduced from the low vitrinite reflectance values is consistent with the high level of preservation of the natural product diterpenoids and their direct diagenetic derivatives.

The preliminary molecular data obtained from the comparative study of the megafossil plant leaves, extant plant samples and the two amber types lead to the following results:

(a) There is absence of abietic and dehydroabietic acids in both types of amber samples. This allows us to reject a relationship between the amber and resin of Pinaceae species. Also, the absence of triterpenoids and labdatriene acids discards the contribution of angiosperms (Anderson et al., 1992; Yamamoto et al., 2006).

(b) In type A amber, the phenolic diterpenoids present in the analyzed samples and the absence of phyllocladane/kaurane type terpenoids discard the contribution of the family Araucariaceae. The presence of phenolic terpenoids (ferruginol, totarol and hinokiol) points to a relation with the extant conifer families Cupressaceae, Taxodiaceae and Podocarpaceae. However, the presence of dehydroabietane (also present in representatives of Pinaceae and Cupressaceae; Otto et al., 2007) points to a relationship with the family Cupressaceae. The presence of callitrisic acid in type A amber reinforces a biochemical relation between the parent resin of amber and modern Cupressaceae, because in modern conifer resins the synthesis of callitrisic acid seems to be restricted to certain genera of Cupressaceae (Anderson, 2006). The analysis of the molecular composition of fossil leaves from the same outcrop shows the presence of key terpenoids, such as ferruginol, in both type A amber and the analyzed *Frenelopsis* fossil leaves, suggesting that this amber could be derived from the genus *Frenelopsis* (Cheirolepidiaceae). For the type A amber, a possible diagenetic route is suggested in Fig. 5 that connects the preserved biological precursors and the major geoterpenoids found in the sample (Otto and Simoneit, 2002; Stefanova et al., 2002; Hauteville et al., 2006; Pereira et al., 2009). Morphological similarities between extinct Cheirolepidiaceae and extant Cupressaceae has been already described, but their phylogenetic relationship remains speculative, mainly due to the lack of molecular evidence (Seoane, 1998; Miller, 1999; Farjon, 2008); there are also paleobotanical data that support close affinities between Cheirolepidiaceae and Araucariaceae mainly based on female cone morphology. The chemotaxonomical affinity between type A amber and the analyzed leaves of *Frenelopsis* (Cheirolepidiaceae), and the affinity between type A amber and Cupressaceae as well, strongly suggests biochemical affinity between the extinct *Frenelopsis* and the modern representatives of the family Cupressaceae.

(c) The overall terpenoid composition of B samples (Fig. 4.2) is represented by non-specific conifer biomarkers. Absence of

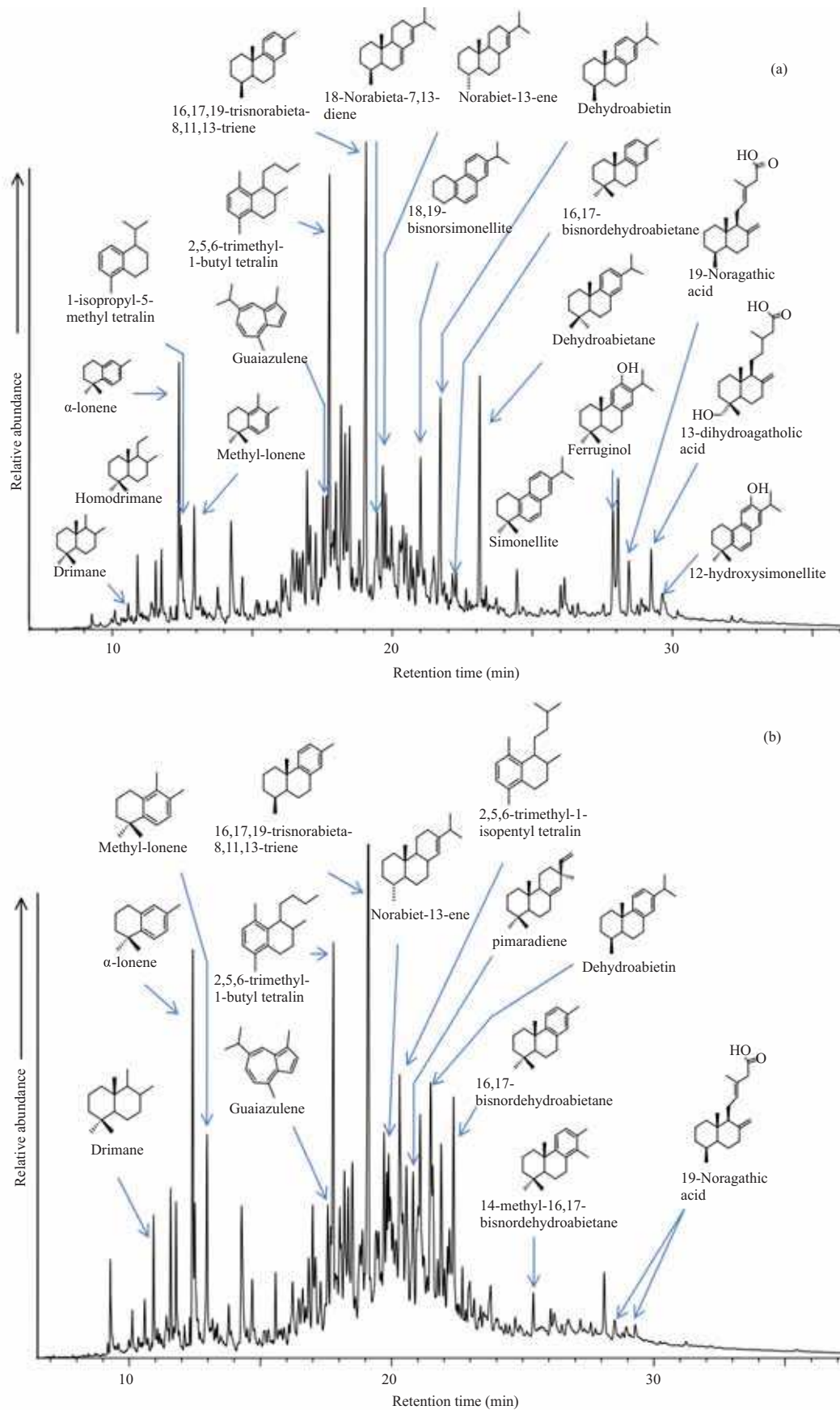


Fig. 4. Gas chromatography-mass spectrometry traces (TIC) analysis and main biomarkers identified in the two types of amber found at El Soplao deposit: type A (a) and type B (b). After Menor-Salvan et al. (2010).

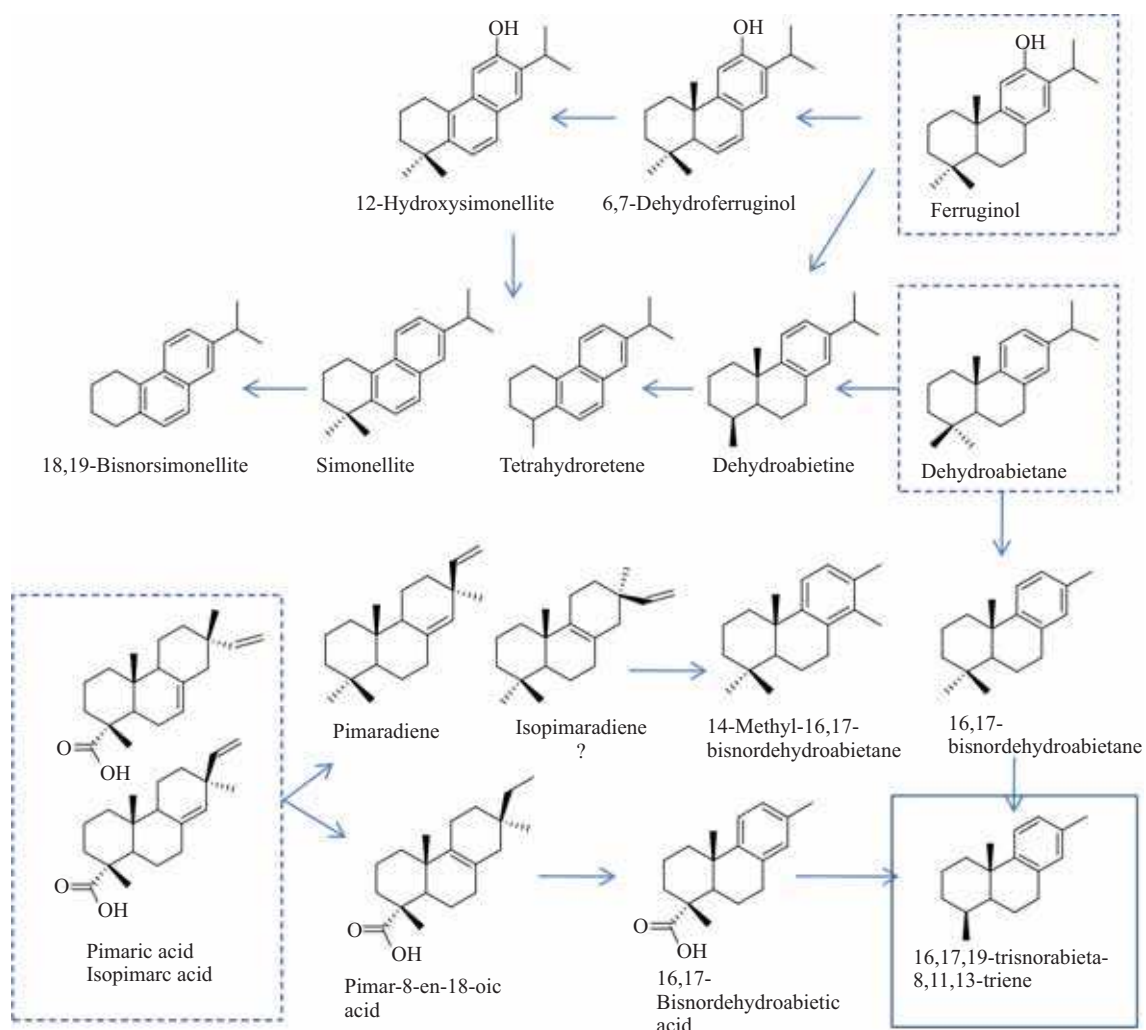


Fig. 5. Diagenetic pathways proposed for the El Soplao amber.
Dotted box: biological precursors. After Menor-Salvan et al. (2010).

phenolic terpenoids and 13-dihydroagathic acid, together with a major presence of diagenetic products of pimarane type terpenoids, saturated and unsaturated norabietanes and alkyltetralines in B samples, points to a different biological origin. Paleobotanical source for this type of amber could not be identified on the basis of the biomarker composition found so far.

Although these results are preliminary and might change when more data become available from analyses of more samples, it is estimated that they already provide a good indication of the potential plant source that originated the El Soplao amber. Future analyses could include fossil wood, leaf cuticle of *Nehvizdya*, more amber samples and conifer resin samples of other taxa.

5 Palynology

This preliminary palynological study complements that based on plant cuticles from the amber outcrop (see Najarro et al., 2009), revealing some additional aspects of the regional paleobotany. The studied samples from Las Pe˜nas Formation

have yielded 32 spore and 29 pollen types (Fig. 6; see Table 1). In addition, undetermined bad-preserved dinoflagellate cysts and lining of foraminifera scarcely occurred. The Pe˜nas-C6breces sample presents more abundant taxa (52) than the Sop-Pe˜nas sample (44). It may be related to a lower palynological richness in the second sample. Both samples present high percentages of conifer pollen grains, especially those of *Classopollis* and *Inaperturopollenites dubius*. The spores of vascular cryptogams are more diverse than pollen grains although they occur in lower amounts. The most usual spores belong to both the trilete and taeniate genus *Cicatricosisporites* and the trilete and psilate genus *Deltoidospora*. Pollen grains of ancient angiosperms occur scarcely in both samples. They are mainly represented by monosulcate and reticulate pollen grains of the genus *Clavatipollenites*.

The Pe˜nas-C6breces sample exhibits high amounts of pollen grains of *Classopollis* (~40%) and *I. dubius* (~30%). Other gymnospermous pollen grains such as *Alisporites* spp., *Araucariacites australis*, *Monosulcites chaloneri* and *Spheripollenites* sp. present lower but remarkable percentages.

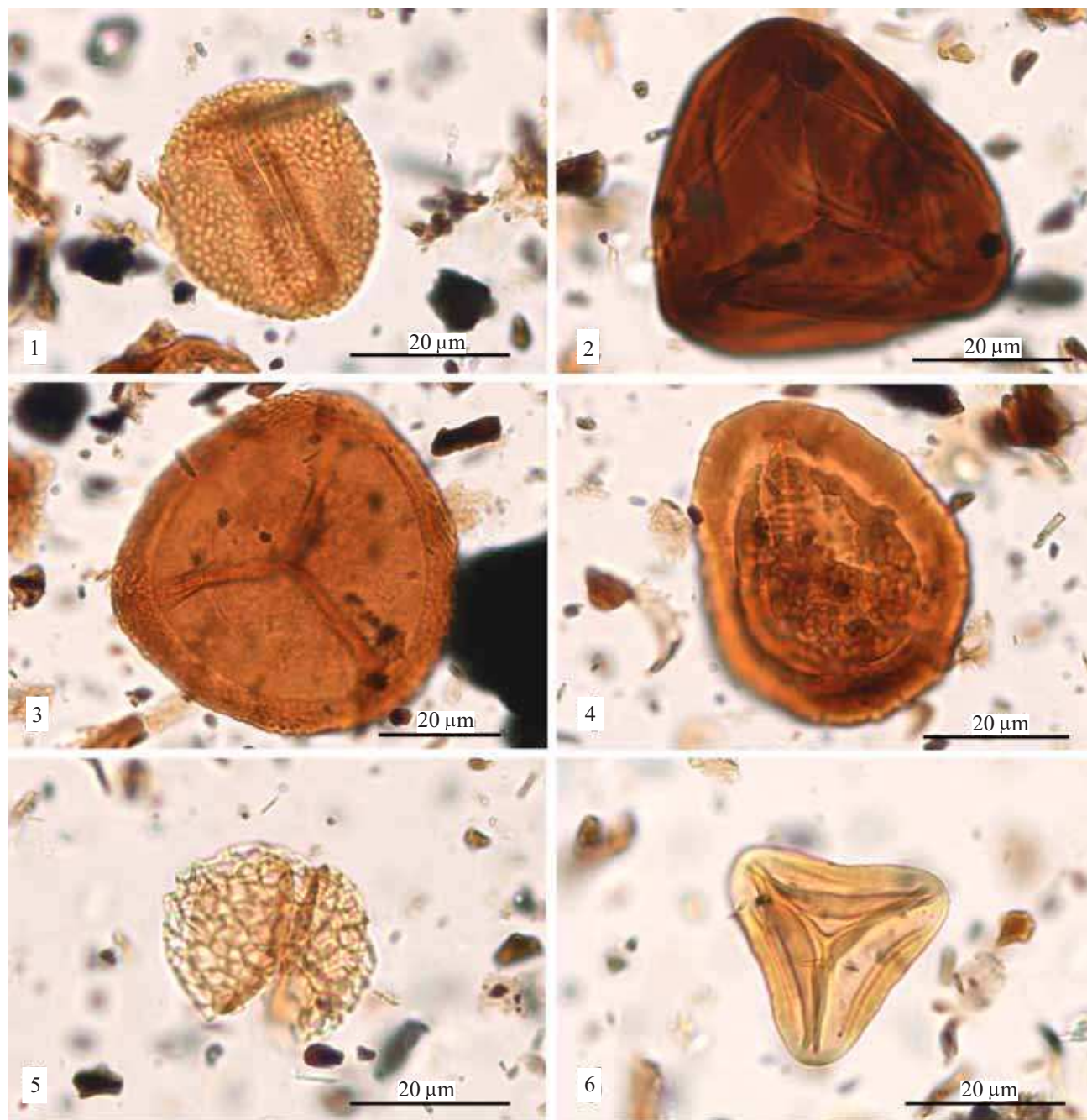


Fig. 6. Miospores from Peñasas-Cóbreces (1–5) and Sop-Peñasas (6) palynological samples.

1: *Liliacidites dividuus* (Pierce) Brenner; 2: *Cicatricosisporites patapscoensis* Brenner; 3: *Densoisporites velatus* Weyland & Krieger; 4: *Taurocosporites segmentatus* Stover; 5: *Pennipollis peroreticulatus* Brenner; 6: *Gleicheniidites senonicus* Ross.

Spores also present low amounts being *Cicatricosisporites* spp. the best represented (~2%). The occurrence of a single tricolpate pollen grain of the genus *Tricolpites* is noteworthy, because up to the Middle Albian this taxon is not abundantly represented.

The Sop-Peñasas sample corresponds to the El Soplao amber outcrop. The palynological assemblage inferred in this sample is characterized by a conspicuous increase in *I. dubius* (~51%) and a marked decrease in *Classopollis* (~11%). The percentage of spores of the genus *Deltoidospora* also increases (~8%). *Cicatricosisporites* scarcely occurs at this sample. Angiosperm

pollen grains are low represented in this sample compared to Peñasas-Cóbreces.

From a biostratigraphical point of view, the occurrence of *Appendicisporites robustus* and *Cicatricosisporites patapscoensis* (Fig. 6.2) indicates a Late Aptian–Middle Albian age. However, the occurrence of *Liliacidites dividuus* (Fig. 6.1) and the low presence of *Tricolpites* sp. indicate an Early Albian age for the Peñasas-Cóbreces sample (Doyle and Robbins, 1977).

Table 1 List of spores and pollen grains recorded from the Early Albian sediments of Las Peñas Formation

	Peñas-Cóbreces	%	Sop-Peñas	%
Spores				
<i>Appendicisporites robustus</i> Kemp 1970	1	0.147	0	0
<i>Appendicisporites dentimarginatus</i> Brenner 1963	0	0	2	0.410
<i>Appendicisporites tricornitatus</i> Weyland & Greifeld 1953	1	0.147	0	0
<i>Appendicisporites</i> spp.	2	0.290	1	0.205
<i>Baculatisporites</i> sp.	0	0	1	0.205
<i>Biretisporites potoniaei</i> Delcourt & Sprumont 1955	3	0.440	3	0.615
<i>Ceratospirites</i> sp.	1	0.147	0	0
<i>Cibotiumspora jurienensis</i> (Balme 1957) Filatoff 1975	0	0	1	0.205
<i>Cicatricosisporites apicanalis</i> Phillips & Felix 1971	1	0.147	0	0
<i>Cicatricosisporites apitereus</i> Phillips & Felix 1971	1	0.147	0	0
<i>Cicatricosisporites patapscoensis</i> Brenner 1963	1	0.147	0	0
<i>Cicatricosisporites recticicricosus</i> Döring 1965	1	0.147	0	0
<i>Cicatricosisporites venustus</i> Deák 1963	2	0.290	1	0.205
<i>Cicatricosisporites</i> spp.	13	1.908	6	1.230
<i>Cingutritetes</i> sp.	1	0.147	1	0.205
<i>Contignisporites</i> spp.	1	0.147	0	0
<i>Crybelosporites</i> sp.	0	0	1	0.205
<i>Deltoidospora australis</i> (Couper 1953) Srivastava 1975	4	0.587	13	2.664
<i>Deltoidospora minor</i> (Couper 1953) Pocock 1970	8	1.174	27	5.533
<i>Deltoidospora</i> sp.	4	0.587	8	1.640
<i>Densoisporites velatus</i> Weyland & Krieger 1953	1	0.147	1	0.205
<i>Dictyophyllidites harrisii</i> Couper 1958	2	0.290	1	0.205
<i>Echinatisporis</i> sp.	1	0.147	0	0
<i>Gleicheniidites senonicus</i> Ross 1949	0	0	4	0.819
<i>Laevigatosporites</i> sp.	0	0	7	1.434
<i>Leptolepidites</i> sp.	0	0	1	0.205
<i>Patellasporites tavadensis</i> Groot & Groot 1962	1	0.147	0	0
<i>Retitritetes</i> sp.	0	0	1	0.205
<i>Stereisporites</i> sp.	0	0	1	0.205
<i>Taurucosporites segmentatus</i> Stover 1962	1	0.147	0	0
<i>Trachysporites</i> sp.	1	0.147	0	0
<i>Triporoletes reticulatus</i> (Pocock 1962) Playford 1971	1	0.147	1	0.205
<i>Todisporites major</i> Couper 1958	1	0.147	0	0
Pollen grains (gymnosperms)				
<i>Alisporites bilateralis</i> Rouse 1959	5	0.734	8	1.640
<i>Alisporites</i> spp.	20	2.937	12	2.460
<i>Araucariacites australis</i> Cookson 1947	12	1.762	25	5.123
<i>Callialasporites dampieri</i> Dev 1961	1	0.147	0	0
<i>Cedripites</i> sp.	1	0.147	0	0
<i>Classopollis classoides</i> Pflug 1953 emend. Pocock & Jansonius 1961	213	31.277	46	9.426
<i>Classopollis</i> spp.	58	8.517	9	1.844
<i>Cycadopites</i> spp.	8	1.174	2	0.410
<i>Eucommiidites minor</i> Groot & Penny 1960	4	0.587	2	0.410
<i>Exesipollenites tumulus</i> Balme 1957	6	0.881	3	0.615
<i>Ginkgoocycadophytus nitidus</i> (Balme 1957) de Jersey 1962	1	0.147	3	0.615
<i>Inaperturopollenites dubius</i> (Potonié & Venitz 1932) Thompson & Pflug 1953	205	30.102	249	51.024
<i>Inaperturopollenites</i> spp.	21	3.084	9	1.844
<i>Monosulcites chaloneri</i> Brenner 1963	22	3.230	8	1.640
<i>Monosulcites</i> sp.	4	0.587	3	0.615
<i>Pinuspollenites</i> sp.	2	0.290	2	0.410
<i>Podocarpidites</i> sp.	0	0	2	0.410
<i>Spheripollenites</i> sp.	12	1.762	5	1.024
<i>Vitreisporites pallidus</i> (Reissinger 1950) Nilsson 1958	2	0.300	1	0.205
Undetermined bisaccate pollen grains	1	0.147	6	1.230
Pollen grains (angiosperms)				
<i>Afropollis</i> sp.	2	0.300	1	0.205
<i>Clavatipollenites hughesii</i> Couper 1958	2	0.300	3	0.615
<i>Clavatipollenites minutus</i> Brenner 1963	5	0.734	1	0.205
<i>Clavatipollenites</i> sp. (trichomosulcate)	4	0.587	0	0
<i>Clavatipollenites</i> spp.	5	0.734	4	0.819
<i>Liliacidites dividus</i> (Pierce 1961) Brenner 1963	3	0.440	0	0
<i>Pennipollis peroreticulatus</i> Brenner 1963	1	0.147	1	0.205
<i>Tricolpites</i> sp.	1	0.147	0	0
Undetermined angiospermous pollen grains	6	0.881	1	0.205
TOTAL miospores	681	100	488	100

The area could have been covered by mixed conifer forests of Cupressaceae and Cheirolepidiaceae that grew near the sea. Their understory was integrated by pteridophytes, cycads and/or Bennettitales. Ponds and swampy areas were mainly occupied by vascular cryptogams and early angiosperms, which could have aquatic habits. The predominance of *Classopollis* and the lower amount of spores observed in the Peñasas-Cóbreces sample could be related to a drier period. The more humid conditions of Sop-Peñasas are indicated by the higher percentages of *I. dubius* and *Deltoidospora* spp. as well as those of *Laevigatosporites* sp., *Alisporites* spp. and *Araucaricites australis*. Considering their composition, the assemblages are similar to those from the Upper Aptian–Lower Albian sediments of the Oliete sub-basin (Iberian Ranges) (Peyrot et al., 2007a, 2007b).

6 Plant Cuticles

Najarro et al. (2009) reported abundant plant cuticles in the amber-bearing beds of El Soplao outcrop, sometimes as levels up to 10 cm thick. This plant assemblage comprises female cones of the genus *Alvinia*, leafy axes of *Brachyphyllum*-type, *Nehvizdya* sp. (and its reproductive organs classified into the genus *Nehvizdyella*), *Pseudotorellia* sp., and mainly *Frenelopsis* and *Arctopitys* (cited as *Mirovia* in that paper, but see Nosova & Wcislo-Luraniec, 2007).

Up to four-time branched shoots of the cheirolepidiaceous conifer *Frenelopsis* have been collected during the last excavation in July 2009 (Fig. 3.5 and 3.6), due to the large area exposed in the prospect hole excavated. The shoots show lateral branches with a single branch per node, a branching pattern similar to that described and discussed by Daviero et al. (2001). These new records are of taphonomic and paleoenvironmental relevance. From the taphonomic point of view, the stiff, articulated leafy internodes of *Frenelopsis* were probably very brittle and fragmented when transport occurred (Gomez et al., 2001, 2002; Riera et al., 2010). At least for some of the plants of the insect-bearing amber assemblage of El Soplao, such an organization suggests a parautochthonous deposition. The representatives of *Frenelopsis* occupied habitats from freshwater wetlands to saline, coastal or estuarine marshes (e.g. Gomez et al., 2001, 2002; Mendes et al., 2010). Such a wide range of habitats suggests possible mangrove-like ecology for the *Frenelopsis* of El Soplao. This paleoecological inference is also supported by the sedimentological context and the occurrence of marine or brackish-water invertebrates in the sediment and on amber surfaces.

7 Charcoal and Charcoalified Plant Fibers

The first record of charcoalified (=fusainized) plant remains indicating paleofires is from the Silurian from Ludford Lane in the Welsh Borders in England (Glasspool et al., 2004). Scott (2000) provided a general view of the Pre-Quaternary history of fire based on charcoalified plant remains, which are especially important in sediments from the latest Jurassic and Early Cretaceous. Charcoalified plant remains are moderately common

in a wide variety of facies, and some notable concentrations of considerable paleobotanical and paleoecological value can occur locally (Nichols et al., 2000).

In Spanish outcrops, amber and charcoal are very scarce except for a few levels in which both appear abundantly. This is the case of all the main outcrops for which this aspect has been explored: Peñacerrada (Suárez-Ruiz, 2003 and López del Valle, per. obs., 2008), La Hoya, San Just (Peñalver et al., 2007, 2008) and El Soplao. In these outcrops there are abundant cuboidal pieces of charcoal (=fusinite) of a few centimeters long (Fig. 7.2), and they are easily recognized with the naked eye even in the field by its silky luster, brittleness and friability. In addition, charcoal pieces are fibrous, black and opaque.

Features of the charcoal associated with amber in El Soplao outcrop using SEM are the undeformed structure and fabric of the tissue with open cell lumina, pits and homogenization of the cell walls (Fig. 7.3–7.5); see Sander and Gee (1990) and Scott (2000, 2010) for general description of charcoal. All of these features revealed by SEM examination are particularly characteristic of a pyrolysis origin. The most important feature is that different layers of the cell wall and adjacent cell walls cannot be distinguished from one to another due to the homogenization during burning. This homogenization of the wall takes place above 300°C and the resulting charcoal is highly resistant to microbiological or chemical degradation during sedimentation and diagenesis (Cope and Chaloner, 1980). In San Just and El Soplao outcrops charcoal pieces with the cell lumina diagenetically filled with framboidal pyrite are found frequently (Fig. 7.6).

The levels of concentration of amber and charcoal in Spanish outcrops, including El Soplao, reveal paleofires in the resinous forests. Grimaldi et al. (2000) reported wood, insect remains and flowers, all charcoalified, and fire-damaged amber from the Upper Cretaceous (Turonian) of New Jersey, and Jarzembowski et al. (2008) reported a single piece of amber with similar features from the Lower Cretaceous of the Isle of Wight. Brasier et al. (2009) reported abundant examples of amber associated with charcoalified wood from the Early Cretaceous (140 Ma) amber deposit in Hastings (Sussex).

In addition to the frequent presence of charcoal associated with the amber in the same beds, a few amber pieces from El Soplao contain charcoalified plant fibers that appear dispersed inside the amber (Fig. 7.1). It is the first time in the fossil record that charcoalified fibers as bioinclusions have been reported. These fibers are small (around 0.7 mm long) and can be recognized as charcoalified fibers due to both their opaque black color and silky luster. This appearance is not due to the fossil diagenetic process because they contrast to other plant fibers in the same amber pieces that have the common translucent clear brown color without silky luster. These small charcoalified plant fibers became included in resin during paleofires moved by convective currents or, after paleofires, transported by the wind to the exposed resin from the soil of burned areas. Scott et al. (2000) reported how abundant finer charcoal material was transported by wind from a charred area of the Frensham Common Country Park (England) several days

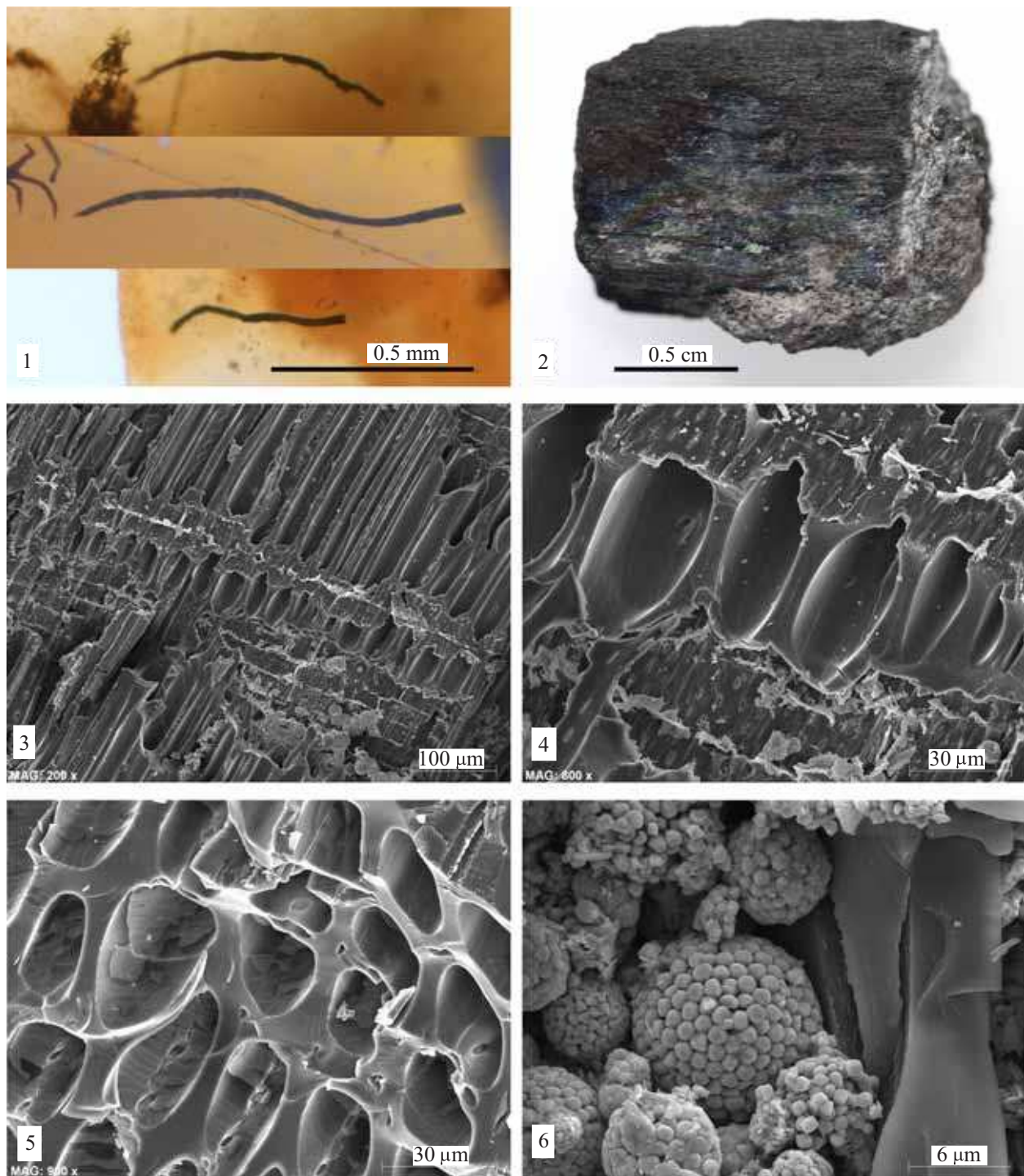


Fig. 7. Fossil carbonized woody tissues (charcoals) from El Soplao outcrop.

1: Three charcoaled plant fibers from the same small amber piece containing numerous of these remains (at the same scale); 2: cuboidal piece of charcoal; 3–5: scanning electron micrographs of charcoals show both pits and cell-wall homogenization in epidermis and peripheral tissues (micrograph 4 is a detail of 3); 6: framboidal pyrite partially filling the cell lumina (micrographs 3–6 are from microsamples of the piece figured in picture 2).

after a wildfire.

The kauri forests (Araucariaceae) of New Zealand most likely are the best extant correlate to the Cretaceous resiniferous forests, except, perhaps, for the fact that the kauri forests do not have a fire ecology (Daniel J. Bickel, pers. comm., 2010). As it

has been observed by us in the kauri forests, very large amounts of organic material occur due to the floor accumulation of litter, including resin pieces; Silvester and Orchard (1999) indicated that litter around a large kauri tree may reach 2 m or more in depth, with a mean residence time of 9–78 years due to a slow

decomposition rate. Although resin burns easily, forest fires only affect the litter superficially, except for ground fires (Scott, 2000). Thus the main part of the accumulated resin usually remains intact. Scott et al. (2000) reported a surface fire in the Frensham Common Country Park that charred only a few millimeters of the organic litter, so the pine crown was practically not affected by the fire. As indicated by Grimaldi et al. (2000) and Martínez-Delclòs et al. (2004), forest fires traumatically induced copious production of resin and they might have been an important factor in the genesis of amber deposits. From this respect, Brasier et al. (2009) reported charcoaled conifer wood from Hastings deposit with cell lumina filled with resin after the paleofire. However, we consider that the joined occurrences of large accumulations of amber and charcoal in Spanish outcrops, and abundant plant cuticles as well, were mainly the consequence of an intensive erosion of the litter in burnt forests, possibly including mass wasting; at this respect, Scott & Stea (2002) reported evidences of post-fire soil erosion in Cretaceous charcoal horizons of Nova Scotia. As summarized by Nichols et al. (2000) from different sources, the removal of vegetation by modern fires can increase erosion rates by weathering and during rainstorms by up to 30 times compared with the sink of pre-fire levels, and single wildfire events may be recognizable as responsible for individual depositional units, for example on alluvial fans.

Nichols et al. (2000) conducted actualistic experiments and concluded that charcoal is an unusual sedimentary material because most fresh material floats, but after prolonged immersion becomes waterlogged and sinks, mainly the small pieces. After the study of an area of heathland in SW England burned by an uncontrolled fire, Blackford (2000) concluded that large particles are not transported long distances and thus are indicative of local fires. The great abundances of centimeter-sized charcoal in amber-bearing beds of San Just and El Soplao outcrops suggest that paleofires in these cases were local or occurred close to the area of deposition. In the scenario we propose, intensive erosion by rivers and storm floods of a burned resinous forest area located close to the deltaic environments of deposition was promoted by the loss of vegetation, and resin plus charcoaled wood were transported together through water to coastal environments.

Another complementary factor that could contribute to the great amber accumulation in El Soplao outcrop was proposed by Najarro et al. (2009) based on sedimentological data: floods during rainstorms eroded and removed the resin and plant remains from the soils of coast-fringing forests. Pieces of resin and wood mixed with mud and sand then were transported by density flows to the coastal and interdistributary bays. Surely, more than one of these three factors might have occurred.

We can assume that wildfires had a great impact on environments and most likely they occurred mainly during the warm, drought season; lightning strike is the most important reported cause of naturally ignited fires today (Cope and Chaloner, 1980; Scott, 2010) and it was probably the same during the Cretaceous. Moreover, levels of atmospheric oxygen during the Cretaceous were among the highest during the

Phanerozoic, close to 30% (vs. 20% today), leading to a much higher prevalence of fires (Robinson, 1989). Secondary evidence of the environmental impacts of wildfires can be obtained from plant and insect fossil records. The tree fern *Weichselia reticulata* has been recorded in some adjacent beds to the Spanish amber outcrops and this taxon can be related to environments disturbed by both floods and fires according to Coiffard et al. (2007) and Scott et al. (2000). More recently Ortega-Blanco et al. (2008) described a new species of anaxyelid woodwasp, *Eosyntexis parva*, from Peñacerrada I amber closely related to the extant anaxyelid taxa that lay eggs in burnt conifer trees shortly after the wildfires.

8 Marine or Brackish-water Invertebrates on Amber Surfaces

El Soplao amber deposit in some levels contains internal marcasite moulds of marine or brackish-water mollusks, and oyster shells that preserve the original calcium carbonate biomineralization. In addition, for the first time in the fossil record we report findings of large amber pieces that show their surfaces colonized by both serpulid worm tubes (Annelida: Polychaeta) (Fig. 8.1 and 8.2) and encrusting bryozoan colonies (Bryozoa: Cheilostomata) (Fig. 8.3); barnacles encrusting Baltic amber surfaces have been reported, but they are recent barnacles (see Grimaldi, 1996). Amber pieces with their entire surface colonized, or only with one side colonized, have been found.

Serpulid worm tubes exhibit four modes of fossilization that sometimes occur in the same amber piece, but in different parts: (1) as limonitic impressions of the tubes (Fig. 8.1); (2) as internal marcasite moulds (Fig. 8.2); (3) as amber tubes (internal fillings with resin during first fossilization phases); and (4) as original carbonate remains (Fig. 8.3). Serpulid tube remains are several millimeters long and internal tube fillings are approximately 0.35 mm diameter.

Bryozoan colonies left marks of the zooid exoskeletons on the amber surface and when original calcium carbonate remains are preserved (Fig. 8.3) they easily fade away during the washing of the pieces. Bryozoan colonies are multiseriate and belong to the Order Cheilostomata; that group produces mineralized exoskeletons and form single-layered sheets that encrust over surfaces. Box-shaped, rectangular zooids are 0.34×0.29 mm in size.

Completeness of these large pieces suggests that they were constituted by polymerized resin when serpulids and bryozoans developed, not fossil resin from eroded strata (reworking process). Resin is comparatively less fragile than amber or copal and, in consequence, more commonly keeps its integrity during marine transport. In any case, this deposit contained a mixture of resin pieces from the litter, and fresh resin pieces directly transported from the trees, together with reworked resin pieces that remained a certain time in saline water. The presence of encrusting bryozoan colonies on the amber surfaces is not surprising because this type of organism is better adapted to shallow, high energy environments. In conclusion, this exceptional record indicates a littoral to coastal marsh

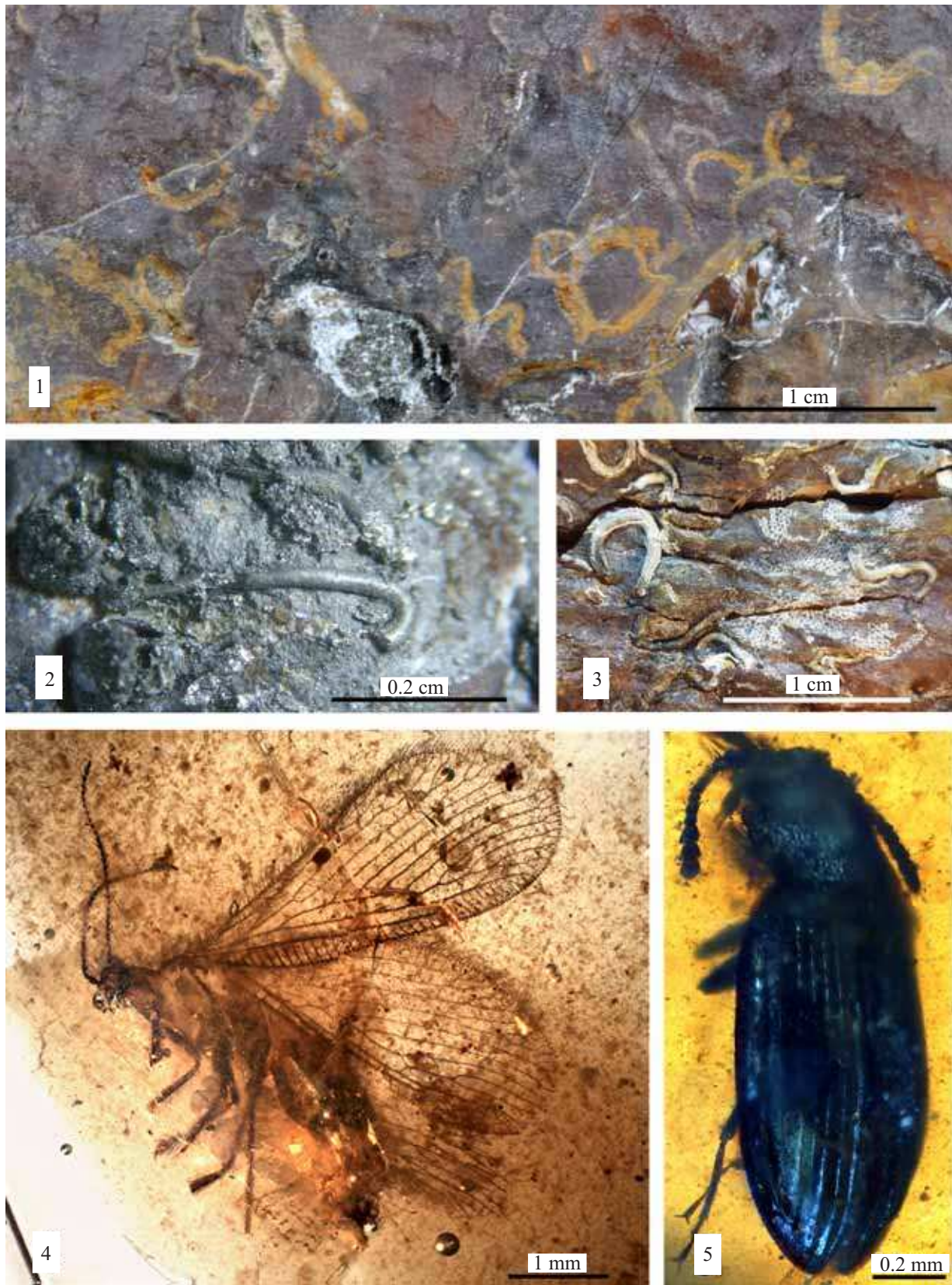


Fig. 8. Marine or brackish-water invertebrates on the surfaces of El Soplao amber and insects as bioinclusions.

1: Detail of the serpulids preserved as limonitic impressions on the entire kidney-shaped amber piece $12 \times 10 \times 5$ cm in size; 2: two serpulid worm tubes preserved as marcasite internal mould of the same amber piece; 3: bryozoan colony and several serpulids preserved as carbonate fossils on the surface of a large amber piece; 4: virtually complete specimen of the family Berothidae (Neuroptera); 5: specimen of Cucujoidea (Coleoptera). Photos 4 and 5 were made with integrated consecutive pictures taken at successive focal planes.

environment of deposition for El Soplao outcrop.

9 New Data on Insect Bioinclusions

Najarro et al. (2009) reported a high abundance of arthropod specimens embedded in the Early Cretaceous amber of El Soplao outcrop, and explained it as a consequence of the unusual concentration of amber pieces indicative of resin flows (drops, crusts and runnels) in this deposit. A significant percentage of amber pieces of this type contains numerous bioinclusions because the original resin was exposed to the atmosphere, was less viscous (it was more easily penetrated by insects), and successive flows encapsulated the trapped insects (Martínez-Delclòs et al., 2004).

Recent paleontological excavations have provided new bioinclusions of significant paleoecological and taxonomical value. To date, El Soplao amber has provided more than 200 bioinclusions, including fungi, plants, and diverse arthropods. Among them, only systematic studies on insects have been already started. The insect inclusions found belong to 11 recognized orders: Blattaria, Isoptera, Psocoptera, Thysanoptera, Raphidioptera, Neuroptera, Hemiptera, Coleoptera, Trichoptera, Hymenoptera, and Diptera; last two groups are the most abundant.

Neuropterid fauna from El Soplao amber is currently being studied. It is composed so far of Neuroptera and Raphidioptera, including the new genus and species of mesoraphidiid described by Pérez-de la Fuente et al. (2010). Neuropterans are represented by two specimens of dustywings (Coniopterygidae) and two beaded lacewings (Berothidae), one of them very complete and interpreted as a new morphotype (Fig. 8.4). Surely, the most outstanding finding is a tentative green lacewing larva (Chrysopidae), which, if confirmed, would be the oldest representative of the family in amber and one of the very few fossil chrysopid larvae ever reported. Its morphology, to be described and discussed elsewhere, is similar to those of the extant trash-carrying chrysopid larvae.

Coleopterans from El Soplao amber are represented only by members of the suborder Polyphaga, and all of them are up to now interpreted as herbivorous or saproxylic forms, most probably living under the bark of the trees. As in other Lower Cretaceous outcrops of surrounding areas in Spain and France, the most abundant forms of beetles from El Soplao belong to superfamilies Elateroidea and Cucujoidea (Fig. 8.5), but there are also present forms of Curculionoidea (?Atelabidae, ?Nemonychidae) and possibly a member of Dascilloidea. The presence of two specimens of Curculionoidea in such small collection of beetles (approximately 10 specimens) is of paleoecological relevance, because this group of beetles in extant ecosystems is closely related to hard parts of trees, in which larval stages developed, and the action damaging trees has also been pointed to as one of the possible causes for the increase in resin-production in trees during the Early Cretaceous and its subsequent record as fossil resins.

Although the hymenopteran assemblage of El Soplao is scarce, it is providing interesting data. It is composed of

parasitoid wasps of several superfamilies as Ceraphronoidea (parasitoids of numerous taxa, hyperparasitoids sometimes), Platygastroidea (insect and spider egg parasitoids), Evanioidea (cockroach egg consumers during larval stage), Ichneumonoidea (commonly hyperparasitoids) and Mymarommatoidea (tiny egg parasitoids). Although ceraphronoids are currently under study, a first approximation allows us to recognize members of a recently described new family of Ceraphronoidea (Ortega-Blanco et al., 2010b). In Spanish amber, Ceraphronoids were only detected in the Peñacerrada amber until date; venation and some antennae aspects of the El Soplao morphotype indicate that it belongs to the same genus that is present in Peñacerrada amber. Platygastroids are the most common hymenopteran insects in El Soplao amber, as in all other Spanish ambers, and the study of this group is in progress. Apparently platygastroids belong to a new species with large, 14-articled antennae. A new parasitoid wasp species of the genus *Archaeromma* (Mymarommatoidea) has been recorded in El Soplao, as it was in Peñacerrada I. Recording the same insect species supports the hypothesis that the age of these ambers was equivalent or at least very close, and suggests that both outcrops had similar paleoecological characteristics.

Dipterans from El Soplao amber are represented by Brachycera and Nematocera forms. One specimen of *Litoleptis* sp. (Spaniidae) has been identified within the Brachycera, similar to one specimen described from San Just amber (Arillo et al., 2009). Other dipteran families present include Hybotidae (Brachycera), as well as Cecidomyiidae and Psychodoidea. The current paleodiversity of biting midges (Ceratopogonidae) has been evaluated, leading to the discovery of five species, including three new species (Najarro et al., 2009). The new species belong to the genera *Archiaustroconops*, Szadziewski, 1996, *Atriculicoides* Remm, 1976, and *Lebanoculicoides* Szadziewski, 1996. All these ancient biting midges would have shown a hematophagous diet which is considered plesiotypic within the family (Borkent, 1995).

10 Conclusions

We present herein data that suggest that amber pieces from El Soplao have at least two botanical sources. Part of these data strongly supports a source related to Cheirolepidiaceae and also suggests a molecular relationship of *Frenelopsis* with extant Cupressaceae. This relationship is based on the chemotaxonomic comparison of biological diterpenes found in amber and plant megafossils and extant Cupressaceae.

The taphonomic significance of wildfires in the origin of the Cretaceous amber accumulations has received little attention and has been clearly underestimated. The potential presence of charcoal, fire-damaged amber pieces and charcoalified plant fibers embedded inside the amber must be considered when studying the origin of the Cretaceous amber deposits. In fact, the occurrence of large accumulations of amber and charcoal in Spanish outcrops were consequence of intensive erosion of the litter in burnt forests, possibly including mass wasting. This scenario has been well documented in the case of the El Soplao

deposit, but similar studies on other Spanish amber deposits should be conducted.

The new data on the biological inclusions from El Soplao amber show a highly diversified entomofauna, mainly represented by dipterans and hymenopterans, with the presence of very specialized groups such as chrysopids or weevils, which may help to better understand the early evolution of some insect groups.

The data previously published and new data herein provided suggest a taphonomic history for the El Soplao amber that may have run approximately as follows: resin was exuded by conifers (perhaps by two different conifers, including Cheirolepidiaceae) closely to the deltaic environments of deposition. Conifer forests were well-present in the region, with understory integrated by pteridophytes, cycads and/or Bennettitales; ponds and swampy areas were mainly occupied by vascular cryptogams and early angiosperms, which could have aquatic habits. A diversified insect fauna, mainly represented by hymenopterans and dipterans, developed around the conifer trees and was abundantly embedded in resin. Coleopterans, which probably lived under the bark or in close contact with the wood of the conifer trees, were abundantly embedded as well. Wildfires in the resinous forests promoted both the resin production and an intensive erosion of the partially burned litter. Resin, fresh leaves, wood and charcoal were shortly transported together through water and accumulated alongside marine or brackish-water mollusks in a restricted tidal channel with low circulation and anoxic bottom-water. The conifer *Frenelopsis*, a potential source of resin as suggested by the biogeochemical analyses, grew close to the deposition area, where articulated branched shoots accumulated (parautochthonous deposition). Resin pieces differed in their biostratigraphic histories because some of them remained a certain time in shallow, high energy saline waters where serpulids and bryozoans grew on the resin surfaces, resulting in a mixed assemblage. Low circulation and anoxic bottom-water promoted an early pyritization of the marine mollusks, charcoal and amber surfaces, including the fixed serpulid tubes. The assemblage was lastly buried by siltstones and sandstones. The maximum temperatures suffered by the amber deposit during diagenesis were in the range of 60–70°C. The inferred low maturity levels were maybe responsible for the good preservation of amber molecular composition and its biological inclusions.

Acknowledgements

This work is part of the Ph.D. Thesis of three of the authors (M.N. -geology-, R.P.F. and J.O.B. -paleobiology-), which are supported by a scholarship from the Instituto Geológico y Minero de España (IGME), an APIF grant of the University of Barcelona, and a FPI grant from the Spanish Ministry of Science and Technology, respectively. This study is a contribution of the IGME Project 491-CANOA 35015 “Investigación científica y técnica de la Cueva de El Soplao y su entorno geológico”, the projects CGL2008-/01237BTE from the MICINN, CGL2008-00550/BTE: “Amber of the Cretaceous of Spain: A multidisciplinary study”, and the ANR Project AMBRACE

BLAN07-1-184190. The contribution is framed in a collaborative agreement among the Cantabrian Government (Regional Cultural, Tourism and Sports Ministry), IGME and SIEC S.A. We thank Gonzalo Nieto and the staff of the Royal Botanic Garden of Madrid for permission and assistance in the sampling of extant conifers. We also express our thanks to Francisco Javier López Marcano (Regional Minister of the Cantabrian Government), José Pedro Calvo Sorando (IGME) and Fermin Unzué (manager of the El Soplao Cave) for their efforts and promotion of the study of the outcrop. We are grateful to Dr Daniel J. Bickel (Australian Museum, Sydney), Dr Vladimir Blagoderov (Natural History Museum, London) and an anonymous reviewer for careful reviews of the manuscript. Thanks are due to all people that participated in the paleontological excavations.

Manuscript received Jan. 4, 2010

accepted April 7, 2010

edited by Fei Hongcai

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