

Supplementary Information (SI)

Cretaceous amber of Ecuador unveils new insights into South America's Gondwanan forests

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Supplementary Data 1. Early Cretaceous amber in Gondwana

Except for the Early Cretaceous amber deposits in Lebanon, Jordan, and Congo that contain bioinclusions, few amber occurrences lacking bioinclusions have been recorded from regions that was once Gondwana¹. Early Cretaceous Gondwanan amber sources include deposits in Brazil at Alter do Chão Formation, in the Amazonas Basin (Albian)², at Ipubi Formation, in the Araripe Basin (Aptian)³, at Crato Formation in the Araripe Basin (Aptian)⁴, and at Maracangalha Formation in the Recôncavo Basin (Berriasian–Hauterivian)⁵. Additionally, amber has also been found in the Kirkwood Formation, in the Eastern Cape, South Africa (middle–upper Valanginian)⁶, in the Pine Island from West Antarctica, at the Amundsen Sea Embayment (mid-Cretaceous)⁷, in Iran at Persian Gulf (Albian)⁸, and in Saudi Arabia, at the Khafji Oil Field at the Eastern Province (Albian)⁹. However, these cited deposits have only yielded anecdotal record of amber and have consistently lacked terrestrial organismal remains (bioinclusions).

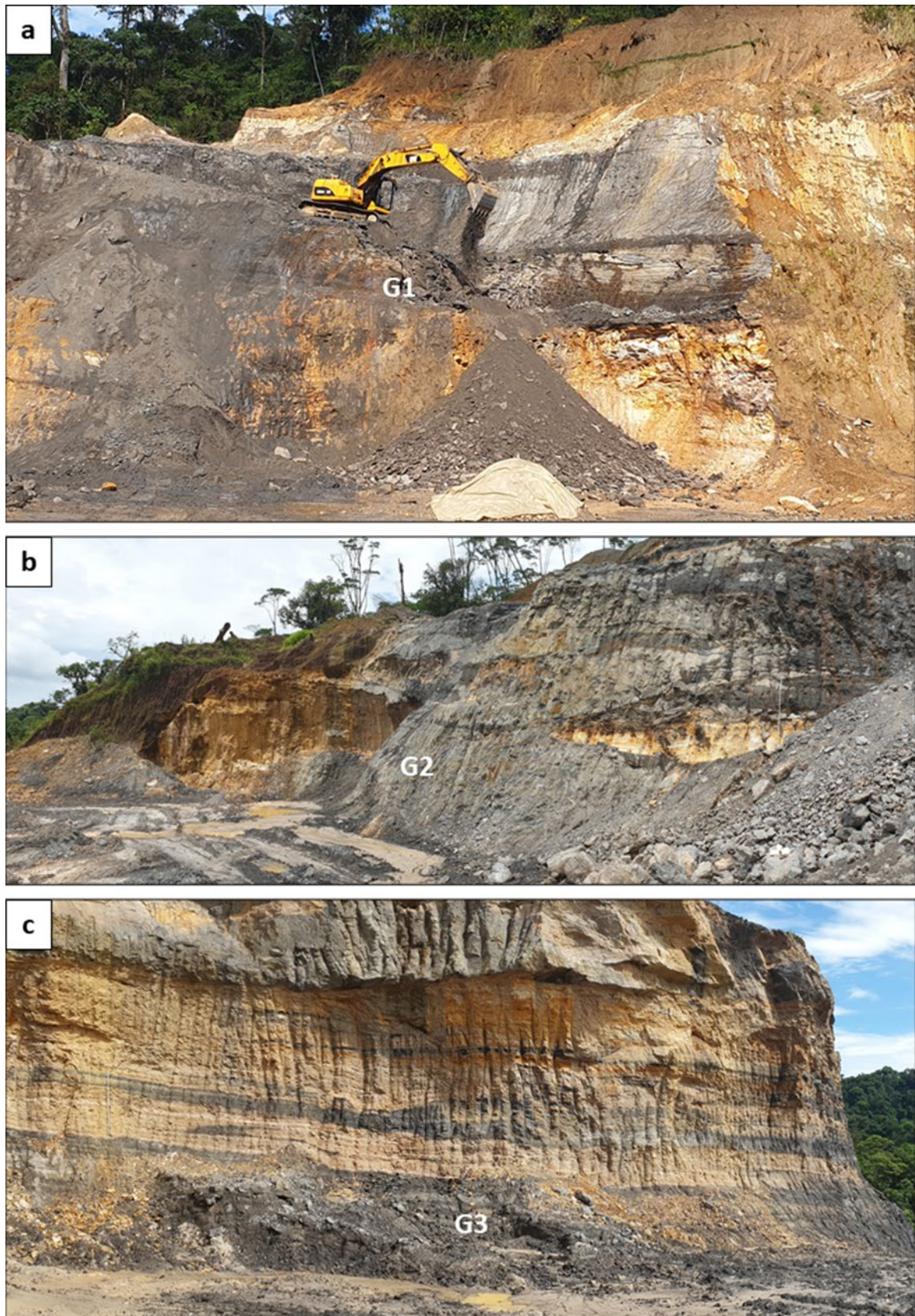
Supplementary Data 2. Geological setting

The Cretaceous Genoveva amber is found in the Oriente Basin, located in the eastern part of the Ecuadorian Andes (Fig. 1). It represents the transarc foreland basin of the Andes, spanning from Colombia to Argentina, north of the convergence between the Central and Northern Andes^{10,11}. The geodynamics of the region is directly conditioned by the subduction of the Nazca Plate beneath the South American continent. Its deformation and structuring result from the tectonic inversion of ancient normal faults associated with a rift system dating back to the Triassic–Lower Jurassic period. These faults, currently reverse and steeply dipping, are mainly oriented N–S or NNE–SSO¹¹.

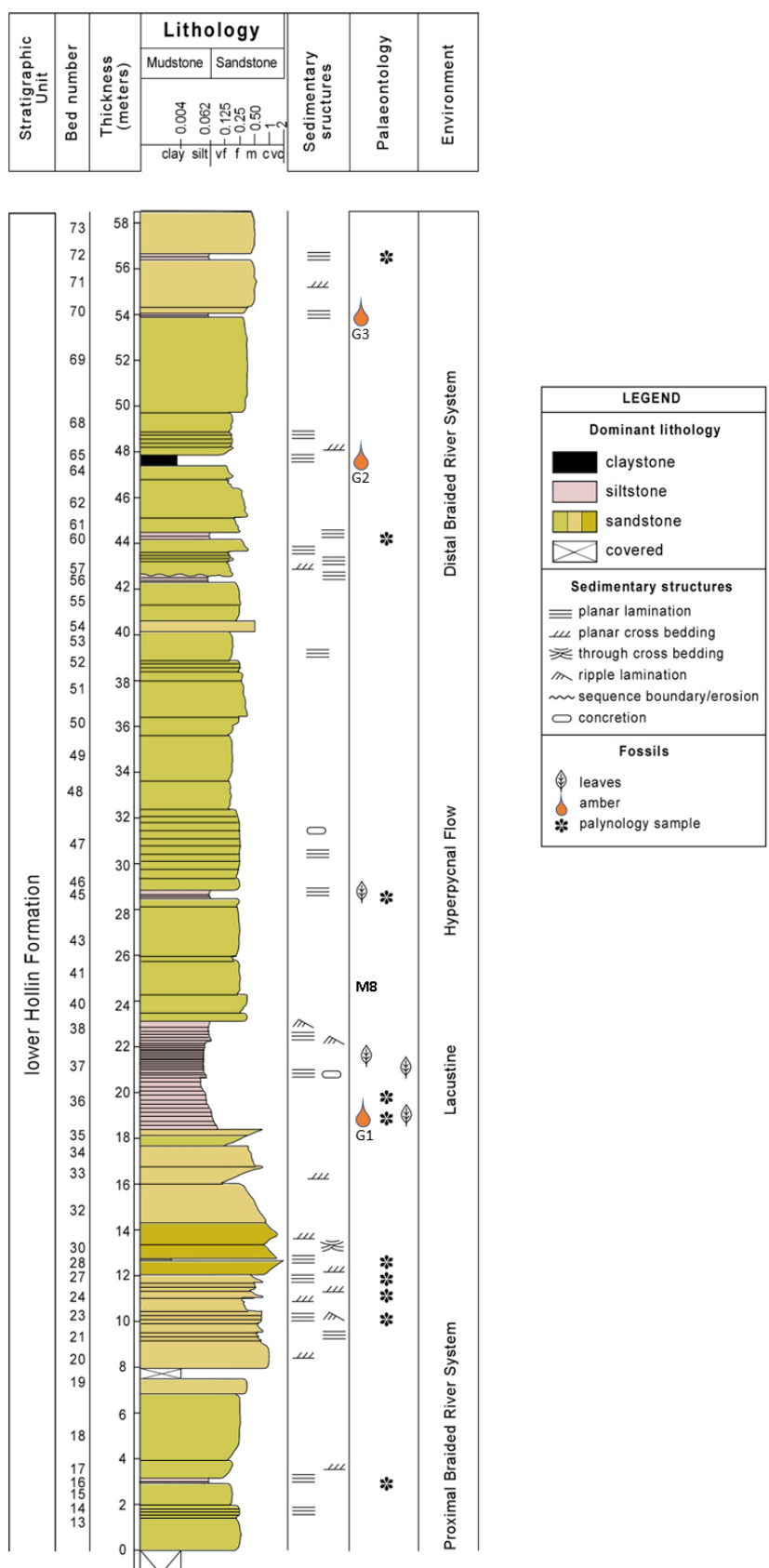
The amber-bearing deposit are located in the Hollín Formation with thicknesses ranging from 30 to 150 m¹⁴. The Hollín Formation age ranges from middle–late Aptian to middle Albian^{15–19} while other authors²⁰ argue an early Aptian–middle Albian age. This formation has been subdivided into two members (Fig. 1b): the lower Member or Hollín Principal (Main Hollín Sandstone)⁴, and the upper Member or Hollín Superior¹⁶, the latter being notably thinner compared to the former. The Hollín Principal Member was deposited unconformably above a pre-Aptian succession. It is characterised by conglomeratic sandstones graded to quartz sandstones with cross-bedding deposited in braided river channels^{15,19}. The Hollín Superior Member is characterised by sandstones intercalated with mudstones rich in organic matter and leaf fragments accumulated in tidally influenced floodplains, featuring swamps in supratidal zones, clay and mixed plains in intertidal zones, and tidal bars and dunes in subtidal zones^{19,21} (Supplementary Fig. 3). The upper boundary of Hollín Formation with the overlying Napo Formation corresponds to a major flooding surface^{19,22}.



Supplementary Fig. 1. Quarries where amber was found in the Napo region in 2022. a Genoveva quarry (-0.71019° / -77.7875°), G1–G3 main amber-bearing levels. **b** Pungarayacu quarry (-0.7065° / -77.7436°). **c** Río Misahuallí quarry (-1.0007° / -77.6735°).



Supplementary Fig. 2. Detail of the three main amber-bearing levels in the Genoveva quarry in 2022. a Stratigraphic level G1 deposited in a lacustrine environment. **b** Stratigraphic level G2 deposited in a distal braided river system. **c** Stratigraphic level G3, located at the top of the quarry, was deposited in the same environment as G2.

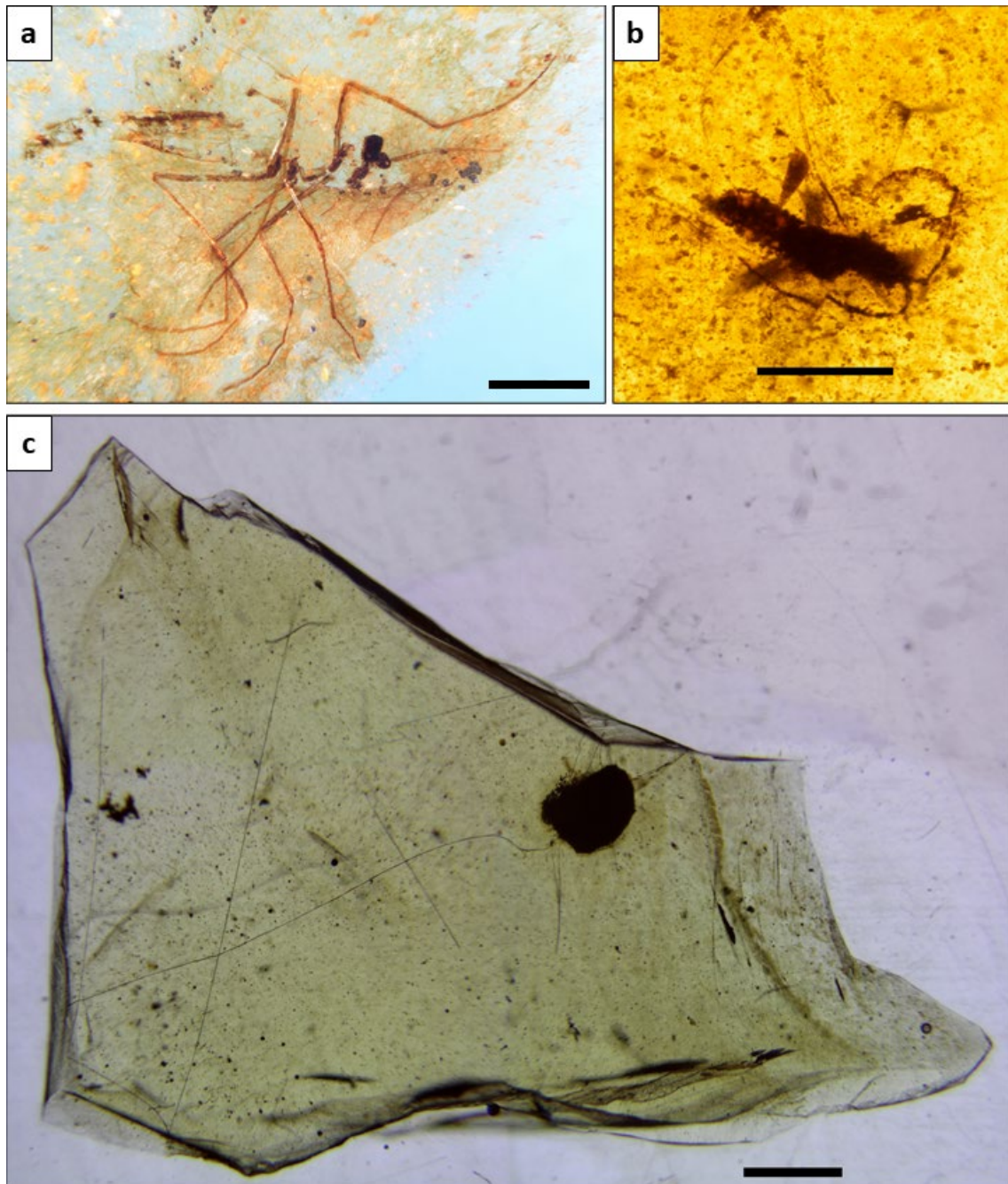


Supplementary Fig. 3. Stratigraphic log of the Genoveva quarry. The levels rich in amber (G1–G3), the 10 sampling points for palynology, the level where sample M8 is obtained for geochemistry analysis, and the levels with plant remains are indicated. Modified from ref. 12. See ref. 13 for more information about sedimentary facies and environment interpretation.

Supplementary Data 3. Arthropod inclusions

Diptera: The better-preserved specimen of the family Ceratopogonidae (Fig. 4a) is placed in the genus *Archiaustroconops* due to several characters: costa vein prolonged beyond R3, presence of two radial cells, five palpal segments, fourth palpal segment about half the length of the fifth, third palpal segment stout and not very elongate (unclear but does not seem swollen so most likely not), it is a male with claws with an inner tooth in legs. The wing has a C vein that continues beyond R3 (costal extension), excluding all Cretaceous biting midge genera except for *Austroconops*, *Jordanoconops*, *Archiaustroconops*, *Fossileptoconops*, and *Archiculicoides*. The r-m vein is not parallel to R, which excludes *Austroconops* and *Jordanoconops*. The male antennae have an erect plume, which excludes *Fossileptoconops* and *Archiculicoides*. *Archiaustroconops* is a genus of the subfamily Leptoconopinae, which includes 13 fossil species, ranging from ~145 Ma (*Archiaustroconops besti* Tithonian–Berriasian Purbeck Limestone) to ~99 Ma (*Archiaustroconops andersoni*, *A. gracilis*, and *A. kotejai* preserved in Cenomanian Myanmar amber). It was previously thought to belong to the Austroconopinae. The specimen of *Microphorites* from the family Dolichopodidae (Fig. 4c) has a wing venation similar to that of *M. extinctus*²³. Other characters are also similar, except for the genitalia, but considering that our specimen is a male and the description of *M. extinctus* is based on a female.

Coleoptera (Figs. 4f–g): The general habitus together with the 5-5-4 tarsal formula place the sole beetle found within Tenebrionoidea. The combination of the following characters place this fossil in Tetratomidae^{24,25}: body surface clothed with distinct hairs or setae; eyes entire, with anterior edge shallowly emarginate around antennal insertions; antennae with 11 antennomeres; well-developed maxillary palpi, with 4 palpomeres; legs long and slender; tarsi simple and narrow, cylindrical; first tarsomere elongated, narrow; and penultimate mesotarsomere distinctly shorter than antepenultimate. In addition, it is possible to place it in the subfamily Eustrophinae based on body small, sides of the body evenly curved, elongate oval body, antennae moniliform, with broadened and transverse antennomeres 3–7; apical maxillary palpomere fusiform and the apex of last abdominal segment exposed dorsally²⁵. Its preservation precludes the accurate identification among both tribes in the subfamily. The fossil record of Tetratomidae includes one Hollomeninae and five Eustrophinae taxa^{26–29}. Members of Eustrophinae are associated with diverse fungi found in and on coarse woody debris in forest habitats, occupying exposed surfaces, on dead wood or on associated fungal bodies^{30,31}. Adults of extant species in Eustrophinae show antennae that range from slightly to distinctly clubbed^{24,30,31}, while the antenna in this fossil appears to lack an apical club. Nevertheless, the fossil species *Synchrotronia idinineteena*, described from the Cretaceous French amber in the subfamily Eustrophinae, showed an antenna without distal club either. In that case, antennomeres 4–10 were distinctly triangular and very similar to each other. Four additional fossil species from the same subfamily in the Cretaceous amber from Kachin were described: *Cretosynstrophus archaicus*, *Thescelostrophus cretaceous*, *Allostrophus cretaceus*, and *Allostrophus yangi*²⁹. The first two fossil species showed a different antenna, filiform, slender and long, with antennomeres longer than wide, which suggests a relationship with Hallomeninae. Nevertheless, the clubbed antenna of *Allostrophus* can be typically seen in extant eustrophines²⁹. It is possible that early eustrophines were much more diverse in terms of antennal morphology than in Recent fauna. Tetratomidae is composed by six different subfamilies³². Traditionally most members of the family were placed in Melandryidae²⁵. Indeed, the subfamilies Eustrophinae and Hallomeninae were transferred from Melandryidae to Tetratomidae^{24,33,34}. Six genera and numerous species of Eustrophinae are widely distributed in most parts of the world except Australia and New Zealand^{25,31}.



Supplementary Fig. 4. Arthropod inclusions found in the Genoveva amber. **a** Diptera: Chironomidae (N-113-JR-2022-20). **b** Hemiptera: Sternorrhyncha Aleyrodidae (N-113-JR-2022-03). **c** A portion of spider web constituted by seven right strands portions; Arachnida Araneae (N-113-JR-2022-15). All will be housed at the Laboratory of Palaeontology, *Escuela Politécnica Nacional*, Quito (Ecuador). Scale bars: 1 mm (c), 0.5 mm (a, b).

The notation proposed by the *Dirección de Áreas Arqueológicas, Paleontológicas y Patrimonio Subacuático* (Directorate of Archaeological, Palaeontological and Underwater Heritage Areas) from the *Instituto Nacional de Patrimonio Cultural*, Ecuador Republic, has been used. This material comes from Genoveva quarry and will be housed at the Laboratory of Palaeontology, *Escuela Politécnica Nacional* in Quito (Ecuador).

N-113-JR-2022-01 Coleoptera Tetratomidae (field sample number (fsn: 41.0.2))
 N-113-JR-2022-02 Diptera Chironomidae (fsn: 41.0.1)
 N-113-JR-2022-03 Hemiptera Sternorrhyncha Aleyrodidae (fsn: 40.0.9)
 N-113-JR-2022-04 Hymenoptera Scelionidae (fsn: 40.0.8)
 N-113-JR-2022-05 Diptera Chironomidae (fsn: 40.3)
 N-113-JR-2022-06 Diptera Ceratopogonidae (fsn: 40.4)
 N-113-JR-2022-07 Hymenoptera indet. (fsn: 40.0.5)
 N-113-JR-2022-08 Hymenoptera indet. (fsn: 40.0.4)
 N-113-JR-2022-09 Hymenoptera Stigmaphronidae (fsn: 40.0.3)
 N-113-JR-2022-10 Hemiptera indet. (fsn: 40.0.10)
 N-113-JR-2022-11 Diptera Scatopsidae (fsn: 40.2)
 N-113-JR-2022-12 Diptera Ceratopogonidae (fsn: 28.6.3)
 N-113-JR-2022-13 Diptera Dolichopodidae *Microphorites* n. sp. (fsn: 28.6.2)
 N-113-JR-2022-14 Diptera Chironomoidea (fsn: 28.6.1)
 N-113-JR-2022-15 Arachnida Araneae -spiderweb remains- (fsn: 28.0.3)
 N-113-JR-2022-16 Diptera Chironomidae (fsn: 28.1.2)
 N-113-JR-2022-17 Diptera Chironomidae (fsn: 28.1.1)
 N-113-JR-2022-18 Trichoptera (fsn: 26.1)
 N-113-JR-2022-19 Diptera Ceratopogonidae: *Archiaustroconops* (fsn: 21.3))
 N-113-JR-2022-20 Diptera Chironomidae (fsn: 40.1)
 N-113-JR-2022-21 Collembola (fsn: 21.1)

Supplementary Data 4. Methods

Fieldwork and material

Sixty aerial amber pieces were obtained after picking in three different levels of the Genoveva quarry (G1–G3, Supplementary Fig. 2a). Genoveva quarry is situated in the area of Cotundo (Napo region, Canton Archidona, Ecuador). The application for administrative permits for the field study of amber deposits was made in October 2021, and field observations and material collecting (aerial amber pieces) in December 2022 (permit: DAAPPS-INPC-Z1/2-026-2022, research code: N-113-JR-2022, *Instituto Nacional de Patrimonio Cultural, Ecuador*). Although all the bioinclusions came from the Genoveva quarry, two other quarries have been researched to obtain amber pieces. These other two quarries are the Pungarayacu quarry and the Río Misahuallí quarry (Supplementary Figs. 2b–c).

Palynology

Palynological samples were prepared following the standard procedure^{35,36} at the *Laboratorio de Palinología y Materia Orgánica* of the *Instituto de Investigaciones en Estratigrafía* (IIES) at *Universidad de Caldas* (Colombia). It consists of acid attack with HCl, HF, and diluted HNO₃ and sieving with different grid sizes (500, 250, 75, 50, and 12 µm). The morphological database of ref. 37, which includes all palynological publications from South America, was used to identify the palynomorphs. The biostratigraphic zonation which was proposed for Northern South America³⁸, was used to establish the age of the samples and amber deposits. An additional zonation for South America³⁹, proposed specifically for Brazil, was not utilised in this study. This zonation primarily encompasses sites from eastern South America, whereas our samples were collected from Western South America. Palynomorphs (Supplementary Table 1) were grouped into four groups including (1) angiospermous pollen, (2) coniferous pollen and other gymnospermous pollen, (3) Gnetales pollen, and (4) Bryophyta/Pteridophyta spores. Gnetales were placed in their own category rather than in other gymnosperms because they are an important component of equatorial Cretaceous floras⁴⁰ as well as climate indicators. Humidity was estimated by comparing the total diversity of dry indicators (*Classopollis*, ephedroid pollen grains, and elater-bearing species) versus humid indicators (fern spores) like in previous studies^{40–42}.

Organic geochemistry and chemotaxonomical analyses of amber

The analyses were conducted following a previously published method, with modifications⁴³. Briefly, 4.005 g of crushed amber pieces, selected to minimize weathering effects and avoiding inclusions, were continuously extracted using dichloromethane (DCM): methanol (3:1, v/v) in a Soxhlet extractor for 8 hours. The crude extract was concentrated and analysed by gas chromatography-mass spectrometry (GC-MS) to determine its bulk composition (Supplementary Fig. 7). The bulk extract was fractionated using silica gel column chromatography into two fractions: a low-polarity fraction, eluted with n-hexane followed by n-hexane:DCM (3:1), and a polar fraction, eluted first with DCM followed by methanol. The polar fraction was dried under a nitrogen stream, and the residue containing polar terpenoids was derivatised with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA)/1% trimethylchlorosilane (TMCS) at 65°C for 3 hours. The derivatised extracts were subsequently analysed by GC-MS as previously described⁴³.

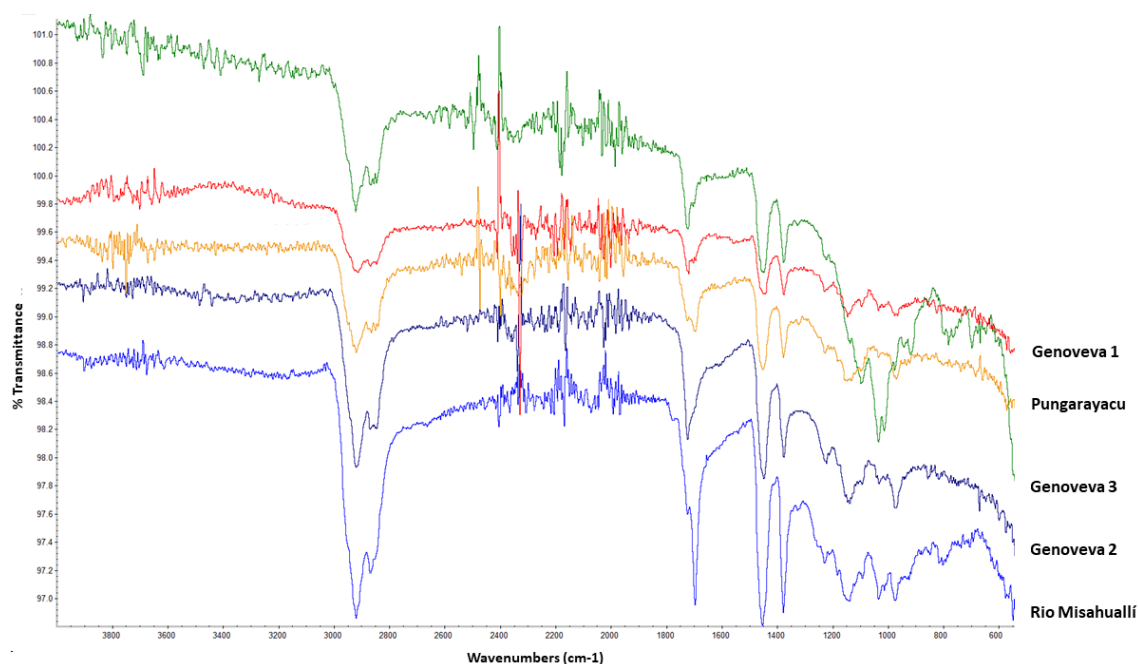
Data acquisition and processing were performed using Agilent MassHunter software. Compound identification was based on comparisons with authentic standards, mass spectral libraries, and relevant literature. Rock samples from amber-containing levels G2 and M8 (30 g each; Supplementary Fig. 3) were extracted using pure DCM in a Soxhlet extractor for 24 hours. The extractable organic material represented approximately 4% of the G2 sample and 6% of the M8 sample. Concentrated extracts (bitumen) were analysed by GC-MS using the same methods as described for the amber samples.

The CPI was calculated to assess the petroleum source and evaluate whether the n-alkane composition in amber was consistent with that of the surrounding bitumen. CPI was determined by integrating n-alkane peaks in the $m/z = 85$ fragmentograms, using the formula:

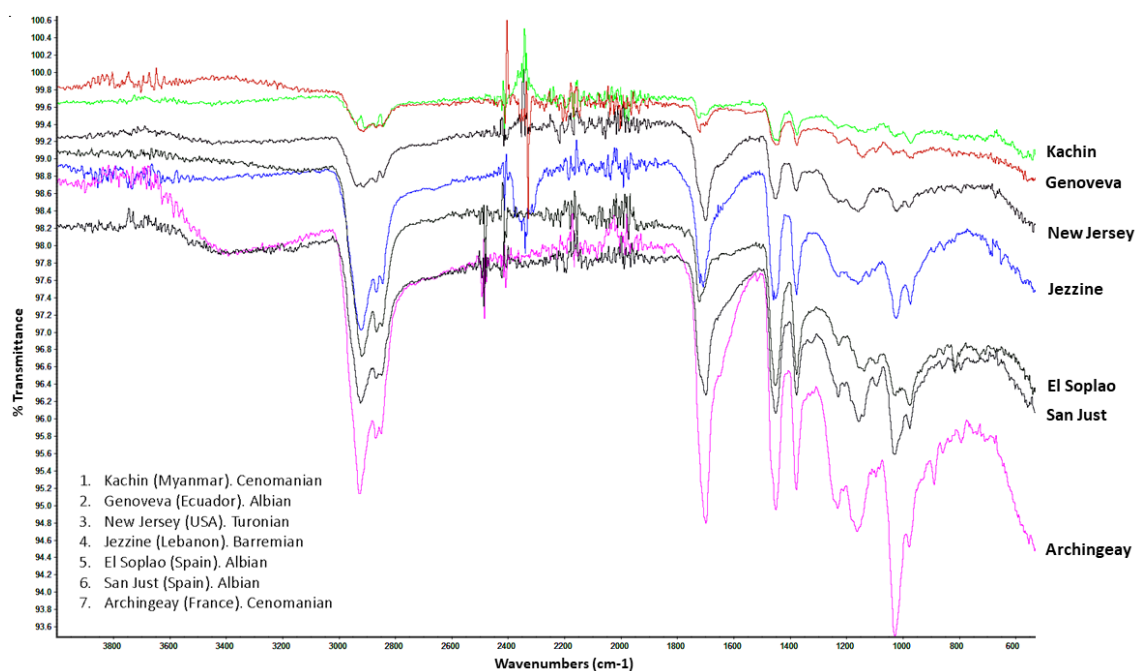
$$CPI = \frac{\sum_{i=5}^{15} C_{2i+1} + \sum_{i=6}^{16} C_{2i+1}}{2 \sum_{i=6}^{16} C_{2i}}$$

Synchrotron imaging (SRμCT)

Synchrotron imaging was performed at DESY in Hamburg (Germany) operated at the Helmholtz-Zentrum Hereon^{44,45}. A total of 5001 projections equally spaced between 0 and π were recorded for a tomographic scan at a photon energy of 18 keV. A custom build 15 MP CMOS detector⁴⁶ with an effective pixel size of 0.456 μm and a sample to detector distance of 49.9 mm was used to record the images. Tomographic reconstruction was done by applying a transport of intensity phase retrieval with a beamline specific workflow⁴⁷ implemented in Matlab (Math Works) using the filtered back projection algorithm implemented in the Astra Toolbox^{48–50}. Raw projections have been binned two times for processing, resulting in an effective pixel size of 0.9 μm in the reconstructed tomographic volume.

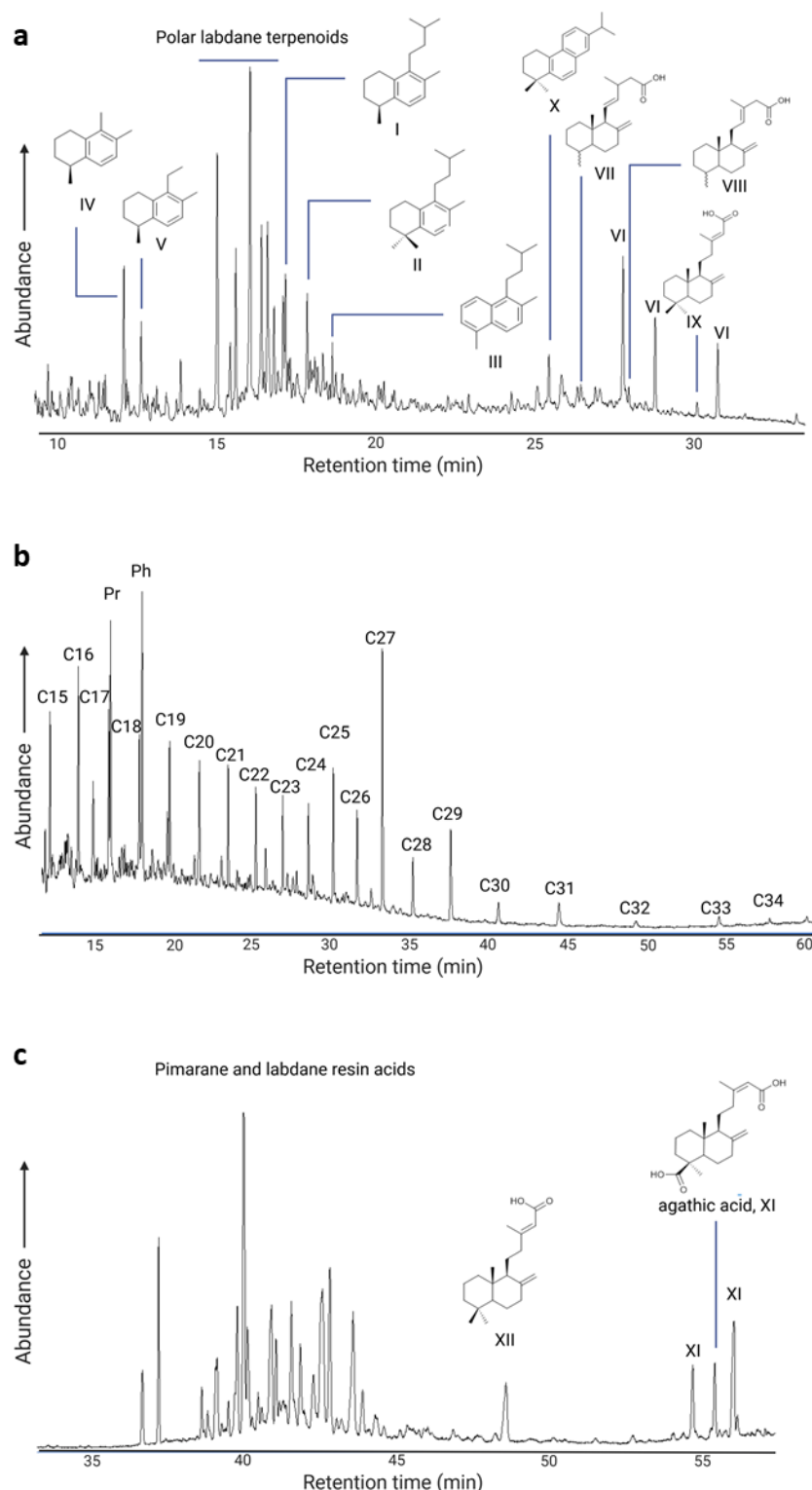


Supplementary Fig. 5. FTIR transmittance spectra of the Genoveva amber from Ecuador. The spectra are of the amber of the three levels (G1–G3) from Genoveva quarry, and from the Río Misahuallí and Pungarayacu quarries as well. The highly different spectra of the sample G3 respect to the other samples between 1100–500 cm^{-1} is due to the high petroleum content of the amber present in that stratigraphic level.



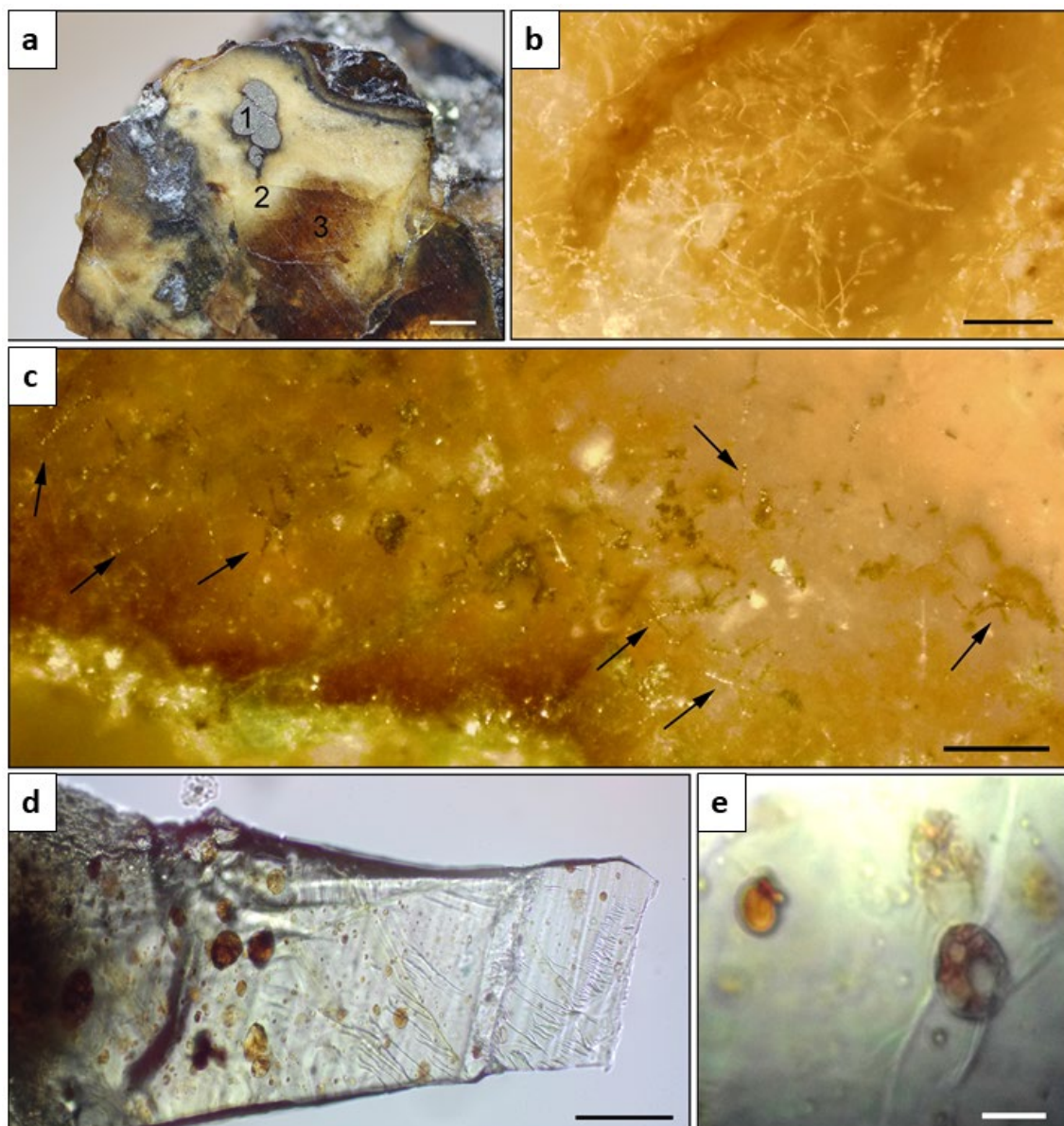
Supplementary Fig. 6. FTIR transmittance spectra of some Cretaceous ambers of different localities around the world to compare with the Genoveva amber. Lower Cretaceous: Jezzine (Lebanon), Genoveva G1 sample (Ecuador), El Soplao and San Just (Spain), Upper Cretaceous: Archingeay (France), Kachin (Myanmar), and New Jersey (USA). Lebanese amber was produced by diverse groups of trees, such as Araucariaceae or Cupressaceae/†Cheirolepidiaceae^{51,52}, Genoveva amber by Araucariaceae (this paper), El Soplao amber by †Cheirolepidiaceae and another non-assigned group (but not Araucariaceae)^{53,54}, San Just amber by Araucariaceae⁵⁵, Archingeay-Les Nouillers amber by Cupressaceae, †Cheirolepidiaceae

or/and Araucariaceae^{56–58}, Kachin amber by Araucariaceae⁵⁹, and the New Jersey amber by Cupressaceae *s.l.*⁶⁰.



Supplementary Fig. 7. Molecular composition of Genoveva amber. **a** GCMS trace of the amber total extract, showing main terpenoids identified. Extract is unusually enriched in polar terpenoids, consistent with extraction of amber hydrocarbons by surrounding petroleum. **b** Fragmentogram at m/z 85 showing the distribution of linear hydrocarbons trapped in analysed amber samples. Pr: pristane; Ph: phytane. **c** GCMS analysis of the derivatised polar fraction. Compounds are identified as their corresponding

trimethylsilyl esters. XI refers to agathic acid and its isomers. Pimarane and labdane resin acids group comprises several isomers, consistent with the diagenesis of original resin acids.



Supplementary Fig. 8. Three types of pseudoinclusions observed in the same amber piece from Genoveva quarry. **a** Polished sample of the amber piece (N-113-JR-2022-46) showing an iron sulphide mineralisation (1), a cortex of resinicolous fungi (2), and concentration of resin-in-sap-in-resin double emulsions or fossilised phloem sap (3). **b** Detail of the fungal mycelia (only the empty core of the hyphae is visible) under optic microscope. **c** Detail of the fungal mycelia under optic microscope in a cortex part where the cores of the hyphae were partially filled with iron sulphide (arrows indicate some sections of the cores that are filled). **d–e** Resin-in-sap-in-resin double emulsions or fossilised phloem sap in a transparent flake from the same piece under optic microscope. Piece will be housed at the Laboratory of Palaeontology, *Escuela Politécnica Nacional*, in Quito (Ecuador). Scale bars: 1 mm (**a**), 0.1 mm (**d**), 0.05 mm (**b, c**), 0.01 mm (**e**).

Presence of the 62 palynomorphs identified in the Genoveva quarry stratigraphic log											
Sample (STRI-ID)		47734	47720	47721	47722	47725	47723	47724	47731	47732	47733
Stratigraphic meter (see Supplementary Fig. 3)		3	10.1	10.9	11.9	12.8	12.8	18.7	19.7	44.5	56.7
Taxon name	Pollen/spore code										
<i>Arecipites</i> sp. 1	Angiosperm						1				
<i>Baculamonocolpites</i> sp.	Angiosperm									1	
<i>Clavatipollenites hughesii</i>	Angiosperm					1					
<i>Cornetipollis perforatus</i>	Angiosperm					1					
<i>Cretacaeiporites</i> sp.	Angiosperm						1				
<i>Dejapollenites</i> sp.	Angiosperm										1
<i>Echimonocolpites</i> sp.	Angiosperm					1					
<i>Liliacidites</i> sp.	Angiosperm								1		
<i>Polyadopollenites</i> sp.	Angiosperm					1					
<i>Proxapertites</i> sp.	Angiosperm	1									
<i>Psilamonocolpites</i> sp.	Angiosperm										1
<i>Retibrevitricolpites</i> sp.	Angiosperm		1								
<i>Retimonoporites</i> sp. 1	Angiosperm										1
<i>Retimonoporites</i> sp. 2	Angiosperm								1		
<i>Retistephanocolpites</i> sp.	Angiosperm						1				
<i>Retitrescolpites</i> sp. 1	Angiosperm									1	
<i>Retitricolpites operculatus</i>	Angiosperm		1								
<i>Reyrea polymorphus</i>	Angiosperm								1		
<i>Rousea</i> aff. <i>miculipollis</i>	Angiosperm	1									
<i>Scabraperiporites</i> sp.	Angiosperm		1								
<i>Senectotetradites</i> aff. <i>varireticulatus</i>	Angiosperm										1
<i>Tricolpites</i> cf. <i>synstriatus</i>	Angiosperm								1		
<i>Tucanopollis</i> aff. <i>crisopolensis</i>	Angiosperm										1
<i>Araucariacites</i> sp.	Conifers_other gymnosperms										1
<i>Callialasporites infirmus</i>	Conifers_other gymnosperms										1
<i>Callialasporites</i> sp.	Conifers_other gymnosperms	1									
<i>Podocarpidites</i> ? sp.	Conifers_other gymnosperms	1									
<i>Taxacites</i> ? sp.	Conifers_other gymnosperms										1
<i>Elaterosporites</i> sp.	Gnetales	1									
<i>Ephedripites</i> aff. <i>regularis</i>	Gnetales								1		
<i>Ephedripites</i> sp.	Gnetales							1			
<i>Equisetosporites ambiguus</i>	Gnetales										1
<i>Equisetosporites fragilis</i>	Gnetales	1									
<i>Equisetosporites laticostatus</i>	Gnetales										1
<i>Steevesipollenites</i> aff. <i>cupuliformis</i>	Gnetales								1		
<i>Steevesipollenites</i> cf. <i>multilineatus</i>	Gnetales		1								
<i>Aequitriradites</i> cf. <i>verrucosus</i>	Bryophyta/Pteridophyta								1		
aff. <i>Staplinisporites</i> sp.	Bryophyta/Pteridophyta										1
<i>Baculatiporites</i> sp.	Bryophyta/Pteridophyta									1	
<i>Clavatisporites</i> aff. <i>clarus</i>	Bryophyta/Pteridophyta							1			
<i>Clavatisporites clarus</i>	Bryophyta/Pteridophyta					1					
<i>Concavissimisporites punctatus</i>	Bryophyta/Pteridophyta					1					
<i>Crybelosporites</i> sp.	Bryophyta/Pteridophyta			1							
<i>Distaltriangulisporites</i> aff. <i>irregularis</i>	Bryophyta/Pteridophyta								1		
<i>Echinatisporis</i> sp.	Bryophyta/Pteridophyta		1								
<i>Gleicheniidites senonicus</i>	Bryophyta/Pteridophyta							1			
<i>Ischyosporites variegatus</i>	Bryophyta/Pteridophyta					1					
<i>Januasporites</i> sp.	Bryophyta/Pteridophyta										1
<i>Leptolepidites</i> sp.	Bryophyta/Pteridophyta			1							
<i>Leptolepidites verrucatus</i>	Bryophyta/Pteridophyta		1								
<i>Microfoveolatosporis skottsbergii</i>	Bryophyta/Pteridophyta					1					
<i>Muerrigerisporis</i> sp.	Bryophyta/Pteridophyta					1					
<i>Osmundacidites wellmanii</i>	Bryophyta/Pteridophyta				1						
<i>Retitriteles</i> aff. <i>eminulus</i>	Bryophyta/Pteridophyta	1									
<i>Scabratriteles</i> sp.	Bryophyta/Pteridophyta							1			
<i>Stoverisporites</i> aff. <i>lunaris</i>	Bryophyta/Pteridophyta		1								
<i>Striatriteles</i> sp.	Bryophyta/Pteridophyta						1				
<i>Verrucatotriteles</i> aff. <i>tortus</i>	Bryophyta/Pteridophyta		1								
<i>Camaronosporites</i> sp.	Bryophyta/Pteridophyta		1								
<i>Cicatricosisporites</i> sp.	Bryophyta/Pteridophyta	1						1			
<i>Clavatisporites</i> sp.	Bryophyta/Pteridophyta			1							1
<i>Retitriteles</i> sp.	Bryophyta/Pteridophyta		1								
TOTAL TAXA: 62		8	10	3	1	9	4	5	8	3	13

Supplementary Table 1. Presence of the 62 palynomorphs identified in the Genoveva quarry stratigraphic log. It includes the number of the 10 samples obtained for palynology and their location in the stratigraphic log. The pollen and spores found are divided in four group: angiospermous pollen grains, coniferous and other gymnospermous non Gnetales pollen grains, Gnetales pollen grains, and spores. Palynology slides are housed at the Smithsonian Tropical Research Institute (Panamá).

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