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Pollen assemblage and environmental DNA changes: a 4300-year-old bat guano deposit from Jamaica

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Abstract:	The discovery of two undisturbed caves in Jamaica with 14C and 210Pb dating indicating that the oldest layers of guano were ca. 200 years old in the first cave (81 cm long core), and as much as 4300 years old in the second cave (129 cm long core) provides exciting possibilities to examine past ecological communities. We analyzed genetic and pollen profiles in these ancient bat guano deposits and revealed DNA sequences most similar to arthropods, mainly insects such as caddisflies (Trichoptera), butterflies (Lepidoptera) and mayflies (Ephemeroptera), suggesting a potential source for past dietary data. Palynological analysis failed to recover well-preserved pollen grains and spores older than ca. 200 years, however in layers preserving pollen, angiosperms were the most abundant plants observed, with a regular occurrence of the potato (Solanaceae) and pepper families (Piperaceae), which are frequently bat-dispersed. In general, changes in frequency of particular plant taxa appear to reflect changes of vegetation and land use in the cave vicinity; however, some changes could be linked to hurricanes, leading to forest canopy damage and promoting the growth of light-demanding species such as guarumo (Cecropia). Higher amounts of mangrove (Rhizophora) pollen have also been recorded in the periods of heavy hurricane activities. Our data highlight the value of bat guano deposits in caves as a resource for the analysis of past ecological systems and stress the conservation values of these deposits.
Suggested Reviewers:	Angela A. Bruch, Dr. Senckenberg Forschungsinstitut und Naturmuseum angela.bruch@senckenberg.de Main research interested devoted, e.g., to climate and Vegetation history and quantification of vegetation parameters and terrestrial palaeoclimate
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	Frank H. Neumann, Dr. University of the Witwatersrand frank.neumann@wits.ac.za His scientific goal is the reconstruction of past environments through mutldisciplinary studies, as well as the understanding of climatic fluctuations and the evolution of biomes using palynology.
	Jens Rydell, Dr. Lunds Universitet jens.rydell@telia.com One of the best known bat specialists working on a wide range of topics, including paleontology, ecology, morphology, entomology, echolocation and conservation.
Response to Reviewers:	

Warszawa, 1 September 2020

Dear Editor,

Enclosed please find our revised manuscript – the result of multidisciplinary research and international cooperation - entitled "**Pollen assemblage and environmental DNA changes: a 4300-year-old bat guano deposit from Jamaica"**. Since only minor changes were requested (all of them were accepted), it did not take much work to correct. We also removed the first sentence from the abstract about the scarcity of sources of ancient climatic and ecological records as there are actually quite several. However, the main point relates to a small chapter (No. 4.6) on the identification of bat DNA, added to the discussion at the request of one of the reviewers.

One thing I am not sure about how to deal with it is the supplementary material (SI). We are not sure about the cost of colour figures and do not want to generate additional costs because of our SI (containing 3 figs.). We may put SI on the Research Gate or on our institutional website, by providing the appropriate link. So far the SI is uploaded as the last of the Figures (and listed as Supplementary Figures).

All the authors read the manuscript and agreed with its content. There is no conflict of interest. Looking forward for your positive reply.

Sincerely yours,

Prof. dr hab. Wiesław Bogdanowicz

On behalf of all authors

Replies to reviewers' comments

Reviewer #1: This manuscript assesses the potential of bat guano for the investigation of past environments by palynological and aDNA methods, and highlights the need for protection of caves with such archives. The ms is very well written and structured. The methods and data are very well described and discussed. The ms is indeed ready for publication after some very minor improvements.
THANK YOU FOR YOUR COMMENT. WE APPRECIATE IT.

Reviewer #1: The only remark I have with respect to the content is the question, why is the result of the bat detection by DNA not discussed? What is the implication of determining a bat species that is not recorded in the region before as given in chapter 2.1? As a reader I expect this issue to be addressed in the discussion.

WE ADDED THE ENTIRE CHAPTER (no. 4.6) DEALING WITH THE IDENTIFICATION OF BAT DNA TO THE DISCUSSION SECTION. IT SOUNDS AS FOLLOWS:

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Jamaica is home to 21 species of bats, with some of them regularly using caves as roosts, including *T. brasiliensis* (NEPA, 2009). We revealed DNA of this species in two layers in SCH Cave: the recent one and one from around 1925. Identification of bats from guano-derived DNA dates back to the early 2000 (Zinck et al., 2004). This approach has been successfully applied to fresh and modern - up to 20 years - samples (e.g., Brown et al., 2017; Walker et al., 2019; Guan et al., 2020). Overall, in all fecal DNA metabarcoding studies, the gold standard is to collect fresh feces for maximum DNA recovery. Research carried out by Walker et al. (2019) revealed that DNA in bat guano, which can be used for host identification, can be recovered up to at least 2.5 years in dry and cool roosts, but at 100% relative humidity, feces that are younger than 6 months are needed. Moisture, particularly in combination with high temperature, promotes the growth of microbes, and thus further DNA degradation.

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Reviewer #1: Technical points are:
There are two versions of abstract in the manuscript. Which one is the correct one? I prefer the one in the online submission form.
Please add a space between numbers and the unit s for second on pages 7 and 8.
Delete both ‚the' in the sentence of the abstract. (The)Higher amounts of mangrove... pollen have also been recorded in (the) periods of heavy hurricanes.
Page 4, Last sentence of first paragraph: should this read ‚from a point of'? and rephrase ‚predating human habitation' as it could read as human habitation is predated...
page 17, last sentence of first paragraph of chapter 4.5: This should be two sentences and a bit

more precisely phrased. the second part ‚For example, the detection...', could read - ‚and as most sequences are from one layer this suggests...',
And a last thing that I would like to leave to the editor to comment on: Personally, I don't like the use of first person grammar in a scientific article. For me, there are far too many ‚we' in the text. Especially in the methods chapter almost every sentence starts with ‚we...'
ALL TECHNICAL POINTS WERE ACCEPTED AND MODIFICATIONS INCORPORATED.

Reviewer #2: This paper reports on 2 bat guano deposits from caves in Jamaica. Carbon and lead dating placed the oldest deposits at 4,300 years old. Pollen analysis reliably revealed grains and spores of mainly angiosperms up to about 200 years ago. DNA study found the presence of primarily insects, but the method did not work well for identifying the bats that lived in the caves. The authors make a connection to the value of bat guano in recovering historical data such as past weather systems such as hurricanes that can alter the ecosystem. Overall, the paper is well written and presented. I don't have any substantial comments or suggestions to make other than perhaps adding insects to the title as this was the main result from the environmental DNA study.

THANK YOU VERY MUCH FOR YOUR POSITIVE REPLY.

Pollen assemblage and environmental DNA changes: a 4300-year-old bat guano deposit from Jamaica

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The discovery of two undisturbed caves in Jamaica with ^{14}C and ^{210}Pb dating indicating that the oldest layers of guano were ca. 200 years old in the first cave (81 cm long core), and as much as 4300 years old in the second cave (129 cm long core) provides exciting possibilities to examine past ecological communities. We analyzed genetic and pollen profiles in these ancient bat guano deposits and revealed DNA sequences most similar to arthropods, mainly insects such as caddisflies (Trichoptera), butterflies (Lepidoptera) and mayflies (Ephemeroptera), suggesting a potential source for past dietary data. Palynological analysis failed to recover well-preserved pollen grains and spores older than ca. 200 years, however in layers preserving pollen, angiosperms were the most abundant plants observed, with a regular occurrence of the potato (Solanaceae) and pepper families (Piperaceae), which are frequently bat-dispersed. In general, changes in frequency of particular plant taxa appear to reflect changes of vegetation and land use in the cave vicinity; however, some changes could be linked to hurricanes, leading to forest canopy damage and promoting the growth of light-demanding species such as guarumo (*Cecropia*). Higher amounts of mangrove (*Rhizophora*) pollen have also been recorded in the periods of heavy hurricane activities. Our data highlight the value of bat guano deposits in caves as a resource for the analysis of past ecological systems and stress the conservation values of these deposits.

Keywords: ancient guano, bats, history, hurricanes, Jamaica, DNA, pollen

1. Introduction

Caves can provide a stable and generally homogenous environment for long periods of time and be a source of protection for artifacts and fossils and soils. In addition, cave sediments can provide a record of paleoclimate (McFarlane et al., 2002; White, 2007; Onac et al., 2014; Cleary et al., 2017). The relatively stable nature of caves provides an important habitat for populations of cave-dwelling species. Bats are common cave-dwelling fauna and their guano (faecal) deposits may accumulate for hundreds or even thousands of years (e.g., McFarlane et al., 2002; Bird et al., 2007; Wurster et al., 2010, 2017; Batina and Reese, 2011; Widga and Colburn, 2015; Choa et al., 2016).

Bat guano is extremely variable, providing numerous microhabitats differentiated by fluctuating temperature, moisture and pH. Variation in the diets of bats affects the nature of guano deposits and associated guanophilic communities (Gnaspini, 1992; Ferreira and Martins, 1999; Moulds, 2006). Based on Richards' (1971) approach to cave biology, guano is one of the four cave ecosystems (besides twilight and entrance zones, dark zone and water systems), but has remained relatively unexplored as a contemporary and historical record of local ecology.

Finding intact guano deposits can be difficult because of commercial and recreational cave exploration as well as periodic inundation and flooding of some cave systems. However, if available, well-stratified guano deposits are important archives for pollen (e.g., Navarro et al., 2001; Carrión et al., 2006; Leroy and Simms, 2006; Maher, 2006; Batina and Reese, 2011; Geantă et al., 2012; Choa et al., 2016) providing information about the vegetation in areas where bats were active (Geantă et al., 2012; Forrey et al., 2015; Marais et al., 2015; Svitavská-Svobodová et al., 2015). Guano deposits can also provide data about entomophilous plants otherwise underrepresented in more traditional pollen sampling sites (Carrión et al., 2006; Leroy and Simms, 2006; Maher, 2006; Svitavská-Svobodová et al., 2015). For more than a decade, guano has also been a target for genetic analysis using metabarcoding (Pompanon et al., 2012) to identify predator-prey relationships and to generate dietary profiles. It remains unclear whether DNA can persist in deep cave deposit conditions.

A total of 21 species of bats occur in Jamaica, about 15 of them commonly roosting in caves (Fenton MB, unpublished data; see also Genoways et al., 2005). There are over 1000 cave systems in Jamaica. However, several face threats to their physical structure and biodiversity due to external (e.g., removal of forest cover) and internal factors (excessive human visitation and mining guano for its nitrogen content.). The abundance of caves and cave-dwelling bats makes Jamaica an ideal location to search for deep, undisturbed deposits of guano as a resource for historical ecology and past climate, as well as assessing their value for conservation efforts.

Here we investigated two intact bat guano deposits in Jamaica. Both had been protected by extremely difficult access requiring specialized breathing and caving gear. To the best of our knowledge these guano deposits are undisturbed and may be a unique

potential repository of data on bat occupancy, diet and surrounding land use change before the time of human habitation of the island.

Our objective was to investigate the value of guano deposits as a record of past ecology. We obtained sequential samples of the guano and analyzed them to determine if layers have remained intact or had experienced mixing. We also processed the guano for any pollen grains and spores and attempted to isolate DNA to identify arthropod material in the examined strata and the bats that left it. We predicted that bat communities occupying the caves are a stable trophic mix, as communities of cave-dwelling bats are well-sheltered. We also predicted that known severe hurricanes would correspond to a jump in pollen concentration of light-demanding plants like *Cecropia*.

2. Materials and Methods

2.1. Study Caves

We targeted guano deposits in Schwallenburgh Cave (SCH Cave) and Home Away from Home Cave (HOM Cave). SCH is in the parish of Saint Ann, in the northern section of the island. The parish is noted for its ca. 60 limestone caves and numerous sinkholes. St. Ann also has considerable wetland (swamp) areas, particularly along the coast. SCH consists of a 49 m shaft leading to a chamber with deep deposits of guano. The chamber occasionally floods during very heavy rains, but when we sampled the deposit in 2012, there was only a minor flow of water along one side of the deposit. HOM Cave is in Cockpit Country in the interior of the island (for both caves, the exact locations are withheld to protect them). Cockpit Country is a karst formation with a Wet Limestone Forest (Asprey and Robbins, 1953). HOM consists of two large chambers. A 20 m vertical descent from the main entrance gives access to the first chamber and the main guano deposit is in the second chamber. This cave does not flood, but water enters by percolation. Although we have no precise census data about SCH cave, an estimated 3000 bats currently occupy HOM cave, with five species identified so far: insectivorous *Pteronotus parnelli*, *Macrotus waterhousii*, and *Mormoops blainvillei*, nectarivorous *Glossophaga soricina*, and frugivorous *Artibeus jamaicensis*. Seven species were recorded in the 2008–2009 bat census of seven caves in Jamaica, three species of

insectivorous *Pteronotus* (*P. parnelli*, *P. macleayii*, and *P. quadridens*), *M. blainvillei*, and *Chilonatalus micropus*, nectarivorous *Monophyllus redmani*, and frugivorous *A. jamaicensis* (NEPA, 2009).

2.1.1. Sample coring and extraction

We (RSS, CG and WB) collected guano samples at the two sites in early March, 2012 and in mid-September 2012. In both the SCH and HOM caves, we dug a narrow trench along the floor of the cave through to the deepest part of the deposit. We intended to sample at the point with the highest rate of accumulation and therefore presumably the highest depth-time resolution. We took samples at ~1cm increments from a vertical column and stored in individual bags using a stainless steel tray measuring 23 cm x 20 cm x 1cm. We wiped the tray clean with paper towel after each sample and rinsed it with high purity acetone every five cm. They were labelled SCH or HOM and numbered sequentially from the top down. We determined the total depth of each deposit (81 and 129 cm, respectively) by pushing a rod to the bottom. In both caves, care was taken to ensure minimal disturbance to the overall deposit and the bats. All waste materials were collected and removed from the caves. For IUCN guano harvesting guidelines see https://portals.iucn.org/library/node/43412.

We determined ^{14}C dates on lipid-untreated samples using accelerated mass spectrometry at the Poznań Radiocarbon Laboratory in Poland using OxCal 4.1.5 and OxCal 2013. For the HOM core, we also used ^{210}Pb dates calculated using the constant rate of supply (CRS) model with corroboration by ^{137}Cs activity. At the HOM core, at 14.5 cm, there was a 580-year difference between the ^{210}Pb date (1900 CE) and the calibrated ^{14}C date (1320 CE – for details see Gallant et al., 2020). Such a difference may be related to two things: either to the dissolution of the limestone cave onto the bat guano deposit, leading to the presence of the old carbon (lacking ^{14}C) or/and to the specific bat diet, where bats preying on aquatic insects can incorporate old carbon from their environment. That is why a 580-year reservoir effect was applied to the HOM core (Gallant et al., 2020). After accounting for this effect, the oldest layer at HOM (128.5 cm) reached 2307 BC, thus making this bat guano deposit ca. 4300-years-old. For the SCH core, the dating model using constant flux and sedimentation rate (CFSR) worked better

than the CRS model. A 2nd order polynomial equation (R^2 = 0.999) was applied to the ^{210}Pb dates and the single ^{14}C date at 78.5 cm (1814 cal CE); dates between 30.5 and 80.5 cm were calculated using said equation (see Supplementary Material — Fig. S1). We did not record any ^{137}Cs activity in this core

We completed ^{210}Pb dating and ^{137}Cs measurements at the University of Ottawa in Canada using an Ortec High Purity Germanium Gamma Spectrometer (Oak Ridge, TN, USA). Certified Reference Materials obtained from International Atomic Energy Association (Vienna, Austria) were used for efficiency corrections, and results were analyzed using ScienTissiME (Barry's Bay, ON, Canada).

2.1.2. Pollen analysis

We obtained samples for pollen analysis of the guano deposit from SCH at 5-cm intervals (depth 0–81 cm) and at HOM at 1-cm intervals (depth 0–13 cm; for high-resolution record for the most recent period) and 7-cm intervals (depth 14–129 cm). We processed 3–5 cm^3 guano from each level using a modified Erdtman's acetolysis method (Moore et al., 1991). Guano samples were sieved using a 5 µm nylon mesh. We prepared 17 samples from SCH, 31 samples from HOM and studied them under a light microscope (at 400× and 600× magnifications) using glycerine jelly as a mounting medium (Fig. 1). In most samples we counted ca. 500 pollen grains and all accompanying spores. Depending on the frequency of pollen grains observed, we studied 1–4 microscope slides from each sample and counted the pollen spectrum.

We used data from spore-pollen spectra to construct simplified pollen diagrams. We calculated percent abundance of each plant taxon from pollen counts relative to the total sum of pollen grains counted. We separately computed the proportion of spores, algae and epiphyllous-saprophyte fungi in relation to the total sum using POLPAL computer software (Nalepka and Walanus, 2003). We estimated pollen and fungal spore concentrations based on counts of *Lycopodium* control spores added during pollen processing (Stockmarr, 1971). Pollen and spore concentrations are the number of pollen grains and spores per cm^3 of sediment. We identified pollen grains to the lowest taxonomic level possible using a combination of reference collections of the W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków, slides of pollen from Cuba

collected by Prof. L. Stuchlik, housed in the same institute, as well as available literature (e.g., Bertrand, 1983; Lozano-García et al., 1995; Melhem et al., 2003; Rull, 2003; Willard et al., 2004; Bauermann et al., 2009; Silva and Santos, 2009; Leal et al., 2011). We also used online keys when needed (Roubik and Moreno Patiño, 2003; Snyder et al., 2007). Where we had identified pollen grains, we obtained further information about the plants from various sources (e.g., Asprey and Robbins, 1953; Adams, 1972). We took microphotographs of selected taxa (Figs. 2 and 3) using a NIKON Eclipse microscope fitted with a Canon digital camera at 1000× magnification.

2.1.3. DNA protocols and analyzes for insect identification

We isolated DNA from guano samples at the Museum and Institute of Zoology, Polish Academy of Sciences in a facility suitable for ancient DNA including a specialized room cleaned with DNA-Erase (MP Biomedicals) and UV irradiated for 60 min. We wore disposable, sterile clothing and performed negative control extractions alongside each batch of extractions. We extracted DNA using the NucleoSpin Soil kit (Macherey-Nagel) applying approximately 2 ml of the starting material and following manufacture's protocol with the use of SL1 buffer, Enhancer XS and elution in 30 µl.

We targeted a 157 bp fragment of the *cytochrome c oxidase* subunit 1 (COI – DNA barcode) to amplify insect DNA using primers ZBJ-ArtF1c and ZBJ-ArtR1c (Zeale et al., 2011). PCR reactions were performed in 25 µl volumes, with 12.5 µl Multiplex PCR Master Mix (QIAGEN), 0.6 µl of each 10 µM primer, 6 µl of sample DNA and H_2O to the final volume. The PCR thermal cycling conditions were as follows: 95ºC for 15 min, then 45 cycles of: 94ºC for 30 s, 47.5ºC for 90 s, 72ºC for 90 s, with the final elongation of 60ºC for 30 min.

We sequenced using the PacBio RS II sequencer and analyzed using the Galaxy platform (Afgan et al., 2018), following the protocols in Clare et al. (2018). We removed all primers and non-target DNA, collapsed the sequences to unique haplotypes and filtered the results to remove sequences longer (>180 bp) or shorter (<100 bp) than expected length (157 bp amplicon).

We used a BLAST analysis to compare the remaining haplotypes to a taxonomically broad reference collection of >600,000 COI sequences extracted from

GenBank. These results were interpreted in MEGAN following Clare et al. (2018). We further screened high BLAST similarity results in the BOLD database.

2.1.4. DNA protocols and analyzes for mammal identification

To assay for bat DNA, we designed five pairs of primers for the amplification of the *cytochrome b* gene fragment. Sequences for primer design were derived from GenBank and came from bats occurring in Jamaica. PCR amplification was performed in two multiplexes (Table 1). The reaction volume was 25 µl, with 12.5 µl of Multiplex PCR Master Mix (QIAGEN), 1.5 µl Primer Mix (2µM of each of the primers), 5 µl H_2O and 6 µl of the sample DNA. The PCR thermal cycling conditions were as follows: 95^{o}C for 15 min, 40 cycles of: 94^{o}C for 30 s, 54^{o}C (for M1) / 50^{o}C (for M2) for 1 min, 72^{o}C for 60 s and final elongation at 60^{o}C for 30 min.

PCR products from each amplification were visualized on a 1.5% agarose gel with Midori Green DNA Stain (NIPPON Genetics EUROPE GmbH). Amplicons were then used for library preparation for sequencing on the PacBio RS II (Pacific Biosciences). The procedure was performed accordingly with manufactures' protocol for short fragments with chemistry C2/P4. Each sample was sequenced in one SMRTcell. Afterwards, we analyzed raw sequences using the Galaxy platform (Afgan et al., 2018), following the protocols proposed by Clare et al. (2018). We removed all primers and non-target DNA, collapsed the sequences to unique haplotypes and filtered the results to the expected size of the amplification product.

We used a BLAST analysis to compare the remaining haplotypes to a reference collection of chiropteran cytb sequences extracted from GenBank. We interpreted these results in MEGAN following Clare et al. (2018).

3. RESULTS

3.1. Palynological investigation

3.1.1 SCH Cave

Pollen spectra from the samples studied were taxonomically diverse, with 40–60 types of pollen identified in most samples. Generally, about 120 different pollen types

from about 65 botanical families and five morphological types of spores were identified (Fig. 4). Indeterminate pollen grains made up less than 5% of the total pollen in most samples analyzed; only in the bottom sample was pollen degradation an issue, where ca. 15% of the pollen grains could not be identified due to severe damage.

Pollen concentration ranged from 2083 to 43466 grains, with an average of 9689 per cm^3 of sediment. Fungal spore concentration ranged from 394 to 6486, with an average 1967 spores per cm^3 of sediment. In addition, we observed single specimens of *Tetraploa* and other epiphyllous-saprophyte micro-fungi, as well as single colonies of *Botryococcus* algae. We found fern spores in 16 samples, making up to 10% of the total sporomorphs in the levels counted. Conifers were represented by a few pollen grains of *Pinus*. Pollen grains of angiosperms were the most abundant in our samples.

Among deciduous trees, shrubs and climbers such as *Cecropia* (up to 65% in one sample), *Piper* (up to 50%), *Rhizophora* (up to 30%), Myrsinaceae (up to 12%) and *Cocos* (up to 10%) were most frequent. We recorded up to 4–5% of pollen grains for *Casearia*, Fabaceae, Melastomataceae/Combretaceae, Sapotaceae, Myrtaceae, and *Zanthoxylum*. We also regularly observed pollen of other taxa, such as *Bursera*, *Cedrela*, other Meliaceae, *Cordia*, *Spondias*, Arecaceae, Bombacoideae (e.g. *Ceiba*), *Citrus*, *Coffea*, Euphorbiaceae, *Psychotria*, Verbenaceae, *Mangifera*, and *Theobroma*.

Herbs were represented mainly by asters (Asteraceae), including *Ambrosia* (up to 12%), other Asteroideae (up to 40%) and Cichorioideae (up to 3%). We also found grasses (Poaceae), including cereals/*Saccharum* (up to 8%), Rubiaceae, including *Borreria*/*Spermacoce* (up to 5%) as well as Amaranthaceae (up to 2%) and Convolvulaceae (up to 2%). Cereal-type grasses are indicators of agriculture activities. We also regularly observed pollen from the potato family (Solanaceae), which are frequently bat-dispersed.

We observed single pollen grains of cf. Bambusoideae, *Begonia*, Brassicaceae, *Cannabis*, Cyperaceae, Malvoideae, Marantaceae/Musaceae, *Passiflora* and *Polygonum*. We also recorded pollen of aquatic and near water plants as single pollen grains of *Nymphoides* and Typhaceae/Sparganiaceae.

3.1.2. HOM Cave

Pollen concentrations and preservation status varied markedly through the profile of this cave. The surface 12 cm intervals of the core had very well-preserved rich pollen assemblage (Fig. 5), but the 13 cm interval (ca. 120 year old) had very few pollen grains. Samples below 14 cm were barren of pollen grains.

For samples from the 0–12 cm levels where good preservation was apparent, pollen concentration ranged from 3220 to 15580 grains per cm^3, with an average 8746 pollen grains per cm^3 of sediment. Fungal spore concentrations in these samples ranged from 223 to 15102, with an average 3734 spores per cm^3 of sediment. We also found some specimens of epiphyllous-saprophyte micro-fungi as well as single colonies of *Botryococcus* algae. In most of these samples, we counted ca. 500 pollen grains and all accompanying spores. For two low frequency samples, this base sum of 500 pollen grains was reduced to about 200–300 grains. Pollen spectra from the well-preserved levels are taxonomically diverse with each sample containing 50–55 types of pollen. We identified ca. 90 different pollen types from about 60 botanical families and 3 spore morphological types.

We found fern spores in all core samples, making up to 8% of the total sporomorphs. Conifers were represented by a few pollen grains of *Pinus* and *Podocarpus*. Angiosperms prevailed in all the intervals that we examined. Deciduous trees, shrubs and climbers included *Cecropia* (up to 40%), Melastomataceae/ Combretaceae (up to 20%), Sapotaceae (up to 20%), Myrtaceae (up to 10%) and Ulmaceae (mainly *Trema*, up to 10%). Pollen grains of Arecaceae (up to 8%), *Bursera* (up to 6%), *Zanthoxylum* (up to 6%), Anacardiaceae (up to 5%), Elaeocarpaceae (up to 5%), Fabaceae (up to 5%), Moraceae/Urticaceae (up to 5%), *Cordia* (up to 4%), Bombacoideae (e.g. *Ceiba*; up to 3%), Euphorbiaceae (up to 3%), Myrsinaceae (up to 3%), were regularly recorded. We also observed some cultivated plants such as *Coffea* (up to 2%) and *Cocos* (up to 1%).

Herbs were represented mainly by Asteraceae, including *Ambrosia* (up to 3%), other Asteroideae (up to 5%) and Cichorioideae as well as Poaceae, including cereals/*Saccharum* (up to 3%) and grasses with smaller pollen grains (up to 4%). Other pollen grains of herbs such as Amaranthaceae, Apiaceae, Araceae, cf. Bambusoideae, *Begonia*, Bromeliaceae (cf. *Vriesea*), *Cannabis*, Convolvulaceae, Cucurbitaceae (cf. *Cayaponia*), Malvoideae, *Passiflora*, Polygonaceae, Rubiaceae (including

Borreria/Spermacoce), Solanaceae and others encountered sporadically. Some of these taxa may be shrubs or trees as well.

3.2. Insect detection by DNA

We recovered DNA sequences from all layers however sequence length varied greatly beyond the expected 157 bp. After processing, 69698 reads were retained from the SCH core and 55193 reads were retained from the HOM core for further analysis. BLAST results interpreted in MEGAN suggest the presence of a variety of insects in most layers (Fig. 6), however to assign sequences we had to lower the minimum score parameter of MEGAN to 50, which can result in false positive assignments and so we raised the MinSupport value to 5 to try and reduce this effect. As a likely consequence, sequences in the HOM 42–43 cm layer show high similarity to Cumacea, an order of small, mainly marine crustaceans suggesting a false positive rate within the data. Other assignments of fragmentary DNA reads are more robust. Many core layers had fragments of approximately 100 bp with matches to known taxa of 88% and 92% similarity. These same taxa are represented in multiple layers of both cores suggesting insect DNA presence. Insect DNA was distributed among ages and in both cores rather than concentrated in the more modern layers.

In the oldest SCH layers we observed DNA with similarity to Lepidoptera, Hymenoptera, and Orthoptera DNA (Fig. 6), whereas in HOM, we observed DNA with similarity to Trichoptera, Coleoptera and Diptera DNA (Fig. 7). At least some of these assignments are robust even by modern DNA standards. For example in the oldest HOM core layer we have a Diptera assignment with a BLAST score of 250 bits representing 149/156 identities (96%) with no gaps and an e-value of 2e-63. Identification in BOLD (Ratnasingham and Hebert, 2007) also resulted in 98.8% similarity to fly DNA (Fig. 7) however it also produced a high similarity match to a beetle precluding a species level assignment and suggesting it may have been chimeric. At the time of analysis there was no taxonomic information for these references in BOLD.

Sequences with bit scores >200 and >95% match over >100 bp to Diptera were found in layers 29–30 cm (1176 yrs AD), 94–95 cm (1151 yrs BC), and 114–115 cm (1988 yrs BC). Similar scoring Blattodea were detected in 29–30 cm (1176 yr AD); 94%

over 147 bp, bit score 228), and similar scoring Hemiptera in 21–22 cm (1235 yrs AD; 94% over 143 bp, bit score 236). Reasonably high scoring Lepidoptera were found in the most recent layer of SCH (93% of 157 bp, bit score 248) and, while this percentage score is low, it is artificially reduced due to a string of *n* bases in the query. When checked in BOLD it generated perfect matches to lepidopteran sequences.

3.3. Bat detection by DNA

Searching for bat DNA was less successful. We analyzed 30 samples and obtained 3 positive PCR amplifications. Positive matches for bat DNA were found in only two layers from the SCH Cave (layer 0–1 cm, recent one — 25 reads and 38–39 cm, around year 1925 – 16702 reads, in both cases with MinScore = 300). They were identified as the insectivorous bat *Tadarida brasiliensis* (family Molossidae), a species that often roosts in caves in Jamaica.

4. Discussion

We analyzed two undisturbed guano deposits in caves in Jamaica used as roosts by several species of bats. Dating with ^{210}Pb and ^{14}C indicated that one of these deposits contain material from more than 4300 yrs BP. We also found that guano layering was not extensively mixed in either the HOM or SCH cave suggesting minimal human activity and minimal mixing from animal activity. We found well-preserved pollen in the upper layers of HOM cave and in all layers of SCH cave, which provide a past environmental record of the surrounding area. We recorded insect DNA in all layers and matched them to major insects groups not normally associated with cave fauna but prevalent in the diet of insectivorous bats. We also have DNA from *T. brasiliensis* in two SCH layers.

4.1. Composition of palynofloras — accuracy of the pollen record from bat guano

Palynological analyses revealed well-preserved pollen grains and spores in almost all layers of the SCH deposit and in the upper layers of HOM. But the oldest layers at SCH were only ca. 200 years old. The absence of truly ancient deposits at SCH could reflect a heavy rain event due to hurricanes. In HOM the oldest guano layers were ca. 4300 years BP, but we could track well-preserved pollen to only 80 years BP. Damage to

pollen may be chemical (e.g., groundwater solutions, oxidation or bacterial destruction) or physical (e.g. abrasion, rupture, or compaction during transportation – Lowe and Walker, 2015). Deterioration at HOM could reflect dry-wet cycles (Campbell, 1994) and destructive influence of water (HOM is more humid than SCH – Tweddle and Edwards, 2010). Disturbance could also reflect higher concentrations of frugivorous rather than insectivorous bats in HOM than in SCH. The former produce guano with higher pH values (Shahack-Gross et al., 2004), and a more alkaline environment (especially above 7.6 pH) reduces the concentration of pollen sharply (Li et al., 2005).

The assemblage depends on such factors as pollen productivity associated with the frequency of zoophilous and anemophilous plant taxa. The pollen assemblages we studied contain a significant proportion of pollen of entomophilous plants (Supplementary Material). Pollen from anemophilous plants and fern spores were also diverse in the HOM and SCH deposits. Some of them (e.g., *Cecropia*, *Piper* and *Rhizophora*) dominate in many of our samples. For example, *Cecropia*, because of its "catkin"-like inflorescences adapted to wind pollination, is assumed to be primarily anemophilous (Bush and Rivera, 2001). The presence of *Cecropia* and *Piper* in bat guano in high proportions is likely caused by high pollen production of these plants, "captured" by insects, and also through their massive consumption by bats (Lobova et al., 2003). It is also possible that fruit-eating bats, such as *A. jamaicensis*, which eat fruits (primarily) and leaves (secondarily; reviewed by Cordero-Schmidt et al., 2016; see also Duque-Márquez et al., 2019), may also digest flowers, pollen, or nectar. The fur of *A. jamaicensis* in Brazil and Costa Rica contained pollen of *Anacardium occidentale*, *Bauhinia pauletia*, *Ceiba pentandra*, *Crescentia*, *Hymenaea courbaril*, *Manilkara zapota*, *Ochroma lagopus*, and *Pseudobombax septinatum* (Gardner, 1977; Ortega and Castro-Arellano, 2001).

Proportions of zoophilous and anemophilous pollen types from SCH varied among samples, but both types were common (Supplementary Material — Fig. S2). Anemophilous pollen was represented by fewer common taxa, but present in higher volumes (Poaceae, cereals, *Rhizophora*, *Cecropia* and *Piper*). These plants are abundant pollen producers with good pollen dispersal. In contrast, most zoophilous taxa show lower values in pollen spectra, but appeared in higher diversity. These results corroborate

other recent palynological studies performed on bat guano and other cave sediments, with similar observations of zoophilous/anemophilous taxonomic ratios (Navarro et al., 2001; Geantă et al., 2012). On the other hand, in samples from HOM, pollen grains of zoophilous plants predominated (Supplementary Material — Fig. S3). These differences may reflect the types of vegetation around the caves.

We also found several non-pollen polymorphs (e.g., epiphyllous and/or saprophytic fungi) in these bat guano deposits (Figs. 4 and 5). *Tetraploa aristata* commonly occurs as saprophyte on grasses (e.g. bamboos) and other monocotyledons on leaf bases and stems just above soil, although it was also recorded on dicotyledons (e.g. *Citrus* and *Eucalyptus*), *Pinus*, and rarely on soil and decaying wood and leaf litter. Conidia of *Tetraploa* often occur as accessory element among airborne fungi in subtropical and tropical regions (Worobiec et al., 2009 and literature cited therein).

4.2. Can changes in vegetation caused by agriculture be detected in bat guano deposits?

Rich soils, a hot climate with a good rainfall and copious underground water foster good crop growth in Jamaica. Agricultural activities occupy nearly 50% of the island, where the most common crops are sugar cane, coconut, bananas, citrus, coffee, maize, cocoa and rice plantation (Blume, 1974; Genoways et al., 2005). Changes in vegetation caused by agriculture influence pollen assemblages. We found pollen of cultivated plants such as *Citrus*, *Cocos*, *Coffea*, *Eucalyptus*, *Mangifera*, *Theobroma*, and cereals/*Saccharum* in our samples (Figs. 4 and 5). Many of these plants are entomophilous and their frequency in the deposit may depend on insects and insectivorous bats.

Changes in the frequency of particular taxa may also demonstrate various changes in vegetation and land use in the cave vicinity — for example the higher presence of *Cecropia peltata* and species such as *Syzygium jambos*, *Mangifera indica*, *Cocos nucifera*, *Spondias*, and *Alchornea latifolia* may reflect a higher degree of disturbance (Camirand, 2002). The vegetation around SCH and HOM is a mosaic reflecting environmental conditions and agricultural use of the land. Pollen spectra are similar in SCH and HOM but there are some important differences. For example, SCH pollen

assemblages are richer in pollen of cultivated plants, such as *Citrus* and *Cocos*, and in pollen of plants such as *Cecropia*, probably deriving from disturbed areas.

In the HOM deposit, we also recorded pollen grains of cultivated plants, such as *Coffea*, *Cocos* and cereals/*Saccharum*, but the pollen assemblage (Fig. 5) mainly reflects the natural vegetation of the Wet Limestone Forest of the Cockpit Country and Dry Limestone Scrub Forest, rich of epiphytes and lianas, aroids, and bromeliads but with mixed forest types between the bare limestone and valley floors. Larger valleys are now under cultivation or pasture with the growing of bananas, sugar cane, yams, coco (*Colocasia*) and cassava (Asprey and Robbins, 1953), with small farms of sugar cane, coffee, bananas, yam, as well as such field crops as breadfruit, coconut and avocado pear (Barker and Miller, 1995). Some of these taxa are also visible in pollen samples at HOM.

The differences in crop production can also be tracked through time. The coffee plant, for example, was introduced to Jamaica in 1728–1730. The American War of Independence (1775–1783) and the Napoleonic wars (1799–1815) hampered the expansion of international trade on the island. This decrease in production can be seen in SCH, where a significant drop in the amount of coffee pollen can be observed around 1810–1830. Some statistics concerning coffee acreages in Jamaica are also available for the years 1877–1953 (Thomas, 1964). They indicate a pronounced acreage depression in the 1930's. In Fig. 4, however, no drop can be noted until 1943 when a gradual decrease up to the year 1962 can be observed. This finding supports the statement of Thomas (1964), suggesting that the figures concerning severe acreage destruction in the 1930's are most probably misleading as coffee is a perennial tree crop and its cultivation has been resistant to short-run changes. According to our data, the decrease in coffee production most probably occurred in the 1940's at the time of WWII and lasted until the end of the 1950's. This observation agrees with the figures provided by the Jamaica Agricultural Commodities Regulatory Authority showing the quantity of coffee exported from Jamaica in years 1791–1957, where a significant drop was noted in the 1940's (see http://www.ciboj.org/index/brief-historical-background-coffee).

4.3. Can hurricanes be detected in bat guano deposits?

One of the main disrupters of local vegetation in Jamaica are hurricanes. Pollen spectra from the bottom samples from SCH (Fig. 4) differ from upper levels. For example the lowermost layer is characterized by high percentage of *Rhizophora* pollen (30%), accompanied by other taxa of mangrove and coastal vegetation (e.g., Melastomataceae/Combretaceae - possibly including *Conocarpus* and *Laguncularia*). The red mangrove (*Rhizophora mangle*) is a common and well-known mangrove in Jamaica which can be self or wind pollinated (Bertrand, 1983). However, the upper samples contain only single pollen grains of this plant. The bottom sample from the beginning of the 19th century probably reflects a heavy hurricane event (two hurricanes occurred in the second half of 1804) which transported pollen from coastal vegetation inland. It could be facilitated by forest clearance in the 18th century for plantation establishment in Jamaica (reviewed by Evelyn and Camirand, 2003). Higher amounts of *Rhizophora* pollen in the SCH core have also been recorded in other periods of the most powerful hurricanes, including Hurricane October 1815, Jamaica 1903, Greater Antilles 1909, Jérémie 1935, Charlie 1951, and Gilbert 1988. The single increase in pollen percentage without a strong support from anemology refers to the middle of the 19th century (Fig. 4).

It is also known that hurricanes damage forest canopy and promote growth of light-demanding species such as *Cecropia*, causing a short-term increase in diversity (Tanner and Bellingham, 2006). Temporal increases in *Cecropia* pollen in the SCH core (Fig. 4) also appears to correspond with hurricane activities on the island. Although the island is a hurricane-active area (e.g., there were 12 recorded hurricanes and tropical storms on the island, from 1950, before the dreaded Hurricane Gilbert of 1988), there was a period of relative stability at the end of the first half and in the second half of the 19th century (see https://www.nlj.gov.jm/history-notes/History%20of%20Hurricanes%20and%20Floods%20in%20Jamaica.pdf). The HOM cave is located far away from mangroves, in less disturbed area, and no clear picture of hurricane activity can be observed, although also in the HOM core, the high percentage of *Cecropia* around 1951 was recorded. In general, our data support the hypothesis that pollen spectra in bat guano may be used as a proxy for tracking hurricane activities in the past.

4.4. Bats and bat-pollinated plants

Bat-pollinated plants in the New World in most cases probably evolved from hummingbird-pollinated ancestors (e.g., von Helversen and Winter, 2003). The pollen of such plants is usually bigger than in their relatives, and pollen size positively correlates with the length of flower style (Stroo, 2000). Some of the pollen grains we recorded could have been deposited directly from bats. Among commercially-valuable plants, bats are responsible for pollinating mango, guava, avocado, fig, and cashew. Among bat-pollinated plants are *Ceiba pentandra*, some Bromeliaceae (e.g. *Vriesea*), Campanulaceae (e.g. *Bermeistra*), some Cucurbitaceae (e.g. *Cayaponia*), some *Passiflora* species as well as members of the families: Anacardiaceae, Bignoniaceae, Euphorbiaceae, Fabaceae, Musaceae, Sapotaceae and others (e.g., Sazima et al., 1999; Stroo, 2000; Hequet, 2003; Muchhala, 2007; Kunz et al., 2011; see also Figs. 4 and 5).

4.5. DNA preservation in guano cores

Although DNA from arthropods was found in all layers of the guano samples, it was highly degraded and sequencing was inefficient. We encountered unusual sequencing outcomes with extreme length variation beyond the expected 157 bp and artefactual sequences with strings of ambiguous bases suggesting both amplification and sequencing problems mostly likely due to sample degradation. Therefore, some regularly used data processing steps were modified or removed to retain as much data as possible in an attempt to match sequences to references. As a consequence of the relaxation of parameters (e.g. in MEGAN), we treat the results conservatively. For example, the detection of a marine crustacean in the inland cave appears improbable, and as most sequences are from one layer, this suggests a contaminating event or a false positive. However, some cumaceans are fresh water (Jaume and Boxshall, 2008) and marine taxa could also be deposited by the marine foraging bats, *Noctilio leporinus*.

Despite the issues of data quality that we encountered, our data provide good evidence for the preservation of DNA from arthropods (mainly insects) in almost every layer of both cores and many of the BLAST similarities are to groups not normally associated with cave systems but routinely found in the diet of modern bats that exist in

these caves (Emrich et al., 2014). This suggests that the caves have been occupied by a stable community of bats throughout their history, which included a rich assemblage of insectivores, and that measuring dietary shifts through time may be possible from these ancient deposits, particularly as sequencing techniques continue to develop.

4.6. *Identification of the bat DNA*

Jamaica is home to 21 species of bats, with some of them regularly using caves as roosts, including *T. brasiliensis* (NEPA, 2009). We revealed DNA of this species in two layers in SCH Cave: the recent one and one from around 1925. Identification of bats from guano-derived DNA dates back to the early 2000 (Zinck et al., 2004). This approach has been successfully applied to fresh and modern - up to 20 years - samples (e.g., Brown et al., 2017; Walker et al., 2019; Guan et al., 2020). Overall, in all fecal DNA metabarcoding studies, the gold standard is to collect fresh feces for maximum DNA recovery. Research carried out by Walker et al. (2019) revealed that DNA in bat guano, which can be used for host identification, can be recovered up to at least 2.5 years in dry and cool roosts, but at 100% relative humidity, feces that are younger than 6 months are needed. Moisture, particularly in combination with high temperature, promotes the growth of microbes, and thus further DNA degradation.

4.7. *Conservation priorities*

In both caves, dates were chronological and thus severe layer mixing has not occurred, with the possible exception of the deepest layer in the HOM cave. Well-preserved pollen was found in one cave and insect DNA was detected in layers from both. Our data provide reasonable evidence that these cave systems have been home to insectivorous and nectarivorous bats for much if not all of the previous 4.3 millennia and still provide stable habitat. Our data also suggest that preserved guano deposits could provide a substantial and highly valuable record of climate and paleoecology from which to measure changes in ecological systems. As a consequence, and given their rarity, their preservation should be a conservation and protection should be a priority.

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Captions to figures

Fig. 1. Example of palynological matter from bat guano (A – fragment of an arthropod, P – pollen grain).

Fig. 2. Examples of examined pollen grains (1. Bombacoideae, 2. *Pithecellobium*, 3. *Ilex*, 4. *Citrus*, 5. *Erythrina*, 6. *Bursera*, 7. *Spondias*, 8. *Cedrela*, 9. Sapotaceae, 10. Malpighiaceae, 11. *Eucalyptus*, 12. *Rhizophora*, 13. *Coffea*, 14. *Casearia*, 15. *Cecropia peltata*, 16. *Theobroma*, 17. *Cocos nucifera*).

Fig. 3. Examples of examined pollen grains (1. Melastomataceae/Combretaceae, 2. Myricaceae/Casuarinaceae, 3. Asteroideae, 4. *Ambrosia*, 5. *Ipomoea*, 6. cf. *Zea mays*, 7. Poaceae, 8. *Borreria/Spermacoce*, 9. *Alternanthera*, 10. *Mimosa pudica*, 11. *Pinus*, 12. Trilete fern spore, 13. Monolete fern spore).

Fig. 4. Palynological diagram from SCH. Red taxa = cultivated plants (those marked by stars are introduced from the Old World); orange taxa = most probably include cultivated plants; squares = most important bat-related plant families (with the highest number of records in the literature — F. Mello, unpublished data); arrows = years of main hurricanes (Gilbert 1988, Charlie 1951, Jérémie 1935, Greater Antilles 1909, Jamaica 1903, October 1815).

Fig. 5. Palynological diagram from HOM. Red taxa = cultivated plants (those marked by stars are introduced from the Old World); orange taxa = most probably include cultivated plants; squares = most important bat-related plant families (with the highest number of records in the literature — F. Mello, unpublished data); arrow indicates the year of the Charlie hurricane strike in 1951 (see also the dark green peak in Urticaceae: *Cecropia* around 1952).

Fig. 6. Arthropods identified in guano in SCH overlaid on a diagram of the taxonomic hierarchy. Proportion of haplotypes assigned are given by pie charts. The oldest

sequences belong to moths and butterflies (Lepidoptera), sawflies, wasps and bees (Hymenoptera), and grasshoppers, crickets and their relatives (Orthoptera).

Fig. 7. Arthropods identified in guano in HOM overlaid on a diagram of the taxonomic hierarchy. Proportion of haplotypes assigned are given by pie charts. Negative values for dating indicate years BC. The oldest sequences belong to caddisflies (Trichoptera), beetles (Coleoptera) and flies (Diptera).

Figure 1

Click here to access/download;Figure;Guano_Fig. 1.jpg

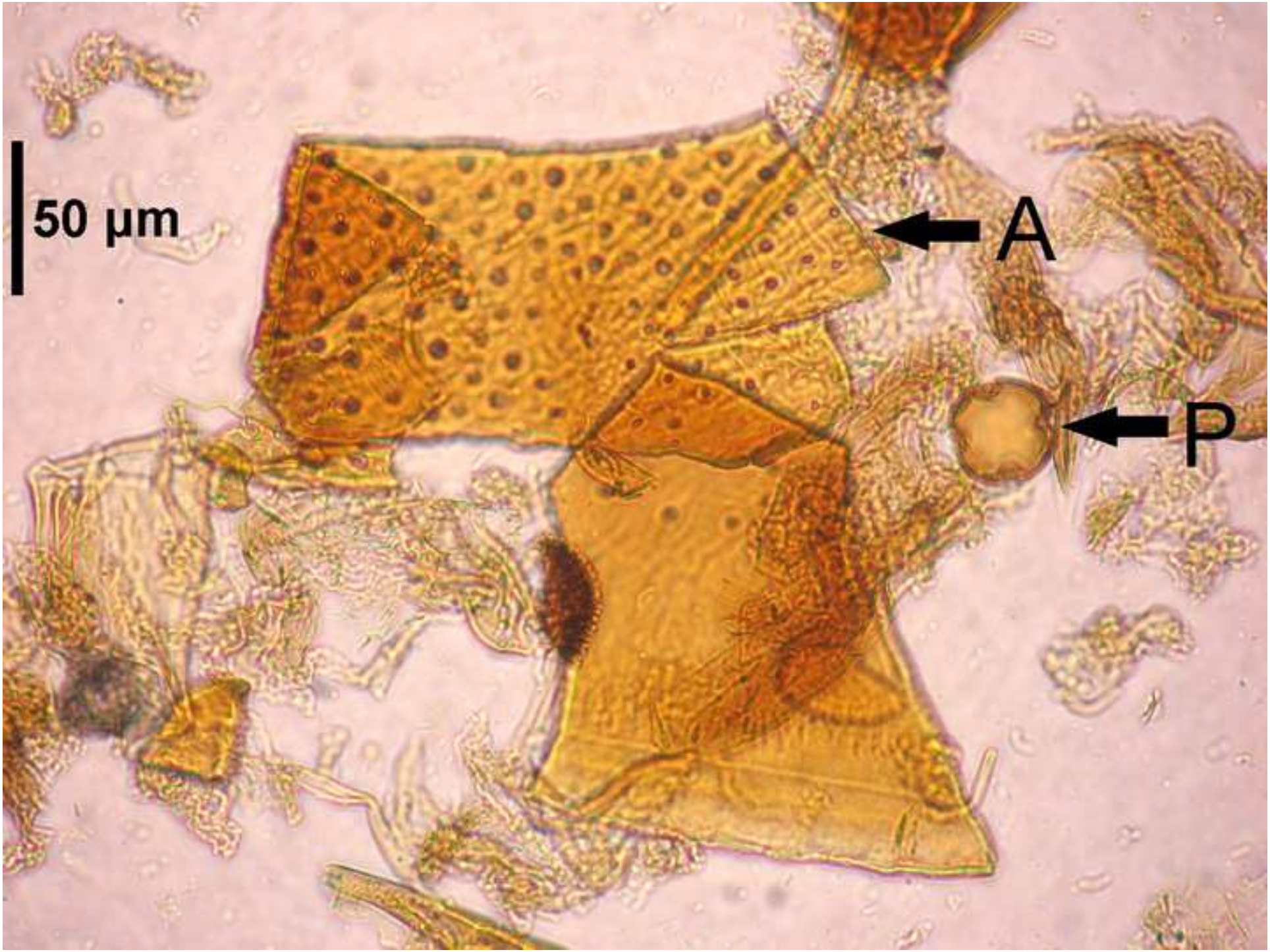

Figure 2

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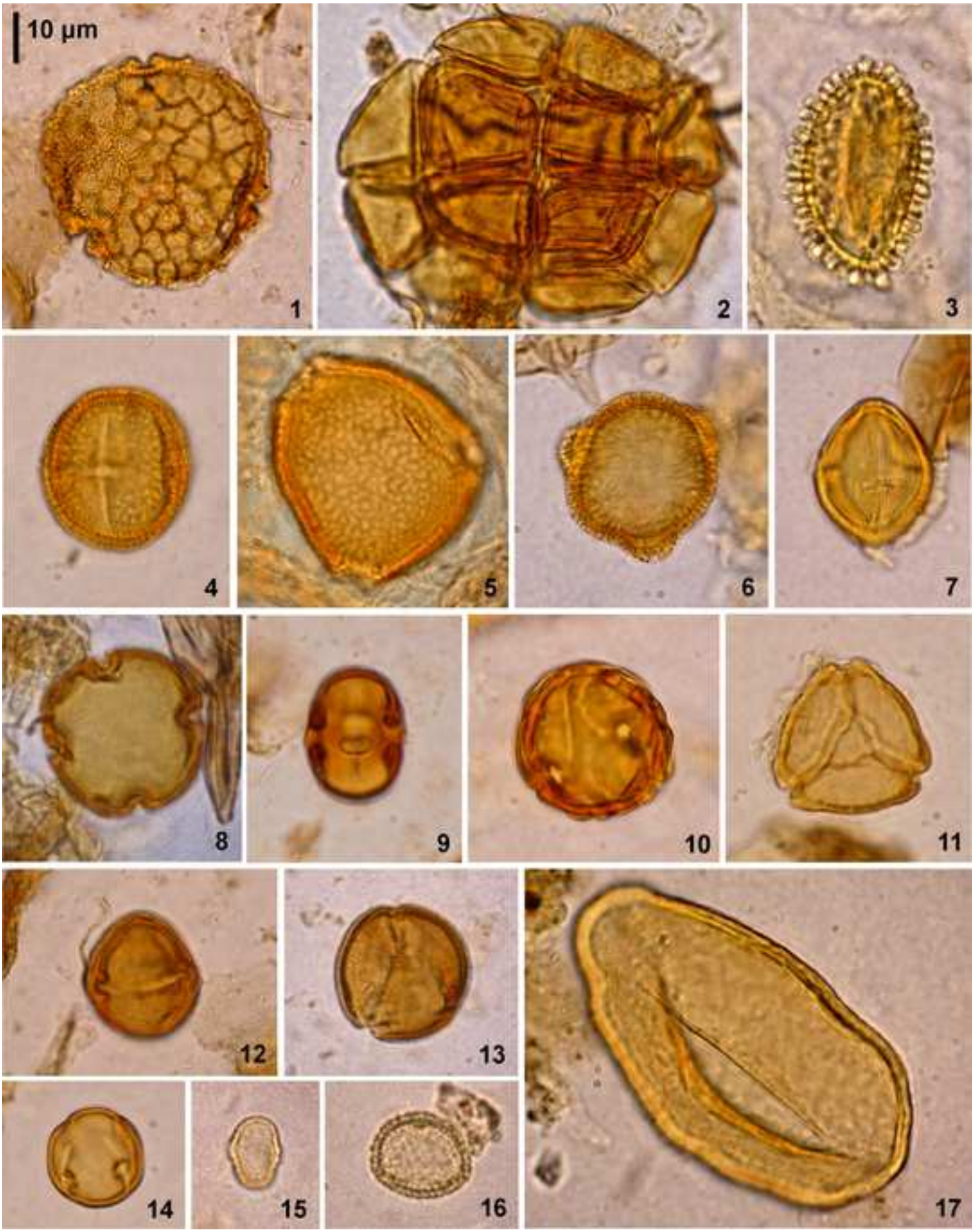

Figure 3

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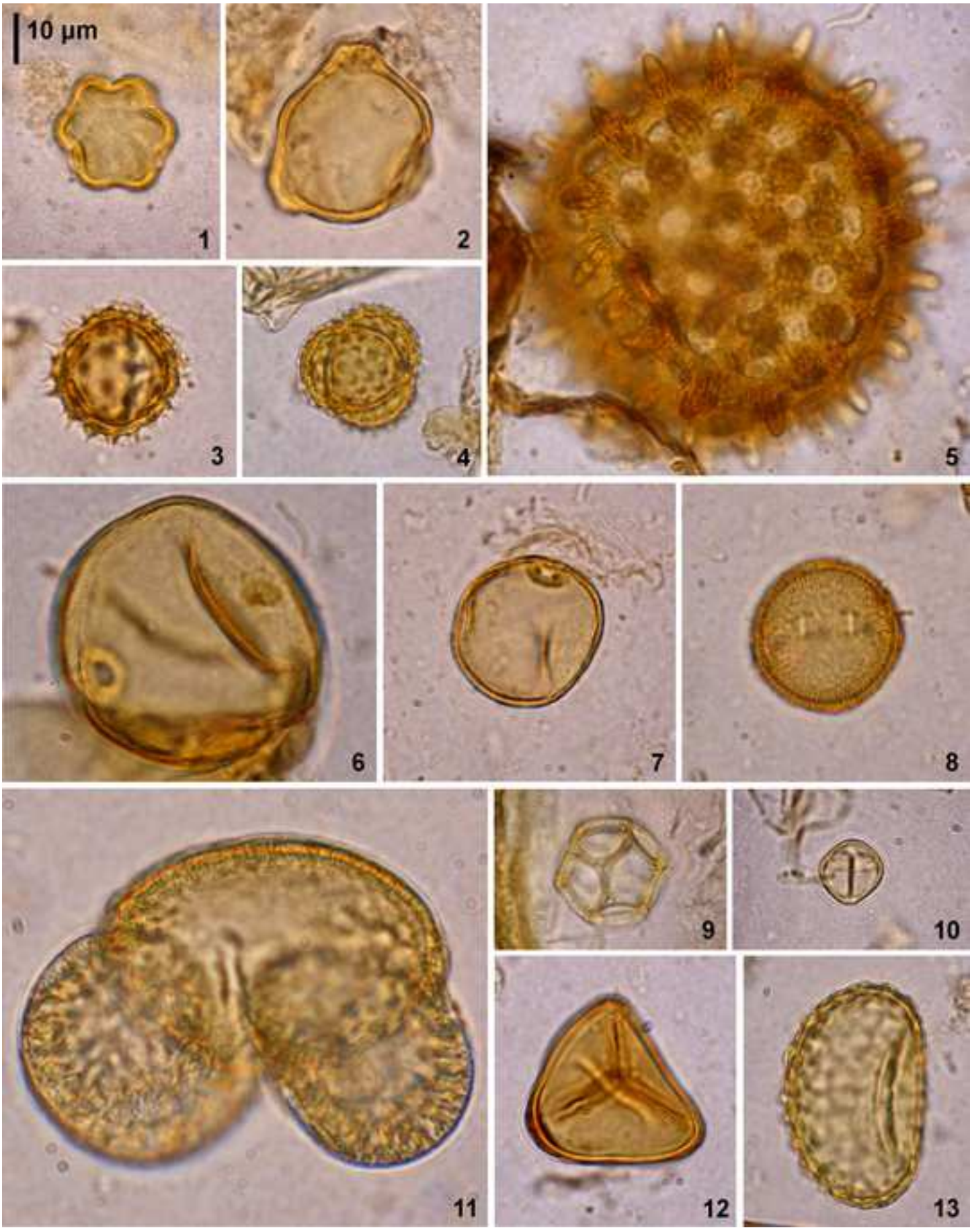

Figure 4

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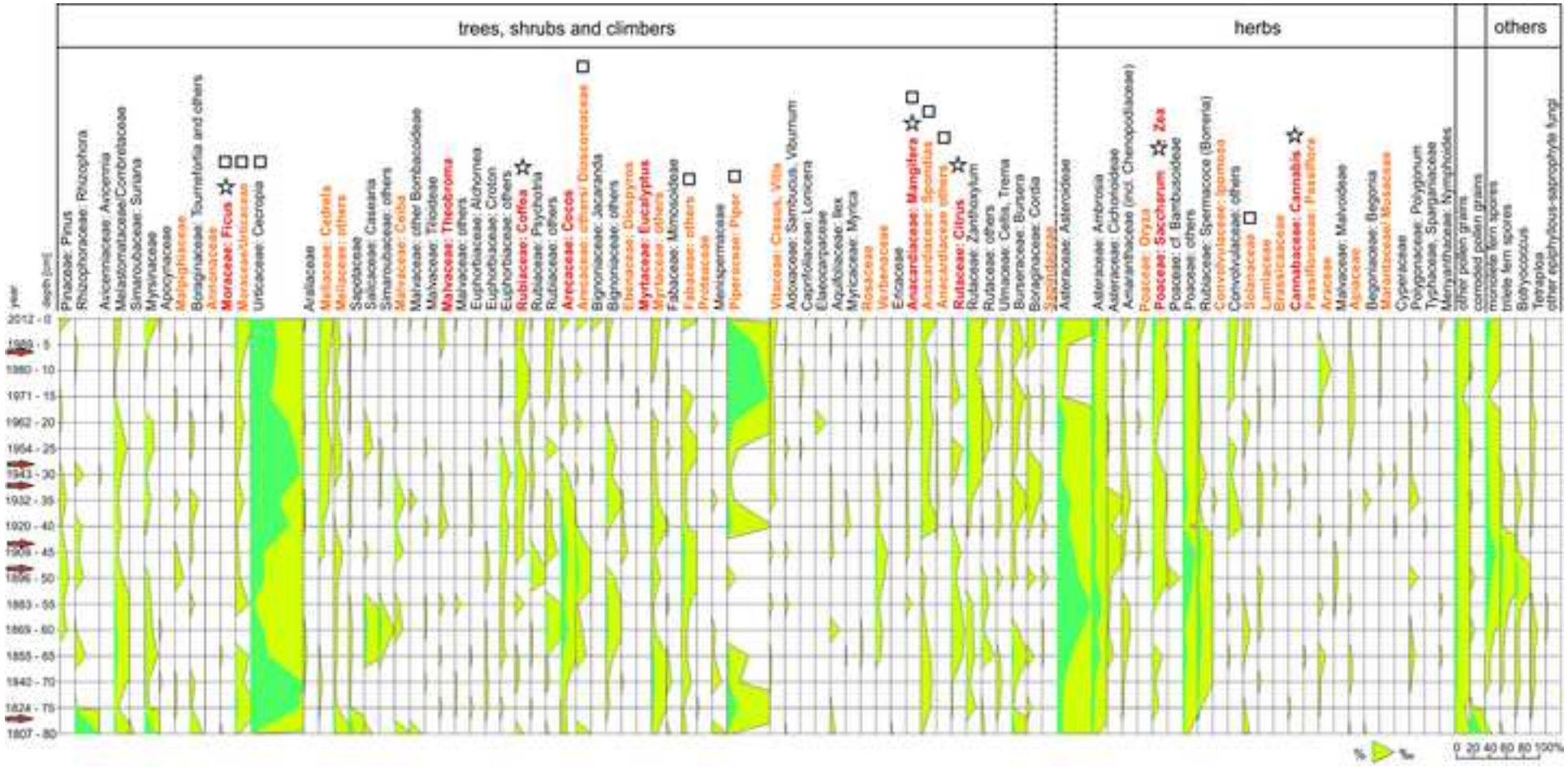

Figure 5

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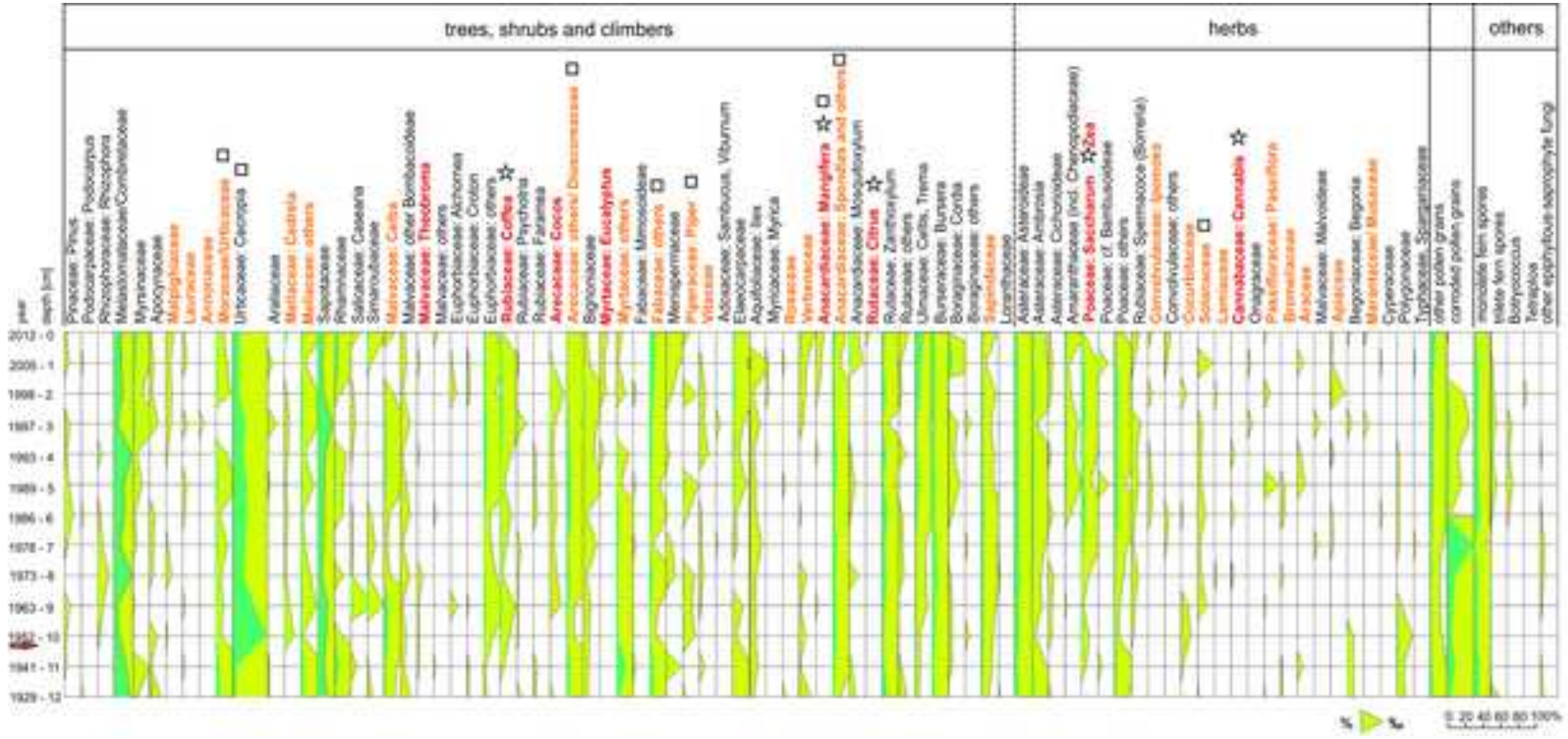
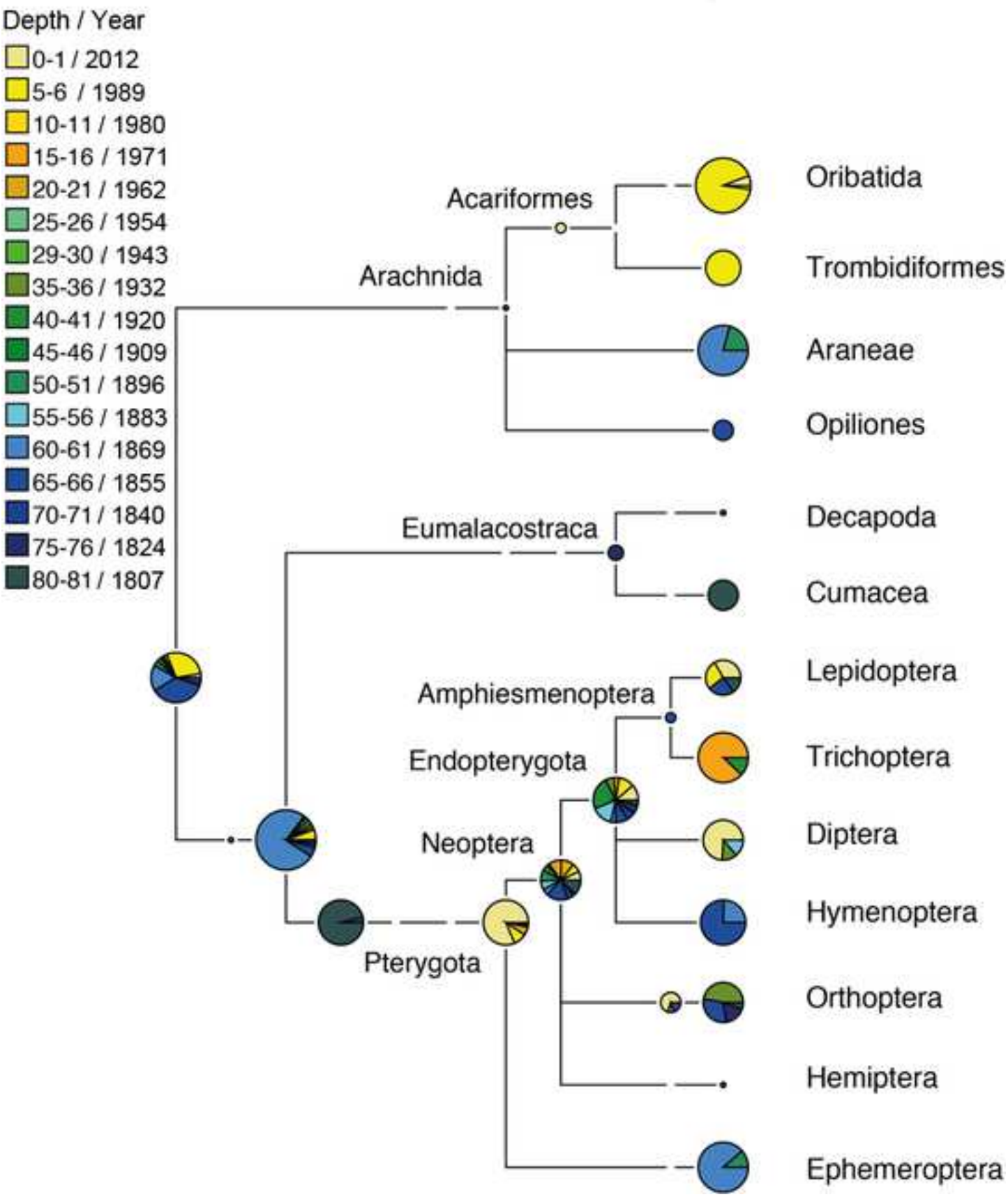


Figure 6



Arachnida
Trombidiformes
Araneae
Eumalacostraca
Decapoda
Cumacea
Amphiesmenoptera
Lepidoptera
Trichoptera
Endopterygota
Coleoptera
Neoptera
Diptera
Hymenoptera
Blattodea
Orthoptera
Hemiptera

Depth / Year

- 0-1 / 2008
- 7-8 / 1978
- 13-14 / 1916
- 21-22 / 1235
- 29-30 / 1176
- 42-43 / 804
- 50-51 / 207
- 56-57 / -164
- 63-64 / -697
- 70-71 / -850
- 77-78 / -947
- 85-86 / -1059
- 94-95 / -1151
- 100-101 / -1556
- 107-108 / -1760
- 114-115 / -1988
- 121-122 / -2320
- 128-129 / -2307

Supplementary Material

Fig. S1. ^{210}Pb dating profile of the SCH core. Two models compared: Constant Flux and Sedimentation Rate (CFSR) and Constant Rate Supply (CRS). A 2nd order polynomial equation ($y = -0.0121x^2 - 1.3606x + 1995.7$, $R^2 = 0.999$) was applied to the ^{210}Pb dates and the single ^{14}C date at 78.5 cm (1814 cal CE) using the CFSR model.

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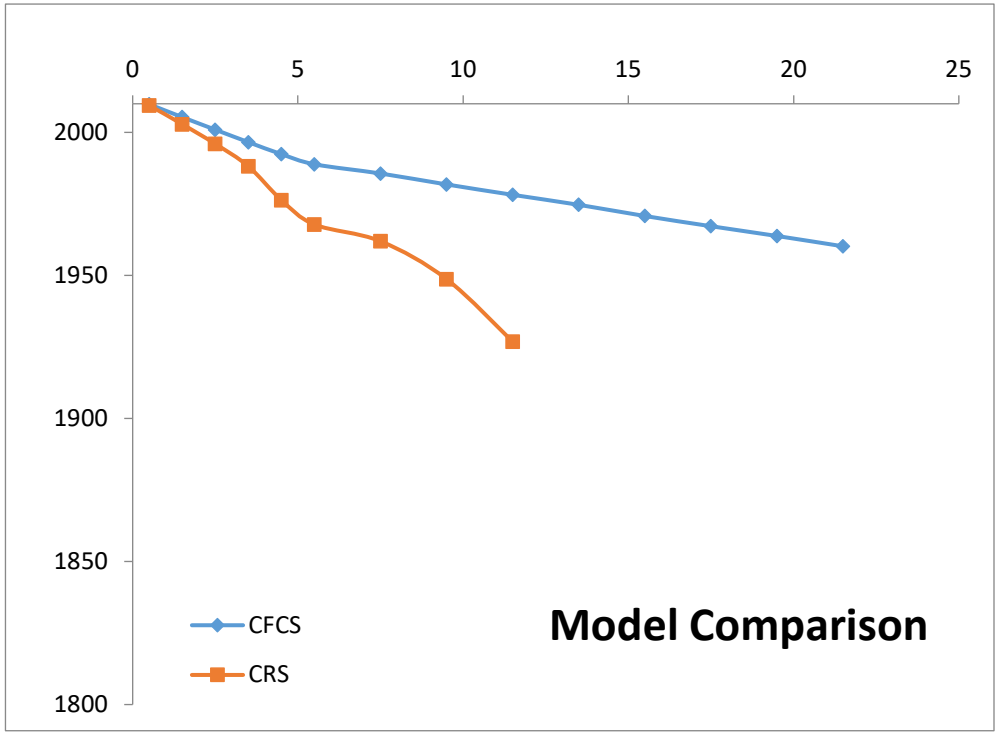
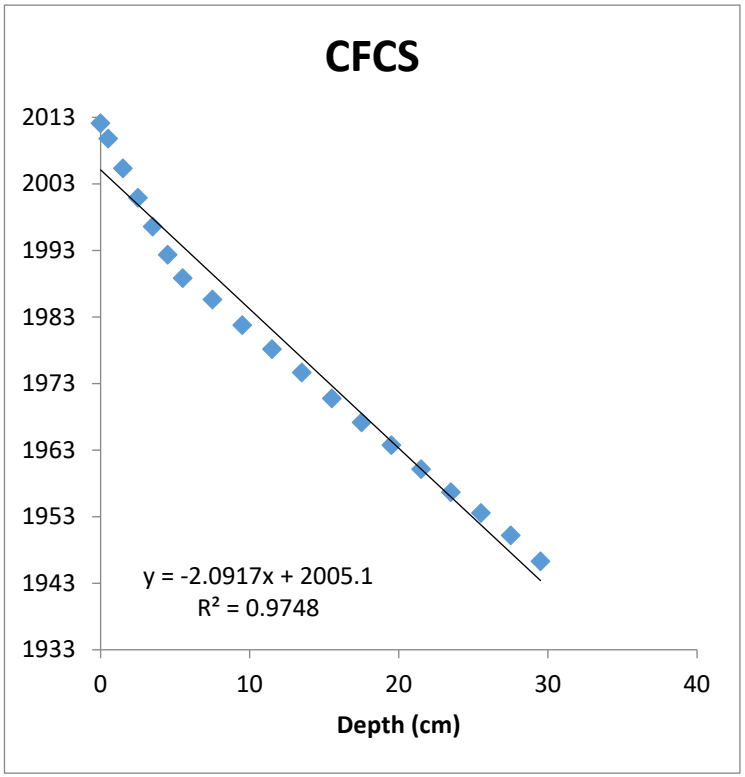
^{210}PB dating profile						Constant Flux & Sedimentation Rate - Profile					Constant Rate of Supply - Fit				
"Midpoint Depth"	Cumul. Drymass	Activity	Error (Activity)	Inventory	Error (Inventory)	Age (CFCS)	Year (CFCS)	Error (CFCS)	Sed. Rate (CFCS)	Error (CFCS)	Age (CRS)	Year (CRS)	Error (CRS)	Sed. Rate (CRS)	Error (CRS)
[cm]	[g/cm^2]	[Bq/kg]	[Bq/kg]	[Bq/m^2/yr]	[Bq/m^2]	[y]	[y]	[y]	[g/cm^2/y]	[g/cm^2/y]	[y]	[y]	[y]	[g/cm^2/y]	[g/cm^2/y]
0	0.00	95.43	20.31	4653.49	117.41	0.00	2012.15	0.00	0.18	0.12	0.00	2012.15	0.00	0.15	0.03
0.5	0.41	90.26	52.38	4271.73	119.39	2.31	2009.84	1.52	0.18	0.12	2.75	2009.40	0.13	0.15	0.09
1.5	1.22	105.57	54.12	3483.99	127.22	6.82	2005.33	4.49	0.18	0.12	9.29	2002.85	0.17	0.10	0.05
2.5	2.00	66.14	52.23	2812.00	133.68	11.21	2000.94	7.37	0.18	0.12	16.18	1995.97	0.41	0.13	0.10
3.5	2.77	90.42	54.26	2204.67	140.24	15.56	1996.59	10.24	0.18	0.12	23.99	1988.16	0.94	0.08	0.05
4.5	3.52	91.24	54.33	1525.99	146.07	19.75	1992.39	12.99	0.18	0.12	35.81	1976.34	1.98	0.05	0.03
5.5	4.15	21.94	49.87	1169.86	149.41	23.28	1988.86	15.32	0.18	0.12	44.34	1967.81	3.00	0.17	0.38
7.5	4.73	45.68	50.81	974.78	152.27	26.52	1985.63	17.45	0.18	0.12	50.20	1961.95	3.91	0.07	0.07
9.5	5.41	50.84	46.30	645.28	155.54	30.35	1981.80	19.96	0.18	0.12	63.45	1948.70	6.63	0.04	0.04
11.5	6.06	47.80	52.02	326.49	159.14	33.98	1978.17	22.35	0.18	0.12	85.32	1926.82	14.54	0.02	0.03
13.5	6.68	57.20	48.72			37.47	1974.68	24.64	0.18	0.12					
15.5	7.38	37.44	52.04			41.37	1970.77	27.21	0.18	0.12					
17.5	8.01	26.16	37.08			44.95	1967.20	29.57	0.18	0.12					
19.5	8.62	29.50	44.28			48.37	1963.78	31.81	0.18	0.12					

21.5	9.26	26.45	41.36	51.97	1960.17	34.19	0.18	0.12
23.5	9.89	44.51	40.54	55.46	1956.69	36.48	0.18	0.12
25.5	10.44	19.40	47.13	58.58	1953.57	38.53	0.18	0.12
27.5	11.04	20.34	46.25	61.95	1950.20	40.75	0.18	0.12
29.5	11.73	36.49	40.50	65.83	1946.32	43.30	0.18	0.12


0
5
10
15
20
25
2000
1950
1900
1850
1800
CFCS
CRS
Model Comparison


CFCS
2013
2003
1993
1983
1973
1963
1953
1943
1933
y = -2.0917x + 2005.1
R² = 0.9748
0
10
20
30
40
Depth (cm)

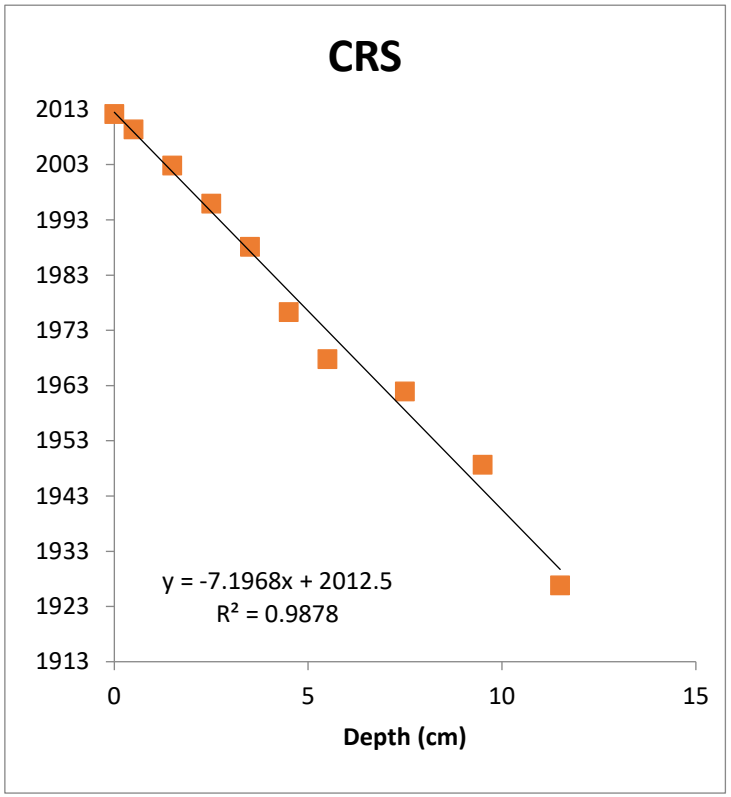

CRS
2013
2003
1993
1983
1973
1963
1953
1943
1933
1923
1913
y = -7.1968x + 2012.5
R² = 0.9878
0
5
10
15
Depth (cm)

Fig. S2. Proportion of anemophilous (blue bars) versus zoophilous (red bars) pollen types from SCH.

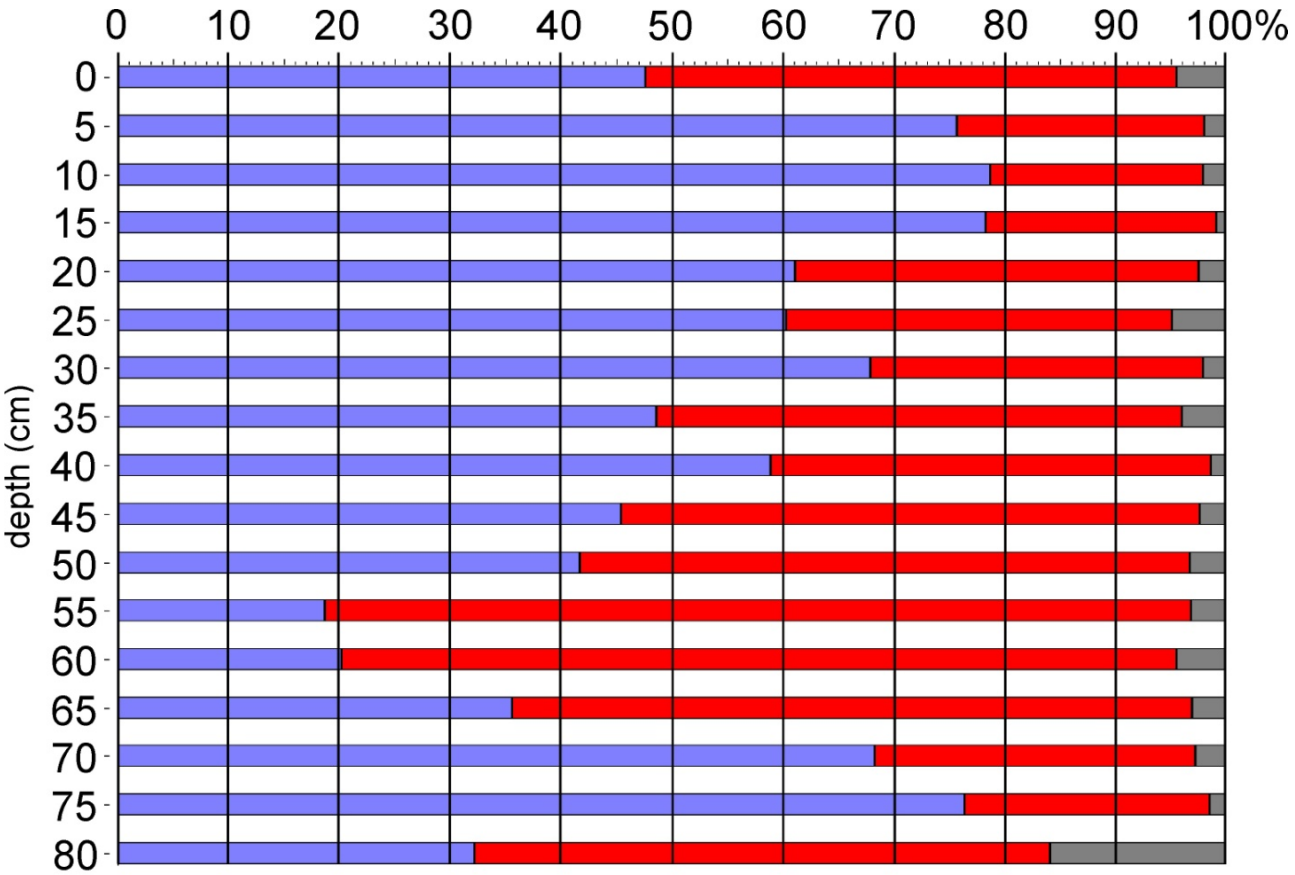
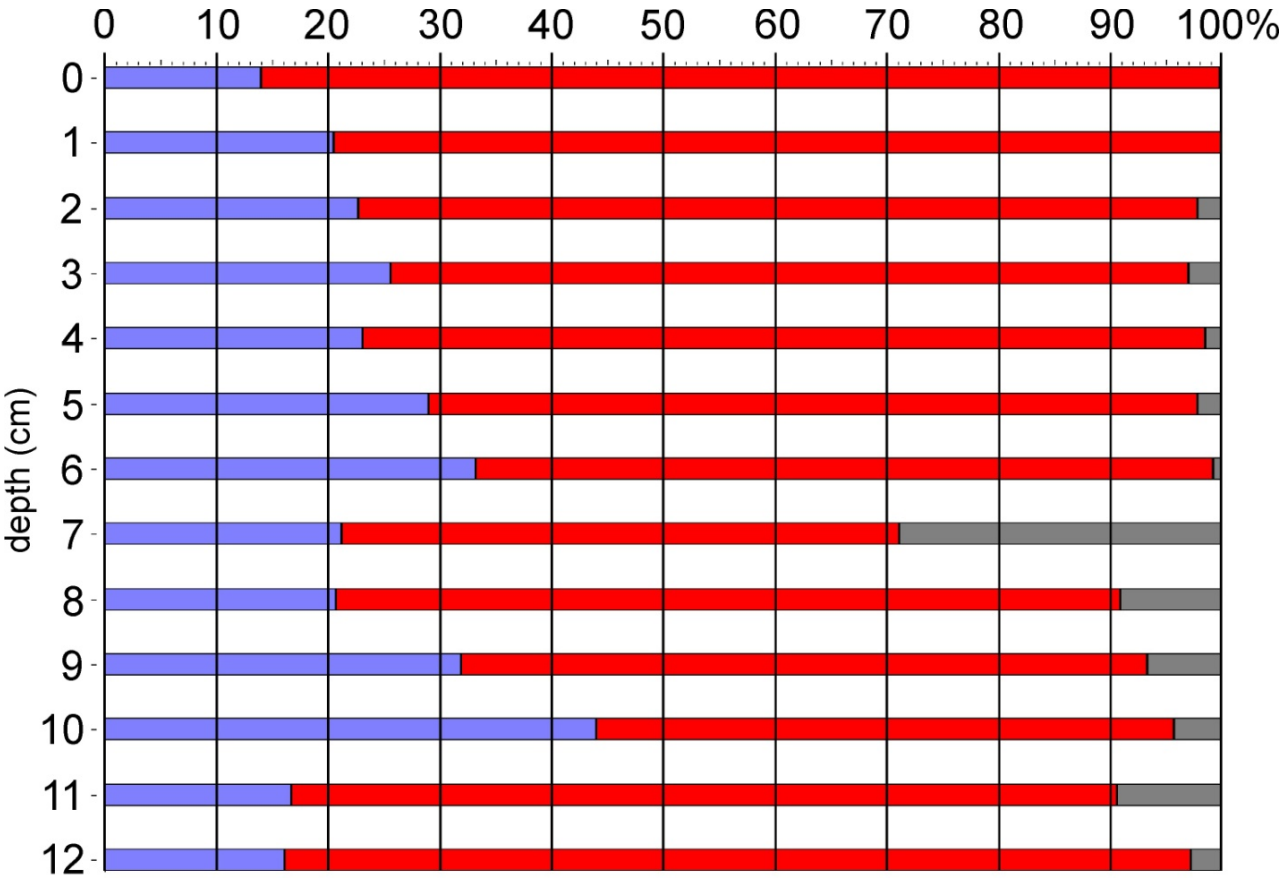


Fig. S3. Proportion of anemophilous (blue bars) versus zoophilous (red bars) pollen types from HOM.



Conflict of Interest

The authors have no competing interests to declare.

Quaternary International

We the authors declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us. We understand that the Corresponding Author is the sole contact for the Editorial process. He is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs.

Sincerely,
Prof. Wiesław Bogdanowicz

On behalf of all authors