

A review of preservational variation in fossil inclusions in amber of different chemical groups

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Running head: Preservation in amber

11 ABSTRACT:

12 Keywords: Fossilization, amber, resin, taphonomy

13 Fossils in amber are a particularly important and unique palaeobiological resource.

14 Amber is best known for preserving exceptionally life-like fossils, including microscopic

15 anatomical details, but this fidelity of preservation is an end-member of a wide-spectrum of

16 preservation quality. Many amber sites only preserve cuticle or hollow moulds, and most

17 amber sites have no fossils at all. The taphonomic processes that control this range in

18 preservation are essentially unknown. Here we statistically analyse the relationship between

19 amber groups and fossil preservation based on published data to determine whether there is a

20 correlation between resin type and aspects of preservation quality. We found that ambers of

21 different chemistry demonstrated statistically significant differences in the preservational

22 quality and the propensity of a site to contain fossils. This indicates that resin chemistry does

23 influence preservational variation; however, there is also evidence that resin chemistry alone

24 cannot explain all the variation. To effectively assess the impact of this (and other) variables

25 on fossilization in amber, and therefore biases in the amber fossil record, a more

26 comprehensive sampling of bioinclusions in amber coupled with rigorous taphonomic

27 experimentation is required.

Exceptional soft tissue preservation is known from amber (fossil plant resin) and copal (subfossil plant resin) from the Triassic (Schmidt et al. 2012) to the Recent (Lambert et al. 1995; Ragazzi et al. 2003; Wunderlich 2004). Amber preservation provides a unique window into ancient ecosystems as it tends to sample organisms that are otherwise underrepresented in the fossil record: small, soft bodied terrestrial organisms (Penney 2002; Labandeira 2014). Fossils in amber also provide an exceptional record of behaviour, including herbivory, parasitism, pollination, mimicry, and mating (Penney & Jepson 2014; Labandeira 2014; Peñalver et al. 2015; Pérez-de la Fuente et al. 2016; Wang et al. 2016). Along with such records the abundance and diversity of fossils preserved in the best amber fossil sites provides ecological data with which fossil communities can be explored (Penney & Langan 2006; Labandeira 2014; Penney & Preziosi 2014; Saint Martin et al. 2014). As such, the data from fossils in amber are a particularly important and unique palaeobiological resource for reconstructing tropical forest ecosystems and predicting consequences of ongoing biotic crises, providing minimum dates for major radiations/extinction events and resolving relationships of modern taxa (Penney 2010; Penney 2016).

There is a wide range of variation in the preservation of fossils in amber. Some of the organisms entombed in amber record high fidelity aspects of morphology such as the internal anatomical features and even parasites (Martínez-Delclòs et al. 2004; Rust et al. 2010; Labandeira 2014; Mazur et al. 2014). The best preservation known occurs in the Dominican and Baltic ambers, and includes insect flight muscles, air sacs, brain and neural tissues, mitochondria with cristae and endoplasmic reticulum (Henwood 1992; Grimaldi et al. 1994), and some biomolecules (Martínez-Delclòs et al. 2004). However, it seems unlikely that DNA is preserved in amber as it was not possible to recover it from the much younger copal precursor (Penney et al. 2013). Incredibly life-like insects are only one end-member of a wide-spectrum of preservation quality in amber (Martínez-Delclòs et al. 2004) as many

amber sites only preserve cuticle and the majority have no fossils (Martínez-Delclòs et al. 2004). The taphonomic processes that control this vast range in preservation quality are almost completely unknown. To get the most from the amber fossil record we need to understand the biases and filters that have operated to preserve (or destroy) animals entombed in resin.

Resin chemistry has been implicated as the principal control on the exceptional preservation of fossils in amber (Henwood 1992; Stankiewicz et al. 1998; Labandeira 2014). The intuitive corollary of this is that the extensive chemical variation seen in both modern resin and fossil amber (Lambert et al. 2008; Lambert et al. 2012; Labandeira 2014; Lambert et al. 2015) may influence variation in preservation occurrence and quality of entombed organisms. Here we examine the literature of fossilization in amber to assess the evidence for and against the hypothesis that resin chemistry influences preservation. The primary purpose of this paper is to statistically compare various amber sites to determine if there is any significant difference in fossilization of entombed organisms between amber of different chemistries. We also qualitatively explore evidence which suggests that resin chemistry cannot account for all the variation in preservation, and remark on other variables that may influence fossilization in amber. Finally, we discuss what further analyses are needed to more carefully test this hypothesis.

1. Resin chemistry

1.1. Resin chemistry and preservation.

The chemical and physical properties of resin (later amber) are thought to prohibit scavengers and decomposition of organisms and result in their exceptional preservation. Resin forms a physical barrier to microbial penetration, dehydrates tissues, and contains antimicrobial and antifungal chemicals (Poinar & Hess 1982; Poinar & Hess 1985; Stankiewicz et al. 1998; Martínez-Delclòs et al. 2004; Labandeira 2014). Experiments to date

79 have tested this general model of amber preservation and have demonstrated that physical
80 sealing alone is not sufficient to inhibit decay; organisms sealed in wax decayed, in some
81 cases more quickly than in unsealed control experiments (Henwood 1992). However, sealing
82 an organism in maple syrup (which is chemically distinct from wax) inhibited decay
83 compared to organisms placed in unsealed control conditions (Henwood 1992). This
84 experiment demonstrated that the chemistry of a sealing medium influences the decay rate of
85 an entombed organism; under some chemical conditions (i.e. in wax) decay is enhanced,
86 whereas in other chemical conditions (i.e. in maple syrup) decay is inhibited. This strongly
87 suggests that the exceptional preservation of bioinclusions in amber must be due, in some
88 part, to the decay-inhibiting effects of resin chemistry rather than to physical sealing
89 (Henwood 1992). Analyses of bioinclusions in amber further supports this, indicating that
90 when an organism is entombed in resin, some of the volatile compounds of the resin infiltrate
91 the tissue of the organism and form chemical cross-links that prevent tissue decay
92 (Stankiewicz et al. 1998). Based on these experiments and analyses of fossils in amber, resin
93 chemistry is generally assumed to inhibit decay and promote preservation. However, resin
94 chemistry is highly variable, and the effects of resins of different chemical compositions on
95 decay have never been robustly tested. Neither wax nor maple syrup is chemically analogous
96 to any form of resin, which are composed of terpenoid and phenolic compounds (Labandeira
97 2014). Paraffin wax is composed of hydrocarbon alkanes and maple syrup is composed of
98 sugars. Some resins may be more analogous to wax in their effects on decay, in that
99 entombed organisms may decay unusually quickly, which could explain amber sites without
100 fossils. It has also been suggested that certain tissues and biomolecules, particularly DNA,
101 degrade more quickly in resin than in other conditions: DNA could not successfully be
102 extracted from inclusions in Recent copal, even though it could be extracted from pinned
103 museum specimens of a similar age (Penney 2013). Other resins may inhibit the decay of

entombed organisms, but to different degrees, resulting in fossils of varying preservational quality. In short, currently it is suspected that resin chemistry may play a significant role in preservation quality, but data to support this are few and no robust analyses across different deposits have been undertaken. To begin to test the hypothesis that variations in resin chemistry can influence the decay of entombed organisms, and thus control preservational quality we evaluate data surveyed from current literature. We compare resin chemistry of amber sites and fossil occurrence and preservational quality within the sites (e.g. no fossil inclusions, poorly-preserved fossil inclusions, well-preserved fossil inclusions). Using our analyses we then review published evidence for and against this hypothesis. If the null hypothesis (i.e. that resin chemistry does not influence occurrence and quality of preservation in amber) is rejected, we predict that there will be little variation in fossil preservation between amber sites of similar chemistry, and more variation in fossil preservation between amber sites of different chemistry.

This is important because if resin chemistry is the principal or only control on preservation in amber, it is unlikely to impart any significant biases on preservation occurrence and quality within a specific amber fossil site, assuming that all amber was sourced from a single tree type and thus had a similar resin chemistry. Any organism entrapped in resin is equally likely to be preserved (although there will still be size and behaviour biases in entrapment), resulting in a preserved assemblage that accurately reflects a known portion of the original life assemblage. Moreover, if resin chemistry does indicate a reliable indicator of preservation quality it would provide a simple diagnostic test to determine whether new sites should preserve exceptional anatomical and soft tissue details. Conversely, if resin chemistry does not control preservation, or if there are other significant factors at play, then the fossil record of amber may not directly reflect an original life

assemblage; more investigation would be needed to determine what processes have filtered the record.

1.2. Chemical classification of resin and amber.

Resin is one of many biological substances secreted by plants (others include gum, wax, sap, latex, oil, and mucilage) which polymerizes over time into a sub-fossil form and then a fossil form – copal and amber, respectively (Lambert et al. 1993; Lambert et al. 2015). The change from resin to copal to amber is a continuum, and although some authors have attempted to define the three substances based on age, chemistry or physical properties, there is no agreed demarcation between the three groups (Anderson 1996; Vavra 2009; Penney & Green 2010). In general, amber is harder and chemically more inert than either copal or resin, and copal is harder than resin but chemically very similar (Labandeira 2014).

Resins are distinguished from other plant exudates by their chemistry; they are complex compounds composed primarily of terpenoids and phenolic compounds, supplemented with a number of secondary compounds (Langenheim 1990; Labandeira 2014). The chemical composition of any specific resin flow is influenced by a number of factors, including the botanical source, the time of year, the water and nutrient conditions in the environment, the metabolic processes of the individual tree, and the plant organ producing the resin (e.g. roots vs. trunk) (Langenheim 1995; Martínez-Delclòs et al. 2004). Despite this, analysis of hundreds of samples of resin and amber suggests that the chemical variation can be roughly encapsulated by a few categories (Labandeira 2014; Lambert et al. 2015). There are primarily two classification schemes, based to some extent on different techniques of analysing the samples, but the resulting categories are basically equivalent, supporting the robustness of amber chemical classification (Lambert et al., 2008; Labandeira, 2014; Lambert et al., 2015).

One classification scheme is based on solid state ^{13}C and solution ^1H nuclear magnetic resonance (NMR) spectroscopy of fossilized amber, which suggests five major chemical groups (A, B, C, D, and E) (Lambert et al., 2008; Labandeira, 2014; Lambert et al., 2015). This classification scheme has also been applied to modern resins (Lambert et al. 2008; Lambert et al. 2012). Group A is found worldwide, from the Triassic to the Recent, and is by far the most common (Table 1) (Lambert et al. 1990; Lambert et al. 1995; Lambert et al. 1993; Lambert et al. 2012; Lambert et al. 2015). Although, in general, it is difficult to determine the botanical source of amber (Langenheim 2003), Group A is most likely produced by a member of the Araucariaceae family; kauri gum, from the modern Araucariaceae tree *Agathis australis* is the most similar modern resin, but there may be a number of trees that produced comparable resin through time (Lambert et al. 1993; Lambert et al. 2015). Group B also has a worldwide distribution and is known from the Carboniferous to the Recent (Bray & Anderson 2009; Lambert et al. 1996; Lambert et al. 2013; Lambert et al. 2015). Comparisons of Group B amber to the chemical signature of modern resins supports a Dipterocarpaceae source (Lambert et al. 2013; Lambert et al. 2015). Group C is the Eocene Baltic amber, one of the best known and most fossiliferous sources of amber which is found in a variety of sites around the Baltic region (Weitschat et al. 2010; Lambert et al. 2015). This amber group is chemically very similar to Group A amber, but the two groups can nonetheless be reliably distinguished (Lambert et al. 2015). The botanical source of Baltic amber is debated because all modern resins have a notable chemical differences to it (Langenheim 1995; Wolfe et al. 2009); it is generally agreed to be from a coniferous source (Lambert et al. 2015). Group D is Miocene Dominican amber (including other very similar Mexican and South American ambers) (Penney 2010; Lambert et al. 2015), which are chemically very similar to the resin produced by the modern (angiosperm) genus *Hymenaea*, and it is thought to have been produced by the extinct species *Hymenaea protera* (Poinar

1991; Poinar & Poinar 1999; Lambert et al. 2015). Group E is a rare and unusual amber composed of fossil polystyrene and is found from the Cretaceous of New Jersey. Due to the similarities between Group E amber and modern *Liquidambar* resin, this amber is also thought to be produced by trees in the Hammelimiidae family (Lambert et al. 2015).

The other classification scheme is based on chemical structural characterization, often (though not exclusively) through pyrolysis gas chromatography (PY-GC-MS), of modern resins and fossil amber, and suggests seven classes of amber and resin (Ia, Ib, Ic, II, III, IV, and V), that overlap almost exactly with the NMR chemical groups (Anderson & Winans 1991; Anderson et al. 1991; Anderson 1994; Beck 1999; Lambert et al. 2008; Labandeira 2014). Class Ia is Group C, Class Ib is Group A, Class Ic is Group D, Class II is Group D, and Class III is Group E (Lambert et al. 2008; Lambert et al. 2015). Classes IV and V are almost unknown in the fossil record, and there are no equivalent groups in the NMR-based classification scheme. The few fossil amber specimens that fall into Class IV or V are considered to be Group A based on NMR analysis (Supplemental Table 1) (Anderson & Botto 1993; Nel & Prokop 2005; Colchester et al. 2006; Lambert et al. 2012).

There is a third, somewhat informal classification scheme that is not based upon chemical analysis, which divides fossiliferous ambers into named types based on similarities of age, geographical location, and entombed biota. Examples of categories in this classification scheme include Dominican amber, Baltic amber, and Lebanese amber (Penney 2010). There are 13-26 (the numbers vary depending on the author) named types of amber, each of which is a subset of a chemical group (Penney 2010; Lambert et al. 2015). The sites within a named type are generally more chemically similar to each other than to other sites in the same chemical group (Lambert et al. 2012).

That resin (and amber) chemistries are grouped based on different schemes, formal and informal, makes it challenging to select categories for statistical analyses of the

relationship - if any - between resin chemistry and preservation. For the analyses herein we use the Group or Class systems (which are equivalent) to categorize amber sites by their chemistry, with a few other analyses based on the informal categories. Although resin chemistries even within Groups will vary, we expect the differences between the Groups to be larger than the differences within the Groups. Moreover, subdividing chemistry-type categories (i.e. within amber Groups) would divide the data to an extent that would preclude statistical analysis. Finally, although some of the chemical Groups are more similar than others (i.e. Groups A and C are more similar to each other than to Group B (Lambert et al., 2008; Lambert et al., 2015)), we employ statistical analyses for categorical data, which makes no assumptions about the degree of similarity of categories. For these types of analyses it does not matter if some chemical Groups are more similar than others.

1.3. Resin chemistry and preservational quality

Many bioinclusions in amber, when examined with the naked eye or light microscopy, look perfectly preserved even down to tiny morphological details (Penney 2010; Labandeira 2014). However, even these apparently perfect specimens vary widely in preservational quality: some are empty voids in the resin, with a thin carbon film providing the appearance of tissue (figure 1 A-C); some retain external tissues such as cuticle remnants, which themselves range from well-preserved to significantly degraded; others have some remnants of internal soft tissues (figure 1 D-G); and some contain well-preserved soft tissues (Martínez-Delclòs et al. 2004; Labandeira 2014). Until recently, assessing the preservational quality of bioinclusions in amber was time-consuming and destructive; typically it required breaking open or dissolving the amber and actually dissecting or chemically analysing the inclusion (Henwood 1992; Grimaldi et al. 1994; Stankiewicz et al. 1998; Penney & Green 2010; Rust et al. 2010; Labandeira 2014). More recently, technology such as CT-scanning and synchrotron tomography has been used to determine the quality of preservation of both

internal and external structures in fossils preserved in amber (Dierick et al. 2007; Lak et al. 2008; Penney & Green 2010; Soriano et al. 2010; Labandeira 2014).

A total of 106 terrestrial arthropod fossils in amber have been investigated using traditional dissecting methods, CT scanning, or synchrotron analysis (Table 2, Supplementary Table 2) and these form the basis of our investigation. A few such studies have also focused on other groups (e.g. flowers, lizards, etc.) (Moreau et al., 2014; del Rosario Castañeda et al., 2015; Serano-Sanchez et al., 2015; Sherratt et al., 2015) but we restrict this analysis to terrestrial arthropods to minimize variation due to the preservation potential of the inclusion. Moreover, we compare site to site rather than inclusion to inclusion, which helps to decrease the effects of inclusion-specific variables on preservation.

Here we consider ‘well-preserved’ specimens to be those with preserved internal soft tissue structures (figure 1 D-G), and ‘poorly-preserved’ specimens as those that are moulds or preserve cuticular anatomy only (figure 1 A-C) (Table 2, Supplemental Table 2). Note that whilst cuticle is nonbiomineralized it is recalcitrant and more decay resistant than the internal soft tissues. Indeed, it has been shown to survive longer than internal soft tissues in laboratory experiments under all conditions tested (Briggs 2003). Assessing preservational quality based on this dichotomy of the presence or absence of internal soft tissue preservation also matches the level of information provided in most of the published literature where preservation quality is not described in any great detail, but the presence or absence of internal soft tissue anatomy is usually recorded (but where more detailed descriptions are provided they are noted in Supplemental Table 2). In some cases where the published description did not mention the presence or absence of internal structures (e.g. Saupe et al. 2012), we were able to examine the synchrotron images to determine whether or not internal structures were preserved (Table 2).

In our survey, most of the specimens (92 out of the 106) could be assigned to one of the chemical Groups A-E based on previous analyses of amber from the same site (Table 2, Supplemental Table 2). All of the groups except E are represented; however, no fossils are known from Group E amber sites, so that specific type of resin is not relevant for this investigation. Fossils in Group B amber have been dissolved out of the amber and dissected, but no information was given in the paper about the presence or absence of internal structures (Mazur et al. 2014), so Group B is also effectively not represented in our dataset.

Each of Groups A, C, and D include specimens with and without preserved internal soft tissues, suggesting that a range of resin chemistries allows for internal soft tissue preservation of bioinclusions. However, the percentage of fossils that preserve internal soft tissue structures (which relates to the average preservational quality of bioinclusions in that chemical group) varies between the three groups: 12%, 55%, and 82% of analysed samples in Groups A, C and D, respectively, preserve internal soft tissue structures. A power analysis, using the program R (Team 2014), indicates we can only statistically compare Group A and each of Groups C and D (Table 3).

Fisher Exact tests reveal that the bioinclusions in Group A amber have significantly lower preservational quality than the bioinclusions in Group C or Group D amber ($p = 0.001$ and $1.86E-08$ respectively) (Table 3). This supports the idea that there is a difference in preservational quality in bioinclusions entombed in resins of different chemistry.

However, the data also appear to suggest that there is a difference in preservational quality between amber sites of similar chemistry (i.e. between sites within the same group), although this cannot be tested statistically due to insufficient sample size (Table 3). There are three exceptions, all among Group A amber: Lebanese amber, with 100% of analysed specimens containing preserved internal structures, can be compared to Charentes amber and New Jersey amber, both of which have 0% of analysed specimens containing preserved

internal structures; and Charentes amber can also be compared to Burmese amber, which has 67% of analysed specimens containing preserved internal structures. The differences between Lebanese and New Jersey amber and between Charentes and Burmese amber are not statistically significant (Table 3) and so do not reveal anything about preservational variation within Group A amber. In contrast, Charentes amber and Lebanese amber have significantly different proportions of bioinclusions with preserved internal structures (Table 3). Therefore, these results suggest that other variables also influence preservational quality. However, with such small sample sizes, even a few new specimens could change the result of our analyses.

There is also other evidence that variables other than resin chemistry influence preservational quality. Perhaps most compelling is one piece of Chiapas amber (Group D) with 8 inclusions, four of which have preserved internal structures, and four of which do not. The chemical variation within one piece of amber is likely to be very small, and therefore other variables other than resin chemistry must control internal preservation in this specific instance (Coty et al. 2014), and potentially across other examples.

These data also offer the opportunity to test the general view that the bioinclusions in Dominican amber are better preserved than the bioinclusions in Baltic amber (Grimaldi et al. 1994). A power test demonstrates that we do have a sufficient sample size to statistically test this assertion, and a Fisher Exact test reveals that a significantly higher percentage of bioinclusions in Dominican amber have internal soft tissue structures preserved than in Baltic amber (Table 3), supporting the consensus view.

1.4. Resin chemistry and presence/absence of fossils

There are 630 amber sites reported in the literature (Table 1, Supplemental Table 1) (Martínez-Delclòs et al. 2004; Lambert et al. 2012; plus references in Supplementary Tables);

of these 106 are known to have bioinclusions, 388 are known not to, and for the remaining 136 this information is unrecorded (Table 1, Supplemental Table 1). If we assume that all sites would have had a living fauna these data suggest either that at some sites insects were not trapped in resin, or that insects were not preserved in the resin as their remains were lost through decay. Studies of modern resin, very young copal, and experimental entrapment in other sticky media suggest that resin and other sticky exudates commonly entrap insects (Penney et al. 2010; Solórzano Kraemer et al. 2015); in particular, some trees produce resin for the purpose of trapping and neutralizing attacking insects (Phillips & Croteau 1999; Becerra et al. 2001; Trapp & Croteau 2001; Villagra et al. 2014). Therefore, although we cannot discount the possibility that some sites did not entomb any organisms, we expect that they are a small part of our data.

In general, about 75% of amber sites (388 out of 494 with data on inclusions) are non-fossiliferous (Table 1, Supplemental Table 1) (Martínez-Delclòs et al. 2004). However, these non-fossiliferous sites are only found among Group A amber sites (of which only 14% are fossiliferous). Groups B-D amber sites are all fossiliferous, and there are no data on inclusions in Group E amber. The data we have offer sufficient power (Table 4) to statistically compare Group A amber sites to Group B, C and D amber sites; the results indicate that a significantly smaller percentage of Group A amber sites have fossils than Group B, C or D amber outcrops (Table 4). This supports the hypothesis that resin chemistry can influence whether organisms once entrapped are preserved or lost through decay processes. It is also consistent with the results presented above that preservational quality is inferior in Group A ambers than in other ambers.

However, there is also some evidence of variation in fossil occurrence within resins of very similar chemistry. For example, for Group A ambers a survey of amber-bearing sites in Spain found that in over 100 outcrops in very close geographic proximity with almost

identical chemistry, seven had bioinclusions (Delclòs et al. 2007). Similarly, of nearly 300 amber-bearing outcrops in Lebanon only 18 have bioinclusions (Azar et al. 2010). Therefore, although resin chemistry may play a role in determining if entombed organisms are preserved other variables are also likely to be influential.

1.5. Conclusions: does resin chemistry influence fossilization?

We have shown that there is statistically significant variation between amber Groups in both the quality of preservation of fossils, and whether or not an entombed organism is fossilized. This supports the hypothesis that resin chemistry does influence the preservation of entombed organisms. However, there are many other factors that we could not test that may also account for correlations between amber Group and preservation. Our analyses shows that to begin to unravel the chemical properties of resin that promote or inhibit preservation, comparison of the chemistry of Group A amber (the only group without fossils, and those with the lowest preservation quality) to group C and Group D ambers (all have fossils, with higher preservational quality) would be most fruitful. All of Groups A, C, and D are formed from polylabanoïd diterpenes and are distinguished by the stereochemistry and the presence or absence of succinic acid (Lambert et al., 2008), as well as other secondary chemical variation (see e.g. Lambert et al., 2008 for examples of the range of compounds found in the different amber chemical Groups): Group A ambers have a regular stereochemical configuration and no succinic acid, Group C ambers have a regular configuration and succinic acid, and Group D ambers have $19\beta,11\alpha,20\alpha$ (non-regular) configuration and no succinic acid. This shows simply that succinic acid and the stereochemistry are unlikely to explain the poor fossilization in Group A ambers; rather, it may be due to more subtle variation in secondary chemical components. Group D ambers also differ chemically from Group A ambers, but the differences are too extensive and complex to assess here. Variations in preservation within the amber Groups may also be

explained by chemical variation within the groups, or equally, by the influence of other variables.

There are some instances in which preservational variation cannot be explained by variations in resin chemistry, suggesting that other variables also influence fossilization in amber. There are a number of other variables that would be interesting to investigate as controls on the preservation of organisms entombed in amber: dehydration of the carcass before being entombed in resin (Henwood 1992; Martínez-Delclòs et al. 2004; Ross; 2010; Coty et al. 2014); permeability of the resin to water, which degrades tissues and biomolecules (Austin et al., 1997; Zschokke, 2003); and variations in the temperature, pressure and other environmental conditions during early diagenesis (Martínez-Delclòs et al. 2004). Furthermore, there may be factors that are entirely unrelated to the immediate environment in which the bioinclusion occurs, as with other fossils, those in amber can be significantly modified by later geological events/processes. These include, for example, orogenesis and the temperature/pressure conditions experienced by the amber.

Our initial analyses shows that resin chemistry may, at least in part, influence preservation quality but what is really required to unravel the role of resin chemistry in preservation are more data – particularly recording data about which sites do not have fossils, which fossils have preserved soft tissue structures, and which fossils show evidence for initial dehydration – this would enable more powerful statistical analyses to test the effect of each of these variables on preservation in amber. Alongside this the chemical variation of amber between and within sites requires more research. In addition, laboratory experiments to determine how resin chemistry and dehydration affect the process of decay and preservation will greatly enhance current understanding of preservation in amber and the biases in the amber fossil record.

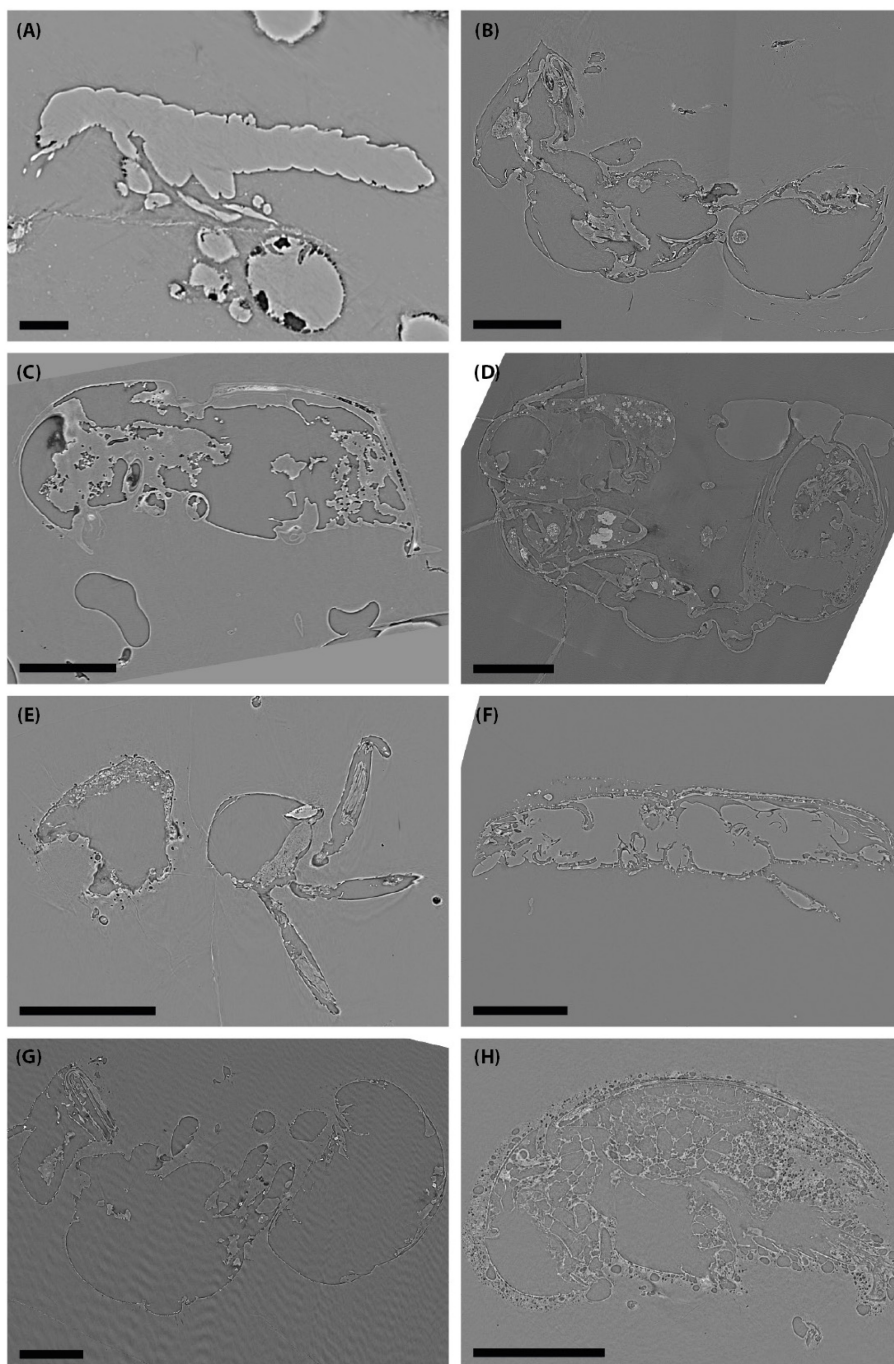
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380 **Figures**

381 **Figure 1:**



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384 **Tables**

385 **Table 1:** Summary table for the presence and absence of fossils in ambers outcrops, divided
 386 into the different chemical groups. See Supplemental Table 1 for details of each outcrop and
 387 references.

Chemical classification	Fossiliferous	Non-fossiliferous	% Fossiliferous	No data on inclusions	Total
Group A (class Ib, IV, V)	62	383	14%	41	486
Group B (class II)	3	0	100%	5	8
Group C (class Ia)	5	0	100%	3	8
Group D (class Ic)	18	0	100%	7	25
Group E (class III)	0	0	?	2	2
No data on group	19	5	79%	77	101
Total	107	388	22%	135	630

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Table 2: Summary of amber specimens that have been analysed with synchrotron tomography, computed tomography (CT scanning), or light microscopy, SEM or TEM after being extracted from the amber. This allows for an assessment of the preservational quality of the cuticle and internal structures. See Supplemental Table 2 for details of each specimen and references. The % with preserved internal structures is based only on those specimens with data about internal structure preservation.

Type of amber	Group	Total specimens analyzed	Inclusions with preserved internal structures	inclusions without preserved internal structures	no data given on internal structures	% with preserved internal structures
Lebanese amber	A	2	2	0	0	100%
Charentes amber	A	22	0	22	0	0%
New Jersey amber	A	7	0	7	0	0%
Burmese amber	A	3	2	1	0	67%
<i>Subtotal Group A</i>		<i>34</i>	<i>4</i>	<i>30</i>	<i>0</i>	<i>12%</i>
Baltic amber	C	27	11	9	7	55%
<i>Subtotal Group C</i>		<i>27</i>	<i>11</i>	<i>9</i>	<i>7</i>	<i>55%</i>
Chiapas amber	D	9	5	4	0	55%
Dominican amber	D	22	18	1	3	95%
<i>Subtotal Group D</i>		<i>31</i>	<i>23</i>	<i>5</i>	<i>3</i>	<i>82%</i>
Oise amber	?	3	3	0	0	100%
Rovno amber	?	1	0	1	0	0%
Spanish amber	?	4	2	2	0	50%
Danish amber	?	2	0	0	2	?
Hell Creek amber	?	4	1	0	3	100%
Total		106	44	47	15	48%

Table 3: Statistical comparisons of preservational quality between ambers in different chemical groups. The observed effect size is calculated from the proportions (p_1 and p_2) of fossils in each group that have preserved internal structures. The measurable effect size is based on a power analysis using the program R, which indicates what effect size can be accurately measured given the sample sizes. There is appropriate power for a statistical comparison if the measurable effect size is smaller than the observed effect size. The Fisher Exact tests were also carried out using the program R; significant p-values (p-values < 0.008, due to a Bonferroni correction for multiple tests) are in bold.

Comparison	Observed effect size = $2\sin^{-1}(\sqrt{p_1}) - 2\sin^{-1}(\sqrt{p_2})$	Measurable effect size (power analysis)	Appropriate power for statistical comparison?	Fisher Exact Test p-value
Group A to Group C	0.963	0.789	yes	0.001
Group A to Group D	1.558	0.715	yes	1.86E-08
Group C to Group D	0.594	0.820	no	NA
Dominican amber to Baltic Amber	1.020	0.898	yes	0.008
Dominican amber to Chiapas amber	1.020	1.134	no	NA
Charentes amber to Lebanese amber	3.142	2.069	yes	0.004
Charentes amber to New Jersey amber	0	1.216	no	NA
Charentes amber to Burmese amber	1.918	1.724	no	0.010
Lebanese amber to New Jersey amber	3.142	2.246	yes	0.028
Lebanese amber to Burmese amber	1.224	2.557	no	NA
New Jersey amber to Burmese amber	1.918	1.933	no	NA

Table 4: Statistical comparisons of fossil presence/absence between ambers in different chemical groups. The observed effect size is calculated from the proportions (p_1 and p_2) of fossils in each group that have preserved internal structures. The measurable effect size is based on a power analysis using the program R, which indicates what effect size can be accurately measured given the sample sizes. There is appropriate power for a statistical comparison if the measurable effect size is smaller than the observed effect size. The Fisher Exact tests were also carried out using the program R; significant p-values are in bold.

Comparison	Observed effect size = $2\sin^{-1}(\sqrt{p_1}) - 2\sin^{-1}(\sqrt{p_2})$	Measurable effect size (power analysis)	Appropriate power for statistical comparison?	Fisher Exact Test p-value
Group A to Group B	2.375	0.845	yes	0.003
Group A to Group C	2.375	0.656	yes	6.42E-05
Group A to group D	2.375	0.351	yes	3.33E-15

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