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The Real Jurassic Park: The Isolation of Proteins From Organisms Preserved in Amber Inclusions for 40 Million Years

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Pressure Symposium, Harvard University, May 21, 2010



- **The mass extinction of the dinosaurs marking the Cretaceous-Tertiary boundary pales in magnitude compared to other lesser known mass extinction events such as the Permian-Triassic.**
- **Five major extinction events over the past 540 million years, have resulted in the extinction of 99% of all of the species that ever lived on earth.**
- **This is furthered by nearly two orders of magnitude, since by even the most conservative estimates, less than 10% of all species presently inhabiting the earth are known, while the vast majority is still undiscovered .**
- **The field of biology is based entirely on what has been learned from fewer than 0.1% of all past and present species.**

Smejkal GB, Poinar Jr GO, Righetti PG, Chu F (2010).
Sample Preparation in Biological Mass Spectrometry, Springer, In press.

An Amber Proteomics Initiative



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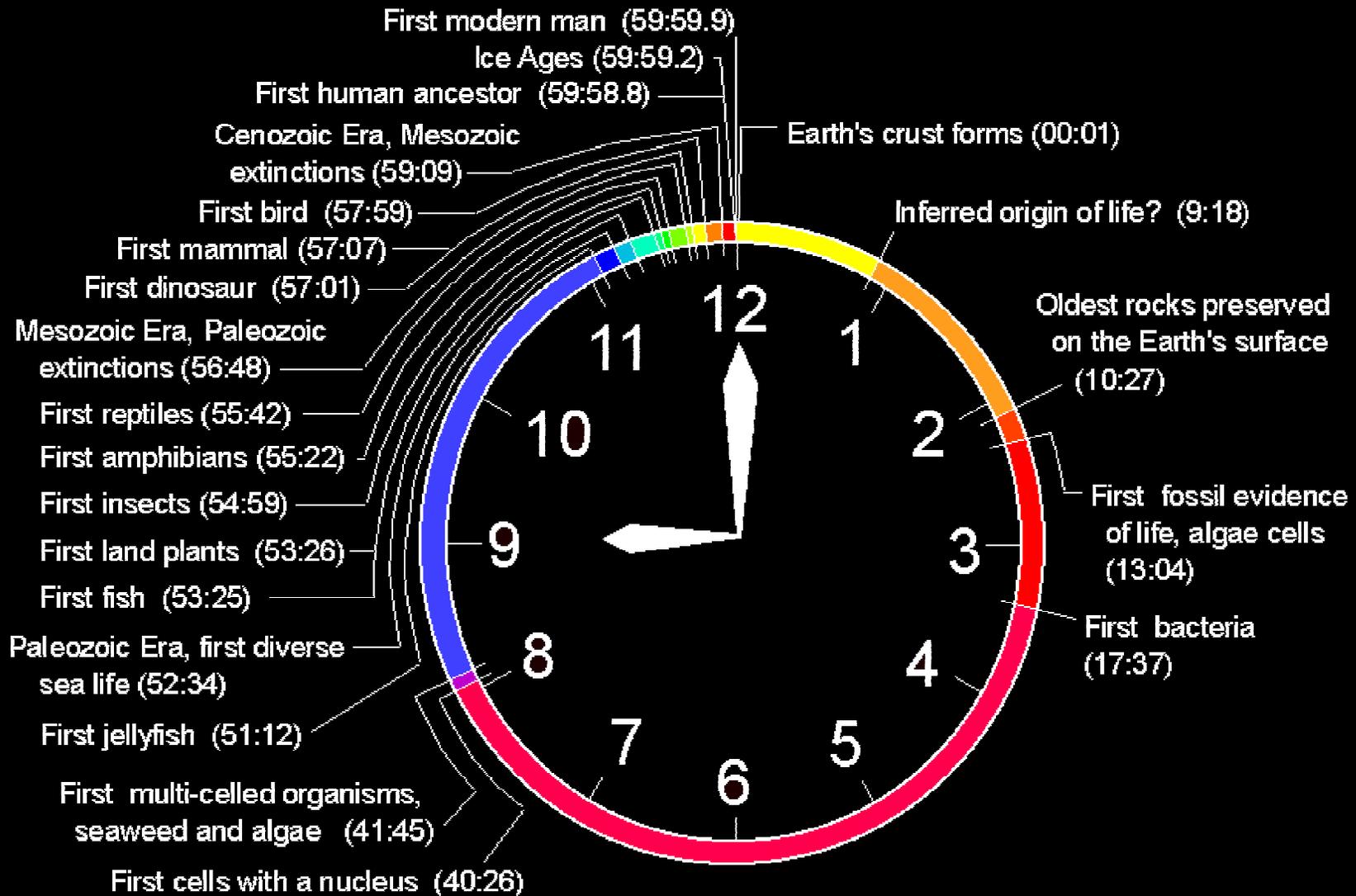
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**Blood-filled female
biting midge in Early
Cretaceous Burmese
amber 100 MYO**

Poinar GO Jr (2008). *Mem. Inst. Oswal.*, 103. 468.

Geological time scale in perspective



4.6 billion years compressed into one hour

Can biomolecules survive geological time spans?

As early as 1954, Abelson reported amino acids recovered from fossils hundreds of millions of years old .

Porphyrins and phycobilins from Precambrian fossils 1.7-2.6 billion years old.

Osteocytes in bone from an 80 million year old *Tarbosaurus bataar* . Carbohydrates and lipids was also observed.

In 1994, Woodward et al. reported the isolation of DNA fragments from a Late Cretaceous dinosaur bone exhumed from bituminous strata.

Non-collagenous proteins in the compact bone of a 120 million year old *Iguanodon bernissartensis*.

- Abelson PH (1954). Carnegie Inst. Wash. Yrbk. 53, 94-101
Kolesnikov MP, Egorov IA (1977). Orig Life 8, 383-390.
Pawllicki R (1977). *Acta Histochem.* 58, 75-78, 1977.
Woodward SR, Weyand NJ, Bunnell M (1994). *Science* 661, 229-232.
Embery G, et al. (2000). *Connect Tissue Res*, 41, 249-259.

Can biomolecules survive geological time spans?

Asara et al. identified peptides with sequence homology to avian collagen from *Tyrannosaurus rex*.

Wick et al. showed the alleged soft tissues found preserved in a 50 million year old bat were due to the replacement of the original soft tissues by lawns of bacteria.

Kaye et al. demonstrated that microbial biofilms form “endocasts” in which three-dimensional structure is preserved with microscopic detail, but in which the original organic material has been totally replaced by minerals.

Dinosaur DNA fragments from a Cretaceous dinosaur egg fossil were found to be more closely related to fungi rather than to reptiles or birds.



- Asara JM, et al. (2007). *Science*, 316, 280-285.
Embery G, et al. (2000). *Connect Tissue Res*, 41, 249-259.
Yin Z, et al. (1996). *Yi Chuan Xue Bao*, 23, 190-195.

Soft tissues are not replaced by minerals in amber

Unfossilized resins are comprised largely of terpenoids, labdanoids, and phenolics which rapidly dehydrate the included specimen.

Possess anti-bacterial, anti-fungal, and anti-inflammatory properties.

Compared to other geochemical environments, the racemization of amino acids has been shown to be curtailed 100,000 times in insects preserved in amber inclusions 40-100 million years old.

Cano et al. isolated Bacillus DNA in fossil bees. Later found viable spores.

DNA isolated from the 30 million year old *Mastotermes electrodominicus* .

The oldest fossil DNA amplified and sequenced were fragments isolated from a 120-135 million year old amber-encapsulated nemomychid weevil.

DeSalle R, Gatesy J, Wheeler W, Grimaldi D (1992). Science, 257, 1933-6.

Cano RJ, et al. (1994). Appl. Environ. Microbiol. 60, 2164-7.

Cano RJ, Borucki MK (1995). Science, 268, 1060-4.

Cano RJ, Poinar HN, Pieniazek NJ, Acra A, Poinar GO Jr (1993). Nature, 363, 536-8.



Preservation of fine structure including individual ommatidia of the compound eye of a formicine in Oligo-Miocene amber 20-30 million years old.

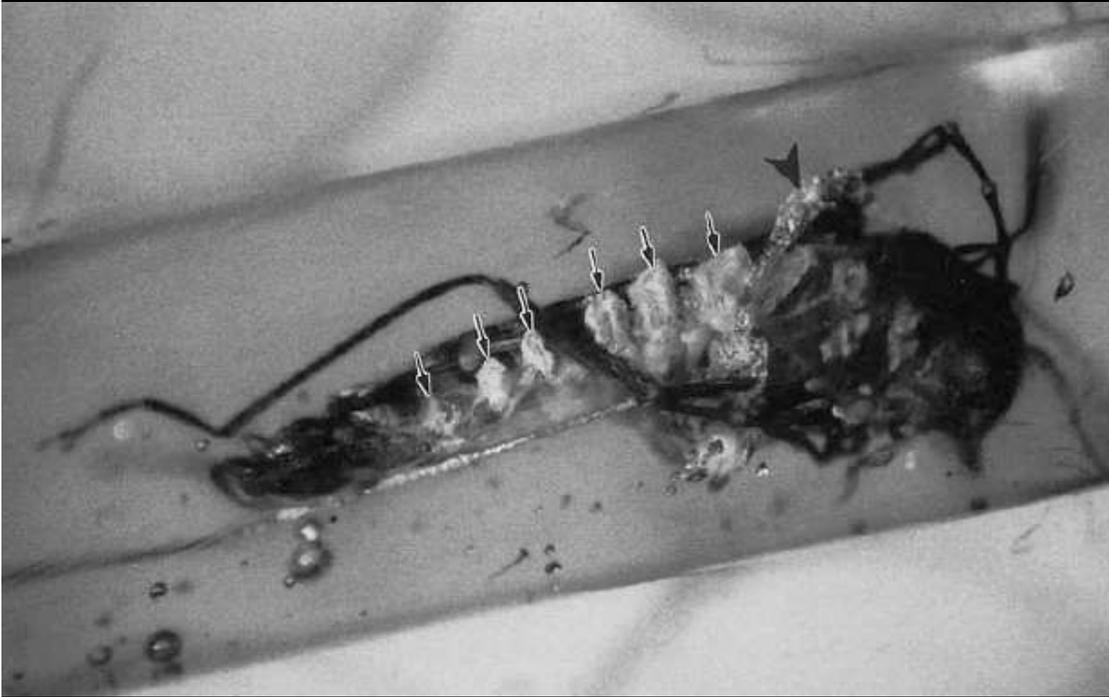
Ants were relatively scarce during the explosive radiations of angiosperms and pollinating insects during the Early Cretaceous, representing less than 1% of all insect species at the time.

Today, over 12,000 species are known, collectively comprising 15-25% of the terrestrial animal biomass.



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**One hundred million years of
chemical warfare by insects**



Poinar GO Jr, Marshall CJ, Buckley R (2007). *J Chem. Ecol.* 33, 1663-1669.

Mermithid partly emerged from abdomen of
sand fly 100-110 MYO

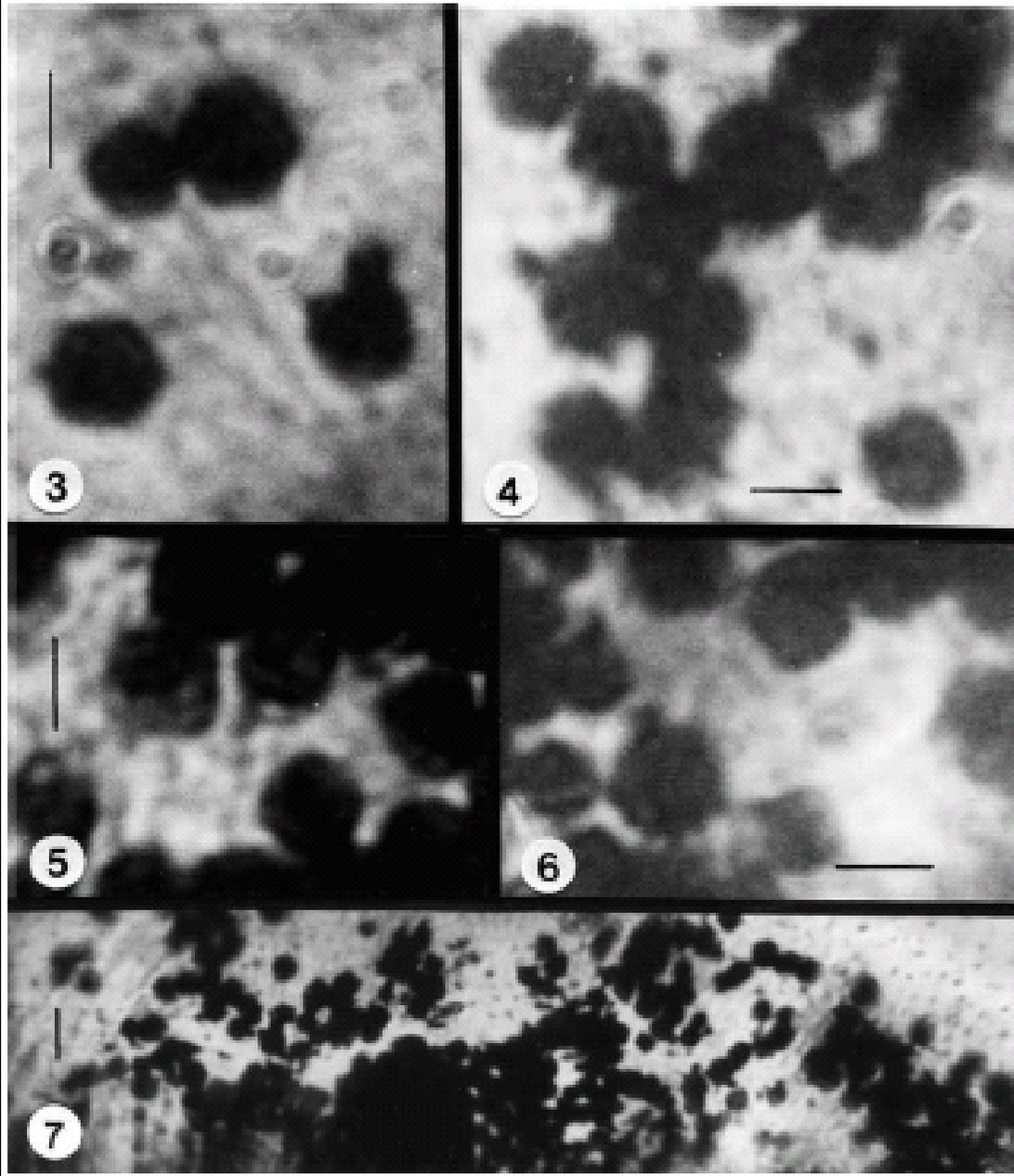


0.5 mm

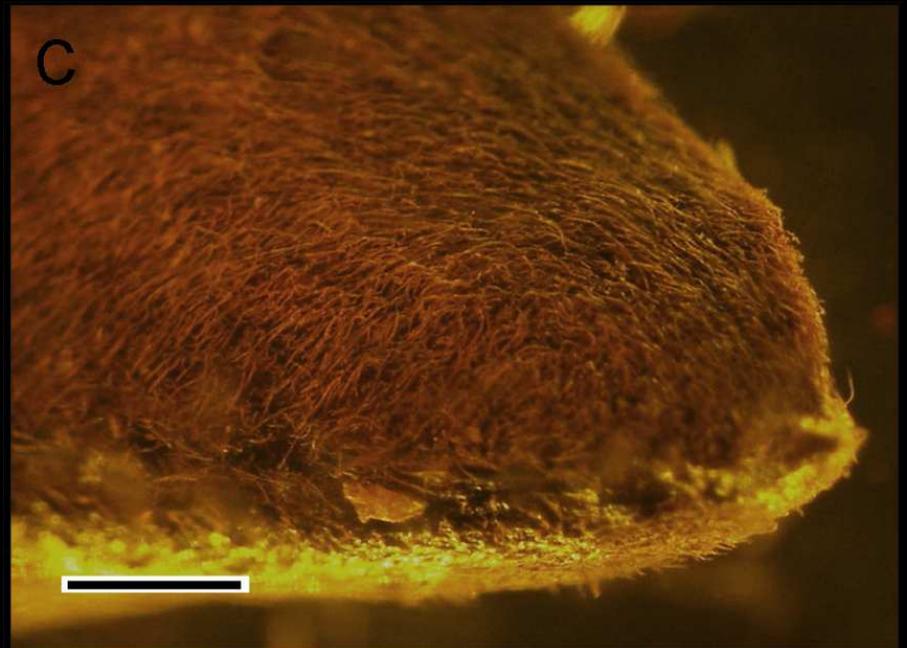
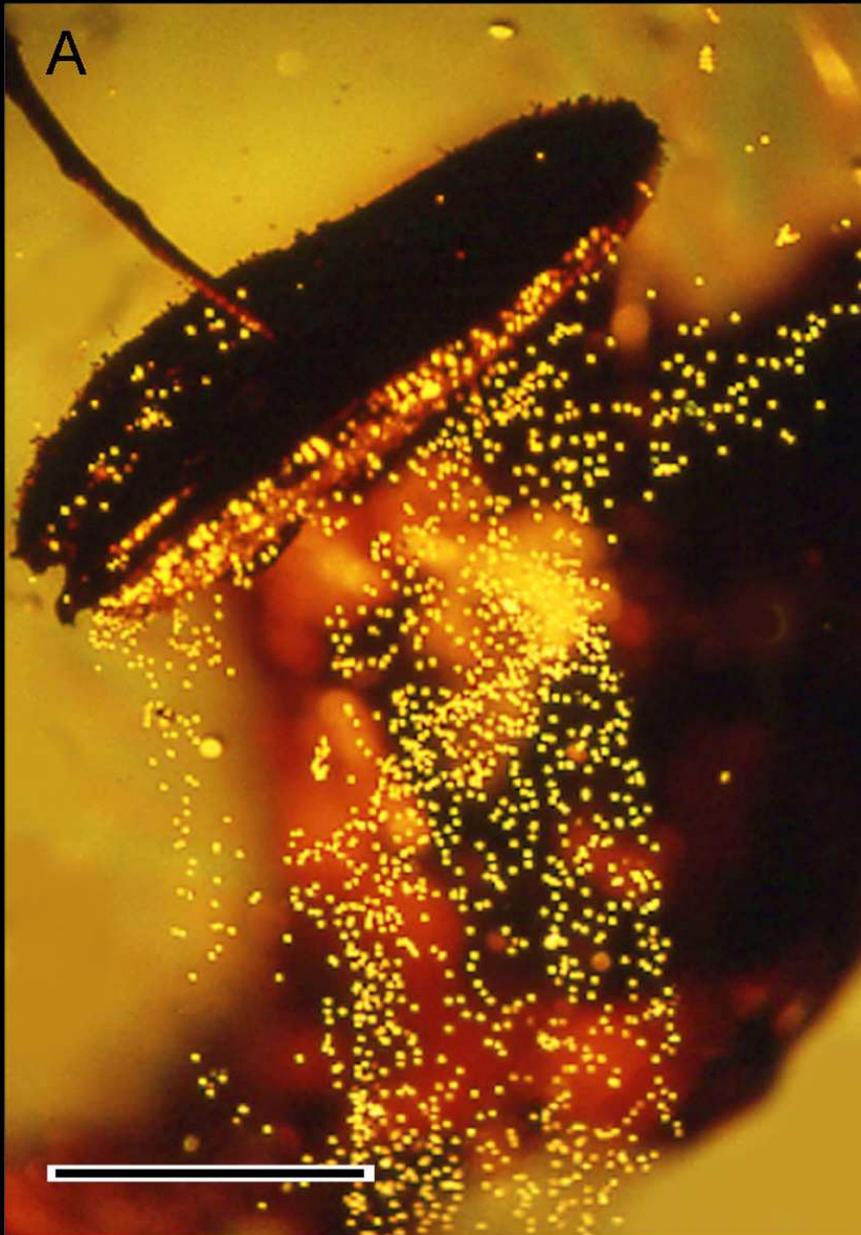
1

Early Cretaceous viruses

Polyhedral inclusion bodies in midgut epithelial cells of Burmese amber biting midge 100-105 MYO.



Poinar Jr GO, Poinar R (2005). *Journal of Invertebrate Pathology* 89, 243–250.



(A) Anther, filament, and pollen grains. Bar equals 0.5 mm. (B) Petal and claw. Bar equals 4 mm. (C) Stipule showing filamentous trichomes. Bar equals 0.3 mm.

Sample processing

Plant triturates were extracted in 125 mM Tris-HCl pH 6.8 containing 2% SDS, 5 mM tributylphosphine, 20 mM aminoethylbenzene sulfonyl fluoride, 10 mM EDTA and 25 mM phenylacetylthiazolium bromide (PTB).

The samples were extracted for 100 X 100 seconds at 35,000 psi.

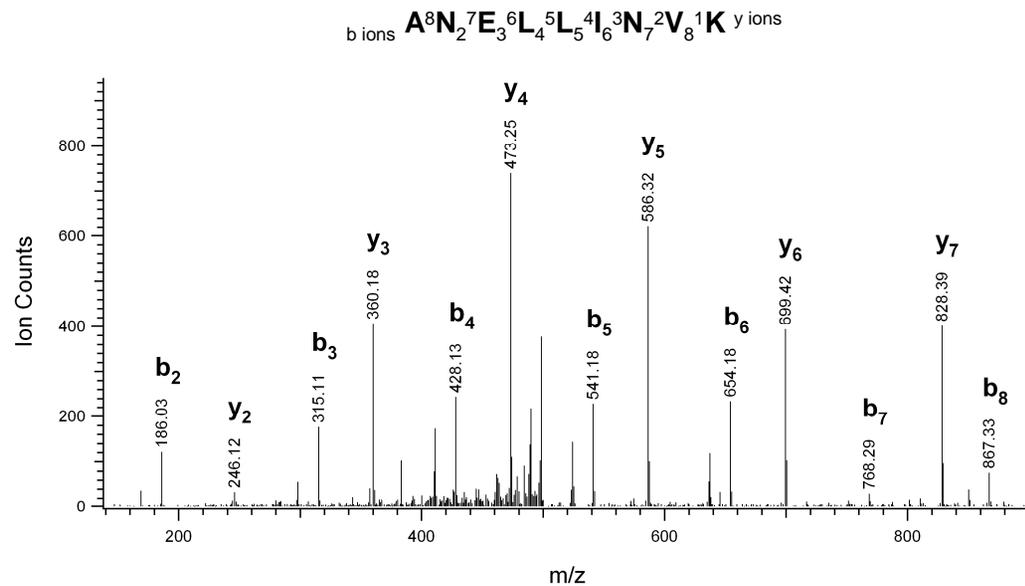
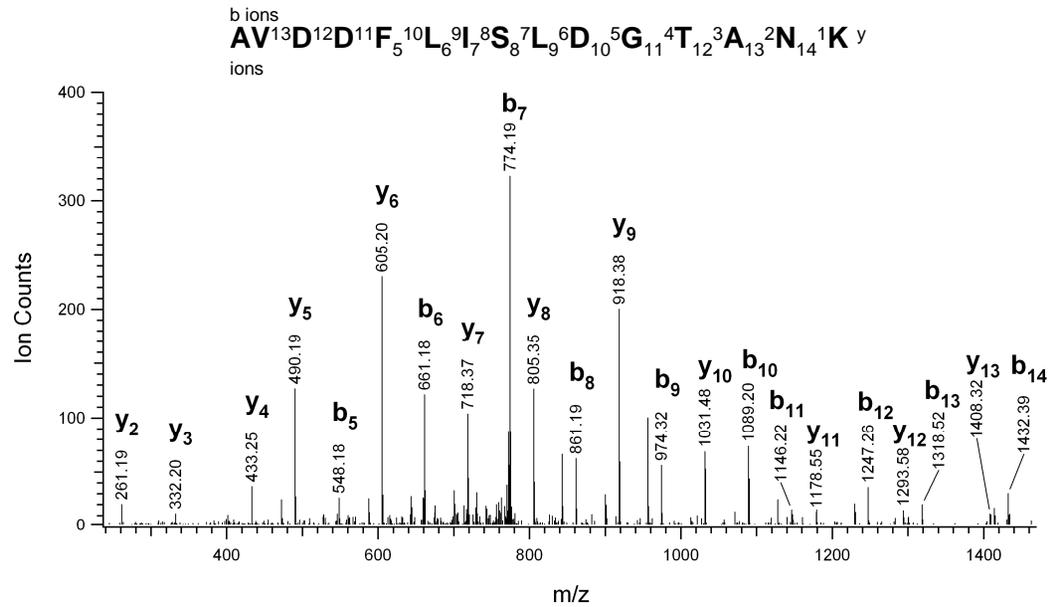
Ultrafiltration yield protein (retentate) and peptide (filtrate) fractions.

Retentates were applied directly to 8-16% PAGE

Trypsin digests were analyzed by LC-MS/MS. Chromatographic separation was carried out on reverse-phase capillary column at a flow rate of 330 nL/min. The HPLC eluent was introduced directly to the micro-ion electrospray source of an LTQ Orbitrap XL mass spectrometer.

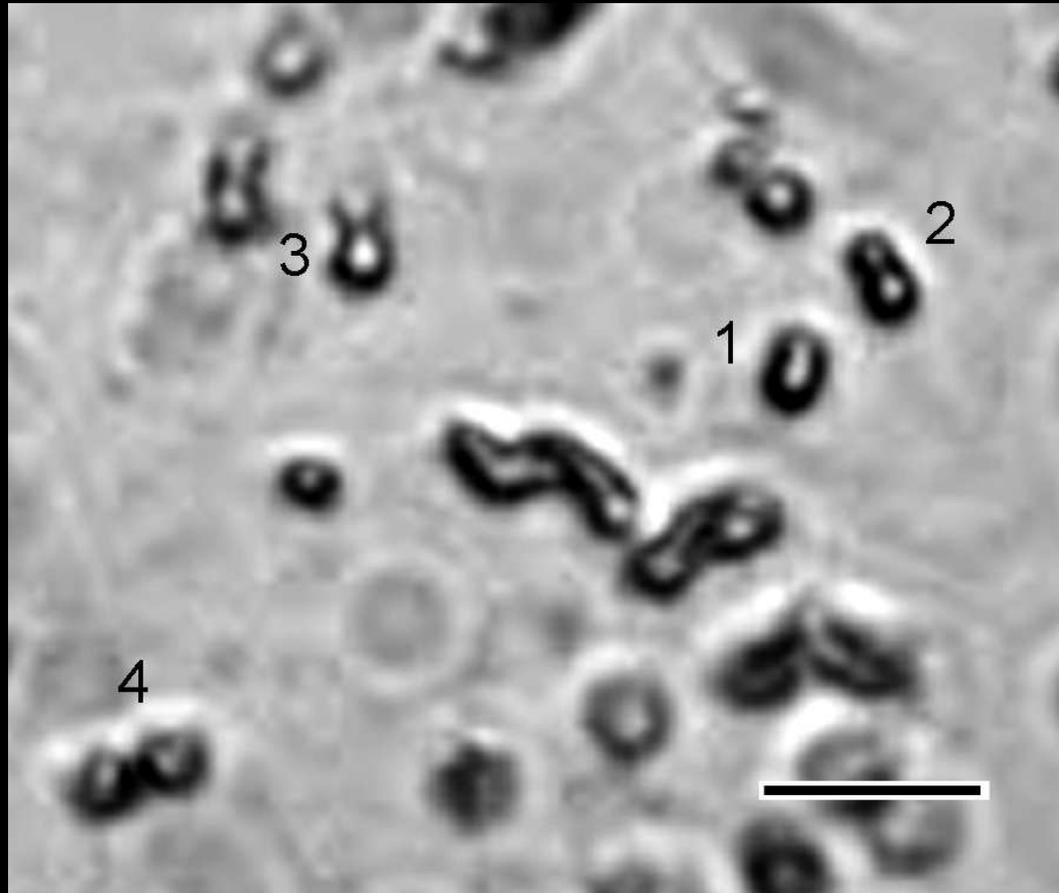


**LTQ-Orbitrap mass spectra
 identifying peptides of
 sequence homology to
Saccharomyces enolase I
 (*top*) and alcohol
 dehydrogenase (*bottom*)
 isolated from 40 million year
 old Dominican Republic
 amber.**



Peptides identified from Oligo-Miocene amber

	protein identification	number of peptides
1	enolase 1	11
2	enolase 2	10
3	alcohol dehydrogenase	9
4	glyceraldehyde-3-phosphate dehydrogenase, isozyme 1	8
5	phosphoglycerate kinase	7
6	translation elongation factor EF-1 alpha	7
7	pyruvate kinase	5
8	glyceraldehyde-3-phosphate dehydrogenase, isozyme 3	3
9	heat shock protein YJM789	3
10	fructose 1,6-bisphosphate aldolase	3
11	phosphoglycerate mutase	3
12	ribosomal L12B	2
13	triosephosphate isomerase	2
14	methionine and cysteine synthase (O-acetyl homoserine-O-acetyl serine sulfhydrylase)	2
15	Ssa1p	2
16	mitochondrial aldehyde dehydrogenase	2
17	rubber elongation factor protein	2
18	actin	2
19	40S ribosomal protein S18	2
20	pyruvate decarboxylase	1
	total	86

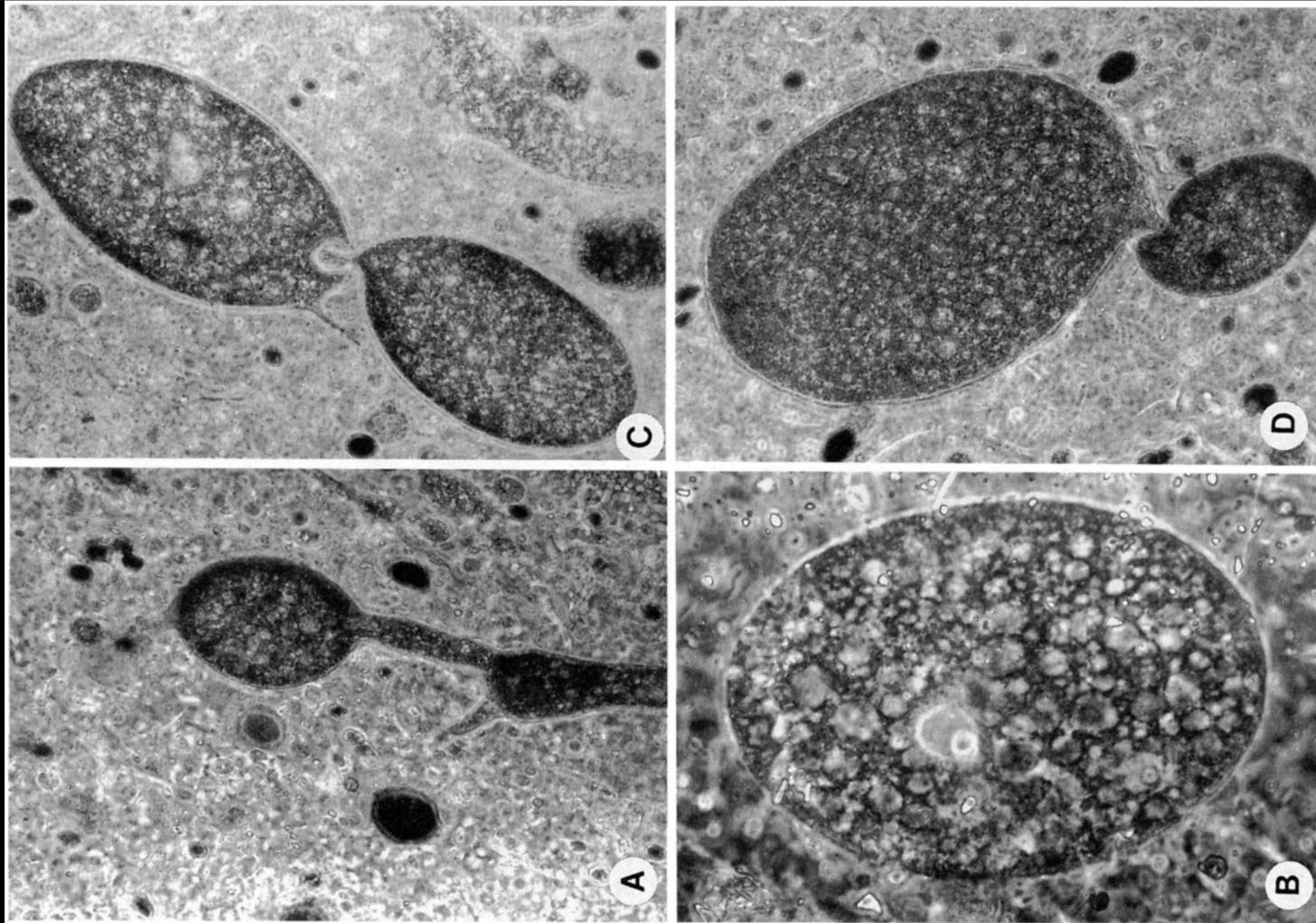


Light microscopy showing budding yeast cells in Dominican Republic amber 20-30 million years old. Stages from single cell (1) to where the progeny cell is nearly equal in size to its parent (4) and intermediary stages (2,3) are shown. Magnification bar equals 6 μ m.

Smejkal GB, Poinar Jr GO, Righetti PG, Chu F (2010).
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Fungi in Lower Cretaceous amber from Israel



Arachnid in Baltic amber 40-50 MYO

