Comment on Franz et al. 2023: A reinterpretation of the 1.5 billion year old Volyn 'biota' of Ukraine, and discussion of the evolution of the eukaryotes

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Abstract. Franz et. al. (Biogeosciences 20, 1901–1924, 2023) report a diverse and three-dimensionally preserved suite of mid-Proterozoic microfossils from miarolotic cavities within the granitic Volyn pegmatite field, a major granitic plutonic

15 complex in NW Ukraine. The biota is dated at between ~1.76 Ga and ~1.5 Ga and includes fungus-like objects. This biota is reported as evidence of organisms living within the continental lithosphere, illuminating part of a ~1.8–0.8 billion year interval of the Proterozoic Eon characterised by relatively low climatic variability and slow biological evolution. We show that at least some of this putative diversity represents modern contamination including plant hairs, a distinctive pollen grain assignable to the extant conifer genus *Pinus*, and likely later fungal growth. Comparable diversity is shown to exist in

20 modern museum dust, this calling into question whether any part of the Volyn 'biota' is biological in origin while emphasising the need for scrupulous care in collecting, analysing, and identifying Precambrian microfossils.

1 Introduction

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Franz et al. (2023) recently published a detailed study on what they claim to be a diverse suite of in-situ Proterozoic microfossils from the Volyn pegmatite field within the Korosten Pluton, a major granitic complex intruded into continental crust and located WNW of Kiev, Ukraine. The fossils were extracted from eight kerite samples and one beryl crystal originating from metre-scale crystal-lined (miarolitic) cavities in the pegmatites (Franz et al. 2023, table 1; see also Franz et

30 al. 2022a, table 1). Kerite is a black, carbon-rich, bitumoid compound found in these cavities in the Korosten Pluton. The origin of kerite has been disputed. Ginzburg et al. (1987) who first described fibrous kerite from the Volyn pegmatite posited an abiogenic origin from volatile hydrocarbons (see also Luk'yanova et al., 1992), although Gorlenko et al. (2000) considered it to represent the remains of cyanobacterial mats probably of hydrothermal origin. The age of the kerite is less than the 1.76 Ga (latest Paleoproterozoic) radiometric (i.e., crystallisation) age of the pegmatites themselves (Shumlyanskyy

35 et al. 2021) and has a minimum radiometric age of 1.5 Ga (earliest Mesoproterozoic) based on a breccia which contains degraded organic matter (Franz et al. 2017, 2022b). This biota therefore falls within the so-called 'boring billion', a 1.8–0.8 Ga interval spanning the Mesoproterozoic characterised by low oxygen levels, relative climatic and tectonic stability, sulphidic oceans, primitive lifeforms, and very slow biological evolution (Mukherjee et al. 2018 and references therein).

The biota described by Franz et al. (2023) comprises a substantial variety of morphotypes classified by these

40 authors as filaments, hollow objects, irregular objects, and spherical objects, as well as flaky objects and agglutinating filaments interpreted as fossil biofilms. Together, these are interpreted as representing free-living as well as sessile forms. This biota is proposed to have lived in large cavities within the pegmatite, therefore representing part of the deep Mesoproterozoic biosphere, and was supposedly fossilised by a rapid influx of hot, mineral-rich fluids associated with a geyser system. These fluids were a mixture of magmatic fluids and meteoric water that caused surficial infiltration of

45 aluminium silicates coupled with encrustations and intergrowths by various minerals, resulting in rapid fossilisation in an exceptional three-dimensional preservational state (Franz et al., 2022a; Franz et al. 2023, fig. 1b). The biota apparently lived relatively close to the surface.

The microfossils were studied and comprehensively illustrated by Franz et al. (2023, their figs. 3–11) using a scanning electron microscope (SEM) and an electron microprobe analyser. Analysis of carbon and nitrogen stable isotopes, and micro-

50 Fourier transform infrared spectroscopy (micro-FTIR) absorption spectra were also undertaken.

We comment on several aspects that point to the inclusion of modern contaminants within the reported Volyn biota, and we present both an SEM analysis of modern museum dust and comparative micro-FTIR absorption spectra, calling into question the high morphological diversity described by Franz et al. (2023).

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2 The Volyn biota in an evolutionary context

The 1.76–1.5 age of the Volyn biota places it within a billion-year interval first identified by Buick et al. (1995) as lacking major biological, geological, and climatic events, and later coined the "boring billion" (Brasier, 2012). This interval is now

60 typically regarded as extending from ~1.8 to 0.8 Ga (latest Paleoproterozoic–earliest Neoproterozoic, or "mid-Proterozoic"). It follows the final assembly of supercontinent Columbia at ~2.0–1.8 Ga but includes the massive Grenville Orogeny and assembly of Rodinia at ~1.3–1.0 Ga (Johnson et al., 2020) that marks the beginning of modern-style subduction tectonics (Santosh and Groves, 2023). However, there were no major glacial events, and atmospheric oxygen levels remained low

throughout. Biologically the interval is significant because eukaryotes had evolved by at least 1.7 Ga (Javaux and Lepot,

65 2018; Miao et al., 2019) and questionably as far back as 2.4 Ga (Barlow et al., 2023), with the appearance of

photosynthesizing eukaryotes estimated at 1.25 Ga (Gibson et al., 2018). Since all eukaryotes require oxygen to metabolise,

the low levels of dissolved oxygen in shallow seas of the mid-Proterozoic (~0.1% of modern surface ocean levels; Tang et

al., 2016) and perhaps other aspects of ocean chemistry including low nutrient trace element concentrations (Mukherjee et

al., 2018) explain their restrained evolutionary diversification through much of this time. Short-lived pulses of oxygenation

70 (≥4% in Shang et al., 2019) do not appear to have significantly affected this overall evolutionary pattern, although a

moderate increase in diversification during the late Mesoproterozoic–early Neoproterozoic (Javaux and Lepot, 2018) reflects

a shift to more favourable conditions. Among these early eukaryotes, the fungi seem to make a relatively late appearance,

dating to just before or within the Neoproterozoic (1010–890 Ma; Loron et al., 2019) judging from the few relatively secure

records available. Within this context of low overall biotic diversity, the Volyn biota with its wide range of reported

75 morphologies including structures ascribed to fungi-like organisms (Franz et al., 2023) represents an anomaly and therefore

calls for scrutiny.

3 Evidence for contamination

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One of the "spherical objects" illustrated by Franz et al. (2023, their fig. 9a–c) is a bisaccate pollen grain assignable to the

extant coniferous genus *Pinu*s (pine). Franz et al. (2023, p. 1910) described this entity as having a "double-ball shape

…clearly grown onto the substrate" and bearing "remnants of a sheath [that] points to cell separation". We note paired air

sacs with subtle surface reticulation that reflects internal structure, joined by a central body with a microrugulate surface; all

85 features that characterise this well-known type of pollen (e.g., Moore et al., 1991, their pls. 5, 59; Cojocaru et al., 2022).

Bisaccate pollen first entered the fossil record in the late Carboniferous (Traverse, 2008; fig. 4 in Riding, 2021). The modern

native distribution of pine across Eurasia including Germany and Ukraine, the occurrence of this pollen type in museum dust

(Fig. 1a–c), and its pristine condition, all allow us to consider it a modern contaminant.

Of the SEM images presented showing curvilinear filaments with smooth surfaces and circular cross section, we

90 note that a specimen illustrated by Franz et al. (2023, their fig. 3j–l) from three views and described as "multiple, conical

filaments with claw-like ends, growing from a common center" strongly resembles an abietiform trichome (Payne, 1978).

Trichomes are appendages covering plant surfaces including leaves, stems and fruits, and are often microscopic outgrowths

of a single epidermal cell (Esau, 1965). Trichomes similar to the specimen shown by Franz et al. are found for example in

extant species of the fagacean genus *Quercus* (Hardin, 1976) and malvacean genus *Alcea* (Arabameri et al., 2020). We do

95 not know if this specimen was studied with a BSE detector or micro-FTIR analysis to determine whether it was mineralised.

However, biomineralization of plant trichomes is a common phenomenon in angiosperms (e.g., Mustafa et al., 2018;

Weigend et al., 2018), with silica, calcium carbonate, calcium oxalate and calcium phosphate among these biominerals

(Lowenstam, 1981; Ensikat et al., 2016). Such biominerals may show complex patterns even within a single-celled trichome (Ensikat et al., 2017), and to the untrained eye might appear similar to the products of fossilisation, especially if only known

100   beyond the context of the plant body. Given that plant trichomes are present in the natural environment and a common component of household and museum dust (Fig. 1d–l), we consider the extremely well-preserved specimen illustrated by Franz et al. to be a modern trichome.

        Franz et al. (2023) present micro-FTIR spectra intended to allow speculation on the biological affinities of the fossils recovered. Micro-FTIR is a powerful non-destructive technique yielding fundamental information on the molecular

105   structure of organic and inorganic components in a sample by using an infrared source and analysing the absorption spectrum typically in the mid-infrared (approximately 4000 to 400 cm^{-1}) region (Chen et al., 2015). Franz et al. measured three 40–60 µm wide translucent dark-brown fragments of kerite from their sample no. 0 (a museum sample from Kiev), all showing similar spectra (fig. 13a in Franz et al., 2023; Fig. 2g). These spectra were compared with that of a chitin standard (commercially supplied deacetylated chitin from shrimp shells) and found to be closely similar if allowing for some thermal

110   maturation of the kerite. The authors take this as evidence of a biological origin for the Volyn biota. Chitin is an essential ingredient of the cell walls of fungi, and its presence would support the claim of Franz et al. (2023) that morphologies displayed within the Volyn biota can be interpreted as fungal in origin. We note, however, that none of the existing micro-FTIR studies published on fossil fungal remains from a variety of ages and thermal maturities (Fig. 2) were included in their analysis or citations. Moreover, no modern fungal chitin standard was shown for comparison. Micro-FTIR spectra of extant,

115   sub-fossil, and fossil fungi (Salman et al., 2010; O'Keefe et al., 2015; Dai et al., 2017; Chen et al., 2013, 2015; Fig. 2) display with increasing thermal maturity characteristic changes including reduction in wave height, reduction and eventual loss of the O-H stretching band of water, reduction in lipids, and loss and/or alteration of carbohydrates and nucleic acids. These changes are largely absent from the spectra produced by the Volyn biota (Fig. 2g) and some other extremely deep-time fungal fossils (Loron et al., 2019; Fig. 2f), excepting the loss of carbohydrates. A spectrum from ~635 Ma fungus-like

120   fossils from China do show the overall reduction in wave height, loss of the O-H stretching band, and reduction in lipids, although changes in chemistry producing lower wave-numbers are obscured by a quartz and pyrite overprint (Gan et al., 2022; Fig. 2e). Very similar results are seen in analysis of early Devonian *Prototaxites* from the Bordeaux quarry (Vajda et al., 2022). Given this variation (Fig. 2), micro-FTIR results should not be used as stand-alone evidence for the presence of fungi. Rock-inhabiting fungi are known from many substrates, including ancient granites with bitumen deposits reminiscent

125   of kerite (Sazanova et al., 2022; Ivarsson et al., 2020a,b, 2021). White-appearing mycelium that is transparent under transmitted light observation is known to be invading rocks that form the Clarkia lagerstätte (Fig. 3). Together, these observations raise concern that the material Franz et al. (2023) have analysed represents younger fungal contamination. The authors note that their specimens were translucent, but they provided no light microscope images that would help discern fossil hyphae/spores from recent contaminants (presence of nuclear materials, lipids, starches, etc.).

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4. Modern museum dust

We used SEM combined with energy-dispersive X-ray spectroscopy (EDX) to examine dust from fossil displays of the
paleobotanical collection in the Goldfuss Museum, Bonn (Fig. 1). EDX analysis is very similar to the EDAX EDS system
used by Franz et al. (2023) on selected cross sections (their fig. 10j–l), but it allows elements comprising or attached to the
observed objects to be visualised over the entire surface as imaged by SEM (Fig. 1). In addition to *Pinus* pollen (Fig. 1a–c), a
number of trichomes (Fig. 1d–l) were observed, along with indeterminate filaments (Fig. 1m–q) reminiscent of the shapes
and diversity depicted by Franz et al. (2023) and with similar surface structures, and with one consisting primarily of silica
showing an opening at its end (Fig. 1o, p vs fig. 11 of Franz et al., 2023). We note in particular the appearance of the
infrareticulum visible on the surface of air sacs in the *Pinus* pollen using a high accelerating voltage (12 kV) similar to the 10
kV used by Franz et al. (2023). The variety of trichomes encountered attests to the ease with which they detach from plants
and become airborne. The samples examined by Franz et al. (2023) under SEM did not receive special cleaning other than
with compressed air on one sample, and in addition to samples collected underground included a specimen from a museum
and another from mine tailings. The underground specimens were simply "picked up" and not separated from the rock
matrix. It seems that all samples were able to acquire airborne "dust" and other contaminants. Had Franz et al. (2023) used
EDX in addition to applying EDAX EDS to selected cross sections, they would have been easily able to determine the
elemental surface distribution for all specimens they imaged using SEM which could have assisted in discriminating extant
contaminants from fossil material.

5. Stable isotopes of carbon and nitrogen

Stable carbon and nitrogen isotope data obtained by Franz et al. (2023) from bulk samples (all samples were analysed) are in
the range of −31 ‰ to −47 ‰ δ13C and of +3 ‰ to +10 ‰ δ15N. Because modern fungi have a range restricted to −19 ‰ to
−29 ‰ δ13C, with the main cluster at −22 ‰ to −28 ‰ δ13C (Mayor et al., 2009; Franz et al., 2023), so Franz et al. (2023)
explained the much lower values of δ13C in the Volyn biota as reflecting incorporation of carbon from methanogenic
bacteria into the fungi-like organisms. These authors concluded that "We exclude an abiotic origin … because of the
extremely low δ13C values and the large variation in morphology." But laboratory experiments have shown that organic
compounds produced by abiotic synthesis under hydrothermal conditions can have 13C depletions in the range of biological
fractionation, with methane for example yielding a value of −50.3 ‰ (McCollom and Seewald, 2006). Nitrogen is an
important component of organic matter, but there is increasing evidence that various carbon-based solid organic compounds
including those containing nitrogen can be synthesised in Fischer–Tropsch-type or Friedel–Crafts-type reactions (Ménez et
al., 2018; Nan et al., 2021) in hydrogen-rich hydrothermal systems. We therefore consider the carbon and nitrogen isotope
data presented by Franz et al. (2023) not to be conclusive evidence of biological affinity for the Volyn kerite.

6. Discussion

170 The Volyn biota at 1.5–1.8 Ga, which apparently includes fungus-like organisms, is claimed to represent life in a highly specialised continental setting within subterranean cavities associated with a geyser system. It cannot therefore be compared directly with published mid-Proterozoic records which are mostly marine and include photosynthetic organisms, as Franz et al. (2023) explain. But the hostile ambient conditions in which this biota is proposed to have lived, including high temperatures, lack of sunlight, and high radiation levels, require exceptional proof for these claims to be accepted, especially

175 given the high morphological variation recorded. It would be necessary to consider and eliminate the possibility of modern contamination, given the pristine state of preservation of many components in the Volyn biota and knowledge that fungal remains associated with bitumen in cavities of brecciated granitoids have been shown to be significantly younger than the rocks themselves (Ivarsson et al., 2020b; 2021). Franz et al. (2022, 2023) do not consider or exclude contamination in any form, yet the age control of other pristine microfossils in igneous rocks is generally lacking, or where present, represents

180 allochthonous material (e.g. Drake et al., 2021).

We identify evidence of modern contamination within the presented Volyn biota, and we raise concern that the presence of chitin is the result of contamination by modern fungi. Franz et al. (2023) discuss the morphological and geochemical similarities of their specimens with fungi, and there indeed exist plausible records of fungus-like fossils extending back 2.4 Ga (Bengston et al., 2017). However, Mesoproterozoic records of such fungus-like fossils (e.g., Loron et

185 al., 2021; Miao et al., 2021) lack the exceptional preservational state of the Volyn biota. While a model was proposed involving hydrothermal fluids to explain rapid preservation for the Volyn biota, this remains a unique process without analogue and therefore requires exceptional supporting evidence.

Documenting evidence of early life requires scrupulous care to minimise and identify any possible contamination. This begins with the careful collection of specimens, ideally from freshly exposed rock surfaces to minimise the possibility

190 of deep penetration by modern fungi. Comparable studies have used petrographic thin sections (Ongeluk Formation, South Africa, 2.4 Ga, Bengston et al., 2017; Grassy Bay Formation, Canada, 1 Ga, Loron et al. 2019), transmission electron microscopy (Bengston et al., 2017; Loron et al. 2019), synchrotron-radiation X-ray tomographic microscopy of discrete blocks (Bengston et al., 2017), or involve splitting (Strelley Pool, Australia, 3.4 G; Alleon et al. 2018) or etching (Onverwacht Group, Australia, 3.5–3.3 Ga; Westall et al. 2001) of the rock prior to its study. Some studies have used light

195 microscopy to reveal the internal structure of the organic component (Loron et al., 2019, 2021; Miao et al., 2021). Airborne contamination can occur in the laboratory, and when pristine three-dimensional fossils are identified these should be checked using light microscopy to ensure there are no fresh cell contents.

Ultimately, all lines of evidence must be weighed, including micro-FTIR spectra or EDX of all specimens, to exclude the possibility of contamination before claims of ancient biotas possessing unique preservation and morphological

200   diversity are made. We conclude that the Volyn biota presented by Franz et al. (2023) incorporates modern contaminants including plant hairs and a pollen grain, all present in modern museum (and presumably laboratory) dust, and the micro-FTIR spectra of specimens are not diagnostic of the thermal maturity that might be expected of in-situ fungal-like organisms, and suggesting the presence of younger fungal contamination. Moreover, the carbon and nitrogen isotope data presented by Franz et al. (2023) do not provide conclusive evidence of biological affinity. We have doubts whether any of the in-situ

205   Volyn 'biota' is organic in origin.

Data availability. All data are as figures in the text.

Supplement. There is no supplement related to this article.

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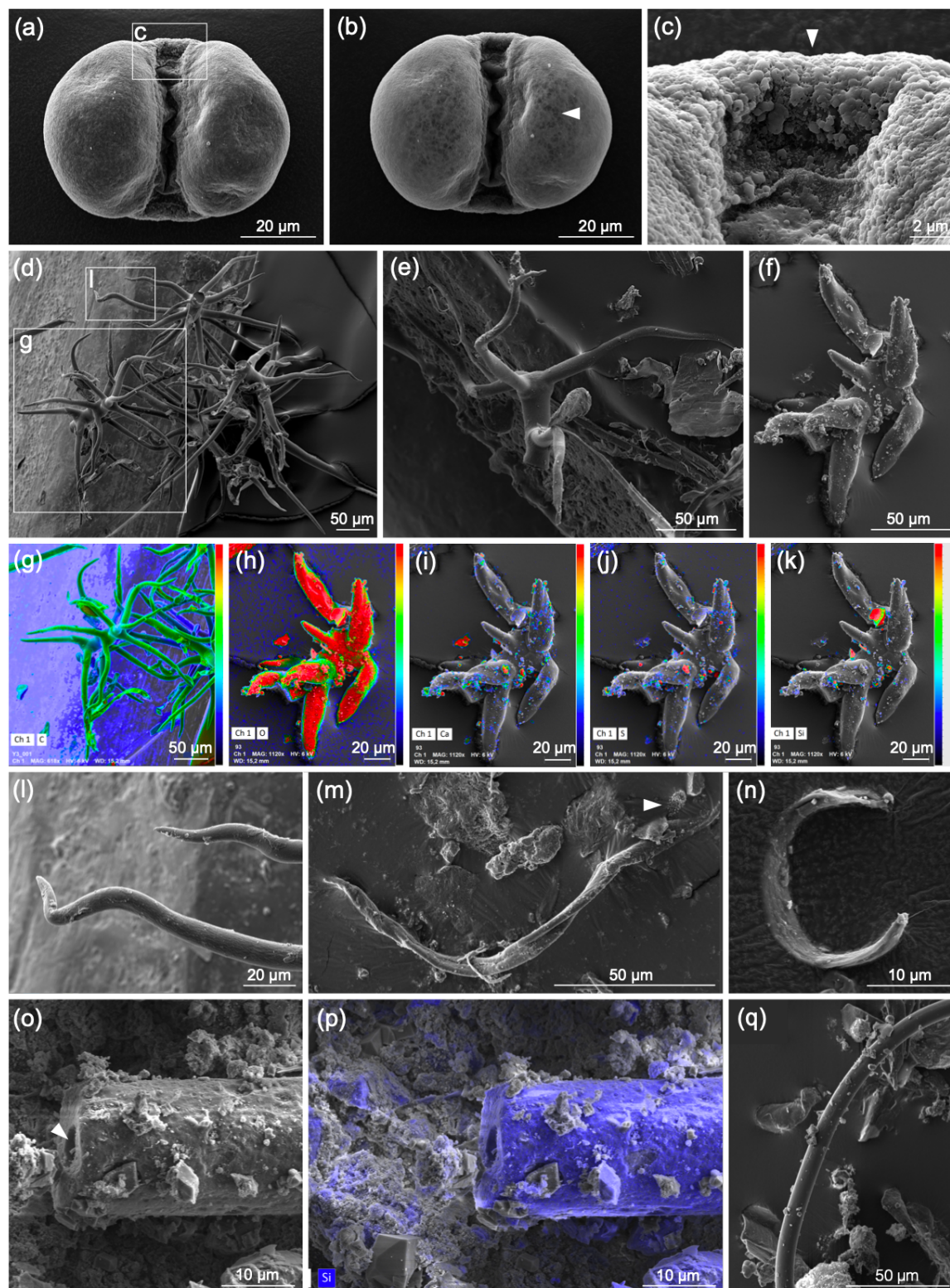
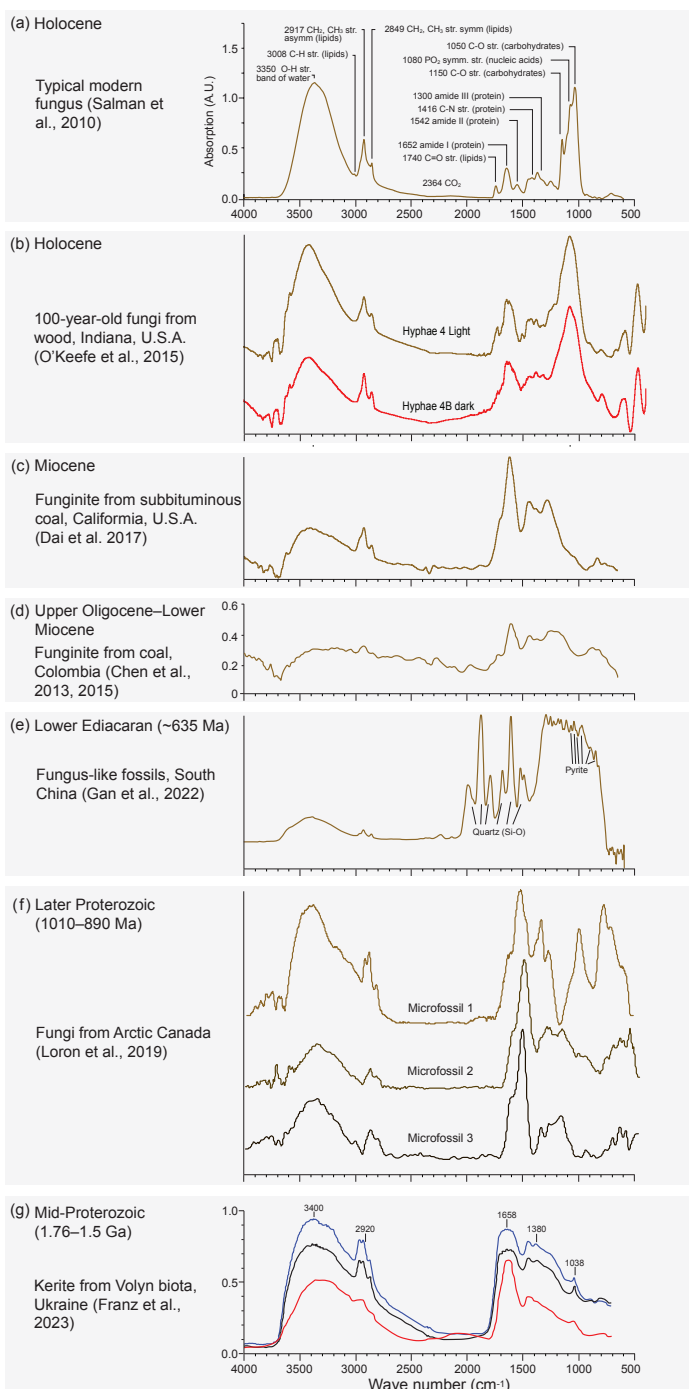


Figure 1. Museum 'dust' under the SEM. (**a–c**) *Pinus* pollen, (**a**) imaged with 6 kV accelerating voltage (AV), (**b**) imaged with 12 kV AV that reveals the infrareticulum of the pollen air sacs (arrowhead), as seen also using a high (10 kV) AV in fig. 9a–c of Franz et al. (2023), (**c**) detail of transitional area between leptoma and cappa with typical microrugulate ornamentation. (**d–l**) Plant hairs (trichomes) showing various morphologies and views, (**g–k**) observed with SEM combined with energy-dispersive X-ray spectroscopy (EDX) where heat maps depict distributions of elements (C = carbon; O = oxygen; Ca = calcium, S = sulfur; Si = silicon) on each object, (**l**) detail of sinuous tapering end of trichome. (**m–q**) Indeterminate filaments, where (m) shows an additional object (arrowhead) comparable to the arrowed specimen in fig. 2i of Franz et al. (2023), (**n**) cryptic specimen, (**o–p**) elongate specimen with a hole (arrowhead) in the middle of the thread-like structure, where (p) reveals mostly silicon (from silicate) under EDX analysis, and (**q**) smooth filament. Dust was mounted on aluminium stubs stickered with conductive carbon tabs, coated with palladium; imaged with a TESCAN CLARA SEM. Images: JG.

435 **Figure 2**. Published Micro-FTIR spectra of (**a**) typical modern fungus spectrum (fig. 1 in Salman et al., 2010) showing suginficant absorption bands and their main functional groups; note large bands at ~1050 cm^{-1} and 3350 cm^{-1}, (**b**) latest Holocene hyphae recovered from a log buried in a migrating sand dune for nearly a century (unpublished spectra from O'Keefe et al., 2015), (**c**) Miocene funginite from subbituminous Sand Bank Coal, Eel River Formation, California (fig. 7A in Dai et al., 2017), (**d**) Late Oligocene–Early Miocene fungal remains recovered from bituminous C coals, Amaga Formation,
440 Colombia (figs. 2 and 4b in Chen et al., 2013, 2015), (**e**) pyritized fungus-like fossils from the ~635 Ma lower Ediacaran of South China showing bands probably representing silica and pyrite (fig. 6b in Gan et al., 2022). (**f**) Fungi from later Proterozoic of Arctic Canada (Loron et al., 2019, their fig. 2 and extended data fig. 3). (**g**) the Volyn study presented by Franz et al. (2023, their fig. 13a). Note the general reduction in absorption with increasing melanization (dark hyphae) (**b**) and age/thermal maturity (**c**–**e**). Note also rising then lowering absorption and minor changes in wavenumber for the amide peak at 1652–1646.
445 In the kerite spectra this peak is at 1658, somewhat higher than in any of the other spectra. Note also the loss of the carbohydrate/lignin peak at 1082–1050 from modern to ancient samples (**a**–**d**). This peak is absent in its entirely in the kerite spectra and microfossils 2 and 3 (**f**) of Loron et al. (2019). The kerite spectra are overall most similar to the spectrum shown in (**c**), although they lack peaks at 1446 and 1277, and to that shown in (**f**), although it lacks saccharides.

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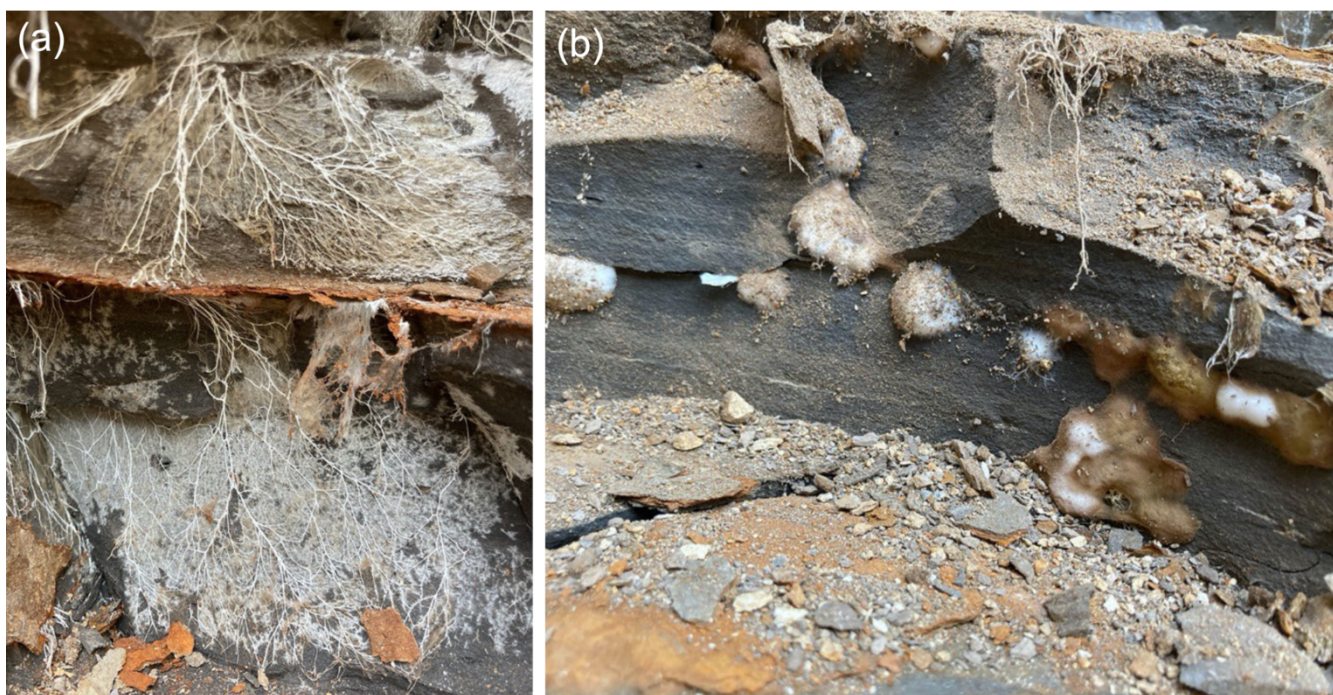
Figure 3. Examples of growth habits of extant lithotrophic fungi from the Clarkia Lagerstätte in Idaho, USA. Fungal mycelium
455   in the form of fan-shaped mats of hyphae along a joint opened up during excavation (a) and fungal mycelium forming bulbous
structures in voids in the rock, some with melanized spores coating the surface (b). Photos JMKO, July 2021.

Sample No.	material	sampling	Sampling date	locality	storage	analysis	Cleaning	preparation
0	kerite	circumstances unknown	unknown	unknown,	Museum, conditions unknown	SEM	none	Mounting on AL stubs with conductive carbon tab, coated with 5nm Ir
1	kerite	"fibre masses between fragments of the wall of the cavities and as larger masses hanging freely on the wall "Picked up with no need for separation from the rock matrix";	2018	from an underground mine shaft (shaft 3) in a depth of approximately 100 m.	stored in plastic bags	SEM, isotopes	none	
2	kerite						none	
3	kerite						none	
4	kerite						none	
5	kerite		2013				none	
6	kerite						none	
7	kerite						none	
8	Beryl cristal with kerite filamentous, flaky and		2008	Mine tailing pegmatite no. 2	No information provided		Dust-cleaned with compressed air	Coated with C
9a, b, c	Topaz with kerite		2018	from an underground mine shaft (shaft 3) in a depth of	No detailed information, presumably like	Only isotopes		

				approximately 100 m.	samples no. 1-7			
BO	Black opal		2018					

460   **Table 2**. Overview of samples analysed by Franz et al. (2023) and associated information. Compiled from Franz et al. (2022a, 2023).