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Early fungi from the Proterozoic era in Arctic Canada

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Supplementary Information

1. **Supplementary Tables**
2. **Supplementary Note**

1. Supplementary Tables

Supplementary information Table I. Average values of characteristic Raman parameters, Raman reflectance and Raman temperature and corresponding average and standard deviation. (N)=number of acquired spectra.

Ourasphaera giraldae	N	ωD1	ωG	ωG - ωD1	RmcRo	T°C Rmc Ro
macerates	**1**	1.349,9	1.598,4	248,5	2,1	196,6
Grassy Bay Fm	**2**	1.348,0	1.597,5	249,5	2,2	198,7
	3	1.348,2	1.596,3	248,2	2,1	196,0
	4	1.345,0	1.596,4	251,3	2,3	202,2
	5	1.349,9	1.595,8	245,9	2,0	191,2
	7	1.347,4	1.597,9	250,5	2,2	200,6
	8	1.346,7	1.598,0	251,4	2,3	202,3
	9	1.349,2	1.598,0	248,8	2,1	197,2
	10	1.352,8	1.597,1	244,3	1,9	187,6
	11	1.347,7	1.598,1	250,3	2,2	200,3
	12	1.347,5	1.598,2	250,7	2,3	201,0
	13	1.340,0	1.595,7	255,7	2,5	210,0
	17	1.351,3	1.600,2	249,0	2,2	197,6
	18	1.351,7	1.599,1	247,4	2,1	194,3
	19	1.353,3	1.600,6	247,4	2,1	194,3
	20	1.346,4	1.588,9	242,5	1,8	183,5
	21	1.348,8	1.594,2	245,4	2,0	190,0
	mean tot	**1.348,5**	**1.597,1**	**248,6**	**2,1**	**196,7**
	std tot	**3,2**	**2,7**	**3,1**	**0,2**	**6,3**
Ourasphaera giraldae	**N**	**ωD1**	**ωG**	**ωG - ωD1**	**RmcRo**	**T°C Rmc Ro**
thin section	**1**	1354,4	1598,7	244,4	1,9	187,8
Grassy Bay Fm	**3**	1356,4	1600,5	244,1	1,9	187,1
	4	1354,7	1599,4	244,7	1,9	188,4
	5	1354,6	1596,7	242,1	1,8	182,4
	6	1340,2	1599,4	259,2	2,7	215,9
	9	1351,9	1598,4	246,5	2,0	192,5
	11	1340,2	1601,7	261,5	2,8	219,4
	12	1352,9	1598,2	245,3	2,0	189,8
	13	1352,7	1609,4	256,7	2,6	211,8
	18	1354,1	1596,1	242,0	1,8	182,2
	19	1352,2	1596,1	243,9	1,9	186,7
	20	1358,3	1598,9	240,6	1,7	178,7
	21 (2)	1350,1	1599,7	249,6	2,2	198,8
	22 (2)	1356,6	1603,8	247,2	2,1	193,9
	23 (2)	1356,6	1596,2	239,6	1,7	176,3
	24 (2)	1356,4	1599,1	242,6	1,8	183,8
	25 (2)	1356,0	1596,4	240,4	1,7	178,1
	26 (2)	1351,4	1599,1	247,6	2,1	194,8
	27 (2)	1352,2	1597,5	245,3	2,0	189,9
	mean tot	**1352,7**	**1599,2**	**246,5**	**2,0**	**191,5**
	std tot	**4,9**	**3,2**	**6,2**	**0,3**	**12,3**

Supplementary information Table II. FTIR assignment of absoption bands (in cm^{-1}) and comparison with standards for chitin, chitosan and β-1,3 glucan. In grey are the typical bands of chitin, chitosan and β-glucan co-occurring in the same band range for the fossil specimen and the standards. vs= very strong; s= strong; m=medium; w= weak; vbr= very broad; br= broad; sh= shoulder.

Micro 1	α-Chitin Std (shrimp)	Chitosan Std	References assignments: [21; 36 - 39]
7347 s, br	3443 vs, br	3443 vs, vbr	O-H stretching
3413 s, br			O-H stretching
3259 s, br, sh	3265 s, br		N-H stretching
3098 m	3108 m		O-H stretching
2961 m	2963 w		C-H stretching (pyranose ring)
2928 m	2931 w		C-H stretching (pyranose ring, **chitin**)
2856 w	2885 w	2880 s	CH2 stretching (pyranose ring)
1670 s, sh	1654 vs, sh	1652 vs	Amide I C=O stretching (**chitin** amide band)
1626 vs, sh	1624 vs		Amide I C-N vibration (chitin amide band)
1592 vs, br		1591 s, sh	NH2 deformation (**chitosan**)
1548 vs, sh	1555 vs		Amide II
1451 s, sh			CH2 bending (pyranose ring)
	1422 m	1422 m	CH bending
1406 vs	1375 s	1378 s	Deformation N-H
1349 vs			Deformation C-H
	1313 s	1319 m	C-O-H bending+ CH2 deformation
	1260 w	1262 w	C-O-H bending+ CH2 deformation
1194, m, sh	1204 w	1201 vw	C-C stretching + C-O stretching + C-H deformation (pyranose ring)
1151 s, sh	1153 s	1151 s	Pyranose C-C
	1113 s		C-O stretching + C-H deformation
			C-O stretching + C-H deformation
1071 vs, br	1069 vs	1069 vs, br	Carbohydrate
	1022 vs	1031 vs	C-OH stretching
984 m, sh			C-C stretching
	952 m, sh		Beta-anomer C-H deformation
	896 w	898 w	Beta-anomer C-H deformation
861 vs, br			Beta-anomer C-H deformation
786 s, sh	747 w		C-H deformation
694 w	698 w		C-H deformation
662 w, sh		664 w	out of plane bending of aromatic C-H

Supplementary information Table III. Oldest known occurrences of main eukaryotic lineages used for Figure 3.

Lineages	Fossils	Geological formation	Locality	Age	Ref
Body fossils:					
EUKARYOTES	*Valeria lophostriata*	changzhougou Fm, Changcheng Gr	China	1673±10-1638±14 Ma	[40]
Fungi	*Ourasphaira giraldae*	Grassy Bay Fm (Shaler Supergr.)	Canada	1013±25-892±13	This study and [14]
Amoebozoa	Vase-shape microfossils	Urucum Fm (Jacadigo Gr.)	Brazil	<889±44 Ma	[41]
Rhizaria	Tests of early Foraminifera	Rasthof Fm	Namibia	746±2-635.6±0.5 Ma	[42]
Stramenopiles	*Jacutianema solubila*	Khatt Fm (El Mreïti Gr)	Mauritania	>1109±22 Ma	[43]
Alveolata	tests of early Ciliates	Tsagaan Oloom Fm	Mongolia	~715–635 Ma	[44]
Rhodophyta	*Bangiomorpha pubescens*	Hunting Fm (Somerset Island)	Canada	1047 +13/–17 Ma	[45]; [46]
Excavata	*Moyeria*	Caradoc	Shropshire (UK)	midle ordovician	[47]; [48]
Biomarkers:					
Metazoans	demosponge steranes	Huqf Supergroup	Oman	>635 Ma	[49]
Rhizaria	rhizarian steranes	Various locality in Australia, Oman, US		~659–645 Ma	[50]

2. Supplementary Note

Precambrian microfossils previously interpreted as possible fungi:

- *Caudosphaera* [51];
- *Huroniospora* [52];
- *Aimia, Eosaccharomyces, Majasphaeridium, Mucorites* and *Mycosphaeroides* [53];
- *Shuiyousphaeridium* [54];
- *Tappania* sp., *Germinosphaera* and *Trachyhystrichosphaera* [10];
- *Arctacellularia/Glomovertella* [55];
- *Tappania*, *Leiosphaeridia*, *Gyalosphaeridium*, *Ceratosphaeridium* and *Germinosphaera* [56];
- Fungus like mycelial fossils in basalt [57];
- Ediacaran *Charniodiscus*, *Dickinsonia*, *Hallidaya*, *Parvancorina*, *Phyllozoon*, *Praecambridium*, *Rugoconites*, *Tribrachidium* interpreted as Lichen [11].

FTIR microspectroscopy:

Chitin in fossils was previously detected successfully with FTIR spectroscopy (e.g. [58, 59, 60]). Within a single fossil assemblage, as in a modern dying microbial community, or even with a single microfossil, preservation can be variable. During degradation of chitin/chitosan, the typical absorption peaks become broader and less distinct [28, 61, 62, 63]. The saccharides bands decrease and become broader. As the degradation continues, they show very little absorbance. The amide region shows also broader and less distinct peaks. A decrease of absorbance is also observed in the O-H and N-H stretching regions.

Spectra of microfossils 2 and 3 presented in the supplementary information figure 3 are comparable to spectra of strongly degraded chitin/chitosan with very weak absorbtion peaks, due to the state of preservation of these microfossils. Analysis of better preserved *O. giraldae* also shows a spectrum representative of degraded chitin/chitosan displayed in Figure 1 in the main text, but with still all characteristic bands of chitin/chitosan detected . The saccharide region is present as a broad strong peak at 1071 cm^{-1} with a medium shoulder peak at 1151 cm^{-1} as in Chitin and Chitosan standard spectra. Overall, all the typical bands are present. The strong typical peak of chitosan at 1592 cm^{-1} is observable, with strong shoulder peak at 1670, 1626 and 1548 cm^{-1} corresponding to the amide bands of alpha chitin (see Table II above).

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