[Supplementary material]

The last meal of Tollund Man: new analyses of his gut content

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OSM1. Detailed description of the method of steroidal biomarker analysis.

Sample preparation

Approximately 1ml of the black suspension from Tollund Man's large intestine was placed in a 10ml glass vial with 5ml dichloromethane:methanol (2:1 vol) solution, mixed on a whirly mixer and ultrasonicated for 10 minutes. The suspension was transferred to glass centrifuge tubes and centrifuged at 3000rpm for ~10 minutes after which the solvent phase was isolated in a new vial. This was repeated three times in total and the combined extract were then evaporated to dryness at room temperature under a stream of nitrogen. This sample was transferred to a 2ml GC vial where 10µl intern standard was added as 10µl methyl tert-butyl ether (MTBE) solution containing 0.56 mg/ml deuteropalmitate followed by the addition of 70µl anhydrous pyridine. Next 70µl of a 99% *N*,*O*-bis(trimethylsilyl)trifluoroacetamide mixture containing 1% trimethylchlorosilane (Sigma Aldrich) was added to the vial and mixed. The vial with closed lid was placed on a heating block at 70°C for one hour. After cooling to room temperature, the solvent was removed by evaporation at room temperature under a stream of nitrogen. The product was dissolved as well as possible in 0.5ml n-hexane by ultrasonication. The vials were then centrifuged for 5 minutes at 5000rpm.

GC-MS analysis and data processing

The supernatant was isolated and transferred to a new GC vial and analysed on a Bruker SCION 456GC-TQMS equipped with a Restek Rtx-5 capillary column (30m, 0.25mm ID, 0.25µm) programmed for a 1ml min^{-1} helium flow. 1µl sample was applied on the PTV (Programmed Temperature Vaporization) injector which was held at 64°C from 0 min to 0.50 min, raised to

315°C at 200°C min^{-1} and held at that temperature for 40 min. The split ratio was 15 the first 0.5 min and then switched to 5. The GC oven temperature was held at 64°C from 0 min to 0.5 min, then raised to 190°C at 10°C min^{-1} and then to 315°C at 4 °C min^{-1} and held at that temperature for 15 min. The EI (electron ionisation) source temperature in the mass spectrometer was 250°C and the ionisation potential was −70 eV. The mass spectrometer was operated in the full scan mode between m/z 45 and m/z 800. Peak assignment was based on NIST database searches, characteristic fragments in the mass spectra (*m*/*z* 213 for sterols and *m*/*z* 215 for stanols) and comparison to pure cholesterol (CAS 57-88-5), sitosterol (CAS 83-46-5) and 5β-stigmastanol (CAS 19466-47-8) reference material (all from Sigma Aldrich).

OSM2. Method of protein analysis.

Sample preparation

Prior to MS analysis the sample was reduced reduced with 5 mM DTT and alkylated with Iodoaceamide. Subseqeuntly trypsin was added (1:50 w/w) and the sample was digested overnight. All incubation was carried out at room temperature except digestion, which was carried out at 37 °C. Digested protein was acidified with 1% formic acid and micro-purified using C18 column material (Empore™) packed in P10 pipette tips (Sarstedt).

LC-MS/MS and data processing

LC-MS/MS was performed using an EASY-nLC 1200 system (Thermo Scientific) connected to either an Orbitrap Eclipse Tribrid Mass Spectrometer or an Orbitrap QExactive+ (Thermo Scientific). Peptides were dissolved in 0.1% formic acid, injected, trapped, and desalted on a trap column (20mm × 100µm inner diameter). The peptides were eluted from the trap column and separated in a 15mm analytical column (75µm inner diameter). Both columns were packed in-house with ReproSil-Pur C18-AQ 3µm resin (Dr. Maisch GmbH, Ammerbuch-Entringen, Germany). Peptides were eluted using a flow rate of 250 nL/min and a 50 min gradient from 5 to 35% phase B (0.1% formic acid and 90% acetonitrile or 0.1% formic acid, 90% acetonitrile). The acquired MS data were peak picked in Proteome Discoverer 2.4 (Thermo Scientific). The .mgf files from Proteome Discoverer was searched against the entire UniProt database restricted to green plants and animal seqeunces using the mascot search engine. Search parameters were trypsin as protease; Precursor Mass Tolerance of 10ppm; Fragment Mass Tolerance of 0.05 Da and dynamic

modifications with oxidation on Met and Pro and deamidation of Gln. All peptides identified were searched in the NCBI nr database to determine if the seqeunces were unique to the indicted species.

OSM3.

Table S1. Results from the macrofossil analysis of Tollund Man's large intestine. Helbæk's original (1951) results are shown as numbers of identified seeds/fragments and estimations of the amount of seeds etc. in the gut sample: xxx: chief component; xx: many; x: one or few.

Latin plant name	English description	Numbers recorded in sub-sample	Estimated numbers in preserved sample	Estimated content in original sample		Helbæk's results (1951)	
		7.4ml	140ml	260ml		50ml	
Cultivated plants				Numbers	Weight	Numbers	Importance
Hordeum vulgare L.	Six-rowed barley	316	6007	11173	335g		xxx
Hordeum vulgare L.	Six-rowed barley, rachis segments	8	152	283		59	
Avena sp.	Oat, glume fragments						x
Linum usitatissimum L.	Flax	92	1749	3253	16g	5+47 frag.	xxx
Linum usitatissimum L.	Flax, seed capsule fragments	14	266	495			
Uncultivated plants							
Brassica rapa L.	Wild turnip	1	19	35	<0.1g		xx
Camelina sativa (L.) Crantz	Gold-of-pleasure	19	361	672	0.8g	3+4 frag.	xxx
Camelina sativa (L.) Crantz	Gold-of-pleasure, seed capsule fragments	3	57	106		4	
Capsella bursa-pastoris (L.) Medicus	Shepherd's-purse	1	19	35	<0.1g	3	x
Chenopodium album L.	Fat hen	12	228	424	0.6g	27	xx
Erysimum cheiranthoides L.	Treacle-mustard					3	x
Fallopia convolvulus (L.) A. Love	Black-bindweed	11	209	389	1.9g	31 frag.	xx
Galeopsis sp.	Hemp-nettles	1	19	35	0.2g		xx
Persicaria lapathifolia s.l.	Pale persicaria	271	5152	9582	29g	97+65 frag.	xxx
Plantago lanceolata L.	Ribwort plantain					1	x

Rumex acetosella L.	Sheep's Sorrel					4	x
Rumex sp.	Docks						x
Setaria pumila (Poir.) Roem. & Schult.	Yellow bristle-grass						x
Spergula arvensis L.	Corn spurrey	32	608	1131	0.6g	27	xx
Stellaria media L.	Common chickweed					1+2 frag.	x
Thlaspi arvense L.	Field penny-cress	¼	5	9	<0.1g	1 frag.	x
Viola arvensis Murray	Field pansy	8	152	282	0.2g	42	xx
Wetland plants							
Epilobium palustre L.	Marsh willowherb	1	19	35	<0.1g		
Juncus conglomeratus L./effusus L.	Compact/soft rush	1	19	35	<0.1g		
Viola palustris L.	Marsh violet	1	19	35	<0.1g		
Sphagnum sp.	Peat moss, leaves	13	247	460	<0.1g		x
Various							
	Food crust	1	19	35			
	Charcoal <2mm	16	304	566			x
	Sand 0.1-2.5mm	617	11729	21816	1.6g		x

OSM4.

Table S2. All raw counts and percentages of NPP from the gut content of Tollund Man.

NPP	Category	NPP count	% of total NPP sum
Taenia sp.	Parasite egg	87	10.9
Trichuris trichiura	Parasite egg	178	22.2
Ascaris sp.	Parasite egg	9	1.1
Linum sp. 1, MM-836	Plant fragment	112	14.0
Hordeum sp., MM-555	Plant fragment	113	14.1
Linum sp. 2, MM-568	Plant fragment	7	0.9
cf. *Camelina sativa*, MM-466	Plant fragment	16	2.0
cf. *Andromeda* sp., MM-461	Plant fragment	2	0.2
cf. *Galeopsis* sp., MM-586	Plant fragment	2	0.2
cf. *Galeopsis* sp., MM-838	Plant fragment	5	0.6
MM-443	Plant fragment	39	4.9
MM-484	Plant fragment	7	0.9
MM-487	Plant fragment	26	3.2
MM-505	Plant fragment	17	2.1
MM-517	Plant fragment	3	0.4
Cf. HdV-217, MM-519	Plant fragment	7	0.9
MM-540	Plant fragment	10	1.2
MM-818	Plant fragment	6	0.7
MM-837	Plant fragment	4	0.5

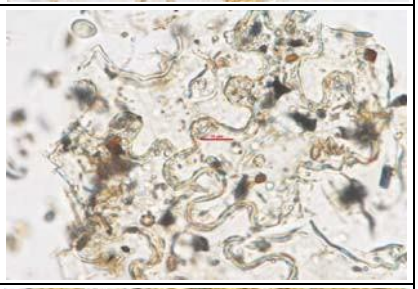
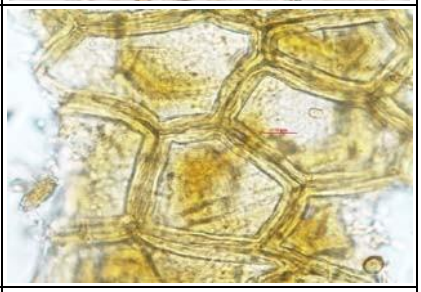
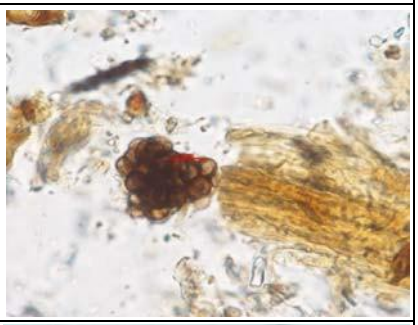
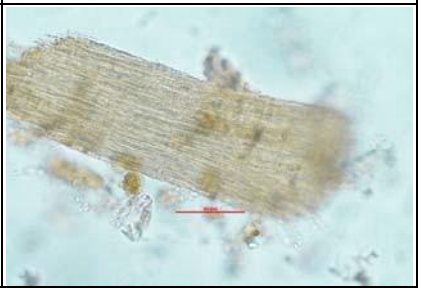
MM-839	Plant fragment	4	0.5
MM-840	Plant fragment	1	0.1
MM-841	Plant fragment	1	0.1
MM-845	Plant fragment	9	1.1
MM-846	Plant fragment	73	9.1
MM-847	Plant fragment	1	0.1
Sphagnum sp., MM-842	Moss	3	0.4
Oocyte undiff., MM-855	Algae	3	0.4
MM-856	Algae	8	1.0
Desmid undiff., MM-549	Algae	1	0.1
cf. *Xanthidium* sp., MM-674	Algae	1	0.1
Fibres undiff., MM-848	Fibre	5	0.6
Fungal spore, MM-356	Spore	2	0.2
Charcoal fragments >20µm	Charcoal	39	4.9
Total NPP sum		**801**	**100**

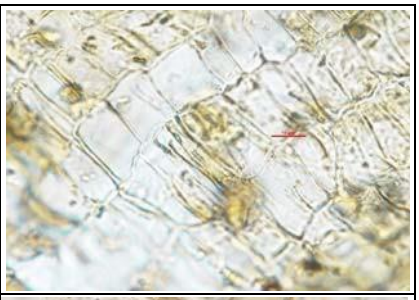
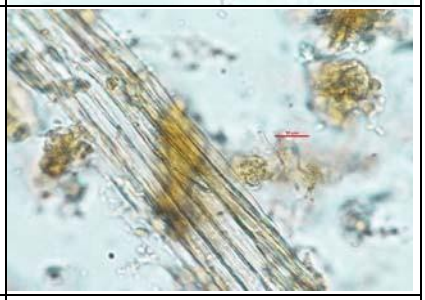
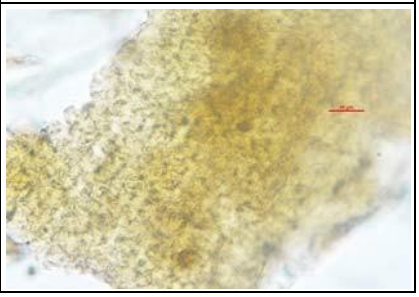
Table S3. All raw counts and percentages of pollen types from the gut content of Tollund Man.

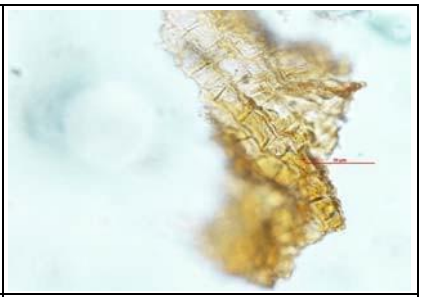
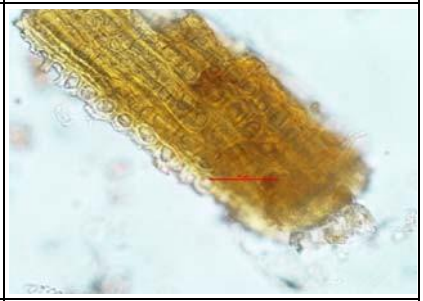
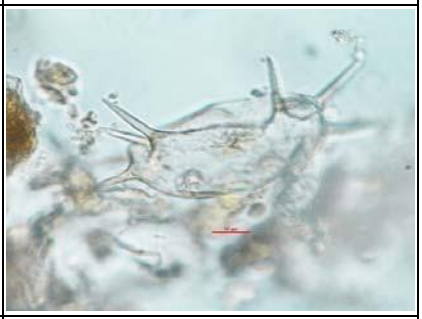
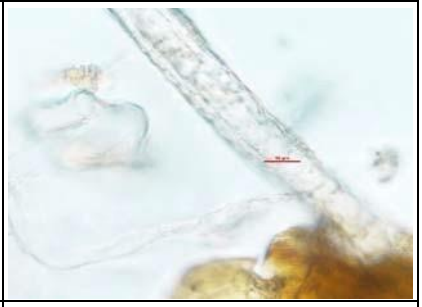
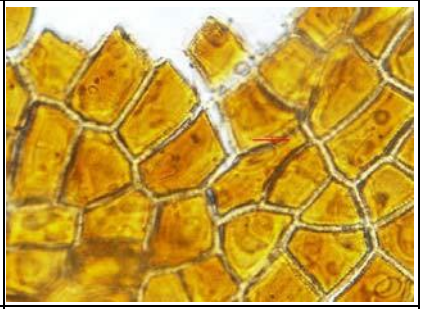
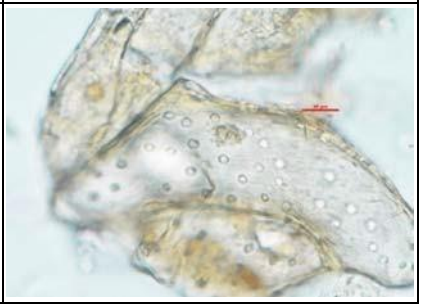
Pollen	Pollen count	% of total pollen sum
Achillea -t.	2	0.2
Artemisia	1	0.1
Betula	6	0.5
Brassicaceae	3	0.2
Calluna vulgaris	5	0.4
Carpinus	1	0.1
Caryophyllaceae	1	0.1
Cyperaceae	1	0.1
Hordeum -t	1142	90.2
Linum usitatissimum	2	0.2
Plantago lanceolata	1	0.1
Poaceae	58	4.6
Polygonum aviculare	13	1.0
Polygonum sect. *persicaria*	4	0.3
Potentilla	1	0.1
Rumex acetosella	9	0.7
Solidago -t.	1	0.1
Spergularia	4	0.3
Succisa	5	0.4
Taraxacum	1	0.1
Undiff.	5	0.4
Total pollen sum	**1266**	**100**

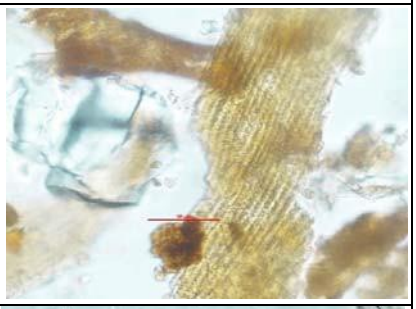
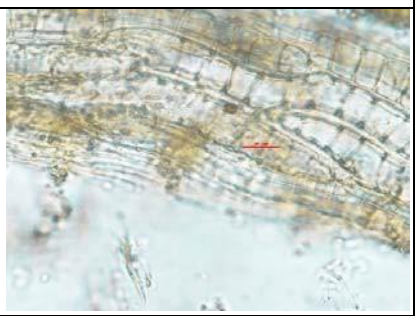
OSM5.

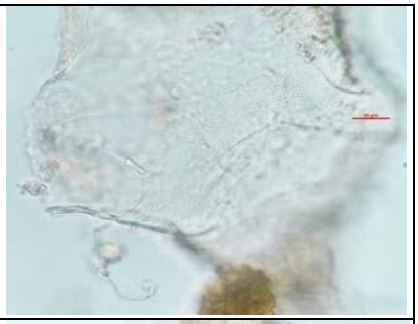
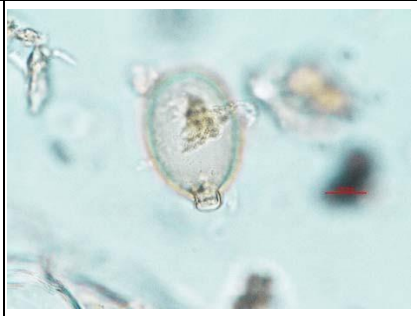
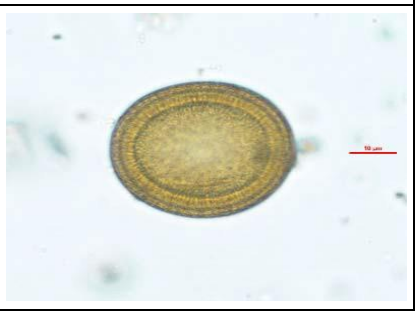
Table S4. Type number, descriptions and photos of all NPP-types found in the gut content of Tollund Man.

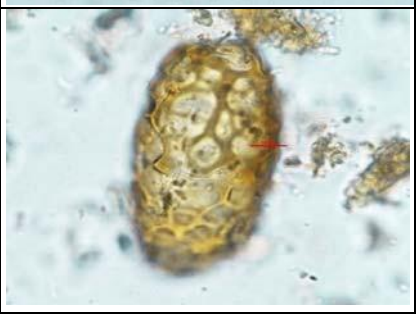
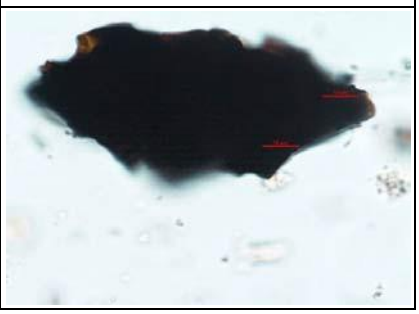
NPP	Description	Reference	Photograph
MM-356	Psilate, brown spore approx. 20µm in diameter and with three pores (e.g. rust fungi cf. *Uromyces appendiculatus* (uredospore)).	McAndrews & Turton (2010)	
MM-443	Plant tracheid with spherical thickenings. Unidentified plant fragment.		
MM-461	Hyaline plant epidermis cells with meandering, thick walls cf. *Andromeda* sp.	Maucouy & van Geel (2007)	
MM-466	Large, very thick-walled (up to 10µm), round to pentagonal plant epidermis cells cf. *Camelina sativa*.	PeterSteen Henriksen *(pers. comm.)*	
MM-484	Cluster of dark brown, round cells. Previously registered in prehistoric and modern freshwater sediments.	Enevold (2018)	
MM-487	Sheet of slim, flattened plant cells. Previously registered in prehistoric and modern freshwater sediments as well as medieval cess pits. Unidentified plant fragment.	Enevold (2018)	

MM-505	Square, hyaline, thin-walled plant cells. Unidentified plant fragment.		
MM-517	Hyaline, round to oval plant cells arranged in a 'brick pattern'. Unidentified plant fragment.		
MM-519	Hyaline spiral cf. HdV-217.	Van Geel et al. (1989)	
MM-540	Long, hyaline, thin-walled plant cells. Unidentified plant fragment.		
MM-549	Hyaline, stacked algal cells. Desmid undiff.		
MM-555	Epidermis cells of *Hordeum* sp.	Helbæk (1951)	

MM-568	Square, medium-walled plant cells similar to MM-505, but not lined up in a row cf. Linum sp. 2.		
MM-586	Palisade plant cells with crystals cf. *Galeopsis* sp.	Helbæk (1951)	
MM-674	Hyaline algal cell (half of a set) with evenly distributed equatorial spikes cf. *Xanthidium* sp.	Gerrath (1982)	
MM-818	Long, hyaline, thick-walled plant fibre (12–15µm) with evenly distributed depressions.		
MM-836	Epidermis cells from *Linum* sp. 1.	Helbæk (1951)	
MM-837	Large, yellow, thick-walled plant cells with evenly distributed pores (1–3µm) in diameter. Unidentified plant fragment.		

MM-838	Large surface plant cells cf. *Galeopsis* sp.	Helbæk (1951)	
MM-839	Long, hyaline, thick-walled plant cells with distinct perforations in the cell wall. Unidentified plant fragment.		
MM-840	Sheet of slim, long plant cells with a striate surface. Unidentified plant fragment.		
MM-841	Plant cell or fibre with distinct patternlike cotton, but with evenly distributedpores.		
MM-842	Hyaline, thick-walled cells with comb-like,transverse lamellae *Sphagnum* sp.	Maucouy & van Geel (2007)	
MM-845	Square, hyaline, thin-walled plant cells. Unidentified plant fragment.		

MM-846	Thin, hyaline sheet of plant epidermis with uneven, thin-walled cells and a scabrate surface. Unidentified plant fragment.		
MM-847	Large, yellow, round, thick-walled plant epidermis cells with indistinct pores in the cell wall. Unidentified plant fragment.		
MM-848	Slim, psilate organic fibres in a bundle.Fibres undiff.		
MM-855	Algal cell or invertebrate egg (oocyte undiff.) with a verythin wall and 2–4 spikes.		
MM-856	Flagellate/algal cell with a very thin wall and a protruding pore.		
Taenia sp.	Round to oval shaped, thick-walled egg.	Flisser *et al.* (2004)	

Trichuris trichiura	Oval shaped, thick-walled eggs with pores at both poles.	Brinkkemper & Haaster (2012)	
Ascaris sp.	Rounded, thick-walled eggs with a mamillated outer layer.	Brinkkemper & Haaster (2012)	
Charcoal fragment >50µm			

OSM6. Results of the protein analysis of gut material from Tollund Man (Excel file).

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