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The diet and environment of mammoths in North-East Russia reconstructed from the contents of their feces

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ABSTRACT

Mammoth feces from northern Yakutia and western Chukotka were investigated in a multidisciplinary study. Radiocarbon dating of the Yakutian mammoth dung yielded ca 42 ka BP and the age of the feces from Chukotka is older than 45 ka BP. The two sites are located about 15,000 km from each other and have a different geological setting. Most plant remains in the dung of both mammoths were grasses and sedges, with some other herbs and dwarf shrubs in addition. The pastures were situated in varying treeless shrubby landscapes: herb–grass associations of meadows, wormwood and shrub biotopes on slopes, in valleys and at watersheds. Besides plant remains and hairs of large herbivore mammals, the feces also contained feathers of Anseriformes, fragments of beetles and flies, ephippia of Cladocera, diatoms, remains of testate amoebae and ascospores of coprophilous fungi from pasture cenoses.

1. Introduction

The diet of the woolly mammoth *Mammuthus primigenius* (Blumenbach, 1799) is known from the intestinal content of remains preserved in the permafrost as well as from paleobotanical remains from the enclosing sediments (Guthrie, 1990; Ukraintseva, 1993, 2002; Tomskaya, 2000; Boeskorov et al., 2007; Van Geel et al., 2004, 2008; Zanina et al., 2011; Kosintsev et al., 2012a,b; Willerslev et al., 2014; Rudaya et al., 2015). The final products of digestion of Ice Age mammals have been rarely used for paleogeographical reconstructions (Mol et al., 2006a,b; van Geel et al., 2011a; Novgorodov et al., 2013). Nevertheless they are valuable sources of information on consumed food and the environment; similar to the coprolites of other fossil animals, or of feces of modern organisms (Chin, 2002; Argant and Dimitrijević, 2007; Owocki et al., 2012).

Mammoth feces preserved in the permafrost represent a particular category. They are not coprolites because they are not fossilized. They are preserved, together with other organic remains including microorganisms (Gilichinsky and Wagener, 1995; Gilichinsky et al., 2008), by the protective influence of the permafrost.

The feces of large herbivores, similar to feces of modern elephants, were found in two river valleys in North-East Russia: the Terekhtyakh River (N. Yakutia), and Maly Anyui River (W. Chukotka) (Fig. 1). Here we report on a multidisciplinary study of these finds that nowadays are part of the collection the Ice Age Museum in Moscow.

Following the zonation of Gvozdetsky and Mikhailov (1978), modern sharply continental climate is typical for the locations where the feces were found. These locations are in Arctic and

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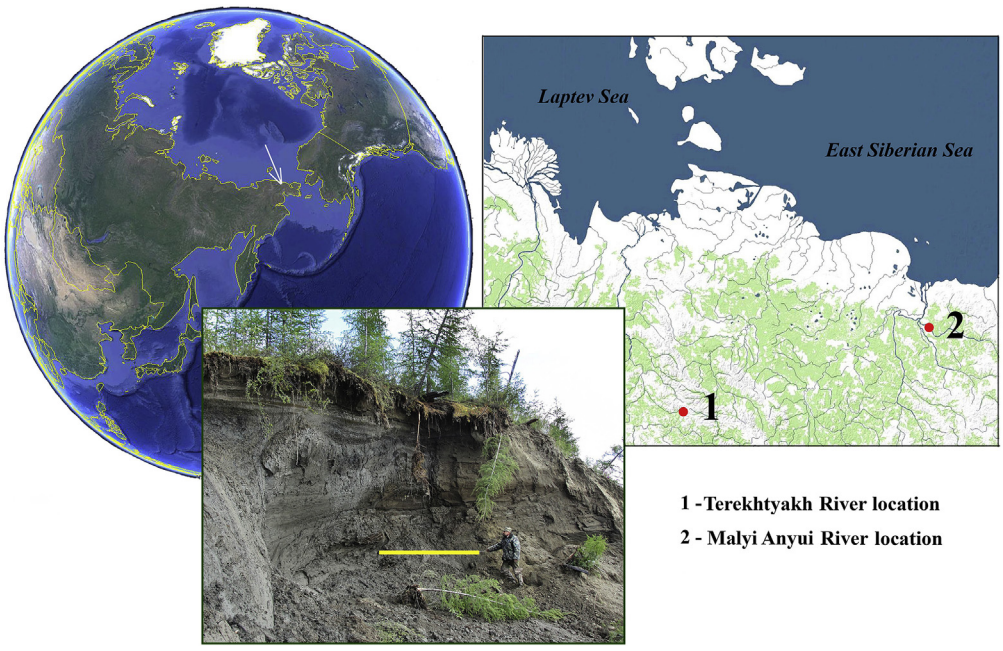

Fig. 1. Localities of the mammoth feces finds. 1 – the Terekhtyakh River, northern Yakutia. 2 – the Maly Anyui River, W. Chukotka. Inset is a picture of a river section in the locality of Sample 2. The line denotes the level of placement for the lens containing feces.

Subarctic climatic regions. The average annual temperature is lower than −10 °C. This severe climate causes deep freezing of formations and permafrost conditions which determine the landscape and preserves organic remains. Such climate also causes soils and vegetation to be relatively uniform.

In northern Yakutia, considerable areas are covered by open larch woodlands with dwarf birch (*Betula* sp.) and willows (*Salix* sp.) in the underbrush, and with cowberry *Vaccinium vitis-idaea* L. and blueberry *V. uliginosum* L. in the ground cover. Small areas of steppe and steppe meadows occasionally occur on the slopes of south facing hills. Treeless steppe communities are formed at slopes of the valleys, where fast and deep defrosting of the ground takes place during spring and early summer, causing a shortage of moisture in the soil during the growing season. Under such conditions, forest vegetation cannot survive. Mountain areas are either occupied by Siberian dwarf pine, or show a mountain tundra or cold stony desert.

In western Chukotka, with its Arctic climate, sparse scattered plants typical for the mountain tundra and open woodlands with dwarfed larch dominate the landscape. It should be noted that now steppe communities are absent in the alpine tundra belt of northern Siberia (Yurtsev, 1973; Gvozdetsky and Mikhailov, 1978).

2. Material and methods

2.1. Material

Sample 1. (Fig. 2A). Collection #F-552. Sample 1A – feces, sample 1B – a fragment of enclosing sediments. The feces were found by a local resident in 2005, in a natural exposure on the left bank of the Terekhtyakh River (right tributary of the Lower Indigirka), 40 km downstream of the settlement Belaya Gora, the administrative center of the Abyiski District of the Republic Sakha (Yakutia) (68°32′ N, 146°11′ E). The material comes from a peat lens situated within icy aleuritics 3–4 m under the ground surface, and 3–4 m above the shoreline. Apart from the mammoth feces the lens also contained bones of Ice Age mammals.

Sample 2 (Fig. 2B). Collection #F-3447. The two studied feces samples (2A and 2B) were found by local resident Lev Meskhi in 2007 at the foot of the river outcrop at the right bank of the Maly Anyui River (68°18′ N, 161°44′ E), 14 km upstream of the settlement Anyuisk, Bilibino District, Chukotka Autonomous Region. The material was melted out of a lens of dark ice, situated below the peat lens, 3–4 m below the ground surface. The material of this lens had been washed out by the river water during spring floods, and formed part of coastal debris along the shoreline. Mammalian remains were not found in this lens. Today, the lens is destroyed by water erosion.

2.2. Methods

To reconstruct the diet of the mammoths and environmental conditions of the two distant localities, we analyzed macrofossils, microfossils, pollen, and other organic inclusions.

2.2.1. Paleobotanical and paleoentomological methods

Subsamples for pollen analysis weighing 3–11 g were taken from the very center of the feces and submitted to specialists. The remaining parts (0.5–1.0 L) were used for the other studies. In addition, samples of the enclosing sediments were used for pollen analysis.

Samples for pollen analysis were prepared using standard techniques according to Faegri and Iversen (1989) and Argant (1990), including the use of heavy liquid. An Olympus BX 51 microscope with magnification 400× and a Leica DM3000 microscope with magnification 500× were used. At least 500 pollen grains were counted in every sample. The percentage of identified pollen and spores was determined. For identification of pollen and spores various sources were used (Moore et al., 1991; Reille, 1992).

Samples for plant macrofossil and paleoentomological analyses were washed through sieves with 0.2 mm mesh, following Nikitin (1969). A Carl Zeiss Stemi 2000-C stereomicroscope with 7×–56× magnification was used for the analysis. The identification of macrofossils was based on reference collections of seeds and other

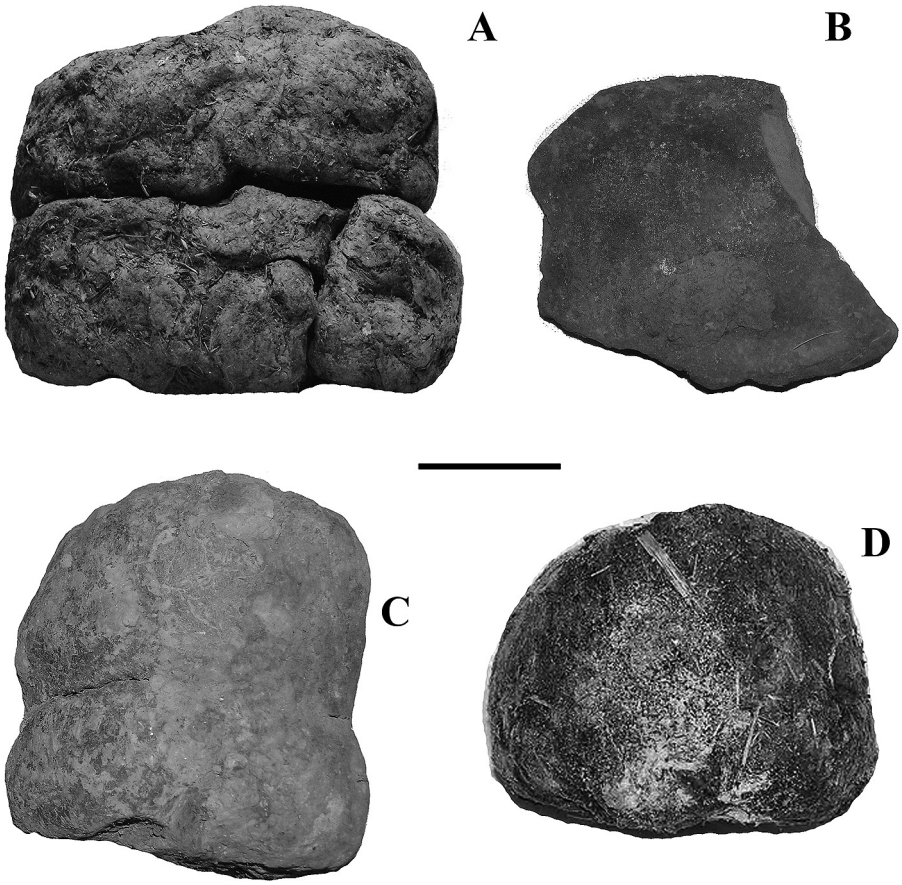

Fig. 2. Objects of the study. A – sample 1, feces. B – sample 1, enclosing sediments. C – sample 2, scale 5 cm. D – feces of the recent Indian elephant from Moscow Zoo.

plant remains at the Institute of Plant and Animal Ecology, Ural Branch, Russian Academy of Sciences (Yekaterinburg, Russia).

The insect remains were extracted simultaneously with plant fragments, using a stereomicroscope. The number of individual insects was determined according to the rule of the minimal number: two elytras, pronotum (or its fragment), and a head of the same species were regarded as one individual.

Phytoliths were extracted from the samples using wet oxidation and heavy flotation techniques. About 5–10 g of a sample was used for each separation. The microfossils were extracted by dissolution of carbonates utilizing oxidation of the organic matter with hydrochloric acid, using 38% hydrogen peroxide at 90 °C. Clay was removed by physical means. Isolation of microfossils from the residue was achieved by separation in a heavy liquid solution with a density of 2.2–2.3 g/cm^3. Microfossil remains were studied using a Carl Zeiss Axiostar optical microscope at 200×–400× magnification, and dry material under a Vega 3 Tescan Scanning Electron Microscope. Phytoliths from the samples were classified in accordance with the International Code for Phytolith Nomenclature 1.0 (Madella et al., 2005).

2.2.2. Extraction and investigation of small crustaceans, feathers, and hairs

Extraction and investigation of small crustaceans, feathers, and hairs was done with a stereomicroscope Leica MZ7.5. To remove soil and bacterial pollution from ephippia and chitinized fragments, we rinsed them with 95% alcohol. The fragments of hairs and feathers were washed, desiccated and degreased in alcohol series of increasing concentrations. After drying and sputtering with gold, the samples were studied under the Electron Scanning Microscopes Tescan Vega (Czech Republic) and JSM 840A (Japan). Electronic graphs were made from the hair surface and of transverse and longitudinal sections of the shaft as well.

2.2.3. Radiocarbon dating

Before the actual ^{14}C measurement, samples were chemically pre-treated in order to isolate the datable fraction, and to remove contaminants (Mook and Streurman, 1983).

The routine treatment of samples consists of the following steps: (i) treatment with acid (HCl) in order to remove soil carbonate and possibly infiltrated humic acids; (ii) treatment with alkali (NaOH) to remove other contaminants e.g. soil humates; (iii) treatment with acid (HCl) to remove any CO_2 absorbed during step ii. This procedure is referred to as the “AAA” treatment.

After the chemical pre-treatment, the samples were combusted and turned into CO_2 by an Elemental Analyzer (EA), coupled on-line with a stable isotope Mass Spectrometer (MS). The EA was also used for purifying of CO_2. In addition, the EA/MS system enables precise measurements of the δ^{13}C-values.

CO_2 was reduced to graphite by reacting under the excess of hydrogen gas (Aerts-Bijma et al., 2001). This graphite was pressed into the target holders which were placed in the ion source of the AMS (Accelerator Mass Spectrometer). The Groningen AMS facility is based on a 2.5 MV accelerator, and measures the ^{14}C concentration in the graphite (Van der Plicht et al., 2000).

The results are presented as conventional dates, which include correction for isotopic fractionation and the use of the conventional half-life (Mook and van der Plicht, 1999). The ^{14}C dates (reported in

BP) are calibrated into calendar ages (reported in cal BP, i.e. calender years relative to 1950) using the recommended calibration curve IntCal13 (Reimer et al., 2013).

2.2.4. 16S rRNA sequencing

Total DNA from 1 g of feces sample was extracted using the DNA Spin Kit for Soil (MO Bio laboratories) as per manufacturer's instructions with additional treatment by PTB (Vasan et al., 1996). The bead-beating was performed using TissueLyser II (Qiagen). The quality of DNA samples was assessed using agarose gel electrophoresis and Nanodrop (Thermo Scientific).

The V3–V4 region of the 16S rRNA genes was amplified with the primer pair 343F (5′-CTCCTACGGRRSGCAGCAG-3′) and 806R (5′-GGACTACNVGGGTWTCTAAT-3′) combined with Illumina adapter sequences, a pad and a linker of two bases, as well as barcodes on the primers (Caporaso et al., 2012). PCR amplification was performed in 50 μl reactions containing 0.7 U Phusion Hot Start II High-Fidelity and 1× Phusion GC buffer (Thermo Fisher Scientific), 0.2 μM of each forward and reverse primers, 10 ng template DNA, 2.3 mM $MgCl_2$ (Sigma–Aldrich) and 0.2 mM of each dNTP (Life Technologies). Thermal cycling conditions were as follows: initial denaturation made at 98 °C for 1 min was followed by 30 cycles of 98 °C for 15 s, 62 °C for 15 s, and 72 °C for 15 s, with final extension at 72 °C for 10 min. A total of 200 ng of PCR product from each sample was pooled together and purified through MinElute Gel Extraction Kit (Qiagen). Library was sequenced on MiSeq Illumina sequencer using the MiSeq Reagent Kit v2 2 × 250 (Illumina) according to the protocol described previously (Caporaso et al., 2011, 2012).

250PEs were overlapped and quality filtered (QV > 20). Any overlapping readings with ambiguous sites and also without primers were removed using CLC GW 7.0 (CLC Bio). RDP Classifier 2.10 (Wang et al., 2007) was used for taxonomic analysis. Before analysis chimera checking was performed by use arch 8.0 (Edgar et al., 2011).

3. Description and results

Specimen 1 (F-552) from the Terekhtyakh River consists of two undamaged well preserved dung heaps (100–115 mm high, 160 mm of maximum diameter, weighing 426 and 439 g) consisting of partly decomposed, sometimes peaty plant macroremains, mainly dark brown in color with notable inclusion (up to 40% of volume) of brownish silty loam saturated with organic matter. The enclosing very dense clay (lacustrine) layers had a similar color. Each sample was structured and consisted of several smaller blocks. Different organic remains were found (Fig. 3). Arboreal detritus and phytoliths of conifers were absent.

Specimen 2 (F-3447) from the Maly Anyui River consists of two well preserved objects with a height of 90 and 125 mm, weight 391 and 410 g, and dimensions 100 × 115 and 95 × 110 mm. It consists mainly of partly decomposed vegetable fragments with parts of bird feathers (Fig. 4) and mammalian hairs belonging to mammoth, bison and horse.

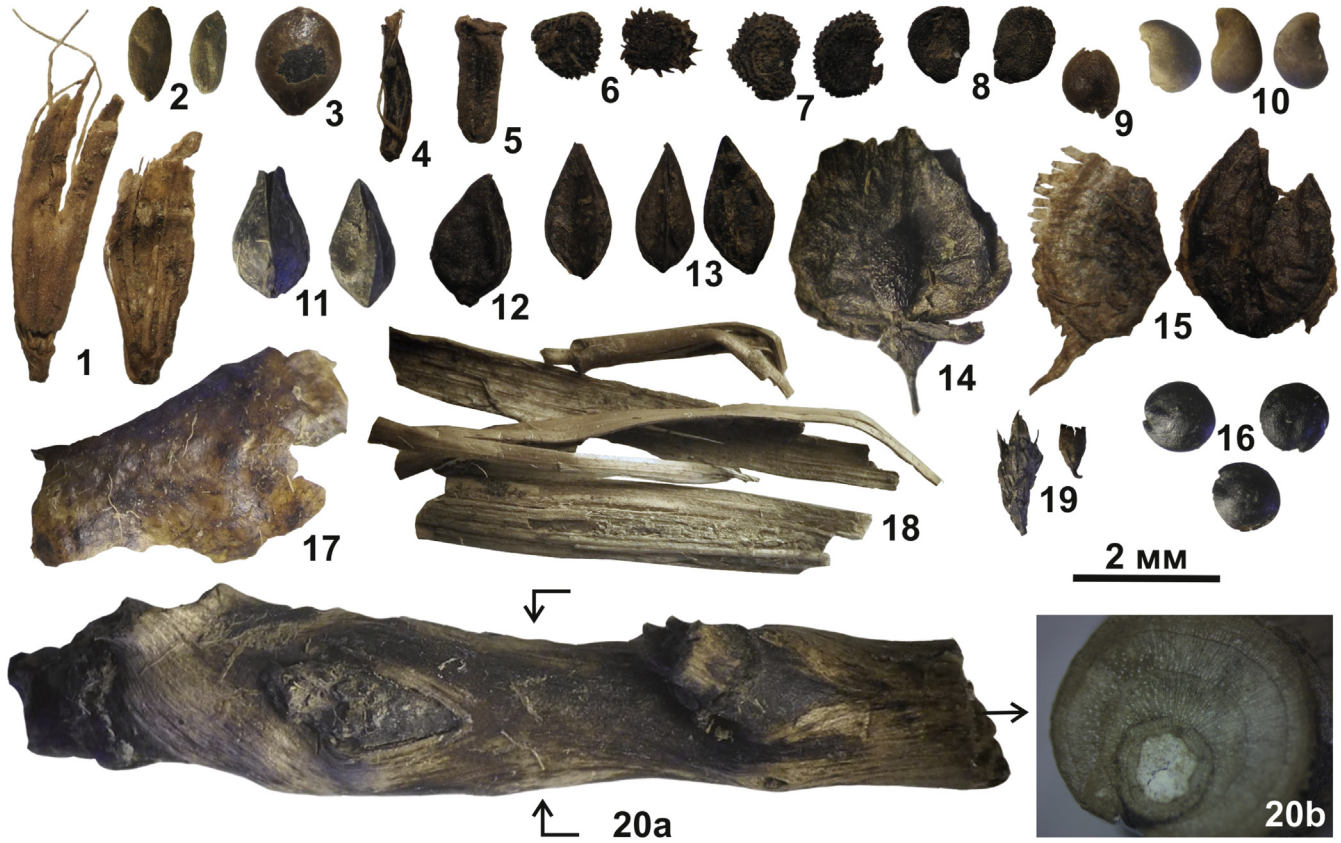

Fig. 3. Macroremains from Sample 1 (F-552). 1 – spikelets of *Hordeum* sp.; 2 – tegmens of *Puccinellia* sp.; 3 – nutlet of *Carex* cf. *melanocarpa*; 4 – seed of *Tripleurospermum hookeri*; 5 – seed of *Artemisia tilesii*; 6 – seeds of *Cerastium* sp.; 7 – seeds of *Silene* sp.; 8 – seeds of *Caryophyllaceae* gen. indet.; 9 – seed of *Rorippa* sp.; 10 – nutlets of *Potentilla* cf. *stipularis*; 11 – nuts of *Rumex sibiricus*; 12 – nut of *Polygonum humifusum*; 13 – nuts of *Rumex acetosa* ssp. *lapponicus*; 14 – perianth of *Rumex acetosa* ssp. *lapponicus*; 15 – seeds of *Corispermum crassifolium*; 16 – seeds of *Chenopodium prostratum*; 17 – mucosal gastrointestinal tissue fragments; 18 – fragments of leaves and stems of Cyperaceae and Poaceae; 19 – sprig, sporangium of Bryales; 20a – sprig of *Salix* sp.; 20b – cross-section of *Salix* sp. sprig.

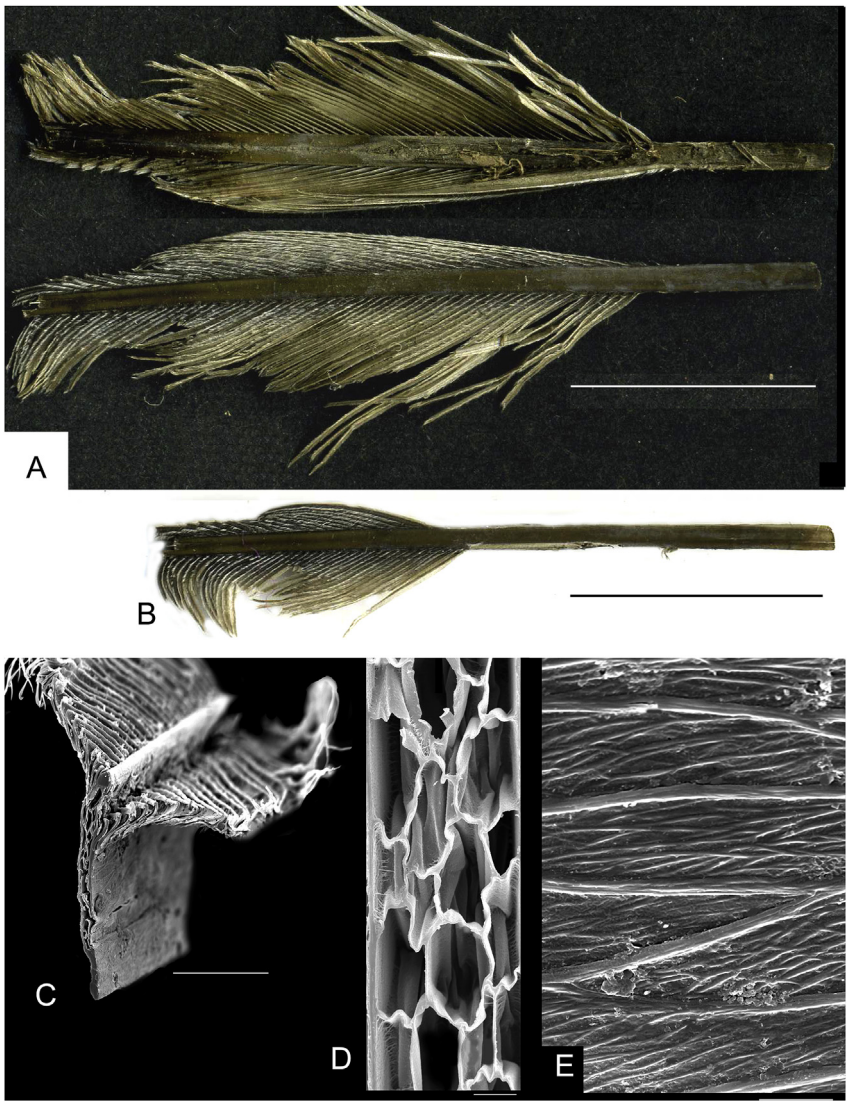

Fig. 4. Bird feathers from Sample 2. A, B – vaned feathers of Anseriformes. C–E – microstructure of a barb from the contour part of the vane: C – transverse section, D – the core, E – cuticular ornament. A, B — scanned image. Scale 10 mm. C–E – scanning electron micrographs. Scale: C – 100 μm, D – 10 μm, E – 1 μm.

Specimen 2 was more consolidated than specimen 1, and the materials it contained were larger in size. The feces of both sites were similar by their dimensions and appearance to those of extant Asian elephants (Fig. 2D).

3.1. Results of paleobotanical studies

Structure and distribution of microfossils within the samples are shown in Tables 1 and 2.

3.1.1. Biomorphs and phytoliths (Fig. 5, Table 1)

Most of Sample 1 consisted of epidermis of Poaceae and other herbs, and their vascular tissues. Remains of arboreal taxa were not found. In addition to vegetable detritus, pollen, spores, stomatal complexes and other plant tissues, spicules of freshwater sponges, shells of testate amoebae, fragments of diatoms and also a small amount of rather similar and badly preserved (possibly underdeveloped) phytoliths were found, as well as remains of insects and small crustaceans, hair fragments of mammoth and reindeer, and fragments of mucous intestinal tissue.

Table 1
Occurrence of phytoliths of various plants in mammoth feces.

Sample №	Number of morphotypes	Groups of phytoliths						
		Dicotyledonous herbs (forbs)	Meadow grasses	Fescue grasses	Sedges	Ericaceae	Conifers	Mosses
Sample 1	10	±	+	–	±	±	–	–
Sample 2	15	+	+	±	–	–	sol.	sol.

«+» – prevail; «±» – occur in noticeable number; «sol.» – solitary instances; «–» – absent.

Table 2
Pollen and plant macrofossil records of samples from mammoth's feces.

Specific definition of the plant macrofossils, pollen and spores		Samples of mammoth's feces from Maly Anyui River (F-3447)						Samples of mammoth's feces and enclosing sediments (peaty loam) from Terekhtyakh River (F-552)					
		Pollen				Macroremains[c]		Pollen				Macroremains[c]	
		Sample 2A[a]		Sample 2B[b]				Feces[b]		Peaty loam[b]			
Family	Genus/species	n total	% total	n total	% total	n total	% total	n total	% total	n total	% total	n total	% total
Apiaceae	Undiff.	3	0.50										
Asteraceae	Undiff.			2	0.13			6	0.58	1	0.2		
	Artemisia sp.	95	15.81	368	24.45			210	20.43	283	56.6	1	0.04
	Artemisia tilesii											1	0.04
	Tripleurospermum hookeri											2	0.08
Betulaceae	*Alnus*	1	0.17										
	Alnus fruticosa			5	0.33	1	0.17						
	Betula sect. *Albae*			3	0.20								
	Betula sect. Nanae			14	0.93	1	0.17	1	0.10	2	0.4		
Brassicaceae	Undiff.	3	0.50					1	0.10			5[d]	0.19
	Erysimum sp.					3	0.51					8	0.31
	Rorippa sp.					1	0.17					1[d]	0.04
Caryophyllaceae	Undiff.	30	4.99	11	0.73	1[d]	0.17	136	13.23			54[d]	2.08
	Cerastium sp.											8	0.31
	Dianthus sp.											6[d]	0.23
	cf. *Minuartia* sp.											10 + 3 t	0.50
	Moehringia sp.					1	0.17						
	Silene sp.											23	0.89
	Stellaria cf. *crassifolia*											3[d]	0.12
Chenopodiaceae	Undiff.			3	0.20			66	6.42	2	0.4		
	Chenopodium album											4[d]	0.15
	Chenopodium glaucum											4	0.15
	Chenopodium prostratum					8	1.36					817 + 23 t	32.35
	Corispermum crassifolium											3[d]	0.12
Cyperaceae	Undiff.	6	1.00	193	12.82			19	1.85			1	0.04
	Baeothryon sp.											1	0.04
	Carex cf. *melanocarpa*											223	8.59
	Carex sp.					88 + 25[d]	19.15					51 + 30[d]	3.12
	Eleocharis sp.					1[d]	0.17						
	Eleocharis palustris											9	0.35
	Kobresia sp.					13 + 6[d]	3.22						
Ericaceae	Undiff.	1	0.17										
Fabaceae	Undiff.	14	2.33	2	0.13			17	1.65				
Papaveraceae	*Papaver* sp.					1	0.17						
Pinaceae	*Picea* sp.			2	0.13								
	Pinus s/g Diploxylon	1	0.17	1	0.07								
Plantaginaceae	*Plantago* sp.	4	0.67										
Poaceae	Undiff.	409	68.05	854	56.74	90[d] + 10 t	16.95	504	49.03	202	40.4	513 t + 27[d]	20.79
	Bromopsis sp.											35[d]	1.35
	Deschampsia sp.											1	0.04
	Festuca sp.					98	16.61						
	Hordeum sp.					5	0.85					50[d]	1.93
	Poa sp.					150	25.42					13[d]	0.50
	Puccinellia sp.					1 t	0.17					56	2.16
Polygonaceae	Undiff.			7	0.47			9	0.88				
	Polygonum sp.					1[d]	0.17						
	Polygonum amphibium	4	0.67										
	Polygonum humifusum											19 + 13[d]	1.23
	Rumex sp.											120[d]	4.62
	Rumex lapponicus											27 + 15[d] + 6 t	1.85
	Rumex sibiricus											17+6[d] + 4 t	1.04
Potamogetonaceae	*Potamogeton* sp.					1	0.17						
Primulaceae	*Androsace* sp.											2	0.08
Ranunculaceae	Undiff.			2	0.13					1	0.2		
	Ranuculus aquatilis	3	0.50										
	Ranunculus pulsatilla	1	0.17										
	Thalictrum	1	0.17										
Rosaceae	Undiff.			33	2.19			53	5.16	7	1.4		
	Comarum palustre					2 + 1[d]	0.51						
	Dryas sp.	2	0.33										
	Filipendula sp.	2	0.33										
	Potentilla sp.	17	2.83			41 + 21[d]	10.51						
	Potentilla cf. *stipularis*											165 + 192[d]	13.75
	Sanguisorba officinalis	2	0.33										
	Sorbus-type	1	0.17										
Salicaceae	*Salix* sp.			3	0.20	2	0.34	4	0.39	2	0.4		
Scrophulariaceae	*Veronica* sp.											1[d]	0.04
Valerianaceae	*Valeriana* sp.							2	0.19				
Urticaceae	*Urtica dioica*					1	0.17						

Table 2 (*continued*)

Specific definition of the plant macrofossils, pollen and spores		Samples of mammoth's feces from Maly Anyui River (F-3447)						Samples of mammoth's feces and enclosing sediments (peaty loam) from Terekhtyakh River (F-552)					
		Pollen				Macroremains[c]		Pollen				Macroremains[c]	
		Sample 2A[a]		Sample 2B[b]				Feces[b]		Peaty loam[b]			
Family	Genus/species	n total	% total	n total	% total	n total	% total	n total	% total	n total	% total	n total	% total
Bryales	Undiff.			2	0.13	16[d]	2.71					23[d] v	0.89
Sphagnaceae	Sphagnum sp.											1 v	0.04
Trilete spore		1	0.17										
Total		601	100.00	1505	100.00	590	100.00	1028	100.00	500	100	2597	100.00
Undeterminate		9	1.50	11	0.73					3	0.6		
Coprophilous fungi		9	1.50	212	14.09			115	11.19	15	3		
Gaeumannomyces hypha					0.00			1	0.10				
Pre-Quaternary spores				1	0.07								

[a] Analysis by Jacqueline Argant.
[b] Analysis by Elena Lapteva.
[c] Analysis by Olga Korona.
[d] Fragment of macrofossil, v – vegetative part, t – tegmen.

Sample 2 contained a large amount of coarse vegetable detritus and phytoliths. The epidermis and vascular tissues of grasses and dicotyledonous herbs dominated. They were well preserved, with clear cellular structure and stomata. The most numerous phytoliths belonged to members of grasses (Family Poaceae; genera *Poa*, *Calamagrostis*, and *Festuca*). Most phytoliths were of distinct shape, and plant tissues were mature, which is typical for the end of the vegetation period. Tissues of mosses and sedges, and remains of arboreal taxa (apart from *Pinus*) were absent. The remains of heathers (Family Ericaceae) most probably belonged to dwarf shrubs such as blueberry, cowberry, or cranberry (*Vaccinium oxycoccos* L.)

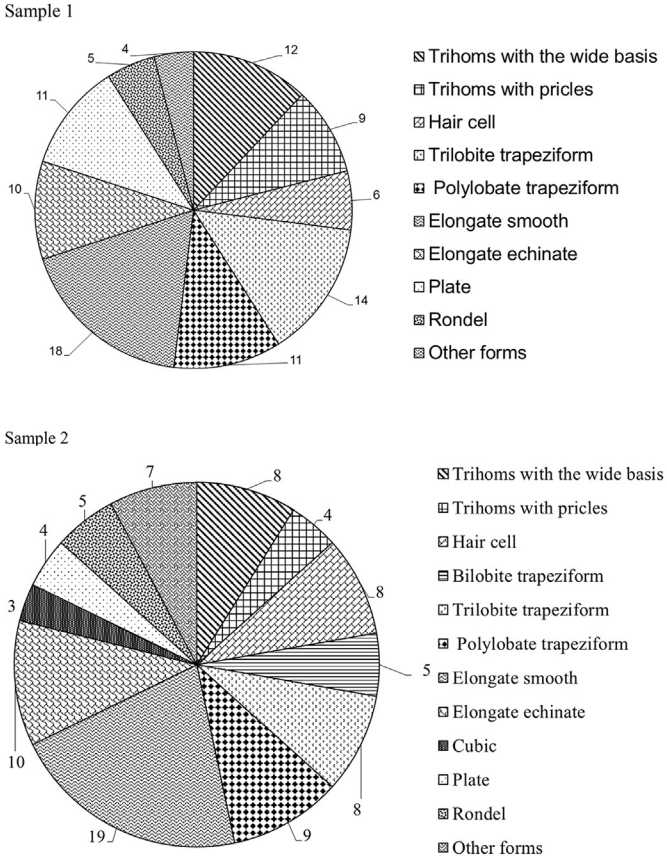

Fig. 5. Comparison of different forms of phytoliths from Sample 1 (northern Yakutia) and Sample 2 (western Chukotka).

3.1.2. Pollen and spores

In the pollen spectra of feces sample 1A herbaceous plants dominate (Fig. 6, Table 2). Grasses prevail (Poaceae — 45.0–50.2%), pollen of *Artemisia* sp. were 20%, pollen of Caryophyllaceae 11.4–15.8%, among other herbaceous taxa species of Chenopodiaceae (4.2–9.0%) and Rosaceae (about 5%) were identified. Isolated pollen grains of shrubs (*Salix* sp.) were found, but pollen of trees was absent. Ascospores of cosmopolitan sordariaceous coprophilous fungi, frequently found at feces of herbivorous animals, were numerous (9.8–13.5%).

In the pollen spectrum of sample 1B (enclosing sediments — peaty loam), also herbs dominate, but *Artemisia* (56.6%) prevail over Poaceae (40.4%). Isolated pollen grains of various herbs and shrubs (*Betula* sect. Nanae and *Salix*) were also found. The number of ascospores of coprophilous fungi was insignificant.

Thus, in the pollen spectra of both samples 1A and 1B, the herbaceous plants were dominating. The largest part of the pollen spectra was composed of grasses (Poaceae), sedges (Cyperaceae), and wormwood (*Artemisia* sp.). The pollen of forbs was not numerous. Pollen grains of shrubs such as *Salix*, *Betula* sect. Nanae, *Alnus fruticosa*, and trees *Picea*, *Pinus* s/g Diploxylon and *Betula* sect. Albae were sporadic. Ascospores of coprophilous Sordariaceae were numerous.

3.1.3. Plant macrofossils (Table 2)

Studied herb fragments were subject to physical and chemical influence inside the alimentary canal of animals. Many were preserved as scraps or fragments, especially grasses. The degree of seed preservation depends on size, shape, and strength of their external coats. Seeds of *Chenopodium prostratum* Bunge preserved best.

Sample 1 contained plant fragments belonging to 38 taxa. Sedges and grasses dominated. Seeds of *Chenopodium prostratum* were abundant. Remains of other herbs such as *Potentilla* cf. *stipularis*, various Polygonaceae (*Polygonum humifusum* Merk. ex K. Koch, *Rumex lapponicus* (Hiitonen) Czernov, *Rumex sibiricus* Hulten, *Rumex* sp.), and Caryophyllaceae (several species of *Cerastium*, *Dianthus* sp., cf. *Minuartia* sp., *Silene* sp., several species of *Stellaria* cf. *crassifolia*, Caryophyllaceae gen. indet.) were scanty. Two fragments of twigs (15–20 mm long, 2–3 mm in diameter) belonging

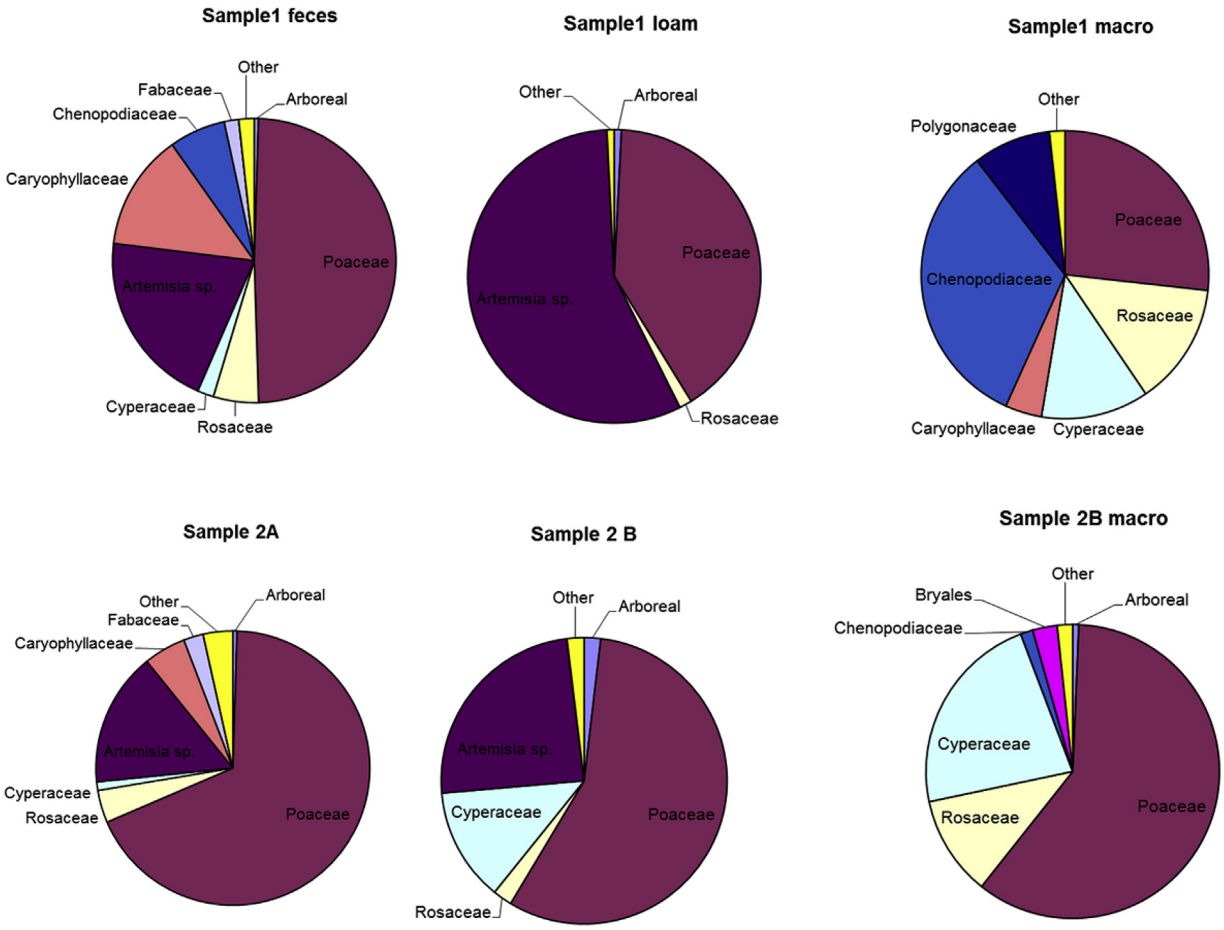

Fig. 6. Spectra of pollen and macroremains from Samples 1 and 2.

to *Salix* sp. were found (identification by L. Gorlanova and V. Kukarskikh).

Sample 2 contained plant fragments from 24 taxa. Remains of monocotyledons (Cyperaceae and Poaceae) dominated. Numerous seeds of *Potentilla* sp. were found. Macrofossils belonging to members of Chenopodiaceae, Polygonaceae, Caryophyllaceae, Papaveraceae, and others are not numerous, remains of shrubs *Betula* sect. Nanae, *Alnus fruticosa* Rupr., and *Salix* sp. were rare.

The macrofossils in our samples which were identified to species or genus level belong to taxa of the modern flora of Chukotka and northern Yakutia (Tolmachev, 1974; Secretareva, 2004).

3.2. Additional inclusions

In addition to vegetation detritus, the mammoth feces also contained indigested matters swallowed during feeding or drinking, such as hairs, feathers, remains of insects, including coprophagous beetles and freshwater cladocerans.

3.2.1. Keratoid derivatives of the vertebrate skin

In sample 1 (F-552), mammoth hairs (3 fragments 3–8 mm in length) and reindeer hairs (about 50 fragments 1–10 mm in length) were found. In sample 2 (F-3447), hairs of mammoth, horse, and bison were identified. They are described below.

A *Mammuthus primigenius*. We found a short fragment of elastic, slightly compressed overhair, deep-brown, almost black in color, 2 cm long and 249 μm thick (Fig. 7A, B). The dark and thin medulla (6–11 μm thick) lies in the center of the shaft. The cuticle is not preserved, and there are numerous long and narrow longitudinal cracks on the surface of cortical layer. This is caused by maceration of the surface layers. But they are not multiple medullary strands mistakenly identified under optical microscopy (Tridico et al., 2014). In fact, fragmentary medulla was found very rarely (Chernova et al., 2015a,b).

B *Bison* sp. Four short fragments of brownish lead hairs 2.5 cm long and up to 136 μm in thickness were found. The shaft is cylindrical, round or oval in cross section (Fig. 7C, D, E). The central cord is displaced to the ventral side, its depth is 35–45% of the shaft depth, accordingly, the cortical layer is rather thick, especially at the dorsal side of the shaft. The medulla is cellular, its air cavities vary in shape but are smaller than 30 μm in diameter. The cavities are separated by thick perforated walls that form several dense leaf-like walls without perforations in the central part of the medulla. At the base of the hair, the walls are situated mainly across the shaft, but further along the shaft they are arranged irregularly. The cuticle is not ring-shaped, i.e. each scale does not wrap the shaft completely. The free edges of rather narrow scales are frayed. The scales are mainly arranged across the shaft, but in some places they may be oriented under an angle of 25–30° to the transverse axis of the shaft. The height of scales relative to the hair depth is 1.0: 3.2 ± 0.1 ($n = 5$), which agrees with the data for the hairs of *Bison* sp. obtained earlier (Chernova and Kirillova, 2013).

C *Equus* sp. The hair is brown, 21 cm long, and up to 60 μm wide. Its shaft is straight and depressed (Fig. 7F, G), without thin basal region, but it widens close to the basis, which is typical for

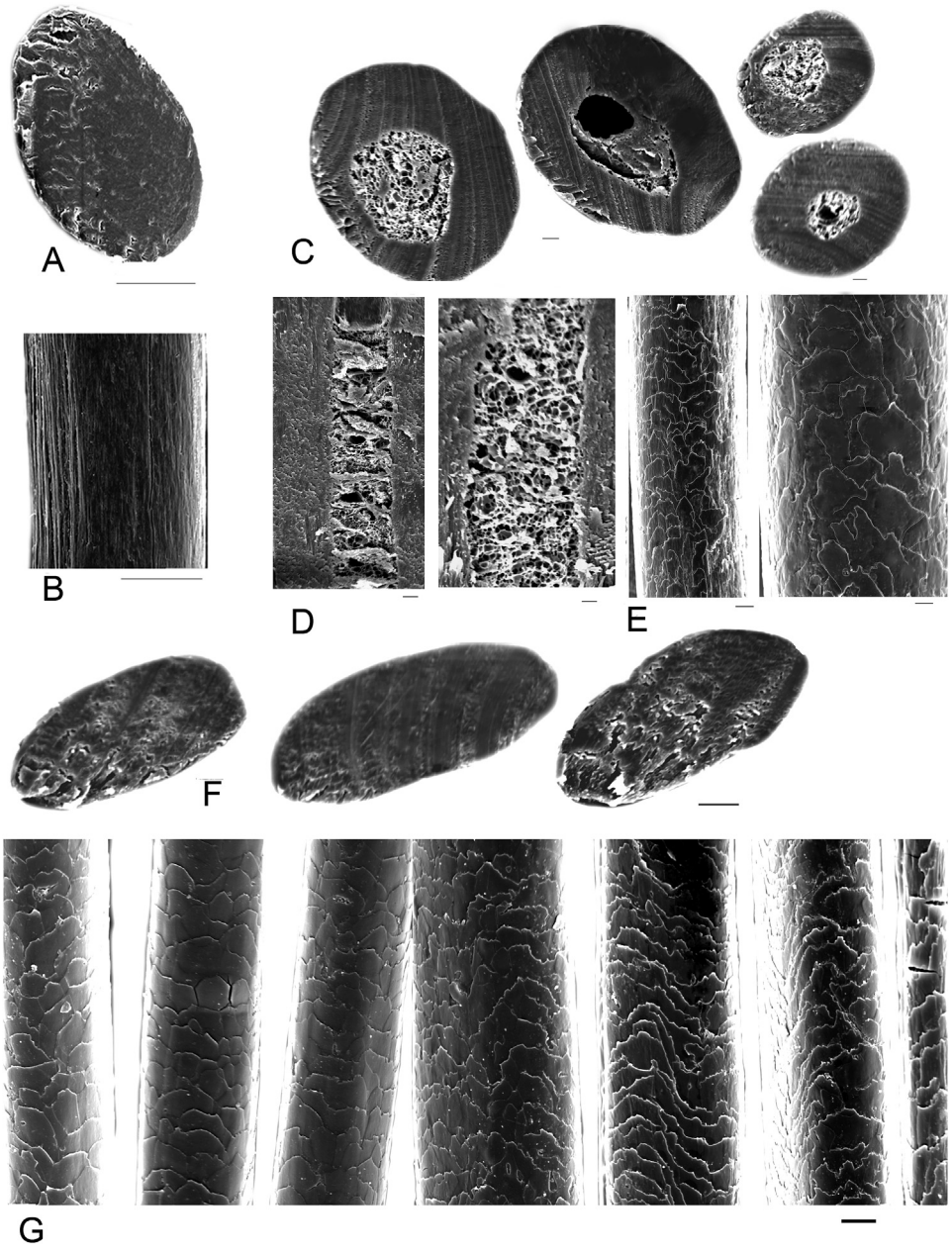

Fig. 7. Mammalian hairs from Samples 1 and 2. A, B – transverse section and surface structure of a thick hair belonging to woolly mammoth. C, D, E – transverse sections, longitudinal sections, and the cuticle of the hair of average thickness belonging to ancient bison. F, G – transverse sections and the cuticle of a thin hair of ancient horse. SEM images. Scale bars: A, B — 100 μm; C–G — 10 μm.

horses. A shallow groove may be noted at some regions of the shaft. The medulla is absent; some fragments without notable structure may be found. The cortical layer has numerous cracks. The ring-shaped cuticle is formed by high large scales with smooth (at the base of the shaft) and indented (further along the shaft) free edges. The height of the scales does not exceed 15 μm; their index is 1.0: 3.6 ± 0.1 (n = 5). The free edge of a scale may have some denticles up to 15 μm high. In the middle regions of the shaft, many scales are arranged in one longitudinal row, extended under the angle of 20–40° to the transverse axis of the shaft.

In Sample 2, two similar feathers with straight tough and thick shaft, and tough dense asymmetric vane were found (Fig. 4A, B). The transverse section of barbs in the contour part of the vane is strongly compressed and lanceolate in shape, its ventral crest being higher than the dorsal one (Fig. 4C). The barbs' medulla consists of three rows of compressed polymorphic cavities elongated along the barb, with undulated fringes and large folds at their walls; internal skeleton of cavities consists of rare thin threads (Fig. 4D). The cuticle of each barb consists of elongated fusiform scales (Fig. 4D); the cuticle relief is the ornament from thick fibers going mainly along the longitudinal axis of the scale. The borders between scales are distinct, appearing like a twisted cord (Fig. 4E). These characters are typical for feathers of geese (Anseriformes), presumably, for members of the genus *Branta* sp., black goose.

3.2.2. Insect remains

Twenty two fragments were found, only in Sample 1 (Table 3): 20 of them belong to beetles (Coleoptera), and 2 are puparia of flies.

The recorded insects are typical for North-East Russia of the Ice Age, and also for the present time (Sher et al., 2005; Sher and Kuzmina, 2007). The presence of fragile remains of *Aphodius* sp. (Fig. 8) and puparia of flies indicate that coprophilous insects penetrated directly into the fresh dung, while other insects could have been eaten by a mammoth together with plants. It is very likely that the dung became part of the permafrost soon after penetration of the insects and remained there till the onset of the river bank erosion.

3.2.3. Small crustaceans

Small crustaceans were found only in Sample 1, namely 26 ephippia of the Cladocera belonging to the Family Daphniidae Straus of the Order Anomopoda Sars. Ephippia have a brownish color, taper posteriorly, have small denticles at almost straight dorsal margins, and contain two eggs with axes perpendicular to the dorsal margin. These features indicate that the ephippia belong to the *Daphnia* (*Daphnia*) *pulex* species group. Eleven ephippia were well-preserved, some of them containing membranes of dormant eggs (Fig. 9).

Table 3
Taxonomic composition of insects' remains found in Sample 1.

Taxon	Number of fragments	Minimum number of individuals
Order Coleoptera		
Fam. Carabidae		
Harpalus sp.	1	1
Fam. Scarabaeidae		
Aphodius cf. *rectus* Motsch., 1866	6	3
Fam. Lathridiidae		
Lathridius sp.	2	1
Fam. Curculionidae		
Notaris aethiops (Fabr., 1792)	1	1
Stephanocleonus sp.	1	1
Order Diptera		
Diptera indet.	2	2

3.3. Radiocarbon dates of the mammoth feces

The results are shown in Table 4.

The sample name, GrA number, ^{14}C age in BP, measurement error (1-sigma), calibrated age in cal BP (1-sigma range), organic carbon content (in %), the stable isotope ratio $\delta^{13}C$ (in ‰), and the chemical sample treatment are shown. Specimen 1A was analyzed in duplicate.

All samples received the full standard AAA treatment. The 3 feces samples appeared to have varying organic carbon content. The duplicate dating for the F-552 feces shows good agreement (the dates overlap within 1-sigma). All stable isotope ratios ($\delta^{13}C$) are within the normal range. The averaged Radiocarbon date of F-522 corresponds to Marine Oxygen Isotope Stage 3 (MIS 3).

The sample from the Maly Anyui River (F-3447) yields a ^{14}C age at background level, taken as >45,000 BP (Van der Plicht and Palstra, 2016).

3.4. The results of 16S rRNA sequencing

The evaluation of microbiological diversity in the feces samples was done using the analysis of the 16S rRNA gene. The results of the taxonomic analysis are shown in Fig. 10. Sample 1 was

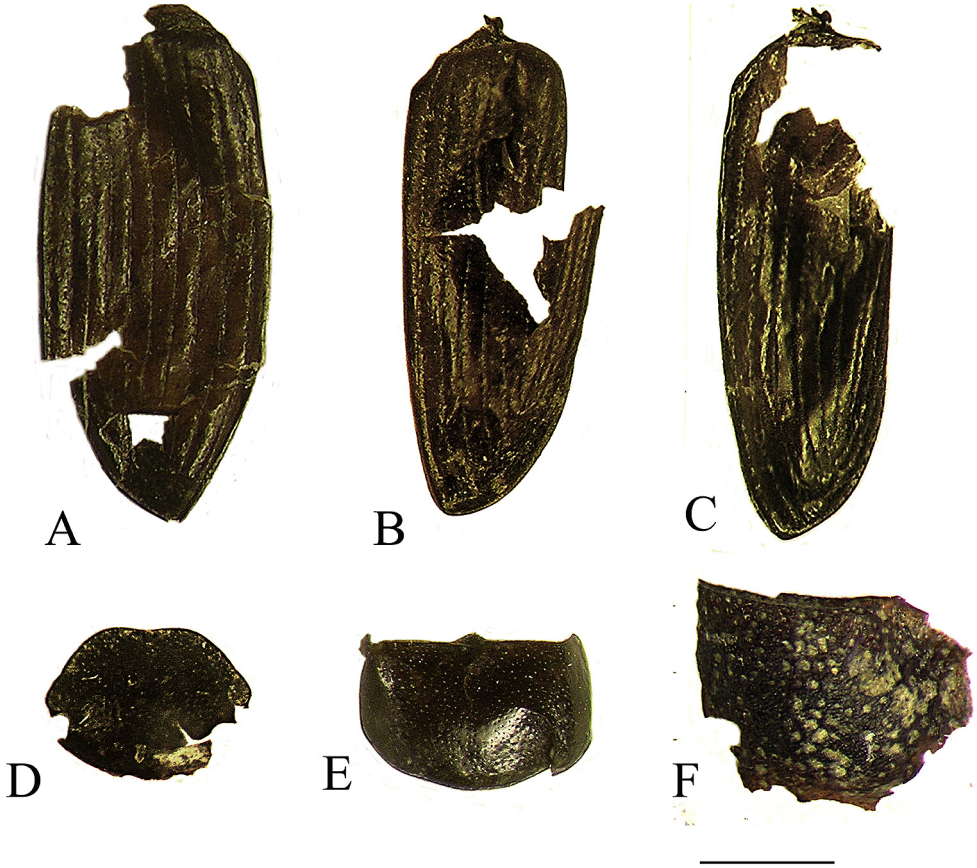

Fig. 8. Remains of *Aphodius* beetles. A – *Aphodius* cf. *rectus*, left elytron; B, C – *Aphodius*. cf. *rectus*, right elytra; D – *Aphodius* sp., head; E – *Aphodius* sp., pronotum; F – *Stephanocleonus* sp., right part of pronotum.

Table 4
Results of ^{14}C measurements of the mammoth feces.

Sample	GrA	^{14}C age (BP)	Calibrated age (cal BP)	%C	$\delta^{13}C$ (‰)	Treatment
1 A	60516	41.490 (−480, +600)	45.470–44.420	9.5	−27.44	AAA
1 A duplicate	60624	42.030 (−460, +550)	45.860–44.900	12.1	−27.17	AAA
2 A	60514	>45.000	>48.000	23.7	−26.08	AAA

dominated by bacteria belonging to the Actinobacteria (58%), Gammaproteobacteria (15%) and Bacilli (16%) classes. In sample 2 the classes Clostridia (53%), Betaproteobacteria (23%) and Gammaproteobacteria (11%) were observed (Fig. 10A). On genus level, the most abundant microbes for sample 1 were Pseudomonas (37%), Promicromonospora (26%) and for sample 2 Clostridium XI (47%), Clostridium sensu stricto (11%) and Pseudomonas (10%) (Fig. 10B).

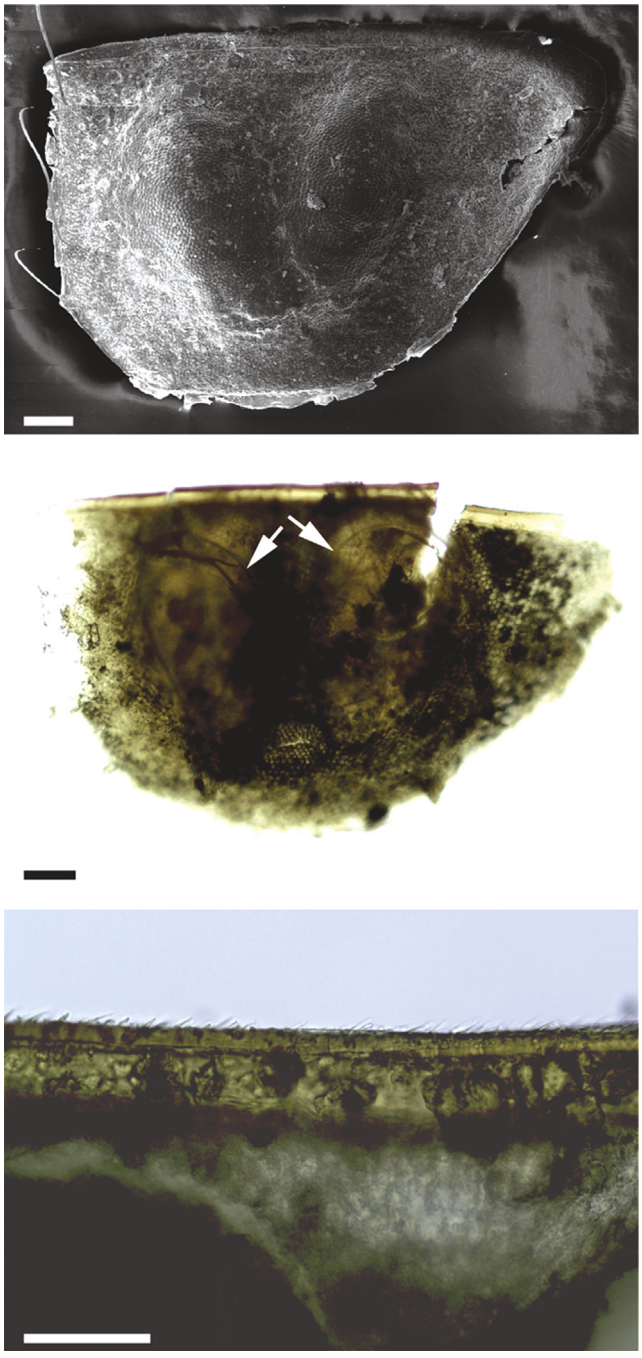

Fig. 9. Ephippia of *Daphnia* (*Daphnia*) *pulex* group from the mammoth feces (Sample 1). A – general view under SEM. B – general view in transmitted light, arrows indicate egg membranes. C – dorsal plate of ephippium. All scales — 0.1 mm.

4. Discussion

4.1. About mammoth feces preservation in permafrost

The burial of feces in frozen sediments most likely takes place when a mammoth visits attractive pastures. Modern elephants and rhinoceroses periodically visit certain places for food, water, or other necessary matters such as mineral salts (Douglas-Hamilton et al., 2006). The same behavior can be assumed for the ancient pachyderms.

Judging from the composition of the sites where the specimens were found, the paleolandscape in both places consisted of a flat surface with small depressions where shallow lakes were formed, containing plants attractive for large herbivorous mammals. The presence of such bodies of water is strongly supported by the discovery of the cladoceran ephippia in Sample 1. After some time, such lakes terrestrialized and turned into swamps. Findings of hairs of reindeer, bison and a horse confirm that these animals also visited such wet sites. Their feces were not found because of their small size, while the large excrements of mammoths are easily discernible in coastal debris and sections. Similarly, numerous bodies and skeletons of large mammals of the Ice Age are found while finds of the much more numerous smaller mammals are occasional. For the site Terekhtyakh, we believe that large herbivores (mammoth, bison, horse and reindeer) visited a shallow lake not only for the sake of pasture, but also because of the enriched with plant organic, very peaty clay sediments. Such lithophagy is well-known for modern mammals (Panichev, 1987, 1990), but any data on lithophagy of fossil animals are scarce (Bgatov et al., 1989; Leshchinskiy et al., 2003; Leshchinskiy, 2006). Our study confirms this lithophagy for the woolly mammoth.

The feces of modern herbivores accumulates sometimes on a large scale. In the area Naran-Bulak (southern Gobi, Mongolia), a narrow valley is the only source of freshwater for hundreds of kilometers around. It is visited by wild and domestic ungulates, and there are many feces (I. Kirillova, personal observation). But during every flood the relief composed of the feces is transformed. There is no chance to be preserved for a long time. At the same time, in some caves in the Mongolian steppe, there are deposits of feces of herbivorous mammals more than 1 m high (Dinesman et al., 1989). Their preservation is maintained by constantly dry air, and by the absence of destroying factors such as water, sun and wind.

At both our sites, at the rivers Terekhtyakh and Maly Anyui, two conditions are responsible for a good preservation of the mammoth feces. These are: (i) particular peculiarities of the landscape, i.e. depressions with developed vegetation, attractive for large herbivores, and (ii) the permafrost, preserving the objects for tens of thousands years. Apparently, the encapsulation of feces into mud that soon became part of the permafrost (certainly for sample 1A) took place soon after its formation.

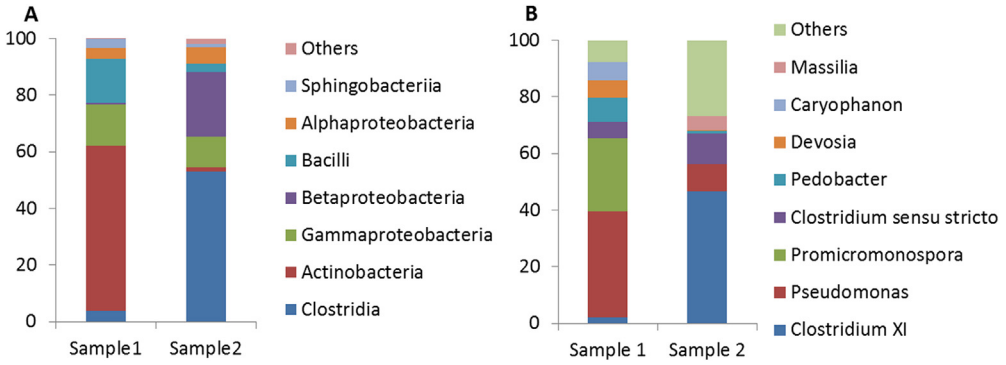

Fig. 10. Relative abundances of different classes (A) and genera (B) of bacteria in the mammoth feces samples.

4.2. Composition and preservation of plant remains

The mammoth feces of both sites contained numerous plant remains (pollen and spores, phytoliths of grasses and dicotyledonous herbs, epidermis of sedges and herbs, stomatal complexes, vascular tissues) which are typical for the content of the gastrointestinal tract of herbivorous mammals (Kosintsev et al., 2012a,b). Plant remains from feces are indicative for the vegetation types on pastures, even when they are not always identical to it in taxonomic and quantitative aspects. Tissues of mosses were not found in the feces, though they can be common in the content of the gastrointestinal tract of mammoths (Kosintsev et al., 2012a,b). Fragments of herbal epidermis contained stomatal complexes, 25–30 μm in diameter, belonging to Ericaceae and Poaceae. Similar stomatal complexes have been described by Carnelly et al. (2004). Conical forms, typical for sedges, were also found. The cubical form and tissues with edged pores typical for needles of *Pinus* sp. were observed as well. The analysis of phytoliths revealed that members of Poaceae belonging to genera *Poa*, *Bromus*, *Calamagrostis* and *Elymus* were dominant among consumed herbs; sedges were rarely found.

The irregular shape and small size of phytoliths from Sample 1 need a special discussion. It is well known that not all plants complete their cycle of reproduction from sprouts to ripe seeds in the tundra zone, switching frequently to a vegetative stage of ontogenesis. When this takes place, the tissues of such plants remain “immature”. If phytoliths did not develop completely, which usually occurs in the middle of the warm season, they are easier subjected to corrosion than the ripe ones. One more possible explanation of such phenomenon is the deficiency of some necessary minerals for the phytolith formation which is a common situation in the tundra environment. The abundance of coprophilous fungi is possibly a consequence of mammoth coprophagy. On the other hand, it may be a sign of an intensive visiting of attractive places by herbivore animals. Unavoidable accumulation of dung could contribute to flourishing of coprophilous fungi at allas grasslands.

Large amounts of spores of coprophilous fungi were recorded by the authors in mammoth fur from the alass deposits close to the Bol'shaya Ghukochya River (Kirillova et al., 2015).

4.3. The diet, pastures, and living environment

Most macroremains from feces found at the rivers Terekhtyakh and Maly Anyui could be identified to genus or species level, and belong to plants of an extant flora, common in Chukotka and northern Yakutia (Tolmachev, 1974; Tomskaya, 2000; Secretareva, 2004). The taxonomic composition is similar in both samples: Poaceae dominate in pollen spectra and as macroremains as well.

Besides grasses and sedges, variable micro- and macroremains of mesophytic species growing mainly in tundra-meadow communities with moderate moisture were found. No remains of typically steppe plants were observed.

Thus, the phytolith spectrum from both samples is indicative for the development of mesophytic meadows with forbs. Near the Maly Anyui River, dwarf birch and willow were common, as well as Ericaceae. Herbal associations of pastures are similar in both localities and consist of meadow grasses of the genera *Poa*, *Bromus*, *Calamagrostis*, *Festuca*, *Elymus*, and variable herbs. The presence of fruits and seeds of xero-mesophytes (*Carex* cf. *melanocarpa*, *Potentilla* cf. *stipularis*), show the presence of stones and stony detritus on the surface, and macroremains of aquatic and swamp species support the presence of appropriate swamped biotopes. Isolated seeds of *Artemisia tilessii* and *Artemisia* sp. were found while the wormwood pollen was abundant. *A. tilessii* usually grows in floodplain meadows or on stony slopes of Arctic and mountain tundra (Andreev et al., 1974; Secretareva, 2004).

Samples 2A and B from the Maly Anyui River contain micro- and macroremains of shrubs *Alnus fruticosa* and *Betula* sect. Nanae in addition to remains of willows (*Salix*). The phytolith spectrum indicates presence of a herbal-sedge group in the region, composed of small meadow herbs and forbs.

Today, plants suitable for grazing are common in the North-East of Russia. These include five species of *Festuca*, including *F. altaica* which prefer willow growth and water-meadows. *Calamagrostis* sp. grows in very wet habitats and is consumed by herbivores all year round. Poaceae and Cyperaceae grow everywhere and are important forage plants, especially during winter and spring months. Ericaceous nutrient species are the Labrador tea (*Ledum* sp.) and blueberry. Sprouts of willows and birch species, widespread in the region, are eaten by modern herbivores during the whole year; they were also found in the intestinal content of the Yuribey and Kirgillyakh mammoths (Tomskaya, 2000). It is reasonable to assume that the composition of plant remains from both samples entirely comply with the extant flora of the alasses. The data obtained from the two studied regions of the Russian North-East are characterized by pastures of varying landscapes (Figs. 1–5 Suppl. 1). Mesophilous meadows with sedge-grass herbage were widespread. Areas with meager soil or without soil cover such as pebble beds, and sediment slopes were occupied by *Artemisia* and members of Chenopodiaceae, Polygonaceae, Caryophyllaceae, and Rosaceae.

Quite a number of findings of mammoth remains are known from northern Yakutia. Paleobotanical studies of their intestinal content and the diet of large herbivores revealed that grasses, sedges, and forbs were dominating (Arslanov et al., 1980;

Tomskaya, 1981, 2000; Ukraintseva, 2002; Willerslev, 2014). Most plant remains found in the intestinal contents of these studied mammoths were also observed in our Samples 1 and 2.

Most of the mammoths date to MIS 3, a period with an arid continental climate, and widespread cryophyte steppes (Tomskaya, 2000). In the pollen spectra from deposits with mammoth remains, pollen grains of herbaceous plants include species of Poaceae, *Artemisia*, Cyperaceae, and Caryophyllaceae, with a contribution of pollen of trees and shrubs (Tomskaya, 1981; Anderson et al., 2002). In the pollen spectrum of the loamy deposits (Sample 2B), which enclose Sample 2, herbs also were dominating (98%), while shrubs are sporadic (Table 1).

4.4. Fossil ephippia as environmental indicators

An ephippium is a transformed dorsal portion of a female shell in the cladoceran crustaceans of the Order Anomopoda. It is strongly chitinized and in addition has a developed surface sculpture. During deposition of dormant eggs, it surrounds resting eggs and serves to protect them from unfavorable environmental conditions (Kotov, 2013). This is the main ontogenetic stage of dispersion in cladocerans from order Anomopoda. Ephippia are easily dispersed by wind, water currents, and by water birds on their feathers, and survive after passing through their guts (Proctor, 1964). They can also be transported by large extant mammals (Vanschoenwinkel et al., 2011). Dormant eggs within the ephippia are protected from UV radiation by a dark pigmentation. They can withstand freezing and total drying, and can survive for a long time. Experimentally, a successful hatching was obtained for eggs from ephippia with an age of 600 years (Frisch et al., 2014). Fossil ephippia were found in the intestinal content of the Yamal baby mammoth (Van Geel et al., 2011b), and in the mature Mongochen mammoth (Kosintsev et al., 2012a). A relatively good preservation of ephippia in feces and within intestinal content indicates that the ephippia, taken in during drinking or via aquatic plants, could retain viability after passing through the intestinal tract of a mammoth. The same phenomenon was shown for extant birds (Proctor, 1964). Even if the ephippia fell into dry environment, the dormant eggs could hatch during a humid season, for example during the seasonal floods, and even after a prolonged period. In this way, large mammals of the Ice Age could participate in colonization of water bodies by small crustaceans.

Fossil ephippia are valuable indicators of paleoecological conditions because they are the evidence of mammoths visiting shallow lakes. The exact type of such lakes could not be precisely identified, but it is known that members of the *Daphnia* (*D.*) *pulex* group prefer stagnant water bodies and are scanty in flowing waters (Benzie, 2005).

4.5. Probable season of the feces deposition (Table 5)

It should be noted that the time of the year relates much less to the calendar than to natural processes which vary from year to year; the difference may be from one-two up to 4 weeks *in extremis*. Also, the vegetation period in the North is much shorter than at middle latitudes, and the warm season does not exceed three or maximally four months. At high latitudes terms such as “spring”, “summer”, or “autumn” are loose concepts and their temporal limits are variable.

Sample 1 from the Terekhtyakh River contained numerous ephippia (which lack in Sample 2 from the Maly Anyui River). Possibly, it was formed during the season of the daphniid sexual reproduction, which is accompanied by mass development of ephippia. This takes place at the end of the warm season. It is necessary to note that fresh ephippia are floating being attached to the water film, but old ephippia sink, and laying at the bottom sediments of a water body, become less available for occasional ingestion by animals. We believe that the high concentration of ephippia in the feces can be explained by their mass presence in the littoral zone (where they were occasionally ingested together with water during its drinking), which happens only during the time of gamogenetic reproduction.

Table 5
Possible season of feces formation.

Items	Sample 1 from the river Terekhtyakh	Sample 2 from the river Maly Anyui
Spores, pollen	–	–
Macroremains of plants	Possibly, warm season	Except the beginning and middle of warm season
Phytoliths	The middle of warm season	Except the beginning and middle of warm season
Beetles	The middle of warm season	(Absent). Cold season
Small crustaceans	The end of warm season	(Absent). Cold season
Object appearance	Structured. Possibly, consumption of green forage. Warm season?	Weakly structured. Possibly, consumption of dry forage. Cold season?

The observed abundance of the sedge macroremains along with the small amount of their pollen and numerous remains of grasses may be explained by the difference in time of flowering and ripening. In the Arctic zone, flowering of sedges takes place from the end of April till early July, and the ripening of their fruits coincides with the flowering of true grasses (from June to August) (Shamurin, 1966). Consequently, the combination of ripe fruits of sedges and large quantity of the pollen of grasses in Sample 1 points to the second half of summer. The fragments of the grass spikelets and good preservation of fruits and seeds point to the formation of feces at the end of the growing season or just after its completion. The presence of the beetle remains, including coprophilous species, also points to the warm season.

Sample 2 possibly formed at the beginning of the warm season, when only parthenogenetic females of *Daphnia* exist in populations, of which bodies are not preserved in deposits, and ephippia are absent. Sample 2 could also be produced in a cold season; this assumption is supported by the absence of remains of beetles.

4.6. Metagenomics

The 16S rRNA metagenomic analysis shows a significant difference between the microbial diversity of two feces samples. The aerobic actinobacteria dominate anaerobic clostridia in samples 1 and 2, and not in sample 2. An explanation for this may be the presence of a large number silty/clayey component (40%) in sample 1.

There are no data for 16S analysis of similar mammoth specimens. Possibly, the nearest analog is 16S analysis of microflora from feces of Indian elephant (Ilmberger et al., 2014). Comparison of the data shows that in mammoth feces and elephant and other extant animals a significant amount of Bacteroidetes which are representatives of the normal mammalian gastrointestinal flora is present. Most probably this difference means that the time before the freezing of the mammoth feces was sufficient for significant changes in microflora composition. It also may be connected with changes of environmental conditions such as drying of the sample.

5. Conclusions

The composition of botanical remains and other organic inclusions in mammoth feces improves our understanding of the

Pleistocene biota of North-East Russia and their spatio-temporal components. The mammoth feces are dated to stage MIS-3. The samples are mainly characterized by taxa of sedge-grasses tundra-like communities with dwarf shrubs (willow, alder and birch). At the same time it is evident that grazing lands expanded to other biotopes: small lakes in coastal plants, growth of shrubs at slopes and watersheds, grasslands associated with slopes and watersheds, and meadow vegetation in depressions. The fragments of mucosal tissue in Sample 1 are indicators of illness and damage of the gastrointestinal tract. A high content of loamy particles in this sample is an indicator of occasional or deliberate lithophagy, because of the need for minerals. In addition, clay minerals have been reported to have beneficial microbiological effects (Bisi-Johnson et al., 2010). The microflora of the samples differs significantly from the bacterial spectrum of the intestinal content and from each other.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.quaint.2015.11.002.

References

Aerts-Bijma, A.T., van der Plicht, J., Meijer, H.A.J., 2001. Automatic AMS sample combustion and CO_2 collection. Radiocarbon 43, 293–298.
Anderson, P.M., Kotov, A.N., Lozhkin, A.V., Trumpe, M.A., 2002. Palynological and radiocarbon data from Late Quaternary deposits of Chukotka. In: Anderson, P.M., Lozhkin, A.V. (Eds.), Late Quaternary Vegetation and Climate of Siberia and the Russian Far East (Palynological and Radiocarbon Database). North-East Scientific Center Far East Branch RAS, Magadan, pp. 35–79.
Andreev, V.N., Nakhabtseva, S.F., 1974. Zonality of Yakutian Tundra. In: Biological problems of the North, vol. 3, pp. 40–50. Yakutsk. (in Russian).
Argant, J., 1990. Climat et environnement au quaternaire dans le bassin du Rhône d'après les données palynologiques. In: Documents du Laboratoire de Géologie de Lyon, vol. 111. France/Neolithic cave/pollen (ancient)/sheep/goat (pp. 132–140)/experiments in modern sheep dung. 32–47/OA.
Argant, J., Dimitrijević, V., 2007. Pollen Analyses of Pleistocene Hyaena Coprolites from Montenegro and Serbia. In: Annales géologiques de la Péninsule Balkanique, vol. 68, pp. 73–80.
Arslanov, X.A., Vereshchagin, N.K., Lyadov, V.V., 1980. About Karga interglacial chronology and Siberian landscape reconstruction based on studies of finds of mammoths and mammoth's fauna. In: Ivanova, I.K., Kind, N.V. (Eds.), Quaternary Geochronology. Nauka, Moscow, pp. 208–213 (in Russian).
Benzie, J.A.H., 2005. The Genus *Daphnia* (Including *Daphniopsis*) (Anomopoda: Daphniidae). Guides to the Identification of the Microinvertebrates of the Continental Waters of the World 21. Kenobi Productions, Ghent & Backhuys Publishers, Leiden.
Bgatov, V.I., Lazarev, P.A., Speshilova, M.A., 1989. Lytophagy and the Mammoth' Fauna. Yakutsk Scientific Center, Siberian Branch, RAS, Yakutsk.
Bisi-Johnson, M.A., Obi, C.L., Ekosse, G.E., 2010. Microbiological and health related perspectives of geophagia: an overview. African Journal of Biotechnology 9 (19), 5784–5791.
Boeskorov, G.G., Tikhonov, A.N., Suzuki, N. (Eds.), 2007. The Yukagir Mammoth. St. Petersburg University, St. Petersburg.
Caporaso, J.G., Lauber, C.L., Walters, W.A., et al., 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proceedings of the National Academy of Sciences of the United States of America 108, 4516–4522.
Caporaso, J.G., Lauber, C.L., Walters, W.A., et al., 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. Isme Journal 6, 1621–1624.
Carnelly, A.L., Theurillat, J.-P., Madella, M., 2004. Phytolith types and type-frequencies in subalpine- alpine plant species of the European Alps. Review of Palaeobotany and Palynology 129, 39–65.
Chernova, O.F., Kirillova, I.V., 2013. Hair microstructure of the late quaternary bison from north-east Russia. Proceedings of Zoological Institute 317 (2), 202–216 (in Russian).
Chernova, O.F., Kirillova, I.V., Boeskorov, G.G., Shidlovskiy, F.K., 2015a. Identification of hair of the woolly mammoth Mammuthus primigenius and woolly rhinoceros Coelodonta antiquitatis using scanning electron microscopy. Doklady Biological Sciences 463, 205–210.
Chernova, O.F., Kirillova, I.V., Boeskorov, G.G., Shidlovskiy, F.K., Kabilov, M.R., 2015b. Architectonics of the Hairs of the Woolly Mammoth and Woolly Rhino. In: Proceeding of the Zoological Institute RAS, vol. 319, pp. 441–460.
Chin, K., 2002. Analyses of Coprolites Produced by Carnivorous Vertebrates. In: Paleontological Society Papers, vol. 8, pp. 43–50.
Dinesman, L.G., Kiseleva, N.K., Knyazev, A.V., 1989. History of the Steppe Ecosystems of Mongolian People's Republic. Nauka, Moscow (in Russian).
Douglas-Hamilton, I., Bhallaa, S., Wittemyera, G., Vollratha, F., 2006. Behavioural reactions of elephants towards a dying and deceased matriarch. Applied Animal Behaviour Science 100 (1), 87–102.
Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27 (16), 2194–2200.
Faegri, K., Iversen, J., 1989. Textbook of Pollen Analysis. Blackburn Press, London.
Frisch, D., Morton, P.K., Chowdhury, P.R., Culver, B.W., Colbourne, J.K., Weider, L.J., Jeyasingh, P.D., 2014. A millennial-scale chronicle of evolutionary responses to cultural eutrophication in Daphnia. Ecological Letters 17 (3), 360–368.
Gilichinsky, D., Wagener, S., 1995. Microbial life in permafrost: a historical review. Permafrost and Periglacial Processes 6, 243–250.
Gilichinsky, D., Vishnivetskaya, T., Petrova, M., Spirina, E., Mamykin, V., Rivkina, E., 2008. Bacteria in permafrost. In: Margesin, R., Schinner, F., Marx, J.C., Gerday, C. (Eds.), Psychrophiles: from Biodiversity to Biotechnology. Springer Verlag, Berlin Heidelberg, pp. 83–102.
Guthrie, R.D., 1990. Frozen Fauna of the Mammoth Steppe: the Story of Blue Babe. The University of Chicago Press, Chicago, London.
Gvozdetsky, N.A., Mikhailov, N.I., 1978. Physical Geography of the USSR. Asian Part. Mysl', Moscow (in Russian).
Ilmberger, N., Güllert, S., Dannenberg, J., Rabausch, U., Torres, J., Wemheuer, B., Alawi, M., Poehlein, A., Chow, J., Turaev, D., Rattei, T., Schmeisser, C., Salomon, J., Olsen, P.B., Daniel, R., Grundhoff, A., Borchert, M.S., Streit, W.R., 2014. A comparative metagenome survey of the fecal microbiota of a breast- and a plant-fed Asian elephant reveals an unexpectedly high diversity of glycoside hydrolase family enzymes. PLoS One 9 (9), 106–707.
Kirillova, I.V., Kotov, A.A., Trofimova, S.S., Zanina, O.G., Lapteva, E.G., Zinoviev, E.V., Chernova, O.F., Fadeeva, E.O., Zharov, A.A., Shidlovskiy, F.K., 2015. Fossil fur as a new source of information on the Ice Age biota. Doklady Biological Sciences 460, 48–51.
Kosintsev, P.A., Lapteva, E.G., Korona, O.M., Zanina, O.G., 2012a. Living environments and diet of the Mongochen mammoth, Gydan Peninsula, Russia. Quaternary International 276–277, 253–268.
Kosintsev, P.A., Lapteva, E.G., Trofimova, S.S., Zanina, O.G., Tikhonov, A.N., van der Plicht, J., 2012b. Environmental reconstruction inferred from the intestinal contents of the Yamal baby mammoth Lyuba (*Mammuthus primigenius* Blumenbach, 1799). Quaternary International 255, 231–238.
Kotov, A.A., 2013. Morphology and Phylogeny of the Anomopoda (Crustacea: Cladocera). KMK, Moscow.
Leshchinskiy, S.V., Burkanova, E.M., Orlova, L.A., 2003. Kochegur – a New Mammoth Locality, an Animal at the South-east of West Siberian Plain. In: Bulletin of Tomsk State University, Suppl. no. 3 (II), pp. 126–128 (in Russian).
Leshchinskiy, S.V., 2006. Lugovskoye: environment, taphonomy, and origin of a paleofaunal site. In: Eurasia. Paleoenvironment. The Stone Age, pp. 33–40.
Madella, M., Alexandre, A., Ball, T., 2005. International code for phytolith nomenclature 1.0. Annals of Botany 96, 253–260.
Mol, D., Shoshani, J., Tikhonov, A., van Geel, B., Sano, Sh., Lazarev, P., Boeskorov, G., Agenbroad, L., 2006a. The Yukagir Mammoth: Brief History, ^{14}C Dates, Individual Age, Gender, Size, Physical and Environmental Conditions and Storage. In: Scientific Annals, School of Geology Aristotle University of Thessaloniki (AUTH), vol. 98, pp. 299–314.
Mol, D., Tikhonov, A., van der Plicht, J., Kahlke, R.D., Debruyne, R., van Geel, B., Pals, J.P., de Marliave, C., Reumer, J.W.F., 2006b. Results of the CERPOLEX/Mammuthus expeditions on the Taimyr Peninsula, Arctic Siberia, Russian Federation. Quaternary International 142–143, 186–202.
Mook, W.G., Streurman, H.J., 1983. Physical and Chemical Aspects of Radiocarbon Dating. PACT Publications no. 8, pp. 31–55.
Mook, W.G., van der Plicht, J., 1999. Reporting ^{14}C activities and concentrations. Radiocarbon 41, 227–239.
Moore, P.D., Webb, J.A., Collinson, M.E., 1991. Pollen Analysis. Blackwell Scientific, London, p. 216.

Nikitin, V.P., 1969. Paleokarpologicheskyi metod (Plant Macrofossil Analysis). Tomsk University Press, Tomsk.
Novgorodov, G.P., Grigorev, S.E., Cheprasov, M.Y., 2013. Prospective location of the mammoth fauna in the Yana River basin. International Journal of Applied and Fundamental Research 8, 255–259.
Owocki, K., Niedźwiedzki, G., Sennikov, A.G., Golubev, V.K., Janiszewska, K., Sulej, T., 2012. Upper Permian vertebrate coprolites from Vyazniki and Gorokhovets, vyatkian regional stage, Russian platform. PALAIOS 27, 867–877.
Panichev, A.M., 1987. Animal Licks of Sikhote Alin: Biological and Geological Aspects. Far East Scientific Center, USSR Academy of Sciences, Vladivostok.
Panichev, A.M., 1990. Lythophagy in the World of Animals and in Humans. Nauka, Moscow.
Proctor, V.W., 1964. Viability of Crustacean eggs recovered from ducks. Ecology 45 (3), 656–665.
Reille, M., 1992. Pollen et spores d'Europe et d'Afrique du nord. Laboratoire de botanique historique et palynologie, CNRS, Marseille, p. 520.
Reimer, P.J., Bard, E., Bayliss, A., Beck, J.W., Blackwell, P.G., Bronk Ramsey, C., Buck, C.E., Edwards, R.L., Friedrich, M., Grootes, P.M., Guilderson, T.P., Haflidason, H., Hajdas, I., Hatté, C., Heaton, T.J., Hoffmann, D.L., Hogg, A.G., Hughen, K.A., Kaiser, K.F., Kromer, Bernd, Manning, S.W., Niu, M., Reimer, R.W., Richards, D.A., Scott, E.M., Southon, J.R., Staff, R.A., Turney, C.S.M., van der, Plicht, J., 2013. IntCal 13 and Marine13 radiocarbon age calibration curves 0–50,000 years cal BP. Radiocarbon 55, 1869–1887.
Rudaya, N., Protopopov, A., Trofimova, S., Plotnikov, V., Zhilich, S., 2015. Landscapes of the 'Yuka' mammoth habitat: a palaeobotanical approach. Review of Palaeobotany and Palynology 214, 1–8.
Secretareva, N.A., 2004. Vascular Plants of Russian Arctic and Adjacent Territories. KMK Scientific Press Ltd, Moscow (in Russian).
Shamurin, V. Ph, 1966. Seasonal rhythm and ecology of flowering of the plants of the tundra communities in the North Yakutia. In: Tikhomirov, B.A. (Ed.), The Vegetation of the Far North of the USSR and its Utilization, Adaptation of Arctic Plants to the Environment, vol. 8. Nauka, Moscow, Leningrad (in Russian).
Sher, A.V., Kuzmina, S.A., Kuznetsova, T.V., Sulerzhitsky, L.D., 2005. New insights into the Weichselian environment and climate of the East Siberian Arctic, derived from fossil insects, plants, and mammals. Quaternary Science Reviews 24, 533–569.
Sher, A.V., Kuzmina, S.A., 2007. Beetle Records. Late Pleistocene of Northern Asia. In: Encyclopedia of Quaternary Science, vol. 1. Elsevier, pp. 246–267.
Tolmachev, A.I. (Ed.), 1974. Key of Vascular Plants of Yakutia. Nauka, Novosibirsk (in Russian).
Tomskaya, A.I., 1981. Cenozoic Palynology of Yakutia. Nauka, Novosibirsk (in Russian).
Tomskaya, A.I., 2000. The Forage of the Yakutia Mammoth in the Late Pleistocene. Mammoth Museum, Yakutsk (in Russian).
Tridico, S.R., Rigby, P., Kirkbride, K.P., Haile, J., Bunce, M., 2014. Megafaunal split ends: microscopical characterization of hair structure and function in extinct woolly mammoth and woolly rhino. Quaternary Science Reviews 83, 68–75.
Ukraintseva, V.V., 1993. Vegetation cover and environment of the "Mammoth Epoch" in Siberia. In: Agenbroad, L.D., Mead, J.I., Hevly, R.H. (Eds.), Mammoth Site of Hot Springs. SD. Inc., Hot Springs, p. 309.
Ukraintseva, V.V., 2002. Vegetation and Climate of Siberia in the Mammoth Epoch. East Siberian Branch of the International Institute of Forest, Krasnoyarsk (in Russian).
Van der Plicht, J., Wijma, S., Aerts, A.T., Pertuisot, M.H., Meijer, H.A.J., 2000. The Groningen AMS facility: status report. Nuclear Instruments and Methods in Physics Research Section B172, 58–65.
Van der Plicht, J., Palstra, S.W.L., 2016. Radiocarbon and mammoth bones: what's in a date. Quaternary International 406 (Part B), 246–251.
Van Geel, B., Pals, J.P., van Reenen, G., Mol, D., 2004. Plant remains in the Yukagir Mammoth dung and an environmental reconstruction. In: The Yukagir Mammoth: Outcome of the First Stage of Research Work. Proceedings of International Scientific Practical Conference. Yakutsk Division of Siberian Branch, RAS Publishing House, Yakutsk, pp. 58–59.
Van Geel, B., Aptroot, A., Baittinger, C., Birks, H.H., Bull, I.D., Cross, H.B., Evershed, R.P., Gravendeel, B., Kompanje, E.J.O., Kuperus, P., Mol, D., Nierop, K.G.J., Pals, J.P., Tikhonov, A.N., van Reenen, G., van Tienderen, P.H., 2008. The ecological implications of a Yakutian mammoth's last meal. Quaternary Research 69, 361–376.
Van Geel, B., Guthrie, R., Altmann, J., Broekens, P., Bull, I., Gill, F., Jansen, B., Nieman, A., Gravendeel, B., 2011a. Mycological evidence of coprophagy from the feces of an Alaskan Late Glacial mammoth. Quaternary Science Reviews 30, 2289–2303.
Van Geel, B., Fisher, D.C., Rountrey, A.N., van Arkel, J., Duivenvoorden, J.F., Nieman, A.M., van Reenen, G.B.A., Tikhonov, A.N., Buigues, B., Gravendeel, B., 2011b. Palaeo-environmental and dietary analysis of intestinal contents of a mammoth calf (Yamal Peninsula, northwest Siberia). Quaternary Science Reviews 30, 3935–3946.
Vanschoenwinkel, B., Waterkeyn, A., Nhiwatiwa, T., Pinceel, T., Spooren, E., Geerts, A., Clegg, B., Brendonck, L., 2011. Passive external transport of freshwater invertebrates by elephant and other mud-wallowing mammals in an African savannah habitat. Freshwater Biology 56 (8), 1606–1619.
Vasan, S., Zhang, X., Kapurniotu, A., Bernhagen, J., Teichberg, S., Basgen, J., Wagle, D., Shih, D., Terlecky, I., Bucala, R., Cerami, A., Egan, J., Ulrich, P., 1996. An agent cleaving glucose-derived protein crosslinks in vitro and in vivo. Nature 382 (6588), 275–278.
Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied Environmental Microbiology 73 (16), 5261–5267.
Willerslev, E., Davison, J., Moore, M., Zobel, M., Coissac, E., Edwards, M.E., Lorenzen, E.D., Vestergård, M., Gussarova, G., Haile, J., Craine, J., Gielly, L., Boessenkool, S., Epp, L.S., Pearman, P.B., Cheddadi, R., Murray, D., Brathen, K.A., Yoccoz, N., Binney, H., Cruaud, C., Wincker, P., Goslar, T., Alsos, I.G., Bellemain, E., Brysting, A.K., Elven, R., Sønstebø, J.H., Murton, J., Sher, A., Rasmussen, M., Rønn, R., Mourier, T., Cooper, A., Austin, J., Möller, P., Froese, D., Zazula, G., Pompanon, F., Rioux, D., Niderkorn, V., Tikhonov, A., Savinov, G., Roberts, R.G., MacPhee, R.D.E., Gilbert, M.T.P., Kjær, K.H., Orlando, L., Brochmann, C., Taberlet, P., 2014. Fifty thousand years of arctic vegetation and megafaunal diet. Nature 506, 47–51.
Yurtsev, B.A., 1973. Phytogeographic zonality and floristic subdivisions of Chukotka. Botanicheskyi Zhurnal 58 (7), 945–964 (in Russian).
Zanina, O., Gubin, S., Kuzmina, S., Maximovich, S., Lopatina, D., 2011. Late-Pleistocene (MIS 3–2) palaeoenvironments as recorded by sediments, palaeosoils, and ground-squirrel nests at Duvanny Yar, Kolyma lowland, northeast Siberia. Quaternary Science Reviews 30, 2107–2123.