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Anatomy, death, and preservation of a woolly mammoth (*Mammuthus primigenius*) calf, Yamal Peninsula, northwest Siberia

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ABSTRACT

A well-preserved woolly mammoth calf found in northwest Siberia offers unique opportunities to investigate mammoth anatomy, behavior, life history and taphonomy. Analysis of the fluvial setting where the specimen was found suggests it was derived from eroding bluffs during ice-out flooding in June 2006. It then lay exposed on a point-bar surface until recovery the following May. AMS dating of bone collagen and plant tissues from the intestine provide age estimates that average about 41,800 ^{14}C yrBP. Anatomical features of interest include a hemispherical mass, apparently composed of brown fat, on the back of the neck. This may have functioned in thermoregulation for the neonate mammoth, born before onset of spring. Abundant subcutaneous fat and milk residues in the alimentary tract demonstrate that this animal was in good nutritional condition before death, making other features of its life history relevant for general studies of mammoth paleobiology. Plant remains from the intestine (mixed with milk residue in a manner consistent with frequent, small meals) show evidence of mastication by adult mammoths, suggesting that this calf ingested fecal material, probably from its mother and presumably to inoculate its intestinal tract with a microbial assemblage derived from a healthy adult. Discrepancies between the season of death we infer (spring) and seasonal indicators from the intestine implicate coprophagy (involving old fecal boli) by the mother. This animal's trachea and bronchi are completely occluded with fine-grained vivianite (hydrated iron phosphate) such as occurs in some lacustrine settings. Because this vivianite does not penetrate the lung beyond the bronchi, we infer that it must have entered as a viscous mass that occluded the airway, causing asphyxia. Nodular vivianite in the cranial region and interiors of long bones must have originated postmortem, but its distribution may be partly controlled by peripheral vasoconstriction, a physiological response to asphyxia. Nodular vivianite may have formed from iron derived from hemoglobin and phosphate liberated by partial demineralization of bones. Demineralization could have been caused by lactic acid, for which the main evidence is loss of tissues dominated by Type 1 collagen (denatured in lactic acid). We propose that this was consequent on postmortem colonization of the body by lactic acid-producing bacteria. These bacteria and their metabolites may have promoted preservation during the time before the body was incorporated in permafrost and could also have inhibited scavenging and bacterial decomposition following recent exposure of the specimen.

1. Introduction

In May 2007, Nenets reindeer herders traveling northward on their annual migratory cycle discovered the frozen body of a female woolly mammoth (*Mammuthus primigenius*) calf exposed on a point bar along the Yuribei River, on the Yamal Peninsula, northwest Siberia. Through a complex series of contacts (partially recounted in the 2009 National Geographic documentary "Waking

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the Baby Mammoth"), this specimen was reposited in the Shemanovskiy Museum and Exhibition Center in Salekhard, administrative center of the Yamalo-Nenets Autonomous Okrug. Recognizing the importance of the specimen, museum officials conferred on it the name Lyuba, honoring the wife of the Nenets reindeer herder, Yuri Khudi, who reported her. As noted by Kosintsev et al. (2010) in the first scientific publication announcing the specimen, Lyuba is essentially complete and in better overall condition than any previously recovered mammoth remains (Fig. 1). Nevertheless, the confidence with which Lyuba's features may be attributed to mammoths in general depends on the degree to which her developmental history, reflected in her perimortem condition and record of dental development, represents that of a normal, healthy individual of her species. Lyuba's cause of death is relevant to this judgment because evidence of antemortem morbidity would raise doubt on the generality of Lyuba's traits; conversely, sudden trauma or mishap would be consistent with a non-selective death unrelated to developmental status. Finally, postmortem history, if it can be reconstructed, could shed light on both cause of death and perimortem condition by clarifying the sequence of events responsible for the current condition of the specimen. Our analysis of Lyuba's discovery, preservation, and anatomy is thus undertaken to discover new features of mammoth biology, to clarify Lyuba's perimortem history, and to elucidate how the body of a young mammoth managed to remain in such a nearly pristine state.

2. History and context of discovery

Details of Lyuba's discovery were first obtained from Shemanovskiy Museum staff. When Lyuba was recovered, the Yuribei River was still solidly frozen, with ice restricted to the deepest part of its channel, marking the low-water stage typical of autumn, when declining temperatures bring to an end the seasonal melting of permafrost and all surface water freezes. The point bar on which Lyuba was found was about a kilometer downstream from the inner margin of a major bend in the river, on the outer curve of which was a high cut-bank where late Pleistocene ice-complex sediments were exposed and where mammoth tusks had been discovered previously by the Nenets. It would not have been surprising for Lyuba to have been found on this bank in a state of partial exhumation, but instead, she was found on "the wrong side of the river" (i.e., on the low-lying point bar rather than the high bank) and with no evidence of either residual or incipient burial by sediment. From the beginning, her occurrence was viewed with suspicion, as if she

Fig. 1. Lyuba, right lateral aspect.

"did not belong" where she was found, but from where, when, and by what agency could she have been moved? All this seemed especially uncanny to the Nenets, because in their worldview mammoths are creatures of an underworld from which they sometimes escape, only to die as they enter onto the plane of our existence – and here was Lyuba, looking almost as if she had just died and were truly a visitor from another world.

Elements of this account clearly needed investigation, but our first opportunity was not until March 2008, when Buigues returned to Yamal to interview Yuri Khudi. On this occasion, Khudi said that after he had reported Lyuba to authorities, he had learned of another Nenets reindeer herder, Nikola Serotetto, who had independently come across Lyuba, though without reporting her, in September 2006. Minor differences in this initially second-hand account (e.g., Nicola had described Lyuba as "in" the sediment, rather than "on" it) implied that these two sightings might have occurred at different places, as well as at different times (because additional erosion during the continuously frozen conditions of winter seemed unlikely). At this point, the best explanation for Lyuba's movement from one location to another seemed to be heretofore-undisclosed human agency. In any case, it seemed possible that interviewing both witnesses "on location" might clarify Lyuba's recent history. Plans were thus made for Buigues and Fisher to meet Khudi and Serotetto in mid-May of 2008, exactly one year after Lyuba's recovery, to revisit the locations where she had been seen.

As Khudi's guests, we journeyed first to the place where he had seen Lyuba, but to our surprise, Nikola Serotetto, who was accompanying us, ready to continue to "his" location, announced that the two places were in fact the same. In retrospect, their differing accounts of Lyuba's relation to her substrate appear to have resulted from seeing her first without, but later with, a cover of snow. This meant that Lyuba must have overwintered (September 2006–May 2007) at the same location from which she was later recovered.

Further investigation (Buigues, Fisher) of the location where Lyuba was found showed the total vertical relief between the low-water level in the channel and the thickly vegetated (by dwarf willow) levee margin to be approximately 4 m. The lowest 1.5 m was snow-covered but unvegetated and relatively steeply sloping toward the channel thalweg. Above this was a zone of intermediate slope, about 1 m from upper to lower margin, the lower portion of which was windswept and unvegetated, with scattered small patches of bare sediment, and the upper portion of which was sparsely vegetated, retaining its snow cover. Next came a broad zone of very low slope, 0.5 m in relief and somewhat more thickly covered by dwarf willow growth and other vegetation (also retaining snow cover) with most individual plants <2 yrs old. Finally, a steep slope of at least 1 m relief rose to the levee margin. Lyuba had been located near the middle of the zone of intermediate slope, just below the lower margin of sparse vegetation.

Excavation into the point bar surface where Lyuba was found revealed it to consist of decimeter-scale fining upward cycles of fine sand to clay, each representing the transition from high flow rates of the sediment-laden Yuribei to the low-energy conditions that follow formation of ice-dams during the brief ice-out flooding events that recur each spring, about the first week in June (at this latitude). At this time of year, sunlight during the rapidly lengthening day drives widespread snow-melt, producing enormous volumes of discharge. In arctic rivers with a northward component to their direction of flow (like the Yuribei), melting in southerly parts of the watershed often occurs before downstream channel thawing. As ice-bound channels are inundated, large rafts of ice break free from channel bottoms and begin to move downstream. However, as water levels rise, ice rafts run aground on the upper reaches of point bars, impinge on levee margins, and crowd together, forming ice-dams. Some of the water impounded behind ice-dams floods beyond the levee margin, but the main volume remains to deposit its sediment load in a fining upward sequence. Episodically, ice-dams break loose, flushing impounded melt-water downstream in a renewed torrent, and the cycle begins again. Sediment deposited within the main portion of a channel is quickly resuspended, but much of the sediment deposited on point bar surfaces remains. The point bar on which Lyuba was found, like point bars generally, was thus an aggradational setting associated with the modern river and was not likely to represent Lyuba's site of death, burial, and preservation. Whether one or several fining upward cycles typically form each spring was not clear, and may vary from place to place. In an attempt to measure depositional rate, two metal stakes were pounded into the frozen surface of the point bar, on the chance that they could be relocated later that year, and the amount of sediment deposited around them measured.

The principal erosional phase of spring flooding of high-latitude rivers occurs when ice-dams break, releasing large volumes of melt-water at once. Under such circumstances, bank incision, especially on the outside curves of meanders, can be dramatic. The present-day bluff margin above such curves exposes large, protruding blocks of permafrost that barely survived collapse into the channel in prior years and "notches" from which comparable blocks have already fallen. Furthermore, the Nenets confirmed seeing enormous blocks fall into the river at times of high discharge. We were not able to observe ice-out on the Yuribei, but Lyuba was probably released from permafrost at this time of year, through upstream incision or bank collapse along bluffs like those located near where she was found. We have, however, little basis for estimating how far she was transported.

Once most snow melts and the resultant volume of water flushes through the system (often in a matter of days), production of additional melt-water slows because it can only derive from permafrost insulated by tundra vegetation. In most years, including 2006 (A. Sokolov, pers. comm.), summer precipitation is limited, and post ice-out water levels decline almost monotonically until autumn, when the annual low-water level is again locked in by freezing temperatures.

In August 2008, several of us (Tikhonov, Fisher, Rountrey and others) returned for additional fieldwork along the Yuribei to prospect for new fossil material and to investigate the modern depositional settings of this landscape in more detail than had been possible under the late winter conditions of our visit in May. Water levels were again near their annual low. Returning to where Lyuba was found, there was no sign of the metal stakes left to measure rate of sedimentation, and the surface of the point bar nearby looked radically reworked, with large areas free of vegetation. Even in a predominantly aggradational setting, scour associated with ice-dam collapse can dramatically alter surface profile. Along this and other stretches of the Yuribei, we found scrape marks where ice rafts had "bulldozed" the point bar surface producing a prominent "spoil pile" at the downstream end of the scrape (Fig. 2A). Overturned, vegetation-bound segments of levee margin marked where ice blocks had been driven into the levee by water impounded behind an ice-dam (Fig. 2B). Large blocks of willow-bound sediment from the levee margin were strewn across some point bar surfaces, showing that transport of an object of Lyuba's size can be trivial indeed. In August, all was quiet, but the record of landforms gave witness to the forces that had been at work.

Synthesizing these observations, Lyuba was found 2 m above the annual low-water level in the Yuribei and well above the water level that would have been observed at any time after ice-out. In our assessment, ice-out flooding in June 2006 is the only natural process available to have moved her to the location where she was found. Nor is it likely that she could have been eroded from nearby outcrops between ice-out and the time of her recovery. However, during ice-out flooding, it is entirely possible that upstream bank collapse introduced Lyuba into the flood-stage Yuribei as part of a block of permafrost. This would have melted rapidly, releasing Lyuba's nearly neutrally buoyant body into the flow. She could then have been swept downstream into an impoundment behind an ice-dam and settled onto the point bar surface, where she remained as water levels dropped. An even better explanation for her position on the channel profile is that she simply ran aground on the flank of the point bar during the waning stages of flow following ice-out. It is remotely conceivable that she was eroded and transported one year earlier, in June 2005, but that only intensifies the mystery of how she could have survived an arctic summer exposed to 24-h sunlight, elevated temperatures, and visitation by scavengers. As it happens, the mammoth specimen known as Mascha, also a female calf from Yamal, is thought to have been exposed for about two years prior to discovery (A. Tikhonov, pers. comm.), but she was recovered in much worse condition than Lyuba. The plausibility of Lyuba's survival for even one year on the present-day landscape is an issue we take up below, but given her excellent condition, one year is easier to explain than two.

3. Condition at time of discovery

No photographs document Lyuba's external condition until after she arrived in the village of Novyy Port. Her transport from the

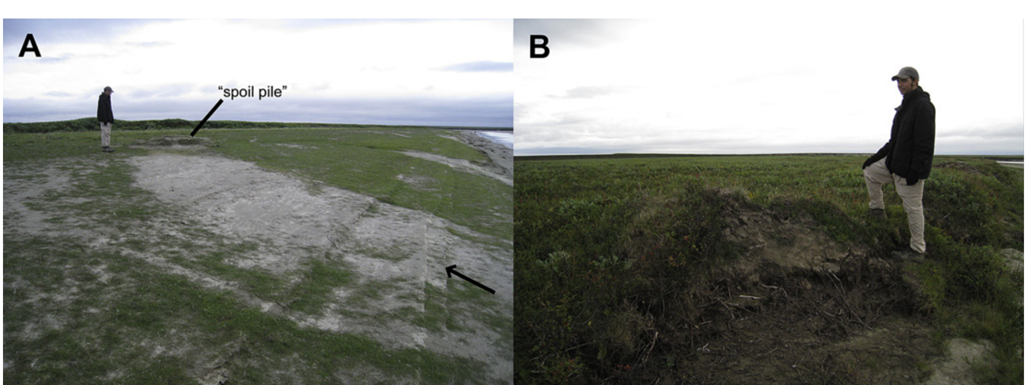

Fig. 2. Surface features along Yuribei River indicative of ice-dam formation. A. Ice-scrape along surface of point bar; arrow indicates direction of movement. B. Over-turned levee margin. ANR for scale in both.

tundra was unfortunately not under the care of Yuri Khudi, and on arrival in Novyy Port, she was left unattended, at which time feral dogs gnawed off most of her tail and her right ear lobe, although most of the pinna (and the entire left ear) was undamaged, as was her trunk tip. Other minor cuts and abrasions are probably attributable to conditions of transport out of the tundra. Most, but not all, of her hair has come loose from the skin and been lost, as have all of her nails. She was identified as female based on her external genitalia (similar to those of female elephants); she had bilateral pectoral nipples but no opening for a musth gland.

Aside from these instances of minor scavenging and physical duress, the most obvious category of degradation on Lyuba's surface involves what appear to be fungal growths affecting small patches of skin. Most of these are on her left side, lowermost as she lay on the point bar, and it seems likely that the higher moisture content of this side, shaded by the rest of her body and located next to the ground surface, was instrumental in allowing more extensive fungal development. The thinner skin of Lyuba's face seems to have been most susceptible to attack, but other loci, especially near points of contact with the substrate, are also affected. We have not yet done histological or biochemical investigations of these features; their identification as fungal is based on their general character and evident relation to moisture. These features have rounded outlines, convex outer surfaces, and porous internal texture with local development of fine-grained, blue vivianite (hydrated iron phosphate) on their surface and interstices. Where underlying surfaces are exposed, it appears that hemispherical depressions have been excavated into the skin surface. Similar features were noted on Blue Babe, a "permafrost mummy" of *Bison priscus* found in Alaska (Guthrie, 1990).

Lyuba's mass soon after recovery was 49.6 kg. Her live-mass would have been greater, as there was clear evidence of water loss during her preservational history. All of her dermal tissues were hard and dry – most notably her trunk, with its high surface-to-volume ratio. In addition, her limb cross sections all had the form of irregularly flattened ellipses, with reduction of thickness apparently driven by water loss from limb musculature (flattening in the lateral direction). Much of this desiccation could have occurred during burial in permafrost, by formation of "segregation-ice" (Hallet et al., 1991), but some could also have occurred during exposure between June 2006 and May 2007.

After recovery, except for a brief period in conjunction with the first dissection and sampling session, Lyuba was at first kept frozen. Initially, this seemed essential, as it was not clear that a specimen this well preserved could ever have been thawed since soon after death. Later, as we realized that Lyuba must have been thawed throughout the summer of 2006, the imperative to keep her frozen became less compelling, but freezing still seemed the safest approach because we did not yet understand her mode of preservation. Her second and last thawing was in preparation for a second dissection session, after which she was put into preservative solutions in preparation for an extended period of exhibition.

4. AMS age estimate

Obtaining an AMS age estimate was one of the first analytical priorities in studying Lyuba. Initially, two small pieces of skin were detached and sent for dating. We realized that external tissue would be most susceptible to contamination by younger carbon, but this was before fieldwork in Yamal had revealed Lyuba's history of exposure. We were months away from our first opportunity to acquire internal samples, so dating skin seemed a reasonable first step, though we now view it as having been misleading (Table 1).

Later, on the occasion of the first dissection, we obtained a bone sample and a sample of plant material from Lyuba's intestine (discussed below). These were much better candidates for dating, and age estimates based on them are both older and congruent with one another. Carbon and nitrogen isotope values on the bone collagen used for GrA-41246 were $\delta^{13}C = -21.91$ and $\delta^{15}N = +8.82$, both of which are normal values for mammoth collagen and consistent with the dentin values reported in Rountrey et al. (in press). Based on these dates, Lyuba lived about 41,800 ^{14}C yrBP.

Table 1
AMS age estimates for Lyuba, Center for Isotope Research, Groningen University, Groningen, Netherlands.

Laboratory number	Age estimate (^{14}C yrBP)	Material dated
GrA-35690	36690 (+320, −280)	Skin
GrA-35859	37150 (+280, −250)	Skin
GrA-41246	41910 (+550, −450)	Bone collagen (rib)
GrA-41861	41700 (+700, −550)	Plant remains from intestines

5. Anatomy

Our first attempt to examine Lyuba's internal anatomy was to collaborate with Naoki Suzuki of Jikei University, Tokyo, Japan, on a Computed Tomography (CT-) scan of most of her body (excepting parts of her back and feet, which did not fit within the 70-cm diameter data volume of this instrument). Although this experience was useful in directing further inquiries, data from this scan were not available for our use.

We next undertook limited tissue sampling and exploratory dissection in June 2008, in Saint-Petersburg, in the Zoological Institute of the Russian Academy of Sciences. All entry was from the left side, because its appearance was already compromised by fungal activity. Lyuba's left dentition was surgically removed to permit analysis (Rountrey et al., in press), an approximately 20 cm (width and height) opening was made in her left abdominal wall to provide access to intestinal contents, and additional parts of her body were sampled by coring or endoscopically-guided biopsy (by Suzuki).

In December 2009, we received permission for a second dissection, at which a larger, single opening into the abdominal and thoracic cavities was made, facilitating direct inspection of heart, lungs, stomach, liver, intestines, and associated tissues. We report here some results of this work, leaving detailed description of histology and biochemistry to subsequent analyses.

In February 2010, we attempted to repeat the CT-scan of Lyuba and subject her to an MRI analysis through collaboration with GE Healthcare Institute, in Waukesha, Wisconsin. Again, Lyuba's size did not permit acquisition of 3D data on her entire body, but we gained insights into those parts we were able to image. Finally, in October 2010, we succeeded in a full-body CT-scan, working with Ford Motor Company, in Livonia, Michigan, on a scanner with a 1-m diameter data volume.

5.1. Cervical fat deposit

One of the most distinctive features of Lyuba's lateral profile is an almost hemispherical mass of tissue on the dorsal aspect of her neck, just behind her cranium (Fig. 1). CT-scans showed this to be low-density tissue that we interpreted as fat. On extracting a core from this mass, it was oily in consistency and had a yellowish-brown color different from the white color of subcutaneous fat (see below). Histologic sections (to be presented in subsequent work) showed this tissue to be well-vascularized and to contain fat cells with a multilocular structure (rather than the unilocular structure of white fat; Lafontan and Berlan, 1993).

All these features suggest that this mass is composed of brown fat, a specialized tissue involved in non-shivering thermogenesis (Lafontan and Berlan, 1993). A cervical mass of brown fat is known in many mammals, including humans, where it serves to maintain core body temperature by warming venous blood returning from the external body surface. Part of a similar-looking hump of fatty tissue was observed on the Oimyakon mammoth calf (Boeskorov et al., 2007), although for that specimen, there was no concurrent histological evaluation. More specimens may be needed to confirm the generality of this feature, but a cervical hump of this nature could have been critical for survival of a young mammoth calf, especially given that our analysis of Lyuba's season of birth (Rountrey et al., in press) places it in spring. At this time of year, a neonate mammoth may have needed the thermoregulatory support of brown fat to maintain a viable core body temperature until weather conditions became more benign, as much as a month later. Seasonally variable accumulations of brown fat could have occurred in older mammoths as well, as noted by Boeskorov et al. (2007), but whether the accentuated head and shoulder profiles sometimes used to depict mammoths in Paleolithic art represent the same feature as Lyuba's cervical fat deposit remains unclear.

5.2. Subcutaneous fat deposits

Incisions made during dissection provided direct access to subcutaneous fat deposits that ranged in thickness from 2 cm to 4 cm (thicker ventrally) on the lateral flank of Lyuba's body. On her face, this layer was <1 cm thick, and CT-scans suggest intermediate thicknesses elsewhere. In contrast to the cervical fat deposit, this fat is white and has a dry, somewhat friable consistency. Infrared spectroscopy by Marianne McKelvey of Dow Corporation, Midland, Michigan, showed it to be saponified, but it clearly represents normal subcutaneous fat, important as insulation and as a caloric reservoir and indicative of an animal in an excellent state of nutritional health.

5.3. General skeletal condition

Kosintsev et al. (2010) report "no internal lesions" evident on Lyuba's skeleton, but during facial surgery, a transverse break on the left coronoid process of the mandible was discovered, and CT-scans reveal another fracture of the mandible, near the symphysis, apparently resulting from lateral compression approximating the two rami. Other parts of Lyuba's body, most notably her skull and pelvis, display plastic deformation reflecting similar compressive forces. On her skull, this deformation has produced shear, displacing features of the left side anteriorly relative to contralateral features. Occurrence of this amount of shear *without* extensive fracture suggests the bones were unusually flexible. This was borne out, following dissection, by direct inspection of rib fragments that were cut to provide access to the thoracic cavity. Even accounting for Lyuba's young age (Rountrey et al., in press), her bones are less fully mineralized than would be expected. However, this leaves unresolved the question of whether this represents her original condition or postmortem alteration.

One other feature of Lyuba's skeleton that was evident on inspection of CT-scans was that bright, radio-opaque loci were scattered throughout the interior of many long bones (Fig. 3) and within or adjacent to parts of her cranium (Rountrey et al., in press, Fig. 3). Only loci associated with the facial region were subject to both direct inspection and evaluation of CT-data (and the latter, only following work at GE), but these turned out to be botryoidal nodules (spheroidal composites with a radial fabric of acicular crystallites) of vivianite (hydrated iron phosphate). Nodules within the oral cavity might be ignored as possible secondary deposits acquired during transport and deposition along the Yuribei. However, during facial surgery, one vivianite nodule with the same structure was encountered within the left infraorbital foramen, and others were embedded within muscle tissue, adjacent to bone. These nodules must represent structures that formed postmortem within tissue, and we suspect that radio-opaque loci within long bones represent an identical phenomenon. Iron needed for forming tissue-hosted vivianite could have come from blood, and phosphate probably came from bones, raising the likelihood that the low degree of mineralization of Lyuba's bones is due to partial demineralization within her burial setting.

Fig. 3. Vivianite nodules inside bones of Lyuba's left hind leg (CT section; anterior toward right); plane of inset marked by lines perpendicular to tibia in larger image; lines in inset indicate plane of larger section.

5.4. Dentition

Lyuba's dentition consists of fully formed and almost-erupted deciduous tusks (dI2), fully formed and just-erupted dP2s, and incompletely formed and unerupted dP3s and dP4s. Kosintsev et al. (2010) refer to her age as "no older than two or three months", but Rountrey et al. (in press) offer a more precise determination of about one month, based on discovery of a neonatal line in her dentin, marking the time of birth, and postnatal dentin increments in her dP2s. Evidence of eruption of dP2s, beyond their position relative to alveoli, includes contact of opposing surfaces along an incipient occlusal plane (Rountrey et al., in press, Fig. 3). The tips of a few conules on each tooth had come into contact (Rountrey et al., in press, Fig. 4). However, this degree of wear was not yet sufficient to allow these teeth to have accomplished the shearing of grass fibers typical of adult mastication (which requires more elongate shearing edges developed along more deeply worn plate margins).

Examination of Lyuba's teeth showed minor, highly localized pitting of enamel (possibly indicating postmortem dissolution), manifesting as absence of a number of adjacent enamel prisms, without evident effect on neighboring prisms. There were also concentrations of vivianite adhering to some external enamel surfaces and to the inner, pulp surface of dentin. Cutting and sampling the teeth for serial isotope analysis (Rountrey et al., in press) revealed that although the enamel was as hard as expected, the dentin was notably soft and yielding to a steel probe or scalpel. Dentin normally mineralizes completely, soon after deposition of its collagen matrix, so this lack of complete mineralization must result from some anomalous condition or postmortem dissolution. Other indicators of dissolution favor the latter alternative.

5.5. Musculoskeletal connective tissue

One of the most surprising observations during dissection was that connective tissue that should have attached muscles to bone was no longer present. This became evident during dissection of the facial region to remove teeth. After cutting through skin, subcutaneous fat, and muscle, blocks of tissue lifted cleanly away from underlying bone surfaces rather than retaining a firm attachment. To confirm this pattern, dissection was extended onto the coronoid process, where temporalis musculature inserts, and onto the ventral aspect of the zygomatic arch, where masseteric muscles originate; all of this tissue lifted freely off the bone. During the second dissection, the same pattern obtained; cut ribs slid freely from intercostal musculature surrounding them, and the entire block of muscles that should have been attached to the scapula lifted cleanly away (Fig. 4A). Likewise, tooth roots should have been suspended within their alveoli by fibers of the periodontal ligament, but instead, they slid freely from alveoli with no trace of periodontal ligament fibers (Fig. 4B).

5.6. Contents of alimentary tract

At the beginning of Lyuba's alimentary tract, her mouth was full of mud, fine sand, and small iron-rich concretions that remain ambiguous in origin. As noted above, this material might have had a primary association with Lyuba and her site of death and/or burial, but it could also have become lodged in her mouth during or after transport down the Yuribei. Excavating into the depths of her oral cavity during the first dissection, there was a transition from more oxidized sediment anteriorly to more reduced, darker-colored sediment deeper in her oral cavity. These contrasting oxidation states probably developed during Lyuba's exposure in 2006, but it is still unclear whether all of this sediment is primary. We left the sediment mass in the back of the oropharyngeal cavity intact, hoping to document it more fully in subsequent work. In the CT-scan done at the GE Healthcare Institute we were able to trace X-ray-dense material back to the larynx, where the cylindrical infilling of the pharyngeal region pinches off (Fig. 5), after which it appears to continue as a solid cylindrical mass extending down the trachea. Just ventral to this, sediment fills a small posteriorly protruding region that must represent Lyuba's pharyngeal pouch (Watson, 1873), the first time this feature has been identified in a mammoth. There is also sediment in the upper part of the esophagus, dorsal to the trachea, but there appears to be some discontinuity between this sediment and that in the oropharyngeal cavity.

The abdominal "window" opened during the first dissection allowed access to both small and large intestine but did not permit extensive palpation in any direction, leaving us with little sense of the location of samples along the entire length of intestine. Samples from the large intestine were analyzed by Kosintsev et al. (2010), and additional samples, from the small and large intestine, were examined by Fisher and Rountrey, with subsamples forwarded to Bas van Geel and colleagues for more detailed analysis, to be published elsewhere.

Kosintsev et al. (2010) interpret their intestine sample as "filled with a mixture of detritus and mineral precipitate". They identify the detritus as "mostly… plant remains", among which "branches of mosses" are especially common, along with spores, pollen, other plant parts, sponge spicules, diatoms, and insect cuticle. Referring to a hypothesis that was discussed freely on the occasion of the first dissection, they mention that "spores of coprophilous fungi (*Sordaria* type and *Sporormiella* type)" could have been ingested by Lyuba "when eating its mother's feces like modern baby elephants do", but they go on to argue that such spores could also have been washed into an aquatic setting. In the end, they appear to prefer this latter scenario, because they claim (p. 211) that the "bottom sediments in [Lyuba's] intestine indicate that [she] drowned in a water body". We discuss this idea further below.

Our preliminary analysis of samples from the first dissection began by sorting and identifying material under a stereomicroscope, followed by sending unknown components for infra-red spectroscopic analysis. Much of the plant material, as recognized by Kosintsev et al. (2010), consisted of monocot stems and leaves, but we noted that many of these were sheared into parallelogram-shaped fragments by parallel cuts at oblique angles

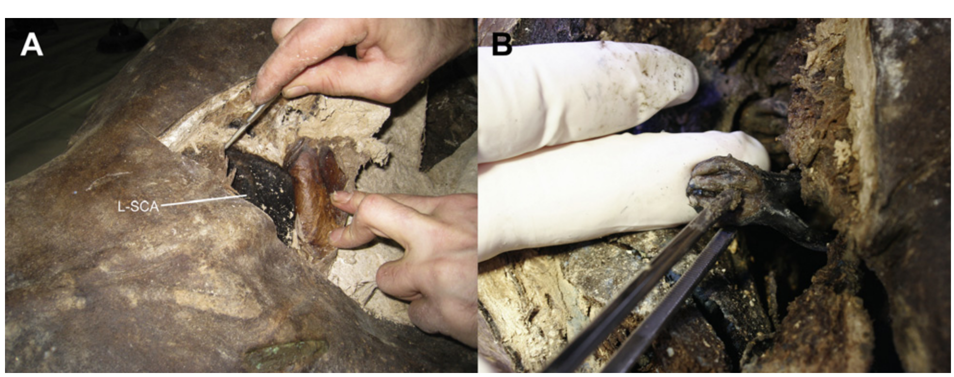

Fig. 4. Soft-tissue consequences of solubility of Type 1 collagen in lactic acid. A. Forelimb musculature detached from left scapula (L-SCA; anterior toward lower left). B. Absence of periodontal ligament on freshly extracted left dP_2.

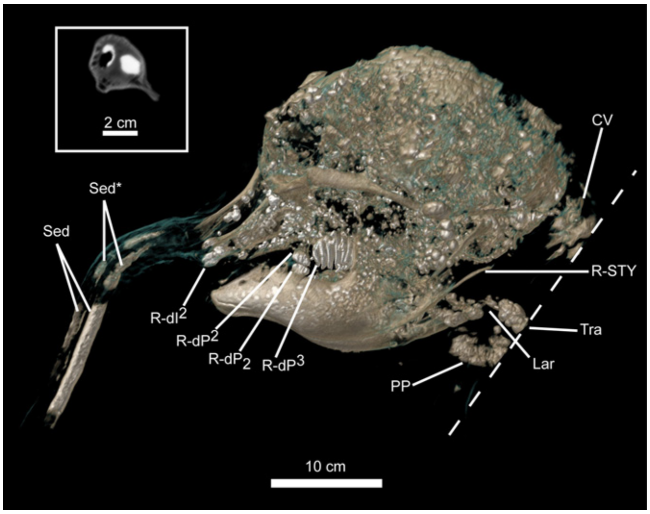

Fig. 5. CT- volume-rendering of Lyuba's head, trunk, and throat. Sed, proximal limit of in situ sediment in trunk. Sed*, sediment from distal portion of trunk, dispersed proximally during chemical treatment. CV = cervical vertebrae; R-STY = right stylohyoid; Tra = trachea; Lar = larynx; PP = pharyngeal pouch. Right teeth (R-…) appear in left lateral view because left teeth were removed previously. Dashed line = data limit on CT. Inset shows trunk cross section at "Sed" location.

to plant margins and conductive elements (Fig. 6A). This pattern is typical of mastication by adult mammoths but could not have been produced by Lyuba's freshly erupted dP2s. Further, much of this material occurred in dense concentrations with a brown fine-grained matrix of organic material, all closely resembling elephant feces. As pointed out by van Geel et al. (2011), occurrences of isolated spores of coprophilous fungi do not necessarily imply ingestion of fecal material (coprophagy), because they tend to be widespread in the environment (and do not form on feces until more than a week after defecation, whereas coprophagy by juvenile elephants tends to involve fresh feces; Leggett, 2004). Nevertheless, given the volume and concentration of intestinal contents resembling fecal material, we believe coprophagy is the best explanation for its presence in Lyuba. The alternative proposed by Kosintsev et al. (2010), that Lyuba acquired this material from the immediate environment of her death, requires either that this material was ingested as bottom sediment during or immediately prior to drowning and then somehow made it all the way into the large intestine before gut motility ceased following death, or that this material entered via the anus and was carried anteriorly as far as the small intestine. We know of no pattern of postmortem gut motility that would permit this type of transport.

In addition to the plant material described above, we observed sand and silt grains that could have been acquired incidentally, during ingestion of fecal material from a sediment substrate. There were also abundant fragments of whitish-brown material in a size range from several millimeters across to sub-millimeter dimensions (Fig. 6B). Marianne McKelvey (pers. comm.) identified these as composed of saponified triglycerides, based in their infra-red spectra, and the best interpretation we can offer for material of this composition in a young mammoth is that it represents remains of partly digested milk. This hypothesis is now under more detailed study by Boris Jansen and colleagues, for separate publication. Provisionally accepting the identification of these particles as derived from milk, they were most likely ingested directly from Lyuba's mother and at the time of her death, were in the process of being transported posteriorly along the alimentary tract. It is thus difficult to see how they could be mixed intimately with "plant detritus" acquired from the environment via the anus.

The second dissection provided access to both thoracic and abdominal cavities, but limits of time and permission precluded comprehensive analysis. Instead, we palpated, but did not open, the esophagus (the distal part was empty), and then opened and sampled contents of the stomach and several sites along the small and large intestine. The stomach was relatively small, cylindrical, and only moderately differentiated morphologically from the small intestine. However, it contained exclusively milk particles like those recovered from the intestine. As noted above, these must have been ingested from Lyuba's mother and must have *followed* the material we identify as fecal down the alimentary tract. This inference refutes the notion that the "plant detritus" was acquired orally during drowning (i.e., she could not have drunk milk *after* drowning, nor could the "plant detritus" have bypassed the stomach without trace).

Our samples of intestinal material from the second dissection replicated the ones we analyzed from the first dissection, except that one section of the large intestine contained a concentration of small, white-to-gray, acicular crystals that we suspected represented vivianite that had never been exposed to light. Shortly after opening this part of the intestine, these crystals began to change color to the bright blue that usually characterizes this mineral. In retrospect, it is likely that this material was responsible for a bright, X-ray-dense portion of Lybua's intestine observed during the first CT-scan, but why vivianite should have concentrated in this area is not clear. This was the only example we found of intestinal contents that could be characterized as a "mineral precipitate"; other mineral components of intestinal material were clearly *clastic* sediment grains. Nevertheless, the report of "mineral precipitate" by Kosintsev et al. (2010) may reflect similar material in their samples from the first dissection.

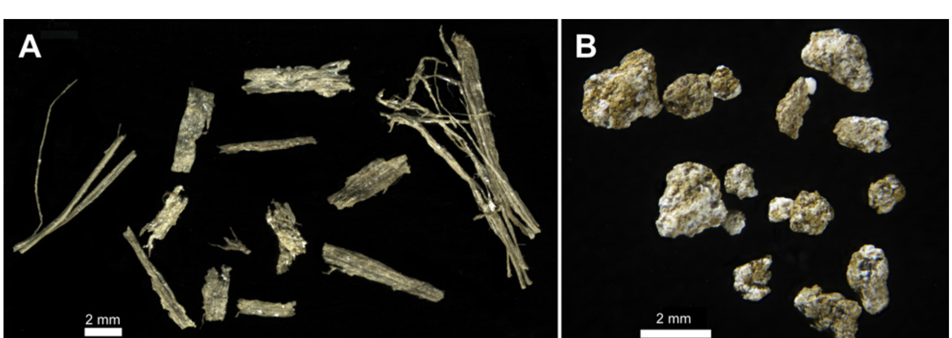

Fig. 6. Intestinal contents. A. Sheared monocot leaves/stems. B. Particles of milk residue.

The second dissection did allow us to do more extensive palpation than had been possible during the first. Only a few portions of the intestine were notably "full", and they were dominated by ingested fecal material. Contents of other portions were more moderate in volume and dominated by milk residue, but much of the intestine was relatively empty. This pattern suggests frequent, small meals, mostly of milk, with occasional coprophagy.

5.7. Respiratory system

One of the first features evident on the original CT-scan done in Tokyo was that both narial tracts within Lyuba's trunk were plugged with sediment (Mueller, 2009, p. 39). This blockage extended closer to the distal end of the trunk on one side than the other, but it ended proximally in both tracts near the level of Lyuba's chin. During chemical processing of Lyuba's body prior to exhibition, submersion in ethanol mobilized some of this material from the distal trunk, allowing it to disperse proximally, closer to the base of the trunk, where it can be seen in the CT-scans from GE (Fig. 5) and Ford, but this translocation was clearly secondary to laboratory treatment. No sediment was found, before or after treatment, within the base of the trunk (dorsal to premaxillae) or anywhere from the boney external nares, high on the forehead, to where the internal nares open on the oropharyngeal cavity (Rountrey et al., in press, Fig. 3). Instead, the lumen of each narial tract within the trunk base appears to have collapsed, pressing the anterior wall of the trunk against the premaxillae and creating a pronounced indentation in this part of Lyuba's face, most evident in lateral profile (Fig. 1).

The original CT-scan also showed that Lyuba's lungs had collapsed, now occupying only a fraction of her thoracic cavity. Later endoscopy (during the first dissection) via small holes bored in her thoracic wall provided an opportunity to view part of the thoracic cavity. The script of the National Geographic (2009) documentary describes this endoscopy and associated biopsies as yielding tissue samples from the lung, and although this seemed plausible then, closer inspection of the video record shows that the tissues sampled were associated with a typical proboscidean system of pleural membranes (West, 2001) located *outside* the lung (in particular, the parietal membrane and pleural connective tissue) and were not parts of the lung itself. In addition, fine sediment distributed diffusely around the thoracic cavity was described as lung contents. This is probably correct, but at the time, it was not clear how this material came to be dispersed.

Lung collapse in vivo is associated with perforation of the lung and thoracic wall, after which the elasticity of lung tissue pulls one or more lobes into a contracted state following loss of pressure differential between the lung interior and the pleural space (perforation of the thorax opens this space to ambient pressure). However, Lyuba shows no external evidence of perforation. A small transcutaneous puncture is located on the left ventrolateral aspect of her chest, in front of her left leg, but it does not penetrate the thoracic wall and probably represents only superficial postmortem damage, perhaps sustained recently, during transport down the Yuribei. An alternative interpretation of Lyuba's condition is that the loss of integrity of the lung could have occurred as part of postmortem decomposition, without breaching the thoracic wall, but this is more complicated than it seems. In most mammals, expanded lungs are held in place only by the "negative pressure" between an intact lung and the adjacent intact thoracic wall, but in proboscideans, the pleural membranes are attached by collagen fibers to the inner wall of the thorax, so this entire connection must be compromised to permit collapse (Short, 1962). This evidently happened sometime following Lyuba's death, but exactly how and why was not yet clear.

During the second dissection, we were able to examine Lyuba's thoracic cavity directly. Even on first opening, before anything was disturbed, many tissue surfaces were covered with the startlingly bright blue of vivianite, and closer inspection revealed where it was coming from. From the time of the first CT-scan, we had realized that Lyuba's trachea and primary bronchi were packed with X-ray-dense material assumed to be sediment. Lifting whole lobes of Lyuba's left lung aside, we were able to examine the bronchial region. Here and there along primary bronchi were openings (obviously postmortem in origin, as the lungs would not have functioned had they been present in the living state) out of which fine, vivianite "powder" seemed to have emerged. This same "powder" packed the entire lumen of the bronchi and presumably continued up the trachea, though we chose not to open this part of the respiratory tract to preserve relationships for additional documentation by CT. The blue vivianite "powder" consisted of extremely fine-grained (clay-size) particles completely different in habit from the acicular crystals recovered from Lyuba's intestine or the radial-fabric nodules found dispersed in her soft tissues and observed by CT inside long bones. To assess the distribution of vivianite "powder" within the lung, we excised part of one lobe, with associated bronchi (Fig. 7). In this specimen, we verified that the bronchi were completely full of this material, except in the immediate vicinity of holes where the bronchial wall was thin and appeared to have torn. Vivianite that had "escaped" from these openings (upon lung collapse?) was apparently responsible for the broadly dispersed blue color of surfaces lining the thoracic cavity. Despite the large volume of vivianite in the basal portion of the bronchial tree, vivianite did not extend into either intermediate or distal parts of the lung. Distal parts of the lung were entirely collapsed, with no macroscopically visible trace of alveolar spaces and notably, no trace of blue color. Instead, cross sections of distal lung tissue were a homogeneous brown color indicating that the vivianite "powder" had never reached this far into the respiratory system.

Lest there be any question about the composition of the fine-grained material within the bronchial system, we later analyzed the "powder" by X-ray diffraction. We expected it to be dominated by vivianite (given its color), with some admixture of clay or other detrital sediment, but in fact, there was no evidence of any other mineral phase.

5.8. Heart

Lyuba's heart was also accessible from her thoracic cavity, after opening the pericardium. It showed a quadrate form in lateral view, due to the same bifid apex seen in living elephants (Mariappa,

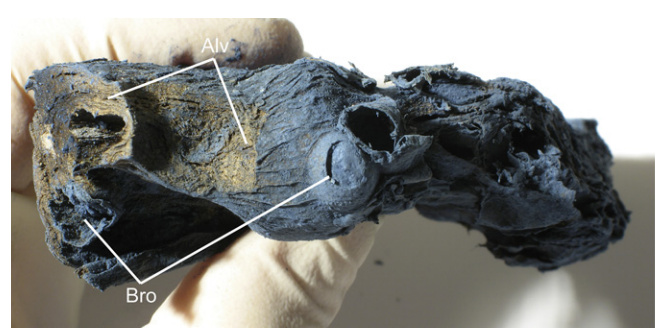

Fig. 7. Lung sample with fine-grained blue vivianite filling bronchi (Bro); alveolar tissue (Alv) is brown, with no vivianite. Blue rendered as white in grayscale version; gloved hand for scale. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

1986). In addition, her heart was richly invested in a deposit of pericardial fat. We did not have permission to do any invasive examination of the heart, but its general condition suggests a vigorous state of good health.

6. Postmortem alteration

Numerous elements of the descriptions above represent departures from the condition expected in a living or recently dead animal and presumably reflect postmortem alteration. Some of these are "normal" in the sense that they would be expected for almost any carcass. For example, loss of most of Lyuba's hair is not unexpected, as the lack of scavenging suggests rapid burial, most commonly associated with wet depositional environments, and slippage of hair from follicles often occurs within days of death for carcasses in wet conditions. Likewise, loss of nails could have occurred by a similar mechanism. Some compression is to be expected due to sediment loading following burial or to other forces, such as those related to formation of ice-wedges within permafrost. Either of these factors could be responsible for some or all of the distortion of Lyuba's skeleton.

Another category of traits that must reflect postmortem alteration includes features that were not familiar to us, but were immediately apparent upon inspection, or investigation with an appropriate modality. For example, the widespread development of tissue-hosted vivianite nodules (especially inside bones) was easily seen in CT-scans and was different from the frequently encountered thin layers of vivianite mineralization on external surfaces of many Pleistocene skeletal remains. Likewise, the lack of attachment between muscles and bone, or between tooth roots and their alveoli, was completely unexpected, but obvious upon dissection. Partial demineralization of Lyuba's bones and teeth (limited pitting of enamel, but pervasive partial demineralization of dentin and bone) was not immediately recognized in the CT-data, but was clear once we subjected teeth and bones to close inspection and handling.

A final aspect of postmortem alteration might never have been recognized except for an associated olfactory cue. During the first dissection, as Lyuba began to thaw and we began to expose soft tissues in close quarters, a distinctive, mildly sour smell became perceptible. This smell was different from the strong, distasteful odor of aerobic soft-tissue decomposition, but was familiar to one of us as a result of earlier experiments on sub-aqueous meat storage (Fisher, 1995). In these experiments, the smell was associated with meat stored underwater (in lakes, ponds, or bogs) and colonized by lactic acid-producing bacteria that occur naturally in many aquatic settings, especially where pH and oxygen levels are low. Lactic acid and carbon dioxide produced during fermentation by these bacteria resulted in increased acidity (lower pH) within the meat mass, which actually enhanced its long-term preservation potential by inhibiting later colonization by bacteria that normally mediate tissue decomposition (Gill, 1983). As one test of the relevance of these experiments to Lyuba, we measured her tissue pH and found it to be about 5.0 (in muscle), consistent with lactic acid-producing bacteria having had a role in her preservational history.

7. Discussion

The observations above are sufficiently multi-faceted that although certain conclusions are obvious, others require additional synthesis. We address below the issues that we believe represent the most important implications of this work for studies of other mammoths, and to some degree, other Pleistocene fauna.

7.1. Coprophagy

van Geel et al. (2008, 2011) argued that the Yukagir mammoth and the Cape Blossom, Alaska mammoth show evidence of coprophagy. In these adult woolly mammoths, this apparently occurred during late winter and may have been an important source of nutrients at a time of year when there was limited access to "fresh" forage. Rountrey (2009) and Clementz et al. (2009) discussed implications of coprophagy in mammoths for isotope composition and variation. Lyuba's coprophagy, given her young age (Rountrey et al., in press) and evident dependence on a milk diet, seems at first to represent a different phenomenon. Although we cannot rule out the possibility that she obtained some nutritional value from ingesting fecal material from an adult mammoth, presumably her mother, a simpler explanation would be to attribute this behavior to the cause suspected for extant elephants. Developing juveniles acquire their intestinal microbial communities as "immigrants" (dare we say "colonists"?) from established communities elsewhere… in the intestines of conspecific adults! Juveniles thus receive a comprehensive "inoculum" by ingesting an aliquot of the mother's feces.

From this perspective, it is interesting, but not surprising, that a juvenile woolly mammoth behaved similarly. The earliest extant elephants are reported to engage in coprophagy is at about 3 months (e.g., Leggett, 2004), but one of us (Fisher, pers. obs., Samburu Hills, Kenya) has seen African elephants (*Loxodonta africana*) as young as one month eating their mother's feces, and a video sequence available on the internet (www.Arkive.org/…) features a young African elephant of about the same age. We therefore do not consider Lyuba's age of about one month (Rountrey et al., in press) as incompatible with coprophagy.

Returning finally to the analysis of Kosintsev et al. (2010), perhaps coprophagy by both adults and juveniles is implicated by evidence coming from Lyuba. Recall that adult coprophagy, as discussed by van Geel et al. (2008) and Rountrey (2009) may have occurred in late winter or early spring and may have involved ingesting "old" fecal boli deposited on the landscape in an earlier season, such as summer or autumn. This means that seasonal indicators derived from fecal material *could* reflect the time of most recent defecation, but they could also reflect an earlier time and a different season. Thus, the late summer to autumn seasonal indicators that Kosintsev et al. (2010) recognize in the seed and phytolith assemblage from Lyuba's intestine need not apply to the time of Lyuba's death, as they imply. Instead, they may simply reflect the season of production of fecal boli that Lyuba's mother had recently ingested, defecated, and passed on to Lyuba. On dental evidence (Rountrey et al., in press), we interpret Lyuba to have died in early spring, but then adult coprophagy – *followed by* juvenile coprophagy – becomes a viable explanation for summer-autumn indicators in the plant assemblage from her intestine.

7.2. Cause of death

Kosintsev et al. (2010) regard Lyuba's cause of death as drowning, and their argument for this position is summarized in their last sentence (p. 211), "The bottom sediments in its intestine indicate that it drowned in a water body". However, as argued above, the absence of sediment in the milk-filled stomach means that even in a protracted death struggle, sediment in the intestine cannot have been acquired by mouth – nor do we regard it as plausible that it was acquired via the anus.

All who have worked on Lyuba agree that her near-pristine condition refutes any idea that she died from musculo-skeletal trauma, because we observe no candidate for a traumatic feature that cannot be better explained as postmortem alteration (e.g., the fractured mandible). Although we attempt below to derive some insight into Lyuba's condition at the time of her death from evidence for her cause of death, these two factors are reciprocally (not circularly) related. At this point in our discussion of cause of death, it is thus relevant that Lyuba's abundant subcutaneous fat, full stomach, and regular feedings rule out abandonment and/or malnourishment as direct causes of death. Potential causes such as disease would be harder to assess (unless through histology or analysis of ancient DNA), but if a strong enough candidate cause is adduced, we may not need to consider explicitly every alternative.

We do not regard cause of death as a question that is necessarily, or even generally, answerable in studies of fossil material, but in this particular instance, data on the condition of Lyuba's respiratory tract provide unusual detail. The single most important factor is the distribution of fine-grained vivianite in her bronchial passages. In drowning, inspired air is typically retained until build-up of carbon dioxide triggers a reflex to exhale and gasp for new air. If the body is submerged, this leads to aspiration of a large volume of water (complete flooding rarely occurs; Edmonds, 1998). If particulate matter, such as the vivianite we are trying to explain, is drawn in with the water, it tends to be distributed throughout much of the lung, especially given the forcefulness of reflexive inhalation. This contrasts markedly with what we see in Lyuba. Following aspiration of water, there may be attempts to clear the airway of fluid, but consciousness is usually lost quickly (Edmonds, 1998). After this, passive flooding may bring some additional particulates into the lung, but the competence of this process for entrainment and transport of sediment is minimal. We have considered the suggestion that the fine-grained vivianite might have been introduced after death by current action, but this process, called "draught filling" (Seilacher, 1971) requires a path for through-going fluid movement (incompatible with the cul-de-sac geometry of the mammalian lung) and thus cannot fill a space completely.

As an alternative to drowning, we propose that Lyuba died of asphyxia or suffocation after forceful, reflexive inhalation of a viscous "mud" composed of the fine-grained vivianite that now occupies her trachea and bronchi. We treat below associated factors that may help to explain circumstances leading to this end, but our central tenet is that there is no force other than the reflexive inhalation of a frantic animal that would be capable of drawing a continuous column of sediment into the airway. If this material had been suspended in a liberal amount of water, it would have been carried more pervasively into peripheral parts of the lung. If that had happened, with sediment or without, we would describe the process as drowning, but if the material being transported is so viscous that it cannot penetrate beyond the bronchi, ending with fatal airway obstruction, then the process is better described as asphyxiation.

One point critical to this argument is that the fine-grained vivianite filling Lyuba's trachea and bronchi (CT-density = 800–1100 Hounsfield units, HU, similar to trunk sediment) is different in character and inferred origin from the vivianite nodules (CT-density>2000 HU) in the rest of her body. We argue below that the nodules of vivianite are postmortem products of a biologically mediated chemical transformation within Lyuba's tissues. In contrast, the fine-grained vivianite in her airway has a different crystal habit. The only reports we have seen of similar material involve lacustrine settings where vivianite mud accumulates in anaerobic conditions (e.g., Manning et al., 1991). This interpretation implies that Lyuba's death involved her falling into such a lacustrine setting and somehow inhaling vivianite sediment. We acknowledge that locating these events in or near a lake raises the question of whether our interpretation is really different from drowning after all. However, when we focus on the actual mechanism by which normal physiological function is compromised, the difference is significant, and the available evidence allows us to distinguish these mechanisms. The only reason ever to have worried about Lyuba's cause of death is to make sure we have an evidentially grounded understanding of circumstances associated with her death, and this requires making all the distinctions that the data permit.

One consequence of this interpretation is that we have a search image for the kind of depositional environment in which Lyuba died and was buried. The chances of locating such an environment may be slim, but knowing what to look for and having a cue as distinctive as vivianite should be of some assistance. While examining stratigraphic sequences exposed along the Yuribei, we saw no vivianite exposures, but they may be uncommon, limited in areal extent, or rapidly weathered.

Another set of factors that could have a bearing on Lyuba's cause of death concerns the distribution of sediment within her trunk and oropharyngeal cavity. The trunk is an airway that is connected to but separate from the mouth, and a mammoth would not suffocate just because its trunk became clogged. Nonetheless, we are considering a subtler sequence of events that would explain an otherwise unusual feature, the occlusion of both narial tracts in the distal two-thirds of the trunk, coupled with the complete absence of sediment from the base of the trunk and the airway descending to the internal nares. Our hypothesis is that Lyuba could have somehow ended up struggling in the wet sediment of a vivianite-producing lake (with or without significant water depth), inadvertently getting a mouthful of sediment, which after snorting and choking could have become partly lodged in her trunk as well. Gagging on the sediment filling her mouth, she could have exhaled forcefully, trying to clear the blockage in her trunk, but because the narial passages become smaller toward its distal end, this was not effective, only impacting the sediment more firmly. She then could have tried the reciprocal response, a forceful, reflexive inhalation to dislodge sediment backward from her trunk. If this had been done with an open oral airway, she would have achieved no significant suction. Whether for this reason, or because her face was already so covered with mud that she did not have oral access to air, we propose that she inhaled forcefully with a closed oral airway … and that was her undoing. A solid column of wet sediment from the larger-diameter proximal third of both narial tracts in her trunk and the passage extending down to the internal nares could have been accelerated backwards straight into her trachea and bronchi. Once there, the sediment mass may have been impossible for Lyuba to expel.

In addition to explaining the distal blockage of Lyuba's trunk and the absence of sediment from its proximal portion, this may explain the facial indentation where the anterior wall of the trunk is compressed against the premaxillae. Under our model, this was caused by the negative pressure generated by forceful inhalation without an alternative route for restoration of ambient pressure. If Lyuba died and was buried in this state, the facial indentation could preserve a direct reflection of her last vigorous movement. We have considered the alternative that the facial indentation reflects only compression by surrounding sediment within the burial environment. However, this would be expected to compress all parts of the trunk that were not already sediment-filled. Furthermore, it leaves the abrupt, bilaterally symmetrical proximal termination of sediment blockage (Fig. 5) as an unexplained coincidence. Under the inhalation hypothesis, this is caused by a diameter-dependent threshold in resistance to shear along the bounding surfaces of narial passages.

We have received comments that a scenario such as this is inconceivable because Lyuba's mother would have rescued her. We acknowledge that mothers often come to the rescue, but not always. In the spring, barely a month after Lyuba's birth, Lyuba and her mother could have been walking across thin ice covering a vivianite-producing lake … when they both fell through. In the struggle, Lyuba's mother might not have retrieved her soon enough, or seen her at all. We do not even know that Lyuba's mother survived the ordeal. Indeed, we can never know many of these details; we can only ask what events are compatible with the observations we make now on Lyuba's condition.

7.3. Condition at time of death

We summarized in Section 7.2 the evidence for Lyuba's excellent nutritional condition at the time of her death. We now need only note that the best-supported hypothesis of cause of death involves events that coalesced in a fatal accident from which no amount of normal development or robust condition could protect her. Beyond this, our conclusion is simply that it is reasonable to take the details of Lyuba's developmental history (Rountrey et al., in press) as normal for other individuals of her species.

7.4. Preservational history

Two factors must have contributed to Lyuba's excellent state of preservation: (1) being buried quickly in sediment that excluded oxygen, prevented access by scavengers, and retarded bacterial degradation; and (2) remaining in the nearly permanently frozen condition typical of Siberia's permafrost. However, our observations on Lyuba's internal condition permit us to go further. We now consider the implications of: tissue acidity; loss of connective tissue attaching muscles to bone, tooth roots to alveolar bone, and pleural parietal membranes to the inner surface of the thoracic wall; partial demineralization of bones and tooth dentin; and vivianite nodules inside bones and within some soft tissues.

Our hypothesis for lowered tissue pH is that Lyuba's body (with the possible exception of parts of her intestinal tract, which would normally have been neutral to basic) could have been colonized after death by lactic-acid-producing bacteria that occur naturally within some aquatic environments. Their activity would have elevated carbon dioxide concentration (lowering pH even on its own) and produced lactic acid (and bacteriocins; Klaenhammer, 1988), inhibiting colonization by bacteria more commonly associated with soft tissue decomposition (most of which require a basic environment; Fisher, 1995). This process could have retarded breakdown of soft tissues, permitting Lyuba's body to remain intact during the interval of time – possibly years long – during which Lyuba's death and burial site was gradually incorporated into permafrost. Lest this seem far-fetched, we have dealt with another case involving a zoo elephant buried in lacustrine clays (in northwestern Ohio, where low temperature was not a contributing factor to the degree it could have been in the arctic; Fisher, pers. obs.). When this animal was excavated 17 years later, its muscle tissue was strongly acidified but remained in morphologically unaltered condition – essentially "acid fixation" of proteins (Gersten et al., 1985). Lyuba's acidic condition persisted even past exhumation, and we propose that this is a large part of why she was not scavenged or subjected to advanced bacterial decay during her exposure throughout the summer of 2006. Only the severe hunger and lowered inhibition of feral dogs in Novyy Port provided incentive enough to overcome an instinctive aversion to the unfamiliar smell presented by Lyuba's carcass (which may also have moderated by May, 2007, and with handling by humans).

Lyuba's loss of connective tissue in particular anatomical contexts may also relate to lactic acid-producing bacteria in that Type 1 collagen, the major structural component of the tendinous attachment of muscles to bone and of periodontal ligament, is denatured by lactic acid (Dung et al., 1994), increasing susceptibility to degradation. We do not know what type of collagen attaches the parietal membrane to the inner wall of the thorax in elephants, but it may be Type 1 collagen as well, and Lyuba's lung collapse might have been consequent on loss of this attachment. The Ohio elephant above did not show comparable detachment of muscles from bone, or loss of periodontal ligament, but perhaps this process is very slow.

Type 1 collagen is also present in bones and tooth dentin, and in Lyuba's case, this must not all have been destroyed because we used it in our AMS age estimate and in isotope analyses of collagen carbon and nitrogen (Rountrey et al., in press). Perhaps Type 1 collagen in mineralized tissues survived longer than in other contexts because the mineral phase partially isolated it from lactic acid exposure. On the other hand, pitting of tooth enamel suggests some dissolution of the mineral phase itself (enamel has almost no organic content). By whatever mechanism, some demineralization of bone and tooth dentin occurred in Lyuba, and perhaps both the mineral phase and the collagen matrix were attacked.

Finally, the vivianite nodules located throughout Lyuba's body required sources of iron and phosphate. If Lyuba died in a place where vivianite was already part of the sedimentary environment, there would have been abundant external sources for these elements. Still, there are questions of how vivianite nodules formed inside Lyuba's body, how they relate to her postmortem chemistry, and what explains their concentration in the interior of long bones (Fig. 4) and especially around her cranium (Fig. 5).

Sourcing of phosphate may be the simplest to explain. With acidification of Lyuba's body by postmortem lactic acid production and partial demineralization of bones, abundant phosphate ions would have been available. Volumetrically, dentin and enamel would have been insignificant relative to bone, and yet, these are plausible sources for vivianite nodules within dental pulp cavities and adherent to external tooth surfaces. Iron also could have come from Lyuba's own body, most notably from blood. Indeed, it is plausible that the distribution of vivianite nodules in Lyuba's body is in part a reflection of the peripheral vasoconstriction that would have accompanied death by asphyxia, augmented by initiation of the "diving reflex" by facial exposure to cold, wet mud, coupled with obstructed breathing (Gooden, 1994). Interestingly, the vasoconstriction that reduces blood supply to skin and muscles over most of the body may not apply to the facial region (Johansen, 1964), and much of Lyuba's cranial vivianite is actually outside of her cranial bones (Fig. 5). Under this model, the lack of vivianite in Lyuba's heart has less to do with iron than with distance from significant sources of phosphate. As for vivianite nodules in long bone interiors, they may reflect proximity to phosphate source and iron stores in marrow (developing erythrocytes and ferritin-containing macrophages; Wulfhekel and Düllmann, 1999), neither of which would be affected by vasoconstriction.

8. Conclusions

The spectacular woolly mammoth calf known as Lyuba was recovered not at the site of exhumation (now unknown), but at a site of secondary deposition following transport by the Yuribei river, associated with ice-out flooding in June 2006. Some alteration of her body reflects exposure during the following summer. Had she not been recovered in May, 2007, she would probably have been retransported, reburied, or destroyed in ice-out flooding in June 2007.

Lyuba offers unique insights into the anatomy of juvenile woolly mammoths and aspects of their developmental history. Because her condition at death, manifested through abundant fat stores and access to milk, shows consistent indicators of excellent nutritional status, what we learn about her life history can probably be generalized to other members of her species. Her cause of death, asphyxia following reflexive inhalation of cold, wet mud, appears to have resulted from an unfortunate accident – not from any debilitating prior condition.

A discrete cervical fat deposit, probably composed of brown fat, is recognized and may have been critical for survival of a neonate calf born at the end of winter. A behavioral feature that might have been anticipated, but for which there was no prior evidence, is coprophagy by juvenile mammoths. As in extant elephants, this presumably inoculated the intestinal tract with a microbial community derived from the mother, but it may also be a precursor to adult coprophagy, for which Lyuba provides circumstantial evidence.

Despite her nearly pristine external appearance, Lyuba's internal state shows abundant evidence of postmortem alteration. Ironically, early postmortem colonization of her body by lactic-acid-producing bacteria may have been critical to retardation of decomposition prior to incorporation of her body in permafrost, but also to inhibition of scavenging and resistance to bacterial decay during the interval of post-exhumation exposure in the summer of 2006. Additional consequences of lactic acid production include denaturing of Type 1 collagen, partial demineralization of bones and some tooth tissues, and formation of vivianite nodules inside long bones and in her cranial region, presenting a syndrome of preservation features not recognized before. The events of Lyuba's life and the circumstances of her death offer new insights into the paleobiology and taphonomy of mammoths.

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References

Boeskorov, G.G., Tikhonov, A.N., Lazarev, P.A., 2007. A new find of a mammoth calf. Doklady Biological Sciences 417, 480–483.

Clementz, M.T., Fox-Dobbs, K., Wheatley, P.V., Koch, P.L., Doak, D.F., 2009. Revisiting old bones: coupled carbon isotope analysis of bioapatite and collagen as an ecological and palaeoecological tool. Geological Journal 44, 605–620.

Dung, S.-Z., Li, Y., Dunipace, A.J., Stookey, G.K., 1994. Degradation of insoluble bovine collagen and human dentine collagen pretreated *in vitro* with lactic acid, pH 4.0 and 5.5. Archives of Oral Biology 39, 901–905.

Edmonds, C., 1998. Drowning syndromes: the mechanism. SPUMS [South Pacific Underwater Medicine Society] Journal 28, 2–9.

Fisher, D.C., 1995. Experiments on subaqueous meat-caching. Current Research in the Pleistocene 12, 77–80.

Gersten, D.M., Zapolski, E.J., Ledley, R.S., 1985. Rapid mechanical *versus* conventional acid fixation in electrophoresis. Electrophoresis 6, 191–192.

Gill, C.O., 1983. Meat spoilage and evaluation of the potential storage life of fresh meat. Journal of Food Protection 46, 444–452.

Gooden, B.A., 1994. Mechanism of the human diving response. Integrative Physiological and Behavioral Science 29, 6–16.

Guthrie, R.D., 1990. Frozen Fauna of the Mammoth Steppe: The Story of Blue Babe. University of Chicago Press, Chicago.

Hallet, B., Walder, J.S., Stubbs, C.W., 1991. Weathering by segregation ice growth in microcracks at sustained subzero temperatures: verification from an experimental study using acoustic emissions. Permafrost and Periglacial Processes 2, 283–300.

Johansen, K., 1964. Regional distribution of circulating blood during submersion asphyxia in the duck. Acta Physiologica Scandinavica 62 (1–2), 1–9.

Klaenhammer, T.R., 1988. Bacteriocins of lactic acid bacteria. Biochimie 70, 337–349.

Kosintsev, P.A., Lapteva, E.G., Trofimova, S.S., Zanina, O.G., Tikhonov, A.N., van der Plicht, J., 2010. The intestinal contents of a baby woolly mammoth (*Mammuthus primigenius* Blumenbach, 1799) from the Yuribey River (Yamal Peninsula). Doklady Biological Sciences 432, 209–211 (original Russian text in Doklady Akademii Nauk 432, 556–558).

Lafontan, M., Berlan, M., 1993. Fat cell adrenergic receptors and the control of white and brown fat cell function. Journal of Lipid Research Volume 34, 1057–1091.

Leggett, K., 2004. Coprophagy and unusual thermoregulatory behavior in desert dwelling elephants of north-western Namibia. Pachyderm 36, 113–115.

Manning, P.G., Murphy, T.P., Prepas, E.E., 1991. Intensive formation of vivianite in the bottom sediments of mesotrophic Narrow Lake, Alberta. Canadian Mineralogist 29, 77–85.

Mariappa, D., 1986. Anatomy and Histology of the Indian Elephant. Indira Publishing House, Oak Park, Michigan.

Mueller, T., 2009. Ice baby. National Geographic Magazine, 30–53.

National Geographic, 2009. Waking the Baby Mammoth (documentary film).

Rountrey, A.N., 2009. Life Histories of Juvenile Woolly Mammoths from Siberia: Stable Isotope and Elemental Analyses of Tooth Dentin. Unpublished dissertation, University of Michigan.

Rountrey, A.N., Fisher, D.C., Tikhonov, A.N., Kosintsev, P.A., Lazarev, P.A., Boeskorov, G., Buigues, B. Early tooth development, gestation, and season of birth in mammoths. Quaternary International, in press.

Seilacher, A., 1971. Preservational history of ceratite shells. Palaeontology 14, 16–21.

Short, R.V., 1962. The peculiar lungs of the elephant. New Scientist 316, 570–572.

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.D., Altmann, J.G., Broekens, P., Bull, I.D., Gill, F.L., Jansen, B., Nieman, A.M., Gravendeel, B., 2011. Mycological evidence of coprophagy from the feces of an Alaskan Late Glacial mammoth. Quaternary Science Reviews 30 (17), 2289–2303.

Watson, M., 1873. Contributions to the anatomy of the Indian elephant: part III. The head. Journal of Anatomical Physiology 8 (Pt. 1), 85–94.

West, J.B., 2001. Snorkel breathing in the elephant explains the unique anatomy of its pleura. Respiration Physiology 126, 1–8.

Wulfhekel, U., Düllmann, J., 1999. Storage of iron in bone marrow plasma cells: ultrastructural characterization, mobilization, and diagnostic significance. Acta Haematologica 101, 7–15. www.arkive.org/african-elephant/loxodonta-africana/video-08d.html. "African elephants eating dung", video accessed 30.01.11.