Preservation of mandibular zinc in a beetle from the Eocene Kishenehn Formation of Montana, U.S.A.

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<th>Journal:</th>
<th>Canadian Journal of Earth Sciences</th>
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<td>cjes-2015-0157.R2</td>
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<td>Manuscript Type:</td>
<td>Article</td>
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<td>Date Submitted by the Author:</td>
<td>05-Jan-2016</td>
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<td>Complete List of Authors:</td>
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<td>Keyword:</td>
<td>fossil insect, taphonomy, zinc, Kishenehn Formation, Staphylinidae</td>
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Preservation of mandibular zinc in a beetle from the Eocene Kishenehn Formation of Montana, U.S.A.

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Abstract

Fossil insects of the 46 million-year-old Coal Creek Member of the Kishenehn Formation exhibit exceptional preservation as evidenced by the preservation of color and the blood-derived pigment heme in a blood-engorged mosquito. In the present study, analysis of a fossil rove beetle (Coleoptera: Staphylinidae) from the Kishenehn Formation, document preservation of zinc, a metal often used to harden the cutting surfaces of mandibles in extant insects, localized to the mandibles of the fossil insect. Scanning electron microscopy and energy-dispersive X-ray spectroscopy demonstrate that the carbonaceous bodies of preserved insects are physically homogeneous, composed primarily of carbon, and are distinct from the adjacent microbial mat within which the insects are thought to have been preserved. The microbial mat that covered the fossil insects is shown to consist of, in part, well-consolidated silicates. This thin layer, while completely transparent when wet, obscures the fossil when dry. The in situ preservation of components such as mandibular zinc and mosquito host blood-derived heme demonstrate that the carbonaceous bodies of Kishenehn Formation fossil insects contain some portion of their original contents. The thin layer of silicate-embedded mat may function to stabilize the fossil and its molecular components and may explain, in part, the exceptional preservation of the Kishenehn Formation fossils.

Key Words: Mandibular zinc, Kishenehn Formation, Staphylinidae, Taphonomy

Introduction
The middle Eocene Kishenehn Formation in Montana, U.S.A., is a large lacustrine deposit exposed along the Flathead River in northwestern Montana. Its insect fossils exhibit exceptional preservation as evidenced by the apparent preservation of color and, in a fossil of a blood-engorged mosquito, the pigment heme, presumably derived from host blood (Briggs 2013; Greenwalt and Labandeira 2013; Greenwalt et al. 2013). The geological record is replete with complex organic molecules, mostly of eukaryotic origin. Although the preservation of biomolecular components, in Cenozoic insects has been reported on a number of occasions the numbers of such compounds is limited (Stankiewicz et al. 1998; Briggs and Summons 2014). Perhaps the best example is the preservation of chitin, the most abundant molecule in insect cuticles, in beetle elytra from the 25 Ma Lagerstätte Enspel in Germany (Stankiewicz et al. 1997). The presence of the pigment melanin, and/or its degradation products, has been reported in Jurassic cephalopod ink sacs and Eocene fish eyes, bird feathers, mammal hair and frog skin (Lindgren et al. 2012; Colleary et al. 2015).

The taphonomic processes by which insects are fossilized in sedimentary rocks can involve replication, by either petrifaction or permineralization, with partial or complete replacement by various types of minerals. Insect fossils composed of CaCO₃, Ca₅(PO₄)₃, FeO(OH), FeS₂, SrSO₄, and SiO₄ have been described (Martinez-Delclòs et al. 2004; Grimaldi and Engel 2005). However, Grimaldi and Engel (2005, p. 43) stated that “Compressions and impressions are the most extensive types of insect fossils”. Insect fossils from the Florissant Formation (34 Ma), one of the great insect Lagerstätten of the American West, are compression fossils that consist of “a residue of organic carbon” (Meyer 2003, p. 37; see also Harding and Chant 2000). Carbonaceous
fossil insects have also been reported from other sites (Wang et al. 2008). Similarly, fossil insects in the oil shale of the more recently described Coal Creek Member of the Kishenehn Formation, dated to the middle Eocene (approximately 46 Ma), have been shown to consist of a body of physically homogeneous carbonaceous material (Greenwalt et al. 2015).

Fossil insects in the oil shale of the Coal Creek Member of the Kishenehn Formation were preserved via unique taphonomic processes—the fossils are found in the remnants of diatomaceous and non-diatomaceous mats, respectively (Harding and Chant 2000; O'Brien et al. 2002, 2008; Greenwalt et al. 2015). The relatively thick layers of carbonaceous material in the Kishenehn Formation insects is presumably derived directly from the original organic components of the insects. The preservation of original organic cuticular material in arthropods, albeit polymerized, has been confirmed experimentally by Gupta et al (2006). Fossil insects of the Kishenehn Formation provide a potentially fertile environment in which to search for biomolecules preserved in situ.

Here we review several taphonomically notable insect fossils from the Kishenehn Formation, including a rove beetle that documents the first report in the fossil record of a beetle incorporating zinc into its mandibles.

**Materials and methods**

All fossil specimens are housed at the Department of Paleobiology, National Museum of Natural History (USNM), Smithsonian Institution in Washington, D.C., U.S.A.
Specimens of extant staphylinids are housed at the Smithsonian Museum Support Center, Suitland, Maryland, U.S.A. Kishenehn Formation fossils were immersed in 95% ethanol for examination and photographed with an Olympus SZX12 microscope equipped with a Q-Color5 Olympus camera. Image-Pro Plus 7.0 software was used to capture and record the images and measurements. Small portions of thin layers of silicate, both unconsolidated and consolidated, covering the fossil insect specimens were removed through use of a Faber-Castell eraser pencil (# 185698). Elemental analysis via standardless, semi-quantitative, energy-dispersive X-ray spectroscopy (EDS) and imaging were performed at the NMNH Department of Mineral Sciences. These analyses were performed on uncoated samples using an FEI NOVA NanoSEM 600 FEG Variable Pressure Analytical Scanning Electron Microscope (SEM) outfitted with a ThermoNoran silicon drift detector energy dispersive spectrometer. EDS spectra were collected at 15kV and 1–2 nanoamp for 2 minutes scanning areas no smaller than 10 by 12 µm. Quantification of these spectra have an analytical uncertainty of approximately 5%. Compositions presented are the average of 3 or more analyses. Some spectra, particularly on carbonaceous material, were collected at 7kV to reduce the excitation volume in order to avoid contribution from underlying material. Hyperspectral x-ray maps were collected for the fossil insects at 15kV, 1–2 nanoamp with a pixel density of 1024 by 882 with a frame time time of 100 seconds and total acquisition time of 120 minutes. The hyperspectral image of the extant insect was collected under the same conditions, but with an acquisition time of 60 minutes.
Results

Fossil insects of the Coal Creek Member of the Kishenehn Formation consist of a visually homogeneous carbonaceous material that, when dried, often cracks, as exemplified by the fossil (USNM 559050) of a mosquito (Diptera: Culicidae) depicted in Figure 1a, b. There is no evidence of the presence of diatomaceous frustules or bacteria, even at high magnification (Fig. 1b). The majority of the abdomen of the mosquito specimen itself is carbon with an estimated wt% of 70%; increased amounts of sulfur (approximately 1.6 wt%, vs. 0.4 wt% in the matrix) were also present in the carbonaceous body of the fossil insect although the nature of this element (elemental vs. molecular) is unknown (Fig. 1c). The shale and fossils are covered by a thin layer of well-consolidated (cemented) potassium aluminum silicate (Fig. 1a, d), which is opaque when dry and makes visualization of the fossil itself difficult. As a further example of this phenomenon, see the fossil (USNM 595151) of a tumbling flower beetle (Coleoptera: Mordellidae) shown in Figure 2. When wetted, however, the silicate layer becomes transparent and the morphological details of the fossil below become more readily observable (Fig. 2b).

In some cases, as in a specimen (USNM 595152) of a fossil parasitic wasp (Hymenoptera: Ichneumonidae), an unconsolidated layer of silicate obscures the fossils, although this material is easily removed with an eraser pencil (Fig. 3a, b). Elemental analysis of the unconsolidated and consolidated silicates reveal similar spectra. However, when the two spectra are overlaid one upon the other, the consolidated silicate material is shown (Fig. 3c) to contain increased levels of calcium (approximately
3 wt% vs. 1 wt%), carbon (approximately 6 wt% vs. 3 wt%), and oxygen (approximately 52 wt% vs. 49 wt%). The specific increases in these three elements in the cemented silicate layer and the very low level of calcium in the unconsolidated material suggest that the differences between the consolidated and unconsolidated silicates are due to the presence of CaCO$_3$ or calcite in the consolidated silicates. The presence of calcite cement, and its mediation of the lithification of the silicates during diagenesis, would explain the consolidated nature of the silicates that overlay the fossils. The presence of unconsolidated silicates is characteristic of fossils that are found on weathered shale surfaces; fossils that are found only after splitting shale to reveal fresh surfaces lack unconsolidated material. In USNM 595152, well consolidated silicates underlie the unconsolidated material.

When examined in cross-section, a fossil (USNM 595154: Fig. 4a) of a water boatman (Hemiptera: Corixidae) revealed a 4–6 µm-thick and visually homogeneous carbonaceous body, composed of approximately 97 wt% carbon, preserved between layers of silicate minerals (Fig. 4b, c). The carbonaceous fossil body does not appear to retain any remnants of original morphology or structure. In cross-section, the upper silicate layer is composed of several distinct undulating layers of elongated silicate grains interspersed with low levels of both carbon and calcium (Fig. 4c,d). This upper layer averages about 2 µm in thickness, but varies from 0.65 µm to over 4 µm. As this upper layer was originally laid down, it covered, and was covered by, other material (e.g., a large crystal of dolomitic calcite [Fig. 4c]).
The final fossil insect documented here from the Kishenehn Formation is a well-preserved example (USNM 595153; Fig. 5) of a rove beetle (Coleoptera: Staphylinidae), identified to the subfamily Paederinae and tribe Pinophilini. This specimen was processed to remove small portions of the consolidated silicate layer that covered the insect’s mandibles (Fig. 5a–c). EDS analysis of several different areas of the exposed fossil mandible (Figs. 5d–f) provides an approximate wt% value for carbon of 39. Zinc is present at a concentration of approximately 13 wt% and is specifically localized to the apex and molar areas of the mandibles. The basal portion of the mandible and adjacent exposed portions of the head and antennae are negative for the presence of zinc. Sulfur is co-localized with zinc and is present at approximately 7 wt%. Atom% values for zinc and sulfur are 4.0 and 4.3, respectively. The approximately 1:1 ratio between the two elements indicates that the zinc may be present in the fossil as ZnS. Sodium is also co-localized with the zinc and sulfur, but was present at a low level (<1 wt%).

For comparison with the fossil rove beetle, a specimen (USNMENT00990243) of the extant staphylinid *Paederus riparius* Linnaeus was examined in an attempt to establish whether extant representatives of the same subfamily as the fossil incorporate zinc into the cutting edges of their mandibles (Fig. 6). Zinc, at about 3 wt%, is present at the tip and cutting surfaces of the mandibles and the edges of the bicuspid molar area. Chlorine, at about 1.4 wt%, is co-localized with the zinc. Given the high solubility of ZnCl₂ in water, it is unlikely that these two elements are present as the chloride salt of zinc. These data are the first to document the presence of mandibular zinc in both an extant species and an unidentified fossil species of the Family Staphylinidae.
Discussion

Given the preservation of the porphyrin molecule heme in the abdomen of a blood-engorged mosquito from the Kishenehn Formation (Greenwalt et al. 2013), the preservation of other original biomolecular components in other insect fossils from the same unit was anticipated. Knowing that many insects strengthen the grinding and cutting edges of their mandibles with the metal zinc (Hillerton and Vincent 1982; Quicke et al. 1998), we originally intended to determine whether or not mandibular zinc was preserved in the Kishenehn Formation fossils. Although examination of the USNM’s collection of Kishenehn Formation fossil insects yielded numerous examples of well-preserved mandibles, removal of the silicate layer covering these fossils proved to be problematic. The more three-dimensional character of the highly sclerotized head and the proximity of the relatively small mandibles to adjacent structures of the head increased the chances of irreversibly damaging the fossils. Of several fossil beetle specimens that were “cleaned”, only the rove beetle (USNM 595153: Fig. 5) reported herein was successfully processed to a point where the consolidated silicate layer was removed from portions of the mandibles and head. Our subsequent analysis of the specimen documented the presence of zinc at the concentration of approximately 13 wt%, a high level assumed to be due to the concentration of the metal as other more degradable materials were lost during the fossilization process. Zinc is concentrated in the apical and cutting/gripping edges of the mandibles, whereas basal portions of the mandibles were negative for the metal (Fig. 5). The zinc X-ray-localization map very
clearly delineates the bicuspid molar portion of the specimen’s left mandible. The mandibular zinc appears to exist as ZnS, possibly sphalerite, a water-insoluble mineral that commonly occurs in nature in crystalline form with low levels of iron. Iron was detected in the specimen, albeit at very low levels, but did not co-localize with the zinc. Why the zinc did not diffuse further into other portions of the head and the adjacent matrix during diagenesis is unknown, but presumably involved stabilization of the metal as ZnS.

Incorporation of metals, specifically zinc and manganese, in insect mandibles was first reported by Hillerton et al. (1982). Since then, studies of numerous orders of insects, including Coleoptera, Blattodea (Isoptera), Orthoptera, Phasmatodea, Hymenoptera, and Lepidoptera, have confirmed the routine use of structure-specific incorporation of metals; representatives of other orders (e.g., Blattodea and Dermaptera) have been reported to lack such metal incorporation, although these studies were limited in scope (Hillerton et al. 1984; Fontaine et al. 1991; Quick et al. 1998, 2004; Morgan et al. 2003; Schofield et al. 2002, 2003; Schofield 2005; Cribb et al. 2008a, b; Stewart et al. 2011; Polidori et al. 2013). Incorporation of zinc in mandibles has been demonstrated to be specific to the cutting and grinding edges/surfaces of insects and has been correlated to the hardness of food sources in both beetles and termites. Incorporation of zinc has been shown to significantly increase the hardness of the mandible in both ants and termites; however, direct evidence for manganese-mediated hardening in both groups is lacking (Hillerton et al. 1984; Schofield et al. 2002; Morgan et al. 2003; Cribb et al. 2008a, b). On the other hand, there is apparently no
relationship between the incorporation of metal in the mandibles and life style in wasps of the families Cynipidae and Figitidae (Quick et al. 1998; Polidori et al. 2013).

Most rove beetles live in decaying leaf litter and are predators of small insects and other arthropods; they are not thought of as having a grinding type of mandible (Thayer 2005). Given that the majority of research done on metal incorporation into beetle mandibles has concentrated on taxa that consume seeds or woody material, examination of staphylinid mandibles for zinc and other metals has not been previously reported. The presence of zinc, specifically localized within the mandibles of the fossil paederine examined in this study, prompted examination of extant specimens of the staphylinid subfamily Paederinae. Of the two species examined, *Lathrobium lineiformis* and *Paederus riparius*, the latter was positive for Zn (Fig. 6), thereby confirming the existence of zinc-mediated hardening of mandibles in at least some extant representatives of this very large subfamily of beetles.

The mechanism by which zinc is localized and bound to the cutting and grinding surfaces of insect mandibles is unknown (Schofield 2005). Zinc may account for 10% or more of the dry weight of some insect mandibles and stoichiometric considerations eliminate reliance on specific metal-binding proteins. However, in the jaws of the polychaete *Nereis*, a preponderance of the Zn-binding amino acids histidine, glutamic acid, and aspartic acid (nearly 40% of total amino acids) has led to the proposal that zinc is localized via Zn(His)$_3$Cl-like units that crosslink the proteinaceous matrix (Quicke et al. 1998; Lichtenegger et al. 2003). Mandibular zinc is associated with chlorine and in termites, the ratio of Zn:Cl has been determined to be 9:2 (Schofield 2005; Cribb et al.
2008a, b). Other studies have demonstrated that zinc localization is not mediated by biomineralization, because zinc does not exhibit crystalline mineral formation (Cribb et al. 2008b). Although Cribb et al. (2008b, p. 9) stated that “both halogen and metal appear integral to the biochemistry and mechanical effects”, the exact nature of the metal-halogen biomaterial involved remains to be elucidated.

In the present study, the carbonaceous bodies of the insects varied in wt% carbon from approximately 35% in the mandibles of the fossil staphylinid to about 97% in the corixid. The latter value may be more accurate, because the electron beam was applied to a newly split cross-section; contamination from residual surface silicates was not possible in this case. In addition, during analysis of “cleaned” surfaces, it was common for the electron beam to penetrate the thin carbonaceous layer of the specimen into underlying material, thereby decreasing the wt% values of the actual components of the carbonaceous layer. Wang et al. (2008) reported carbon wt% values from 7% to 47% in fossil insects from the Middle Jurassic Daohugou Formation of Inner Mongolia. The exact nature of the molecular form(s) of the carbon present in the Kishenehn Formation fossils is unknown. We assume that the carbon is original to the insect—the presence of heme and mandibular zinc in Kishenehn Formation insects supports this view. The carbon may be in the form of biopolymerized degraded derivatives of original carbon-based components (Briggs 1999). Proteins may or may not have survived, although the presence of protein moieties has been reported in Oligocene beetles (Stankiewicz et al. 1997). Gupta et al. (2006) provided evidence for the in situ polymerization of cuticular lipids in fossil arthropods.
The carbonaceous bodies of the Kishenehn Formation fossil insects are covered by a thin, calcite-cemented layer of silicates approximately 2 µm in thickness. It is this layer that renders the fossils nearly invisible when dry. The opaque nature of the dry silicate layer is a defining characteristic of the insect fossils of the Kishenehn Formation and may well explain why they were not discovered earlier in the 20th Century. This layer must be removed prior to analysis of the fossil by secondary-ion mass spectrometry and energy-dispersive X-ray spectroscopy. Unfortunately, removal of this strongly adherent layer is difficult and fraught with the potential to damage and even destroy the fossil. As a result, a broader and more in-depth study of mandibular metals in fossil insects, that could potentially shed light on the origin and evolution of metal-based hardening of insect cuticular structures, is not currently feasible.

Fossil insects of the Kishenehn Formation include well-preserved, but often very small, insects such as fairy wasps (Hymenoptera: Mymaridae) and mosquitoes (Diptera: Culicidae), that are rarely collected at other compression fossil Lagerstätten in North America (Huber and Greenwalt 2011; Harbach and Greenwalt 2012). The taphonomic basis for this size bias is poorly understood. Although fossil insects of the Kishenehn Formation are thought to be preserved by microbial mats, their carbonaceous bodies are not surrounded by diatomaceous frustules, and they show no evidence of mineralization as reported in other fossil biofilms and/or bacterial mats (Peñalver et al. 1996; Harding and Chant 2000; Wolfe and Edlund 2005; O’Brien et al. 2002, 2008; Wang et al. 2008). The silicate-rich layer appears to have formed as part of a fossilized microbial mat that played an integral role in both the observed size bias and the preservation of original components.
Greenwalt et al. (2015) showed that the fossiliferous Kishenehn Formation shale consists of very thin varves (100–500 µm) composed of a basal thick layer of heterogeneous, often cuboidal sedimentary particles (quartz, feldspar, calcite, etc.) and an overlying thin carbonaceous layer derived from a microbial mat. The mat consists of undulating layers of carbonaceous material within which are interspersed small sedimentary particles, many of which are rectangular in cross-section, laid down as layers parallel to and/or within the undulating carbonaceous layers. The sedimentary clasts are assumed to have accumulated at the surface of the mat, either at the water’s surface or at the bottom of the lake. In the corixid fossil examined herein (Fig. 4), the mat layer has been displaced by the carbonaceous body of the fossil itself. We speculate that the organization of this layer of silicates into undulating layers of long thin silicate particles may provide the basis for the splitting of the shale. The shale never splits within the thick basal layer of cuboidal sedimentary particles and the Kishenehn fossils are never present as part/counterpart pairs that would indicate splitting through the insect fossil itself.

The silicate layer certainly serves to protect the fossils in the present time although, when exposed to air and/or water for long intervals, the calcite cement gradually dissolves. Shale that was split and then stored for five years has not developed a surface residue of whitish unconsolidated silicate. In a taphonomic sense, the questions are: 1) when did the cemented silicate layer form and 2) did it have a role in the preservation of the fossils over deep time? Formation of calcite cement is thought to involve the flow of large volumes of groundwater over small grains of sediment and the chemical precipitation of calcium carbonate to form new crystalline material inside
sediment pores. Depending on the proximity of the source and the speed of transportation, the cementation process may require a prolonged interval of time (Walderhaug and Bjørkum 1998). A source of calcium and carbonate ions appears to have been available in fossil Lake Kishenehn, based on the observation that a high percentage of varves in the fossiliferous shale of the Coal Creek Member contain layers of pure calcium carbonate thought to have formed as the result of spring warming-induced precipitation of carbonate-saturated lake water (Greenwalt et al. 2015). Unfortunately, it is not currently possible to tell if the cementation process occurred at a taphonomic stage that would have affected the preservation potential of the insects directly.

The geochemistry of the Kishenehn Formation shales and the insect fossils found therein is distinct from those of the Florissant and Green River formations. However, fossil insects from the Miocene Rubielos de Mora in northeast Spain are very similar to those of the Kishenehn Formation in both their appearance and the observation that they are essentially invisible until wetted (Peñalver 2002; Peñalver and Engel 2006). The dehydrated and cracked carbonaceous bacterial biofilm that defines portions of the fossil tadpoles from the adjacent Miocene Libros Lagerstätte in Spain superficially resembles the Kishenehn Formation fossil insect bodies, but the former are much thicker and are composed of what were interpreted as readily visualized, fossilized bacteria approximately 1 μm in diameter (McNamara et al. 2010); such structures are not present in the Kishenehn shale fossil insects. Comparative analyses of the shales and fossils from the Florissant, Green River, Rubielos de Mora, and other
deposits will further refine our understanding of the taphonomic processes involved in the preservation of insects in lacustrine deposits.

Acknowledgements

We thank the organizers of the 2014 GSA session Eocene Northern North America: Biotic Change and Environmental Context for organizing this Special Issue and the two anonymous reviewers whose comments significantly improved the manuscript. At the National Museum of Natural History, we also thank Conrad Labandeira (Dept. of Paleobiology) for his sponsorship and administrative support, Finnegan Marsh (Dept. of Paleobiology) for his ongoing assistance, and Floyd Shockley (Dept. of Entomology, USNM) for access to the Museum’s collection of staphylinids. This is contribution number 286 of the Evolution of Terrestrial Ecosystems Consortium of the National Museum of Natural History in Washington, D.C.

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Hemoglobin-derived porphyrins preserved in a Middle Eocene blood-engorged 

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Figure captions

**Fig. 1.** Scanning electron micrographs and elemental analyses of the abdomen of a fossilized, blood-engorged mosquito (Diptera: Culicidae), USNM 559050, from the middle Eocene Kishenehn Formation, Montana, U.S.A. For an image of the entire specimen see (Greenwalt et al., 2013, Fig. 1). (a, b) Close up micrographs of the surface of the fossil abdomen; scale bars = 100 µm (a) and 10 µm (b). (c, d) Graphs depicting energy-dispersive X-ray spectra of two surfaces in the specimen: (c) the carbonaceous surface (arrow in “a”) of the abdomen, exposed by removal of the overlying layer of consolidated silicates, and (d) remnants of the original, overlying silicate layer (arrowhead in “a”). The former (i.e., carbonaceous layer) is dominated by a large peak for carbon, whereas the latter (i.e., silicate layer) is dominated by three peaks (from left-to-right) for oxygen, aluminum, and silicon. keV = kiloelectron-volts.

**Fig. 2.** Photographs of a fossil tumbling flower beetle (Coleoptera: Mordellidae), USNM 595151, from the middle Eocene Kishenehn Formation, Montana, U.S.A. Many insect fossils from this deposit are obscured by a thin layer of well-consolidated potassium aluminum silicate that renders the underlying fossils barely visible (a) when dry, but they become more readily visible (b) after immersion in 95 % ethanol. Images at same magnification; scale bar = 0.5 mm.

**Fig. 3.** Micrographs and elemental analysis of an intact fossil wasp (Hymenoptera: Ichneumonidae), USNM 595152, from the middle Eocene Kishenehn Formation, Montana, U.S.A., before and after physical removal (“cleaning”) of unconsolidated
silicate layer. (a) The original fossil, prior to “cleaning”; scale bar = 2 mm. Note how only some portions of the specimen are visible through the overlying, thin silicate layer. Arrowhead denotes area analyzed by energy-dispersive X-ray spectroscopy (EDS); see graph in “c”. (b). The same fossil, after “cleaning”, and at same scale as previous image. (c). Graph depicting elemental analyses of fossil surfaces, at point denoted by arrowhead in “a”. The spectrum of the opaque unconsolidated silicate layer is overlaid by the spectrum of the transparent consolidated silicate layer. Increased peak areas for carbon (C) and calcium (Ca) are indicated by arrows at the far left and far right, respectively. Spectra were magnified in order to emphasize smaller peaks and, as a result, the tip of the oxygen peak is not shown (The three largest peaks, from left to right represent oxygen, aluminum and silicon, respectively.). keV = kiloelectron-volts.

**Fig. 4.** Structural and elemental analyses of the fossil of a water boatman (Hemiptera: Corixidae), USNM 595154, from the middle Eocene Kishenehn Formation, Montana, U.S.A. (a) Light micrograph of entire specimen, in dorsal aspect; scale bar = 1.0 mm. Dotted white line denotes location of cross-section shown in next image. (b) Scanning electron micrograph of a thin section cut transversely across specimen (along dotted line in previous image); scale bar = 10 µm. From left-to-right, arrows denote a calcite crystal, the carbonaceous body of the insect, the cemented silicate layer, and the epoxy used to stabilize the specimen for thin sectioning. (c) X-ray image localization of carbon (yellow), silicon (red) and calcium (green) in a higher magnification image of the left one-third of the image in “b”. (d) Close up SEM image of the parallel and thin layers of silicates in the cemented layer at the far left of the image in “b”.
Fig. 5. Localization of zinc in the mandibles of a fossil rove beetle (Coleoptera: Staphylinidae: Paederinae), USNM 595153, from the middle Eocene Kishenehn Formation, Montana, U.S.A. (a) Light micrograph of entire specimen, in dorsal aspect; scale bar = 2.0 mm. (b) Light micrograph and close up view of mandibles; scale bar = 0.2 mm. (c) Scanning electron micrograph of mandibles, at approximately the same magnification as previous image. (d–f) X-ray images of mandibles, at lower magnification than previous two images, showing localization of carbon (d), zinc (e), and sulfur (e).

Fig. 6. Localization of zinc in the mandible of an extant rove beetle *Paederius riparius* (Coleoptera: Staphylinidae: Paederinae), USNMENT00990243 (Westphalia, Germany). (a) Scanning electron micrograph of mandibles in dorsal aspect. (b) X-ray image of mandibles in dorsal aspect, showing localization of carbon (yellow) and zinc (red). Scale bar for both images = 0.1 mm.
Fig. 1. Scanning electron micrographs and elemental analyses of the abdomen of a fossilized, blood-engorged mosquito (Diptera: Culicidae), USNM 559050, from the middle Eocene Kishenehn Formation, Montana, U.S.A. For an image of the entire specimen see (Greenwalt et al., 2013, Fig. 1). (a, b) Close up micrographs of the surface of the fossil abdomen; scale bars = 100 µm (a) and 10 µm (b). (c, d) Graphs depicting energy-dispersive X-ray spectra of two surfaces in the specimen: (c) the carbonaceous surface (arrow in "a") of the abdomen, exposed by removal of the overlying layer of consolidated silicates, and (d) remnants of the original, overlying silicate layer (arrowhead in "a"). The former (i.e., carbonaceous layer) is dominated by a large peak for carbon, whereas the latter (i.e., silicate layer) is dominated by three peaks (from left-to-right) for oxygen, aluminum, and silicon. keV = kiloelectron-volts.
Fig. 2. Photographs of a fossil tumbling flower beetle (Coleoptera: Mordellidae), USNM 595151, from the middle Eocene Kishenehn Formation, Montana, U.S.A. Many insect fossils from this deposit are obscured by a thin layer of well-consolidated potassium aluminum silicate that renders the underlying fossils barely visible (a) when dry, but they become more readily visible (b) after immersion in 95 % ethanol. Images at same magnification; scale bar = 0.5 mm.
Fig. 3. Micrographs and elemental analysis of an intact fossil wasp (Hymenoptera: Ichneumonidae), USNM 595152, from the middle Eocene Kishenehn Formation, Montana, U.S.A., before and after physical removal ("cleaning") of unconsolidated silicate layer. (a) The original fossil, prior to "cleaning"; scale bar = 2 mm. Note how only some portions of the specimen are visible through the overlying, thin silicate layer. Arrowhead denotes area analyzed by energy-dispersive X-ray spectroscopy (EDS); see graph in "c". (b). The same fossil, after "cleaning", and at same scale as previous image. (c). Graph depicting elemental analyses of fossil surfaces, at point denoted by arrowhead in "a". The spectrum of the opaque unconsolidated silicate layer is overlaid by the spectrum of the transparent consolidated silicate layer. Increased peak areas for carbon (C) and calcium (Ca) are indicated by arrows at the far left and far right, respectively. Spectra were magnified in order to emphasize smaller peaks and, as a result, the tip of the oxygen peak is not shown (The three largest peaks, from left to right represent oxygen, aluminum and silicon, respectively.). keV = kiloelectron-volts.

863x635mm (72 x 72 DPI)
Fig. 4. Structural and elemental analyses of the fossil of a water boatman (Hemiptera: Corixidae), USNM 595154, from the middle Eocene Kishenehn Formation, Montana, U.S.A. (a) Light micrograph of entire specimen, in dorsal aspect; scale bar = 1.0 mm. Dotted white line denotes location of cross-section shown in next image. (b) Scanning electron micrograph of a thin section cut transversely across specimen (along dotted line in previous image); scale bar = 10 µm. From left-to-right, arrows denote a calcite crystal, the carbonaceous body of the insect, the cemented silicate layer, and the epoxy used to stabilize the specimen for thin sectioning. (c) X-ray image localization of carbon (yellow), silicon (red) and calcium (green) in a higher magnification image of the left one-third of the image in "b". (d) Close up SEM image of the parallel and thin layers of silicates in the cemented layer at the far left of the image in "b".
Fig. 5. Localization of zinc in the mandibles of a fossil rove beetle (Coleoptera: Staphylinidae: Paederinae), USNM 595153, from the middle Eocene Kishenehn Formation, Montana, U.S.A. (a) Light micrograph of entire specimen, in dorsal aspect; scale bar = 2.0 mm. (b) Light micrograph and close up view of mandibles; scale bar = 0.2 mm. (c) Scanning electron micrograph of mandibles, at approximately the same magnification as previous image. (d–f) X-ray images of mandibles, at lower magnification than previous two images, showing localization of carbon (d), zinc (e), and sulfur (e).
Fig. 6. Localization of zinc in the mandible of an extant rove beetle Paederius riparius (Coleoptera: Staphylinidae: Paederinae), USNMENT00990243 (Westphalia, Germany). (a) Scanning electron micrograph of mandibles in dorsal aspect. (b) X-ray image of mandibles in dorsal aspect, showing localization of carbon (yellow) and zinc (red). Scale bar for both images = 0.1 mm.