Current Biology

Report

New Canadian amber deposit fills gap in fossil record near end-Cretaceous mass extinction

Highlights

- Big Muddy amber is the most diverse amber fauna close to the K-Pg mass extinction
- The deposit was produced in an ancient subtropical swamp near remnants of a marine seaway
- Two partial crown ants are preserved, filling a 16-million-year gap in the ant fossil record
- The assemblage suggests a faunal turnover among insects prior to the K-Pg mass extinction

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In brief

Loewen et al. describe the most diverse amber deposit near the end-Cretaceous mass extinction. This newly discovered deposit, formed 1 million years before the extinction event, supports a faunal turnover among insects prior to the impact. Common Cretaceous insect families and genera appear to be replaced by modern taxa for lineages such as ants.

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Report

New Canadian amber deposit fills gap in fossil record near end-Cretaceous mass extinction

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SUMMARY

Amber preserves an exceptional record of tiny, soft-bodied organisms and chemical environmental signatures, elucidating the evolution of arthropod lineages and the diversity, ecology, and biogeochemistry of ancient ecosystems. However, globally, fossiliferous amber deposits are rare in the latest Cretaceous and surrounding the Cretaceous-Paleogene (K-Pg) mass extinction.[1–5] This faunal gap limits our understanding of arthropod diversity and survival across the extinction boundary.[2,6] Contrasting hypotheses propose that arthropods were either relatively unaffected by the K-Pg extinction or experienced a steady decline in diversity before the extinction event followed by rapid diversification in the Cenozoic.[2,6] These hypotheses are primarily based on arthropod feeding traces on fossil leaves and time-calibrated molecular phylogenies, not direct observation of the fossil record.[2,7] Here, we report a diverse amber assemblage from the Late Cretaceous (67.04 ± 0.16 Ma) of the Big Muddy Badlands, Canada. The new deposit fills a critical 16-million-year gap in the arthropod fossil record spanning the K-Pg mass extinction. Seven arthropod orders and at least 11 insect families have been recovered, making the Big Muddy amber deposit the most diverse arthropod assemblage near the K-Pg extinction. Amber chemistry and stable isotopes suggest the amber was produced by coniferous (Cupressaceae) trees in a subtropical swamp near remnants of the Western Interior Seaway. The unexpected abundance of ants from extant families and the virtual absence of arthropods from common, exclusively Cretaceous families suggests that Big Muddy amber may represent a yet unsampled Late Cretaceous environment and provides evidence of a faunal transition before the end of the Cretaceous.

RESULTS AND DISCUSSION

Studied material

The first 47 arthropod inclusions from Big Muddy amber fill a critical gap in the arthropod fossil record and provide direct observational data of the composition of arthropod communities just 1 million years before the K-Pg mass extinction. Amber was collected from the Upper Cretaceous Battle Formation within the Big Muddy Valley in southwestern Saskatchewan, Canada (Figure 1B). This organic-rich laminated shale unit (Figure 1C) was deposited after a recession of the Western Interior Seaway (WIS) approximately 70 Ma (see site geology in STAR Methods).[8,9] Bentonite within the upper portion of the Battle Formation has been dated using high-precision radiometric analyses to 66.936 ± 0.047 Ma,[10] tightly constraining the age of Big Muddy amber to ∼67.20–66.88 Ma.[10]

Diversity and biases

The arthropod fauna in Big Muddy amber is dominated by Hymenoptera and Diptera (56% of all inclusions), as is common among amber deposits worldwide, with a significant number of Hemiptera (13%) (Figures 2 and 3; see amber inclusions in

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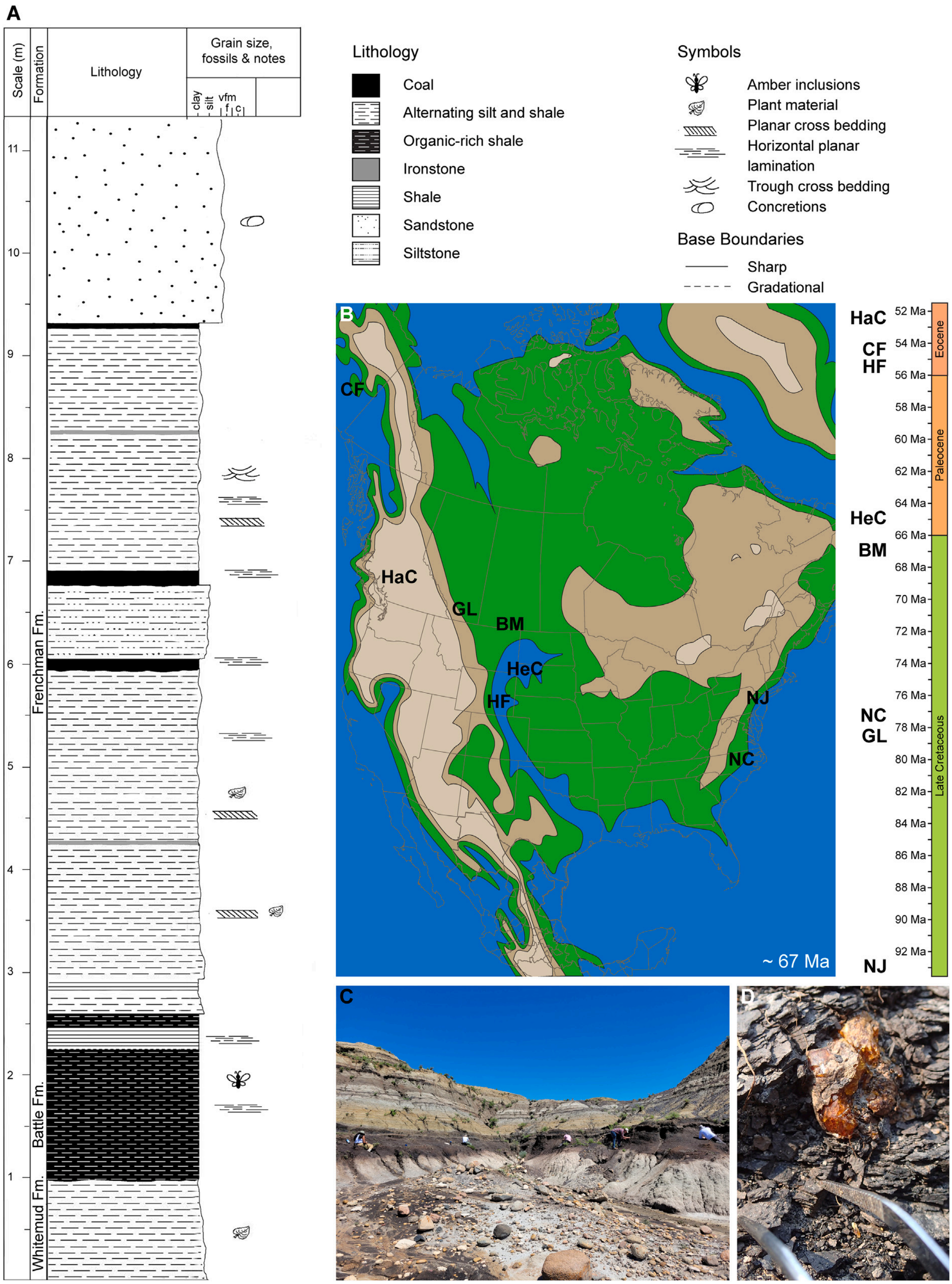

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STAR Methods; Table S1). Small-bodied representatives from these orders dominate the assemblage, likely due to their high abundance in the subtropical swamp ecosystem and because their size and behaviors increased their likelihood of entrapment.[11–13] The small size and friability of Big Muddy amber may further bias the size and completeness of inclusions toward smaller-bodied arthropods and fragmentary remains[13,14]; the majority of pieces are 5 mm or less in diameter. However, one of the ants preserved in Big Muddy amber has a total body length in excess of 3 mm, indicating the potential to preserve larger-bodied insects within the expected range for Cretaceous amber deposits, which predominately trap tiny arthropods less than 5 mm long (see sample collection and processing in STAR Methods).[11]

For a relatively small deposit, Big Muddy amber contains higher ordinal diversity and evenness than Cretaceous deposits of a similar size. For instance, in Tilin amber (~72.1 Ma, Kabaw Formation, Myanmar), Hymenoptera and Diptera account for 80% of the 34 insect inclusions,[5] while in amber from the Hell Creek Formation of South Dakota (~65.76 Ma; Figure 1B), dipterans account for all but one of the 22 documented inclusions (95% of all inclusions).[4,15] As more samples are recovered from all these deposits, including Big Muddy, it will be noteworthy to see if the assemblage compositions change and whether these initial trends are representative of the overall deposit assemblages or the result of sampling bias. The ordinal fossil diversity and richness of the initial Big Muddy amber assemblage also differs significantly from Grassy Lake amber (~79–78 Ma), a large and well-studied Late Cretaceous deposit located nearby in Alberta (Figure 1B).[16–18] In a random sample of Grassy Lake amber, hemipterans accounted for 31% of 765 arthropod inclusions, whereas Diptera and Hymenoptera accounted for 33%.[18,19] The large number of hemipterans in this sample was influenced by several pieces of amber, ranging in size from less than 0.1 g to 1 g, containing monospecific aphid accumulations[18]—a phenomenon not yet observed in Big Muddy amber. In combination with the paleoenvironmental characterization of the amber (see environmental reconstruction), this suggests that Big Muddy amber may be sampling a different environment and arthropod community than other Cretaceous deposits, even those that are in relatively close spatial and temporal proximity such as marginal marine Grassy Lake amber and South Dakota amber.

Big Muddy amber is one of few deposits close to the end-Cretaceous mass extinction event (Figure 1B), making it an important reference for the time interval. Comparisons between this new deposit and discoveries from other amber deposits close to the K-Pg boundary such as South Dakota amber (~65.76 Ma),[4,20] Hat Creek amber (~55–50 Ma, British Columbia),[21] Hanna Formation amber (~58–55 Ma, Wyoming),[3] and Chickaloon Formation amber (~54.65 Ma, Alaska)[22,23] may eventually allow us to flesh out our understanding of terrestrial diversity surrounding the extinction event (Figure 1B). However, very few arthropods are known from these deposits. Currently, Big Muddy amber offers the last diverse arthropod assemblage prior to the end of the Cretaceous, with Tilin amber from Myanmar providing the next closest diverse assemblage, near 72 Ma.[5]

Arthropod survivorship

Most insect families identified in Big Muddy amber are well-represented in Cretaceous and Cenozoic ambers (Table S1), including Mymaridae (Figure 2G), Rotoitidae (Figure 2H), Chironomidae (Figures 3B and 3D), Ceratopogonidae (Figures 3A and 3C), and Cecidomyiidae (Figure 3F). Less common families identified in Big Muddy amber include Formicidae (Figures 2A–2F), Mesozoicaphididae (Figure 3G), and Platygastridae (*sensu*[24]; Figure 2I). The latter record is noteworthy because Scelionidae has historically been recovered as the dominant platygastroid group in other Cretaceous deposits.[25–27] Even more noteworthy is the absence of the exclusively Cretaceous wasp family Serphitidae, the second most abundant wasp lineage in Grassy Lake amber,[25] and the distinctly low abundance of flies in Ceratopogonidae compared to other amber deposits.[18,28,29] These discrepancies suggest that Big Muddy amber may represent a previously unsampled Cretaceous environment, or that some of these taxa disappeared prior to 67 Ma. Most Serphitidae are under 1.5 mm long,[30] so the smaller-than-average size of Big Muddy amber pieces should not be a limiting factor to their entrapment. It is worth noting that Serphitidae were also not among the first 34 insect inclusions documented from Tilin amber (~72.1 Ma),[5] nor were they reported in amber from South Dakota (~65.76 Ma).[4] Additionally, only two specimens of Ceratopogonidae in *Culicoides* (as *Protoculicoides*) were recorded from Tilin amber.[5] Detailed taxonomic work has not been published for the 21 dipterans identified in South Dakota amber,[4] but this material may serve as a spatially and temporally close comparison point for Big Muddy amber.

The new deposit extends the range of the aphid *Mesozoicaphes parva* (RSKM_PAL_A33, Figure 3G), which until now was only known from Grassy Lake amber (~79–78 Ma) in Alberta.[16,31] Mesozoicaphididae is currently the only extinct family represented in Big Muddy amber—all other insect taxa belong to extant families and genera. This is the first time this pattern has been reported for a Cretaceous amber deposit. As more inclusions are discovered in Big Muddy amber, it will be interesting to see if these patterns change and whether they are representative of the whole deposit or only this limited first sample. The Tilin amber deposit in Myanmar is the only other known deposit that comprises mainly extant families.[5] However, Tilin amber contains several more extinct families and genera.

Figure 1. Brief stratigraphic description and map showing location of the study area in the Big Muddy Valley, Saskatchewan, Canada

(A) Stratigraphic column of the study area showing horizons with amber inclusions.

(B) Map of North America during the Late Cretaceous (~67 Ma) showing the location of known fossiliferous deposits. Map colors represent generalized paleoelevation. Age of each deposit is indicated on the geologic timescale. HaC, Hat Creek amber; CF, Chickaloon Fm. amber; HF, Hanna Fm. amber; HeC, Hell Creek amber; BM, Big Muddy amber; NC, North Carolina amber; GL, Grassy Lake amber; NJ, New Jersey amber.

(C) Photo of the outcrop exposure (dark shale layer).

(D) Drop-shaped surface amber.

See also Figure S2.

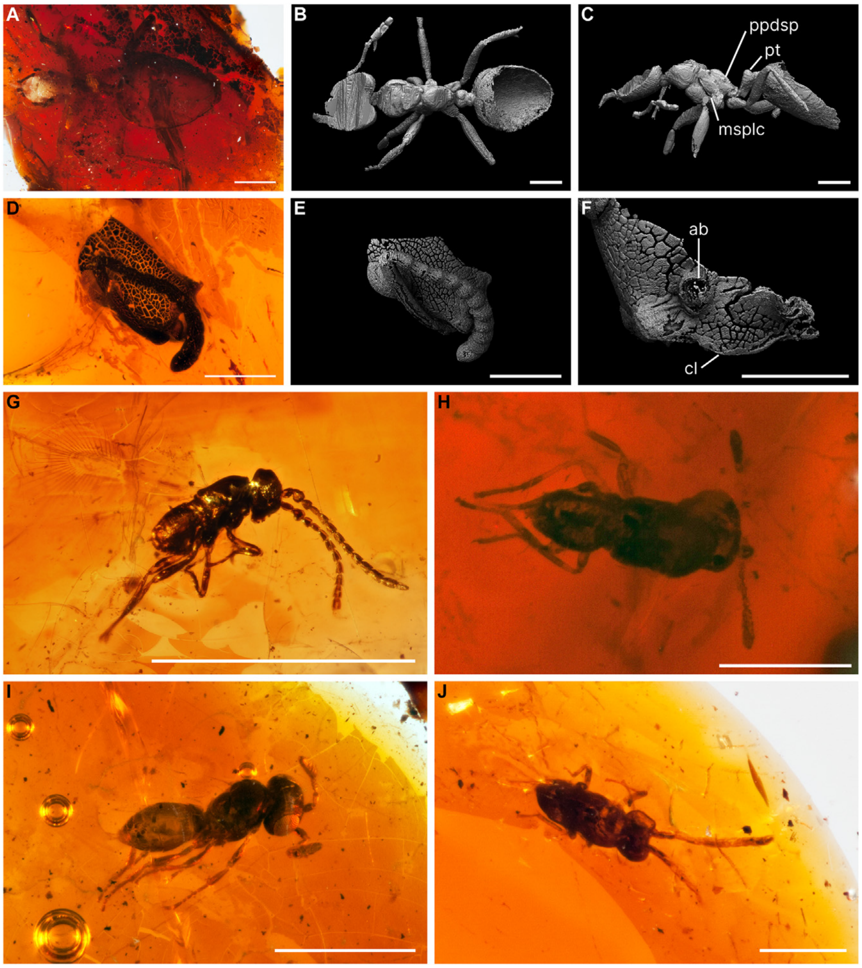

Figure 2. Hymenoptera in Big Muddy amber

(A–C) Image and digital visualization of crown Formicidae body fossil, RSKM_PAL_A18.

(D–F) Image and digital visualization of partial crown Formicidae head, RSKM_PAL_A17.

(G) Mymaridae, RSKM_PAL_A2.

(H) Rotoitidae, RSKM_PAL_A3.

(I) Platygastridae, RSKM_PAL_A22.

(J) Hymenoptera, RSKM_PAL_A21. Abbreviations: ab, antennal base; cl, clypeus; msplc, mesopectus longitudinal carinae; ppdsp, propodeal spiracle; pt, petiole.

Scale bars, 0.5 mm (A–C, G–I), 0.75 mm (D, E), 0.625 mm (F), 0.25 mm (J). See also Table S1.

Other notable discoveries in Big Muddy amber include the presence of two ants (RSKM_PAL_A17, Figure 2D; RSKM_PAL_A18, Figure 2A), which are extremely rare in Late Cretaceous amber deposits in North America.[32,33] Both partial ants can be diagnosed as crown-group taxa (i.e., diverged from the common ancestor of living ants). RSKM_PAL_A18 (Figures 2A–2C) has angular longitudinal carinae along the anterolateral margins of the mesopectus, round propodeal spiracles, and complete tergosternal fusion of the petiole; all are plesiomorphies of the crown clade of Formicidae (*sensu*[34,35]). RSKM_PAL_A17 (Figures 2D–2F) can be identified as a crown ant based on the plesiomorphic condition of the integrated clypeal disc, location of the antennal bases, and geniculate antennae with an elongated scape and shortened flagellum (*sensu*[34,35]). Lower-level taxonomic work is ongoing to identify the partial ants to subfamily.

While a sample size of two ants is hardly representative of overall formicid diversity, it is anomalous to find two crown-group ant inclusions among the first specimens from Big Muddy amber (4% of inclusions). In comparison, Grassy Lake amber only contains 11 ant specimens out of 6,000 inclusion-bearing pieces,[25] and all but one of these 11 are stem ants (i.e., ants that diverged before the last common ancestor of living ants); the ants represent less than 0.2% of all inclusions.[36] Despite the most recent common ancestor of living ants originating an estimated 120 Ma,[37] stem ants are far more common than crown ants in Cretaceous deposits.[32,33] Crown-ant fossils become more common in the Eocene, presumably coinciding with increased abundance and ecological dominance over stem ants, who are absent from fossil deposits after a critical 16-million-year gap in the ant fossil record that spans the K-Pg mass extinction (~72.1–55 Ma).[33] The sole presence of crown-group ants in Big Muddy amber is similar to the pattern noted within the initial work on Tilin amber,[5] and it may support the hypothesis that the stem ant to crown ant faunal turnover was well underway prior to the end of the Cretaceous.[38] In Asia, the transition from stem- to crown-ant-dominated assemblages appears to have taken place in Myanmar somewhere between the formation of Kachin amber (~98.79 Ma),[39] which contains both stem and crown ants, and Tilin amber (~72.1 Ma),[5] which reportedly contains only crown ants.[38,40] The new ant records from Big Muddy amber may suggest that the transition in North America occurred sometime between the formation of North Carolina amber (~83.6–72.1 Ma, previously the youngest known North American Cretaceous deposit to contain ants[38,41]) and Big Muddy amber (~66.93 Ma). Even with these new discoveries in Big Muddy and Tilin amber, ants are poorly represented in the latest Cretaceous, obscuring the exact geochronological timing of the stem ant to crown ant faunal turnover. We know that the ant faunal transition was complete by at least the Eocene, as evidenced by numerous crown-ant inclusions in Baltic amber and other Eocene fossil deposits.[32,33] If more ants can be found in Big Muddy amber, whether crown or stem ants, the deposit may prove to be a key benchmark for refining our understanding of ant evolution in the latest Cretaceous.

The parasitoid wasp family Rotoitidae, whose extant relatives are only found in Chile and Australia,[42] is also found in Big Muddy amber (RSKM_PAL_A3, Figure 2H). Local Cenozoic extinctions, or perhaps extinctions across the K-Pg boundary, may have led to the family's relictual distribution.[43] Gumovsky et al.[43]

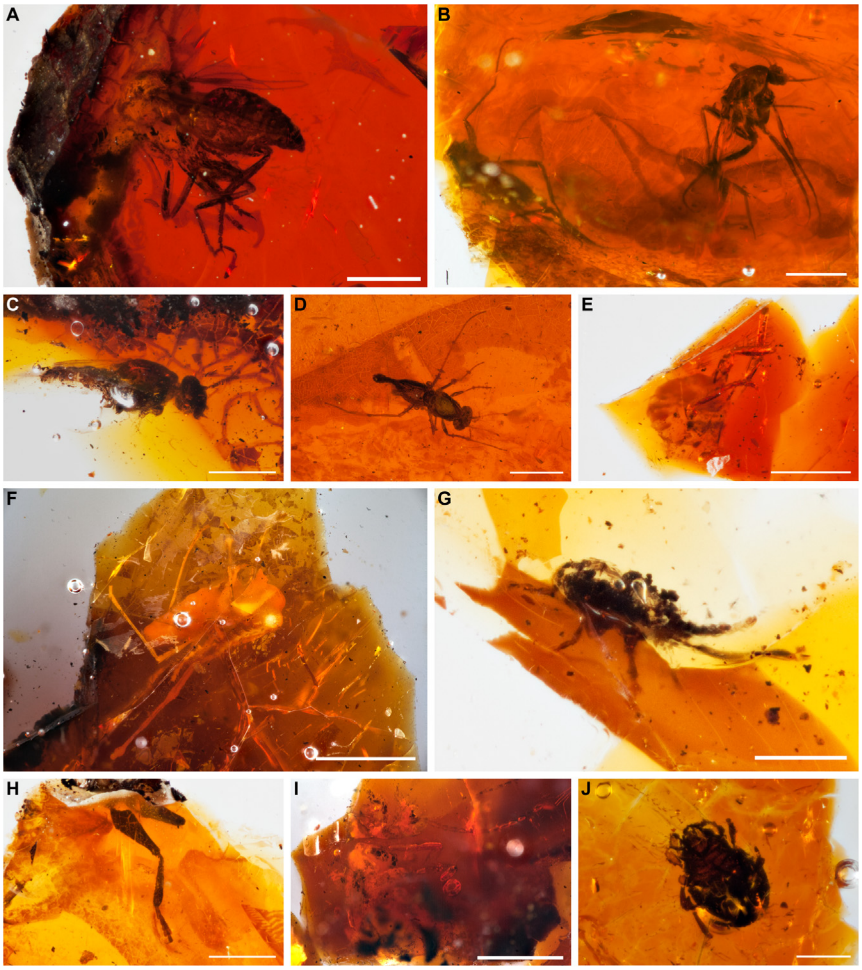

Figure 3. Diptera, Hemiptera, Blattodea, Coleoptera, and Arachnida in Big Muddy amber

(A) *Stilobezzia* (Ceratopogonidae), RSKM_PAL_A11.
(B) Male and female Chironomidae, RSKM_PAL_A1.
(C) Ceratopogonidae, RSKM_PAL_A4.
(D) Chironomidae, RSKM_PAL_A8.
(E) Incomplete Blattodea, RSKM_PAL_A34.
(F) Cecidomyiidae, RSKM_PAL_A7.
(G) *Mesozoicaphes parva* (Mesozoicaphididae), RSKM_PAL_A33.
(H) Coleoptera leg, RSKM_PAL_A35.
(I) Araneae with unidentified syninclusion, RSKM_PAL_A29.
(J) Acari, RSKM_PAL_A40.
Scale bars, 0.5 mm (A–F, H, I), 0.25 mm (G, J). See also Table S1.

hypothesized that the survival of Rotoitidae lineages may be dependent on the low ant abundance in these regions. They proposed that northern amber sites in the Cretaceous fell within the "*Baeomorpha* Realm," a faunal region characterized by high aphid and Rotoitidae abundance and depauperate ant faunas.[43] If additional formicids are recovered from Big Muddy amber, they may test the faunal patterns that characterize the "*Baeomorpha* Realm" and their extent throughout the Cretaceous.

Environmental reconstruction

With the limited number of inclusions currently available, much of the data for paleoenvironmental reconstruction stems from the chemistry and composition of Big Muddy amber and the surrounding geology. The regional extent of the Battle Formation in southern Alberta and as far east as the Big Muddy Valley in Saskatchewan, combined with the presence of *Aulacoseira* sp. (primarily freshwater diatoms), Fragilariales diatom species, and carbonized roots,[44] supports an environment of shallow, highly productive marshes and swamps. However, Battle Formation outcrops in Alberta lack coaly beds and amber, suggesting that the environment was more vegetated and perhaps more humid to the east. This vast swamp system was likely shallower and drier near the Big Muddy region, promoting the development of extensive vegetation under subtropical climatic conditions.

The Big Muddy deposit is dominated by aerial amber retaining delicate droplet and runnel morphologies, although larger oblong pieces lacking bioinclusions are occasionally encountered. These larger pieces may represent *in situ* resin production by roots, which has been observed on modern trees[1] and interpreted for the newly described Cretaceous Ariño amber deposit.[45] The predominance of delicate amber morphologies with uneroded surfaces suggests minimal transport.

Beyond regional geology (see site geology in STAR Methods), the inclusions in Big Muddy amber point toward a freshwater habitat. The presence of a new species of fly in the extant Ceratopogonidae genus *Stilobezzia* (RSKM_PAL_A11, Figure 3A) supports a lacustrine environment, as modern immatures live in freshwater aquatic habitats where larvae predate other invertebrates. The female *Stilobezzia* specimen was identified based on the single tarsal claw on each of the three legs and the presence of two radial cells on the wings, which is typical for this genus.[46] The Big Muddy specimen is distinct from the three other described Cretaceous *Stilobezzia* species based on significant differences in the size of the second radial cell and the proportions and shape of the claw and tooth.[46–48] It likely represents a new Cretaceous species. The genus arose in the Late Cretaceous, with the earliest representative known from New Jersey amber (94–90 Ma).[46] The new material represents only the fourth known *Stilobezzia* species from the Cretaceous.[46–49] Adult female *Stilobezzia* capture and feed on other small nematocerous Diptera, generally from swarms of Chironomidae, which are also present in Big Muddy amber (Figures 3B and 3D).

Based on the Fourier-transform infrared (FTIR) absorption spectra of Big Muddy amber, it was likely produced by trees in the family Cupressaceae or Araucariaceae (i.e., resin primarily composed of labdane-based diterpenes; Figures 4C and 4D; see FTIR analysis in STAR Methods).[50] Strong spectral similarities between Big Muddy amber and other ambers from the Cretaceous of western North America, particularly in the

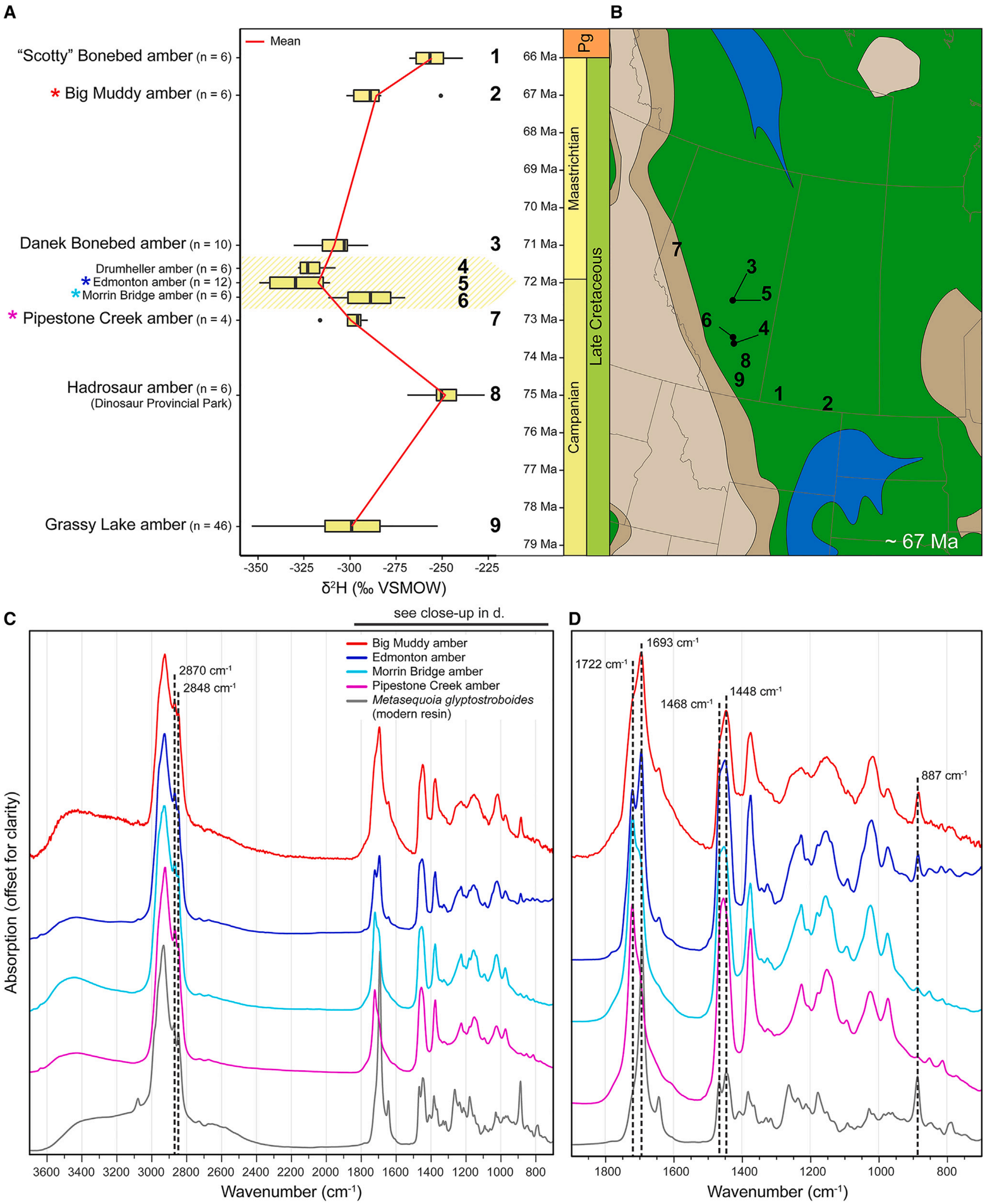

Figure 4. Chemical analysis of Big Muddy amber

Boxplot of variation in δ^2H between Big Muddy amber and surrounding ambers, and Fourier-transform infrared (FTIR) spectrum of Big Muddy amber compared to some of the same ambers and modern *Metasequoia* resin.

(legend continued on next page)

wavelength range 800–1400 cm^{-1} (Figure 4D), suggest production by the same family of trees.[8] The most likely source is a cupressaceous conifer in the genus *Parataxodium,* due to the presence of its foliage in amber deposits with similar chemotaxonomy[16,51]; however, the genus requires taxonomic revision.[52,53] *Parataxodium* has close affinities to modern bald cypress (*Taxodium* sp.) and dawn redwood (*Metasequoia glyptostroboides*), which are extremely water-resistant deciduous conifer trees.[52] This fits with interpretations of the source tree for ambers in the Western Sedimentary Basin as a swamp- or floodplain-dwelling cypress tree.[16]

Big Muddy amber fragments were analyzed using isotope ratio mass spectrometry to determine the carbon (Figure S1) and hydrogen stable isotope composition (Figure 4A; see stable isotope analysis in STAR Methods). The hydrogen values recorded in Big Muddy amber suggest that remnants of the WIS still had a strong influence on regional precipitation patterns in paludal settings but that water had undergone a few evaporation and rainout cycles before reaching the trees. Throughout much of the Cretaceous the WIS bisected North America,[54] leaving traces of its fluctuating coastline and marine influences in the geologic and amber records.[54,55] The mean δ^2H of the Big Muddy amber is −285.7‰ ± 18.6‰, with values ranging from −302.0‰ to −250.8‰ (Table S2). These values are high compared to other Late Cretaceous ambers from Canada (Figure 4B).[56,57] This is consistent with geological data from seiche deposits in North Dakota that suggest the WIS coastline was close to Saskatchewan at the time of the K-Pg extinction.[20] Assuming an isotopic discrimination factor of 229‰ between local water sources and the resin produced by trees,[58] the mean δ^2H value of precipitation received by the Big Muddy amber forest was −56.7‰. Modern precipitation in North America shows similar δ^2H values on the northern Atlantic coast, as well as farther inland in the USA (where the Gulf of Mexico exerts a strong influence on precipitation).[59,60] It is noteworthy that the influence of the WIS appears to have been stronger in the overlying Frenchman Formation, when amber was deposited within the "Scotty" *Tyrannosaurus* bonebed (∼66.46 Ma),[56] than it was when Big Muddy amber was being formed (67.20–66.88 Ma). This contrasts with conventional paleoenvironmental reconstructions that place the final regression of the WIS at approximately 72 Ma.[55] However, the findings agree with preliminary analyses of microvertebrate fossil assemblages in the Late Cretaceous Frenchman Formation (∼65.5–65 Ma) that suggest increasing marine influence toward the K-Pg boundary.[61] Coastlines need to be validated through additional proxies, but amber measurements provide a good first approximation of surface water composition, using material unlikely to have suffered from weathering effects or isotopic overprinting.[62]

The geological and chemical data from Big Muddy amber suggest that the site is sampling a unique environment through the combination of its floristic components, environmental features, and proximity to the WIS. The new deposit offers a rare glimpse into the diversity of arthropods living in the conifer-dominated swamps of the latest Cretaceous. These ecosystems demonstrated the greatest survivability across the K-Pg extinction.[63,64] Compression fossils of foliage from the Paleocene Ravenscrag Formation (∼65.5 Ma) in the Big Muddy Valley indicate that shortly after the extinction deciduous conifers *Metasequoia occidentalis* (Cupressaceae) and *Glyptostrobus dakotensis* (Cupressaceae) once again dominated, with an understory of ferns, broadleaf angiosperm shrubs, and small trees.[65] The Late Cretaceous conifer swamp in the Big Muddy area likely had similar vegetation, providing a test bed for survivorship into the Paleogene.

Conclusion

Overall, the Big Muddy arthropod assemblage seems unique, with notable ant inclusions and the absence of nearly all extinct Cretaceous insect families and genera. Although our sample size is still limited, the composition of the initial assemblage suggests that there may have been a faunal transition among insects from ancestral extinct lineages to extant ones prior to the K-Pg mass extinction, as has been suggested for some insect pollinator lineages[66] and inferred by fossil-calibrated molecular models.[2] Big Muddy amber provides a benchmark for arthropod diversity before the K-Pg mass extinction, allowing us to directly observe the responses of arthropod lineages to environmental changes that occurred throughout the latter part of the Cretaceous.

North America has one of the most continuous amber fossil records for Late Cretaceous and Eocene arthropods from a single region, with diverse assemblages spanning the continent.[16,21,27,38,41,64] Big Muddy amber and adjacent deposits provide a stable backdrop to observe arthropod evolution and changes in arthropod diversity and paleoenvironmental conditions through time. Future research will synthesize isotopic and biodiversity data from North American amber with geological, palynological, and vertebrate paleontological data, characterizing large scale paleoenvironmental changes in the Late Cretaceous and across the K-Pg boundary more precisely.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY

(A) Boxplot range of δ^2H in each deposit using Vienna Standard Mean Ocean Water (VSMOW) as a standard. The box has lines at the lower quartile and median and upper quartile values. The lines extending from each end of the box represent the extent of the rest of the data; black dots represent outliers. The green-yellow diagonal-line patterned pentagonal arrow indicates that Drumheller amber, Edmonton amber, and Morrin Bridge amber are all approximately 72 Ma. The red line is the mean δ^2H through time; n indicates the number of samples per deposit. Colored stars indicate ambers that were used as comparison spectra for FTIR. Carbon isotope results are presented in Figure S1; see also stable isotope analysis in STAR Methods and Table S2.

(B) Map of western Canada during the Late Cretaceous (∼67 Ma) showing location of each numbered amber deposit. Map colors represent generalized paleoelevation.

(C) FTIR spectral range 3700–700 cm^{-1}.

(D) Close-up of the spectral range 1900–700 cm^{-1}. See also FTIR analysis in STAR Methods.

  - Lead contact
  - Materials availability
  - Data and code availability
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
- METHOD DETAILS
  - Site geology
  - Sample collection and processing
  - Amber inclusions
  - FTIR analysis
  - Stable isotope analysis

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2024.03.001.

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AUTHOR CONTRIBUTIONS

E.J.T.L., M.A.B., and R.C.M. conceptualized the project. M.A.B., J.M.R., and M.V.C. analyzed site geology. J.M.R. drew the stratigraphic column. R.T. conducted the FTIR analysis. P.C., K.M., and R.C.M. conducted the isotope analyses. E.J.T.L., M.A.B., A.B., C.L., M.S.E., and R.C.M. identified arthropod inclusions. E.J.T.L. and C.L. reconstructed synchrotron μCT-scans. E.J.T.L. and M.A.B. took pictures. E.J.T.L. designed visuals. E.J.T.L., M.A.B., and R.C.M. wrote the manuscript with contributions from all co-authors.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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REFERENCES

1. Seyfullah, L.J., Beimforde, C., Dal Corso, J., Perrichot, V., Rikkinen, J., and Schmidt, A.R. (2018). Production and preservation of resins – past and present. Biol. Rev. *93*, 1684–1714.
2. Condamine, F.L., Clapham, M.E., and Kergoat, G.J. (2016). Global patterns of insect diversification: Towards a reconciliation of fossil and molecular evidence? Sci. Rep. *6*, 19208–19213.
3. Grimaldi, D.A., Lillegraven, J.A., Wampler, T.W., Bookwalter, D., and Shedrinsky, A. (2000). Amber from Upper Cretaceous through Paleocene strata of the Hanna Basin, Wyoming, with evidence for source and taphonomy of fossil resins. Rocky Mt. Geol. *35*, 163–204.
4. DePalma, R.A., Cichocki, F., Dierick, M., and Feeney, R. (2010). Preliminary notes on the first recorded amber insects from the Hell Creek Formation. JPS *2*, 1–7.
5. Zheng, D., Chang, S.C., Perrichot, V., Dutta, S., Rudra, A., Mu, L., Thomson, U., Li, S., Zhang, Q., Zhang, Q., et al. (2018). A Late Cretaceous amber biota from central Myanmar. Nat. Commun. *9*, 3170–3210.
6. Schachat, S.R., Labandeira, C.C., Clapham, M.E., and Payne, J.L. (2019). A Cretaceous peak in family-level insect diversity estimated with mark–recapture methodology. Proc. Biol. Sci. *286*, 20192054.
7. Labandeira, C.C., Johnson, K.R., and Wilf, P. (2002). Impact of the terminal Cretaceous event on plant-insect associations. Proc. Natl. Acad. Sci. USA *99*, 2061–2066.
8. Street, H.P., Bamforth, E.L., and Gilbert, M.M. (2019). The formation of a marine bonebed at the Upper Cretaceous Dinosaur Park - Bearpaw Transition of West - Central Saskatchewan, Canada. Front. Earth Sci. *7*, 1–15.
9. Dawson, F.M., Evans, C.G., Marsh, R., and Richardson, R. (1994). Uppermost Cretaceous and Tertiary strata of the Western Canada Sedimentary Basin. In Geological Atlas of the Western Canada Sedimentary Basin, G.D. Mossop, and I. Shetsen, eds. (Canadian Society of Petroleum Geologists and Alberta Research Council), pp. 387–406.
10. Eberth, D.A., and Kamo, S.L. (2019). First high-precision U–PB CA–ID–TIMS age for the Battle Formation (Upper Cretaceous), Red Deer River Valley, Alberta, Canada: Implications for ages, correlations, and dinosaur biostratigraphy of the Scollard, Frenchman, and Hell Creek formations. Can. J. Earth Sci. *56*, 1041–1051.
11. Martınez-Delclòs, X., Briggs, D.E.G., and Peñalver, E. (2004). Taphonomy of insects in carbonates and amber. Palaeogeogr. Palaeoclimatol. Palaeoecol. *203*, 19–64.
12. Solórzano Kraemer, M.M., Delclòs, X., Clapham, M.E., Arillo, A., Peris, D., Jäger, P., Stebner, F., and Peñalver, E. (2018). Arthropods in modern resins reveal if amber accurately recorded forest arthropod communities. Proc. Natl. Acad. Sci. USA *115*, 6739–6744.
13. Solórzano Kraemer, M.M., Kraemer, A.S., Stebner, F., Bickel, D.J., and Rust, J. (2015). Entrapment bias of arthropods in Miocene amber revealed by trapping experiments in a tropical forest in Chiapas, Mexico. PLoS One *10*, e0118820.
14. Pike, E.M. (1993). Amber taphonomy and collecting biases. Palaios *8*, 411–419.
15. Nel, A., DePalma, R.A., and Engel, M.S. (2010). A possible hemiphlebiid damselfly in Late Cretaceous amber from South Dakota (Odonata: Zygoptera). Trans. Kans. Acad. Sci. *113*, 231–234.
16. McKellar, R.C., and Wolfe, A.P. (2010). Canadian Amber. In Biodiversity of Fossils in Amber, D. Penney, ed. (Siri Scientific Press), pp. 149–166.
17. McKellar, R.C., Wolfe, A.P., Tappert, R., and Muehlenbachs, K. (2008). Correlation of Grassy Lake and Cedar Lake ambers using infrared spectroscopy, stable isotopes, and palaeoentomology. Can. J. Earth Sci. *45*, 1061–1082.
18. Pike, E.M. (1994). Historical changes in insect community structure as indicated by Hexapods of Upper Cretaceous Alberta (Grassy Lake) amber. Can. Entomol. *126*, 695–702.
19. Pike, E.M. (1995). Amber taphonomy and the Grassy Lake, Alberta, amber fauna. Doctoral thesis (University of Calgary).
20. DePalma, R.A., Smit, J., Burnham, D.A., Kuiper, K., Manning, P.L., Oleinik, A., Larson, P., Maurrasse, F.J., Vellekoop, J., Richards, M.A., et al. (2019). A seismically induced onshore surge deposit at the KPg boundary, North Dakota. Proc. Natl. Acad. Sci. USA *116*, 8190–8199.

21. Poinar, G., Archibald, B., and Brown, A. (1999). New amber deposit provides evidence of early Paleogene extinctions, paleoclimates, and past distributions. Can. Entomol. *131*, 171–177.

22. Grimaldi, D.A., Sunderlin, D., Aaroe, G.A., Dempsky, M.R., Parker, N.E., Tillery, G.Q., White, J.G., Barden, P., Nascimbene, P.C., and Williams, C.J. (2018). Biological inclusions in amber from the paleogene Chickaloon Formation of Alaska. Am. Mus. Novit. *3908*, 1–37.

23. Triplehorn, D.M., Turner, D.L., and Naeser, C.W. (1984). Radiometric age of the Chickaloon Formation of south-central Alaska: Location of the Paleocene-Eocene boundary. Geol. Soc. Am. Bull. *95*, 740–742.

24. Talamas, E.J., Johnson, N.F., Shih, C., and Ren, D. (2019). Proterosceliopsidae: A new family of Platygastroidea from Cretaceous amber. J. Hymenopt. Res. *73*, 3–38.

25. McKellar, R.C., and Engel, M.S. (2012). Hymenoptera in Canadian Cretaceous amber (Insecta). Cretac. Res. *35*, 258–279.

26. Grimaldi, D.A., Engel, M.S., and Nascimbene, P.C. (2002). Fossiliferous Cretaceous amber from Myanmar (Burma): Its rediscovery, biotic diversity, and paleontological significance. Am. Mus. Novit. *3361*, 1–71.

27. Grimaldi, D.A., Shedrinsky, A., and Wampler, T.P. (2000). A remarkable deposit of fossiliferous amber from the Upper Cretaceous (Turonian) of New Jersey. In Studies on fossils in amber, with particular reference to the Cretaceous of New Jersey, D.A. Grimaldi, ed. (Backhuys Publishers)), pp. 1–76.

28. Borkent, A. (2000). Biting midges (Ceratopogonidae: Diptera) from Lower Cretaceous Lebanese amber with a discussion of the diversity patterns found in other ambers. In Studies on fossils in amber, with particular reference to the Cretaceous of New Jersey, D.A. Grimaldi, ed. (Backhuys Publishers), pp. 355–451.

29. Borkent, A. (1995). Biting midges in the Cretaceous amber of North America (Backhuys Publishers).

30. McKellar, R.C., and Engel, M.S. (2011). The serphitid wasps (Hymenoptera: Proctotrupomorpha: Serphitoidea) of Canadian Cretaceous amber. Syst. Entomol. *36*, 192–208.

31. Heie, O.E., and Pike, E.M. (1992). New aphids in Cretaceous amber from Alberta (Insecta, Homoptera). Can. Entomol. *124*, 1027–1053.

32. Barden, P. (2017). Fossil ants (Hymenoptera: Formicidae): ancient diversity and the rise of modern lineages. Myrmecol. News *24*, 1–30.

33. LaPolla, J.S., Dlussky, G.M., and Perrichot, V. (2013). Ants and the fossil record. Annu. Rev. Entomol. *58*, 609–630.

34. Bolton, B. (2003). Synopsis and classification of Formicidae. Mem. Amer. Ent. Inst. *71*, 1–370.

35. Boudinot, B.E. (2015). Contributions to the knowledge of Formicidae (Hymenoptera, Aculeata): A new diagnosis of the family, the first global male-based key to subfamilies, and a treatment of early branching lineages. Eur. J. Taxon. *120*, 1–62.

36. McKellar, R.C., Glasier, J.R., and Engel, M.S. (2013). A new trap-jawed ant (Hymenoptera: Formicidae: Haidomyrmecini) from Canadian Late Cretaceous amber. Can. Entomol. *145*, 454–465.

37. Borowiec, M.L., Rabeling, C., Brady, S.G., Fisher, B.L., Schultz, T.R., and Ward, P.S. (2019). Compositional heterogeneity and outgroup choice influence the internal phylogeny of the ants. Mol. Phylogenet. Evol. *134*, 111–121.

38. Sosiak, C.E., Cockx, P., Suarez, P.A., McKellar, R.C., and Barden, P. (2024). North American amber from the Late Cretaceous reveals prolonged faunal turnover and asymmetric extinction in ants. Curr. Biol. *34*.

39. Shi, G., Grimaldi, D.A., Harlow, G.E., Wang, J., Wang, J., Yang, M., Lei, W., Li, Q., and Li, X. (2012). Age constraint on Burmese amber based on U–Pb dating of zircons. Cretac. Res. *37*, 155–163.

40. Perrichot, V. (2019). New Cretaceous records and the diversification of crown-group ants (Hymenoptera: Formicidae). In 8th International Congress on Fossil Insects, Arthropods and Amber, P.C. Nascimbene, ed. (International Paleoentomological Society), p. 67.

41. Krynicki, V.E. (2013). Primitive ants (Hymenoptera: Sphecomyrminae) in the Campanian (Late Cretaceous) of North Carolina (USA). Life Excit. Biol. *1*, 156–165.

42. Gibson, G.A.P., and Huber, J.T. (2000). Review of the family Rotoitidae (Hymenoptera: Chalcidoidea), with description of a new genus and species from Chile. J. Nat. Hist. *34*, 2293–2314.

43. Gumovsky, A., Perkovsky, E., and Rasnitsyn, A. (2018). Laurasian ancestors and "Gondwanan" descendants of Rotoitidae (Hymenoptera: Chalcidoidea): What a review of Late Cretaceous *Baeomorpha* revealed. Cretac. Res. *84*, 286–322.

44. Siver, P.A., Velez, M., Cliveti, M., and Binda, P. (2018). Early freshwater diatoms from the Upper Cretaceous Battle Formation in Western Canada. Palaios *33*, 525–534.

45. Álvarez-Parra, S., Pérez-de la Fuente, R., Peñalver, E., Barrón, E., Alcalá, L., Pérez-Cano, J., Martín-Closas, C., Trabelsi, K., Meléndez, N., López Del Valle, R., et al. (2021). Dinosaur bonebed amber from an original swamp forest soil. Elife *10*, e72477.

46. Borkent, A. (2000). Further biting midges (Diptera: Ceratopogonidae) from Upper Cretaceous New Jersey amber. In Studies on fossils in amber, with particular reference to the Cretaceous of New Jersey, D.A. Grimaldi, ed. (Backhuys Publishers), pp. 453–472.

47. Borkent, A. (2012). Further biting midges (Diptera: Ceratopogonidae) in Canadian Cretaceous amber. Can. Entomol. *144*, 834–842.

48. Choufani, J., Perrichot, V., Girard, V., Garrouste, R., Azar, D., and Nel, A. (2013). Two new biting midges of the modern type from Santonian amber of France (Diptera: Ceratopogonidae). In 6th International Congress on Fossil Insects, Arthropods and Amber, D. Azar, M.S. Engel, E. Jarzembowski, L. Krogmann, A. Nel, and J. Santiago-Blay, eds. (Brill), pp. 73–95.

49. Szadziewski, R., Arillo, A., Urbanek, A., and Sontag, E. (2016). Biting midges of the extinct genus *Protoculicoides* Boesel from Lower Cretaceous amber of San Just, Spain and new synonymy in recently described fossil genera (Diptera: Ceratopogonidae). Cretac. Res. *58*, 1–9.

50. Tappert, R., Wolfe, A.P., McKellar, R.C., Tappert, M.C., and Muehlenbachs, K. (2011). Characterizing modern and fossil gymnosperm exudates using micro-fourier transform infrared spectroscopy. Int. J. Plant Sci. *172*, 120–138.

51. Cockx, P., McKellar, R., Tappert, R., Vavrek, M., and Muehlenbachs, K. (2020). Bonebed amber as a new source of paleontological data: The case of the Pipestone Creek deposit (Upper Cretaceous), Alberta, Canada. Gondwana Res. *81*, 378–389.

52. Rothwell, G.W., Stockey, R.A., and Smith, S.Y. (2020). Revisiting the Late Cretaceous *Parataxodium wigginsii* flora from the north slope of Alaska, a high-latitude temperate forest. Cretac. Res. *116*, 104592.

53. Arnold, C.A., and Lowther, J.S. (1955). A new Cretaceous conifer from northern Alaska. Am. J. Bot. *42*, 522–528.

54. Storer, J. (1989). Geological History of Saskatchewan. (Regina, Canada (Saskatchewan Museum of Natural History, Government of Saskatchewan)).

55. Blakey, R.C., and Ranney, W.D. (2018). Ancient Landscapes of Western North America (Springer Nature).

56. Cockx, P., Tappert, R., Muehlenbachs, K., Somers, C., and McKellar, R.C. (2021). Amber from a *Tyrannosaurus rex* bonebed (Saskatchewan, Canada) with implications for paleoenvironment and paleoecology. Cretac. Res. *125*, 104865.

57. McKellar, R.C., Jones, E., Engel, M.S., Tappert, R., Wolfe, A.P., Muehlenbachs, K., Cockx, P., Koppelhus, E.B., and Currie, P.J. (2019). A direct association between amber and dinosaur remains provides paleoecological insights. Sci. Rep. *9*, 17916.

58. Wolfe, A.P., Csank, A.Z., Reyes, A.V., McKellar, R.C., Tappert, R., and Muehlenbachs, K. (2012). Pristine Early Eocene Wood Buried Deeply in Kimberlite from Northern Canada. PLoS One *7*, e45537.

59. Bowen, G.J., Wassenaar, L.I., and Hobson, K.A. (2005). Global application of stable hydrogen and oxygen isotopes to wildlife forensics. Oecologia *143*, 337–348.

60. Terzer, S., Wassenaar, L.I., Araguás-Araguás, L.J., and Aggarwal, P.K. (2013). Global isoscapes for $\delta^{18}O$ and $\delta^{2}H$ in precipitation: improved prediction using regionalized climatic regression models. Hydrol. Earth Syst. Sci. *17*, 4713–4728.

61. Bamforth, E.L., Tokaryk, T.T., and Larsson, H.C.E. (2017). Marine influence as a hidden driver of paleobiodiversity: an example from the latest Cretaceous (66 Ma) of Southwest Saskatchewan, Canada. In Society of Vertebrate Palaeontology August 2017 Abstracts of Papers 77th Annual Meeting, A. Farke, A. MacKenzie, and J. Miller-Camp, eds. (Society of Vertebrate Paleontology), p. 77.

62. Nissenbaum, A., and Yakir, D. (1996). Stable Isotope Composition of Amber. In Amber, Resinite, and Fossil Resins, K.B. Anderson, and J. Crelling, eds. (American Chemical Society), pp. 32–42.

63. Johnson, K.R. (2002). Megaflora of the Hell Creek and lower Fort Union formations in the western Dakotas: Vegetational response to climate change, the Cretaceous-Tertiary boundary event, and rapid marine transgression. In The Hell Creek Formation and the Cretaceous-Tertiary boundary in the northern Great Plains: An integrated continental record of the end of the Cretaceous, J.H. Hartman, K.R. Johnson, and D.J. Nichols, eds. (Geological Society of America), pp. 329–391.

64. Vajda, V., and Bercovici, A. (2014). The global vegetation pattern across the Cretaceous–Paleogene mass extinction interval: A template for other extinction events. Glob. Planet. Change *122*, 29–49.

65. Postnikoff, A.C.L. (2009). Flora of the Ravenscrag Formation of the Big Muddy Valley, Willow Bunch Lake map area (72H), Saskatchewan. Doctoral thesis (University of Saskatchewan).

66. Labandeira, C. (2014). Why did terrestrial insect diversity not increase during the angiosperm radiation? Mid-Mesozoic, plant-associated insect lineages harbor clues. In Evolutionary Biology: Genome Evolution, Speciation, Coevolution and Origin of Life, P. Pontarotti, ed. (Springer), pp. 261–299.

67. Nascimbene, P.C., and Silverstein, H. (2000). The preparation of fragile Cretaceous ambers for conservation and study of organismal inclusions. In Studies on fossils in amber, with particular reference to the Cretaceous of New Jersey, D.A. Grimaldi, ed. (Backhuys Publishers), pp. 93–102.

68. Faragó, T., Gasilov, S., Emslie, I., Zuber, M., Helfen, L., Vogelgesang, M., and Baumbach, T. (2022). Tofu: a fast, versatile and user-friendly image processing toolkit for computed tomography. J. Synchrotron Radiat. *29*, 916–927.

69. Cignoni, P., Callieri, M., Corsini, M., Dellepiane, M., Ganovelli, F., and Ranzuglia, G. (2008). Meshlab: an open-source mesh processing tool. In Eurographics Italian Chapter Conference, V. Scarano, R. De Chiara, and U. Erra, eds. (The Eurographics Association), pp. 129–136.

70. Wickham, H. (2016). ggplot2: Elegant Graphics for Data Analysis (Springer-Verlag). www.ggplot2.tidyverse.org.

71. R Core Team (2024). R: A Language and Environment for Statistical Computing (R Foundation for Statistical Computing). www.R-project.org.

72. McKellar, R.C., Wolfe, A.P., Muehlenbachs, K., Tappert, R., Engel, M.S., Cheng, T., and Sánchez-Azofeifa, G.A. (2011). Insect outbreaks produce distinctive carbon isotope signatures in defensive resins and fossiliferous ambers. Proc. Biol. Sci. *278*, 3219–3224.

73. Tappert, R., McKellar, R.C., Wolfe, A.P., Tappert, M.C., Ortega-Blanco, J., and Muehlenbachs, K. (2013). Stable carbon isotopes of C3 plant resins and ambers record changes in atmospheric oxygen since the Triassic. Geochim. Cosmochim. Acta *121*, 240–262.

74. Dal Corso, J., Schmidt, A.R., Seyfullah, L.J., Preto, N., Ragazzi, E., Jenkyns, H.C., Delclòs, X., Néraudeau, D., and Roghi, G. (2017). Evaluating the use of amber in palaeoatmospheric reconstructions: The carbon-isotope variability of modern and Cretaceous conifer resins. Geochim. Cosmochim. Acta *199*, 351–369.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Big Muddy amber	This paper	Accession 21044
Software and algorithms		
R Software	https://www.r-project.org/	N/A
Adobe Photoshop	https://www.adobe.com/ca/products/photoshop.html	N/A
Adobe Illustrator	https://www.adobe.com/ca/products/illustrator.html	N/A

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Elyssa Loewen (ejl377@uregina.ca).

Materials availability

The Big Muddy amber collection is housed in the Royal Saskatchewan Museum (RSM [RSKM specimen numbers]) Palaeontology Collection in Regina, Saskatchewan, Canada under accession number 21044. Individual catalogue numbers for specimens are provided in Table S1. Specimens were collected under Saskatchewan Heritage Conservation Branch Permits 14-002P, 15-002P, 16-002P, 17-002P, 18-001P and 19-002P. Samples for FTIR and isotope analyses were consumed. Detailed site locations and GPS coordinates are available through the Royal Saskatchewan Museum.

Data and code availability

All data supporting the findings of this study are available within the manuscript and its supplemental information. All newly generated carbon and hydrogen isotope ratios for Big Muddy amber are provided in Table S2. This paper does not report original code. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

The experimental subjects are insect specimens and amber collected from the Battle Formation in the Big Muddy Valley, Saskatchewan, Canada (Figure S2). The collection is housed in the Royal Saskatchewan Museum Palaeontology Collection in Regina, Saskatchewan, Canada. Specimen details, including sex if known, are listed in Table S1.

METHOD DETAILS

Site geology

The study site is located in the Big Muddy Valley in southwestern Saskatchewan, Canada (Figure S2). The Upper Cretaceous Whitemud, Battle and Frenchman formations and Paleocene Ravenscrag Formation are all extensively exposed in the study area (Figures 1 and S2). The boundaries between the different stratigraphic units are not easily distinguished, with only the Battle and Whitemud formations having a marked contact in the section. An outcrop of approximately 10 m thickness was measured at the study site (Figure 1A).

The measured outcrop is comprised of alternating layers of mainly clay, silt, and sandstone with some organic-rich content. There is evidence of cross- and planar-lamination in various layers throughout the measured section. The Whitemud Formation forms the basal layer and consists of white claystone with a minor proportion of silt. The Battle Formation sits atop the Whitemud Formation with an abrupt contact. The Battle Formation contains the amber occurrence and is composed mainly of an organic-rich laminated shale. It also contains a small layer of purplish-mauve-brown friable shale with a minor component of botanical material in a low-grade stage of coalification. Above these organic-rich layers, is the Frenchman Formation. It contains several alternations between gray shale and thin coal seams, followed by two organic shale layers alternated with gray shale and a laminate layer with white silt and brown clay. At the very top of the measured section, there are more gray-brownish siltstone layers and a thin layer of ironstone followed by black shale. The K-Pg boundary is found between the Frenchman and Ravenscrag formations near the top of the measured section and approximately 10 m above the Battle Formation; however, the contact is not easily distinguished in the weathered parts of the hillside. Another section 140 m to the west was also described and found to be consistent with the main measured section, except for some sandstone channel structures cutting down into the siltstone layers as part of the Frenchman Formation.

Sample collection and processing

Big Muddy amber was surface collected with forceps from weathered outcrops of the Battle Formation at the study site. The friable amber was prepared at the Royal Saskatchewan Museum and the procedure followed a slightly modified version of the technique developed by Nascimbene and Silverstein.[67] The amber was embedded in mineralogical-grade epoxy resin (Epo-Tek 301) under vacuum conditions (91 kPa) followed by polishing using a lapidary wheel, wet sanding, and a felt polishing wheel with aluminum oxide powder. The epoxy resin was used to preserve the samples, avoid oxidation, support the fragile pieces, fill fractures, and reduce the refractive indices. The epoxy-embedded samples were screened under a stereomicroscope, in some cases using glycerin or water to temporarily mount the polished blocks to slides, or as a viewing bath. When inclusions were found, they were cut out of the larger support block using a razor saw or lapidary saw, polished again to obtain better anatomical views, then glued to petrographic slides using the same epoxy resin as in the embedding process.

As a friable amber, Big Muddy amber is quite fragile and often crumbles into smaller pieces. This favours the preservation of small inclusions as they are less likely to have surface exposures in small amber pieces or be damaged as the amber breaks apart. This taphonomic bias affects most amber deposits worldwide.[11] On average, Big Muddy amber pieces are 5 mm smaller than amber from the two largest Cretaceous amber deposits in North America: Grassy Lake amber (~79–78 Ma)[16] and New Jersey amber (~94–90 Ma).[27] The variation in the size of collected amber pieces may be attributable, in part, to different collecting methods. Grassy Lake amber, in particular, is primarily surface collected from large tailings piles resulting from pit mining operations rather than from the *in situ* source.[16] This may increase the average size of the amber collected because smaller pieces may not be visible at the surface.

The Big Muddy amber deposit is less productive than other known North American amber deposits collected under similar conditions: Grassy Lake amber has yielded approximately 148 arthropod inclusions per kilogram of amber (combined screened and picked samples),[14,19] whereas Big Muddy amber has yielded approximately 20 arthropod inclusions per kilogram of unprocessed amber. However, in the context of Cretaceous ambers, the Big Muddy site is comparatively productive—recent work on gem-grade amber from Tilin, Myanmar (~72.1 Ma) has yielded 34 arthropod inclusions in 5 kg of amber or approximately 7 arthropods per kilogram of amber.[5] The preparation process for Big Muddy amber is more labor intensive than gem-grade material as it requires embedding the amber in epoxy, prior to scanning for inclusions. The 2015–2019 Big Muddy amber collection comprised 78 centrifuge tubes each containing ~25–50 mL (~10–30g) of amber pre- embedding in epoxy.

Amber inclusions

Arthropod, botanical, and other inorganic and organic inclusions are abundant in Big Muddy amber. The arthropod fossils are diverse, including arachnid orders Araneae (9% of inclusions) and Acari (9%), and at least 11 families of Insecta in Hymenoptera (26%), Diptera (30%), Hemiptera (13%), Coleoptera (2%) and Blattodea (2%) (Table S1). Big Muddy amber also contains a myriad of non-diagnostic organic amber inclusions predominantly identified as shredded wood fragments. These wood fragments sometimes show ray and tracheid structures. Often the shredded plant material is clumped into pellets, as part of insect frass, but these pellets do not have a distinctive morphology that would allow them to be attributed to a particular insect taxon. Fungal mycelia or bacteria are also abundant as organic inclusions growing from inside (i.e., original inclusions trapped within the amber) and through the amber (i.e., resinicolous or taphonomic fungal traces that have subsequently penetrated the amber).[11] In smaller quantities, lichen fragments, silk strands, insect fecal pellets, and moss microphyll have also been identified. Inorganic inclusions have been identified as isolated mineral growth coming from within the amber, as well as from external sources penetrating the amber through cracks.

Inclusions were observed using a Leica MZ 16 stereomicroscope and an Olympus CH30 compound microscope capable of both dark- and light-field observations under a wide range of incident and transmitted lighting configurations. Photomicrographs were obtained using a Visionary Digital imaging station that uses a Canon 5D Mark III digital camera and Canon MP-E 65mm f/2.8 1-5X lens, fitted to a macro rail (Cognisys Inc., www.cognisys-inc.com), to take high-magnification stacks of images. The resulting photographs were combined using Helicon Focus 5.1 software. Final figures were prepared with Adobe Photoshop and Illustrator software.

The two ant specimens (RSKM_PAL_A17; RSKM_PAL_A18) were X-ray microtomography (μCT) scanned on the Biomedical Imaging and Therapy wiggler-based beamline (BMIT-ID) at the Canadian Light Source (CLS) in Saskatoon, Saskatchewan. The beam energy was set to 35 keV. The slides were mounted with the glass perpendicular to the beam at the start of the scan to minimize glass interference. The effective voxel size is 1.5 μm. The tomographic data was reconstructed using the image processing package UFO-tofu.[68] The reconstructed image stacks of each specimen were segmented and visualized using Dragonfly software, Version 2022.1 (Comet Technologies Canada Inc.; www.theobjects.com/dragonfly). Mesh files were generated from the segmented ants and processed using the ambient occlusion filter in MeshLab, Version 2022.02.[69] Renderings of the mesh files were photographed in the software ZBrush Version 4.0 developed by Pixologic (www.maxon.net/en/zbrush).

FTIR analysis

Eight untreated amber pieces were set aside for chemical analyses. Flakes of amber were obtained from the newly fractured surfaces of three of these untreated amber pieces, and analyzed using FTIR micro-spectroscopy. The flakes were less than 50 × 50 μm in outline and less than 10 μm thick within the region of interest. Flakes were placed on an infrared-transparent silicon wafer (orientation 200, 450 μm thick, type N/Phosphorus, resistivity > 10 Ω-cm) for analysis. A Thermo Nicolet Continuum FTIR microscope attached to an 8700 FTIR main bench at the University of Alberta was used to collect absorption spectra in the wavenumber range 650 to 4000 cm^{-1}. The spectral resolution was set to 4 cm^{-1} in transmission mode. Both the background and sample were scanned 128 times, using a square beam size of 100 × 100 μm. OMNIC ver. 8.3 software was used to collect the spectra and view the data. Baseline correction was performed manually.

The presence of distinctive absorption peaks at 2848 cm^{-1}, 1448 cm^{-1} (Figure 4C), and 887 cm^{-1} (Figure 4D) provides conclusive evidence that Big Muddy amber is derived from resin secreted by trees in the families Cupressaceae or Araucariaceae (i.e., the resin is primarily composed of labdane-based diterpenes).[50] Minor spectral differences between the ambers can be linked to different degrees of degradation, with Big Muddy amber representing one of the best-preserved ambers from the Cretaceous of North America.

Stable isotope analysis

The carbon and hydrogen stable isotope compositions of eight untreated amber pieces were analyzed at the University of Alberta using a Finnigan MAT 252 dual-inlet mass spectrometer. Chips of amber were sealed in quartz glass tubes with ∼1–2 g of CuO, and any water vapour or volatiles were removed from the sample tubes by repeatedly heating to ∼90°C under vacuum before sealing. Samples were combusted at 800°C for 12 h, then H_2O(g) and CO_2(g) products were extracted and separated. Before analyses, H_2O was converted to H_2 via a reaction with zinc. One of the eight δ^2H measurements (−387.8‰) is anomalously low and had to be excluded from the dataset because it resulted from an error in loading zinc into the reaction tube for H_2 extraction. Results are expressed in delta notation: $\delta^{13}C$ and δ^2H, with Vienna Pee Dee Belemnite (VPDB) and Vienna Standard Mean Ocean Water (VSMOW) as standards, respectively (Table S2). Data are presented in a boxplot to compare the distribution of δ^2H and $\delta^{13}C$ values in Big Muddy amber to published δ^2H and $\delta^{13}C$ values from other surrounding amber deposits (Figures 4A, 4B, and S1). Boxplots were created using the package ggplot2 (www.ggplot2.tidyverse.org)[70] in R Studio version 2023.09.1 + 494 (www.posit.co/download/rstudio-desktop) with R version 4.3.1 (www.R-project.org).[71]

Big Muddy amber displays $\delta^{13}C$ values ranging from −24.6‰ to −21.7‰ with a mean value of −23.1‰ ± 1.0‰ (Figure S1; Table S2). These values are lower than those observed within the overlying Frenchman Formation of Saskatchewan, at the "Scotty" *Tyrannosaurus rex* quarry (mean −22.1‰ ± 1.3‰, range −24.2‰ to −20.6‰; 66 Ma), but the ranges overlap extensively.[56] The $\delta^{13}C$ values from both Big Muddy amber and the 'Scotty' bonebed amber are 0.8‰ to 1.6‰ higher than those observed in older Cretaceous amber deposits from Alberta.[51,56] The inclusions and isotopic composition of Big Muddy amber do not suggest an increase in $\delta^{13}C$ values due to major ecological stress[27,72]; therefore, the difference is likely due to variations in atmospheric $\delta^{13}C$ or the partial pressures of CO_2 and O_2 in the atmosphere through time, which trend toward higher values throughout the Maastrichtian.[73,74]