Preliminary Notes on the First Recorded Amber Insects from the Hell Creek Formation

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ABSTRACT

Insects, the most diverse of living organisms today, inhabit virtually every terrestrial and freshwater ecosystem on earth. Yet the strata of the Upper Cretaceous Hell Creek Formation, although deposited in a luxuriant subtropical biome during the initial diversification of flowering plants, until now have revealed practically no insect fossils. Here, we provide a preliminary report on the discovery of the first amber insects from the Hell Creek Formation. This well-preserved assemblage of amber insects includes members of the Diptera (Suborders Nematocera and Brachycera) and Odonata (Suborder Zygoptera). The discovery will enable future studies to develop a better paleoecological understanding of the Hell Creek that includes the essential role of insects.

INTRODUCTION

The Hell Creek Formation, a fluvially deposited clastic wedge dating from the last ~2 million years of the Cretaceous (Frye, 1969; Hicks et al., 2002; Murphy et al., 2002), has been a focus of Upper Cretaceous research for over 100 years. Because it contains the Cretaceous-Tertiary (K-T) boundary, and its preponderance of well-preserved vertebrate remains, Hell Creek studies have focused on vertebrate faunas and their change across the K-T boundary (Fastovsky, 1987; Lehman, 1987; White et al., 1998; Russell and Manabe, 2002). In the past 20 years, the flora of the Hell Creek Formation has also been intensely studied, adding to our understanding of the subtropical Upper Cretaceous environment as well as the dynamics of insect herbivory based on insect-mediated damage (Labandeira et al., 2002; Nichols, 2002; Shockley and Oboh-Ikuenobe, 2010). Although some research has looked at the distribution and abundance of whole vertebrate faunas, especially in relation to the Western Interior Seaway (Bartlett, 1999, 2004; DePalma, 2010), insects have been conspicuously absent from most Hell Creek paleoecological analyses, and significant Maastrichtian amber insect localities are globally virtually nonexistent (Labandeira et al., 2002; David Grimaldi, Pers. Comm., 2010). Only recently have investigators begun to explore the role of insects in Hell Creek ecosystems. The main reason for this deficiency is that Hell Creek insect fossils are extremely rare, occurring mostly as traces, such as insect-mediated leaf damage and burrows (Labandeira et al., 2002). Although a handful of poorly preserved body fossils have been found in fine-grained strata, until now none have been reported from Hell Creek amber (Labandeira et al., 2002; David Grimaldi, Pers. Comm., 2010).

Expeditions led by Robert DePalma and Terry Smith in 2004 and 2005 led to the discovery of extensive amber deposits in the Upper Cretaceous Hell Creek Formation in Harding County, South Dakota. These contain the first reported Hell Creek amber insects (DePalma, 2010). The insect-bearing Hell Creek amber occurs in finely laminated, organic-rich siltstone deposited in paleo oxbow lake and marsh environments adjacent to a contemporaneous river and its tributary. Stratigraphic context of the site places the amber in the late Maastrichtian Upper Hell Creek regime (DePalma, 2010), more specifically in the HC II floral zone of Johnson (2002), which is less than 20 m (less than 1 million years) below the K-T boundary. Other evidence suggests that the western shoreline of the Western Interior Seaway was in close proximity at the time of deposition (10 km or less; DePalma, 2010).

METHODS

We systematically excavated the amber-bearing deposits after removing ~1m of overburden. The amber-bearing siltstones were excavated in 6cm-thick subsets, and the amber, plants, and vertebrate remains separated. The amber nodules were 0.5-6 cm in diameter (Fig. 1), and cohesive enough to remain intact without cyanoacrylate impregnation. We immediately placed amber removed from the matrix in opaque plastic or glass collecting vessels to exclude light. Damp cloth inserted in the collecting vessels served initially to maintain the moisture level close to that of the siltstone matrix. The samples were then allowed to dry slowly in the containers over a period of several weeks, until the moisture content equilibrated with the ~20% humidity of the

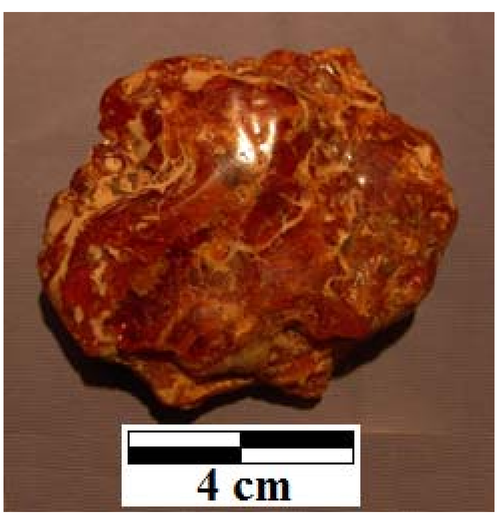

Figure 1. *An example of the unusually large and stable amber from the study area (PBMNH.P.09.001). This piece has been partially polished.*

laboratory. Exposure to sunlight and extreme temperature changes was prevented to avoid physically degrading the amber. Amber containing insect inclusions was reposited in the Palm Beach Museum of Natural History.

Initial inspection of the amber was facilitated by its inherent clarity and remarkable preservation, often with the original surface morphology of droplets and runnels intact. We separated amber specimens that either contained visible inclusions, or were suspected to do so, from the composite collection. We polished these specimens first, and, if necessary, examined the inclusions with Environmental Scanning Electron Microscopy (ESEM), and/or Micro-Computed Tomography (Micro-CT).

Because the amber had little to no surface crazing, we began the polishing process with 150-grit abrasive paper, gradually working down to 1200-grit paper. Final buffing with a cotton wheel rendered the amber surface transparent. In most cases, after such polishing, specimens were clear enough to study insect inclusions directly. However, internal fracturing in some specimens obscured the detail of insect inclusions, and such specimens were imaged with Micro-CT scans at the Centre for X-ray Tomography at Ghent University, Belgium. Scans of the amber samples were made on a 1µm resolution scanner with a transmission type X-ray source set at 70 kV and 100µA. The magnification was adjusted for each sample to maximize the resolution, resulting in a voxel size between 10 and 5 micrometers. The in-house developed reconstruction software Octopus (Vlassenbroeck et al., 2007) was used to calculate the cross-sections from the projection data.

Some insect inclusions were discovered in natural fractures in the amber. Even though such insect fossils were thereby damaged, this afforded an opportunity otherwise unavailable to examine internal soft anatomy using Environmental Scanning Electron Microscopy (ESEM).

RESULTS

We identified a total of 22 individual insects and numerous other inclusions in the ~400g of amber collected. We are currently conducting detailed taxonomic investigation of all insect inclusions, however at least 11 different insect morphotypes are already apparent based on gross anatomy.

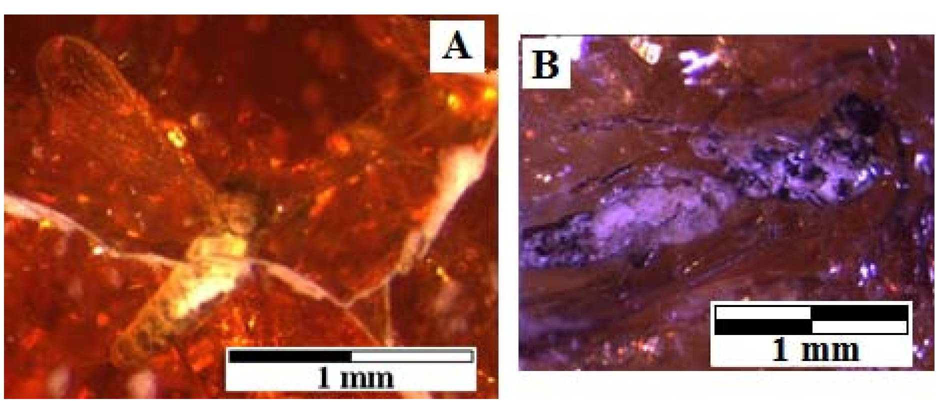

Figure 2. *Two dipterans located during the initial stages of visual inspection. (A) is a brachyceran (PBMNH.P.09.007), and (B) a nematoceran (PBMNH.P.09.010).*

Initial inspection revealed 10 whole or partial insects, comprising nine dipterans (six morphotypes) and one zygopteran (Michael Engel, Pers. Comm., 2009, 2010; Andre Nel, Pers. Comm., 2009). Polishing revealed five additional insects (two additional morphotypes), all of which were dipterans (Fig. 2). Four pieces of highly fractured amber containing insects were examined using Micro-CT, revealing seven more dipterans (three additional morphotypes) belonging to both the Nematocera and Brachycera (Fig. 3). We also examined insects that were naturally bisected and partially encased in broken amber fragments by ESEM, which revealed the extent of the excellent soft tissue preservation. Delicate tissues such as internal organs, muscle fibers, and proximal anchoring points of setae, were preserved in fine detail (Fig. 4).

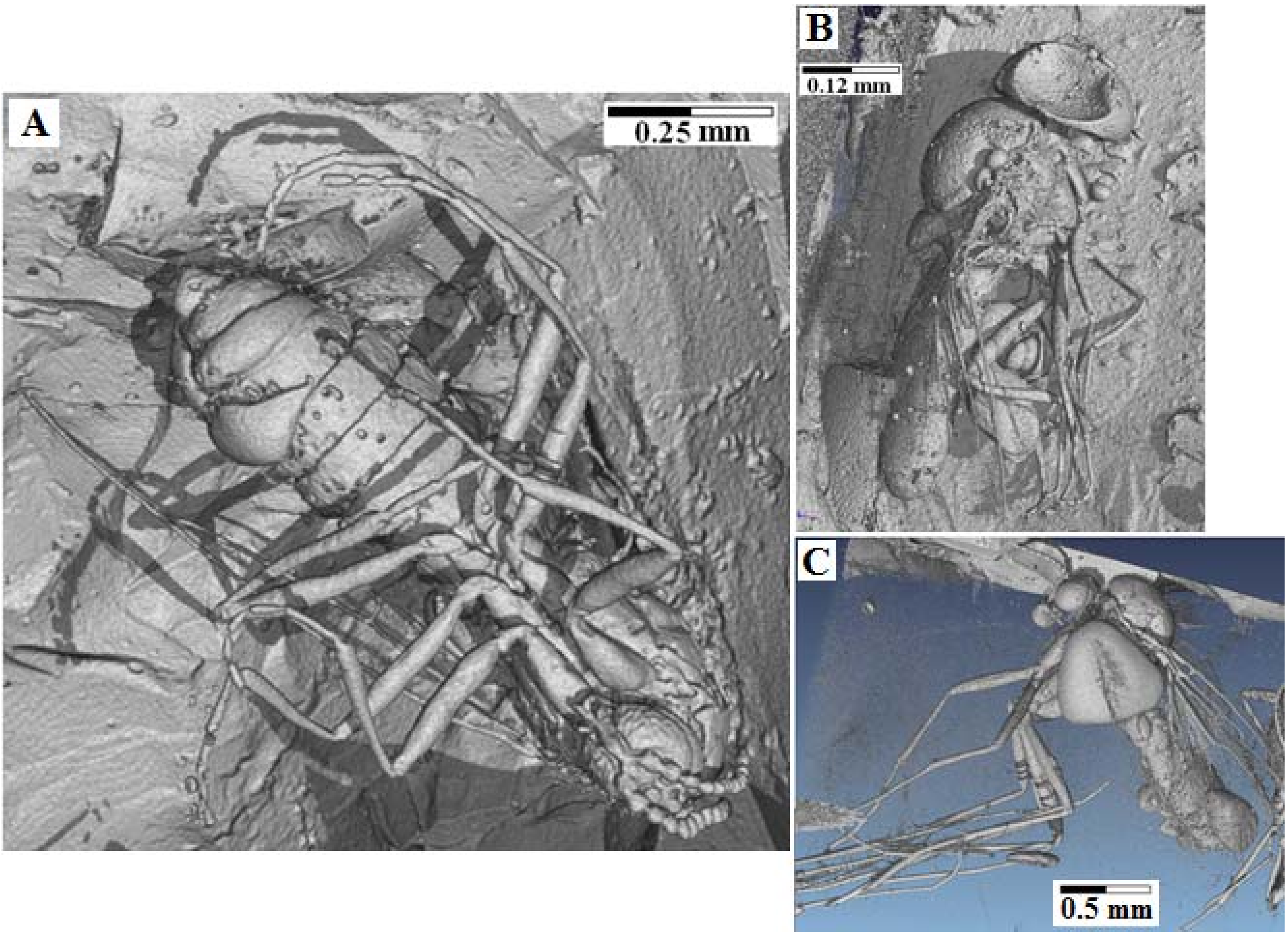

Figure 3. *Insects imaged using Micro-CT (PBMNH.P.09.020). Lateral and ventral views of nematocerans in (C) and (A), respectively. A brachyceran in lateral view (B). The outstanding preservation and completeness of the insects is immediately evident.*

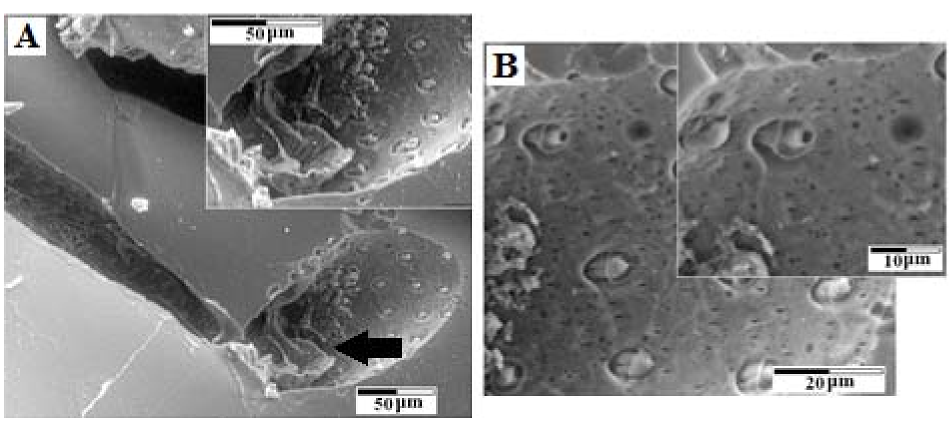

Figure 4. *Environmental Scanning Electron Micrographs (ESEM) of a fractured nematoceran leg, showing preservation of internal structures (PBMNH.P.09.019). Muscle fibers can be seen in (A), and anchoring points of the setae can be seen in (A) and (B).*

DISCUSSION

Our success in finding and visualizing high-quality insect fossils in Hell Creek amber is due to careful collection, preparation, and sophisticated imaging techniques. One critical step in collecting good amber specimens is to use opaque storage containers and to maintain an internal humidity close to that of the siltstone matrix so as to reduce subsequent fracturing due to rapid drying and exposure to sunlight. Ensuring that specimens are not exposed to extreme heat or cold also aids in long-term preservation. Because the amber containing insect fossils is much more stable than typical Hell Creek amber, we employed simple polishing to search for insect inclusions. Although polishing was extremely helpful in revealing such inclusions, it did not eliminate the obscuring effects of internal fractures or opaque clouds of microbubbles and organic smears. For this reason, Micro-CT was invaluable for revealing details of insect anatomy. Micro-CT scans produced spectacular three-dimensional images of the superbly preserved insects, and provided views that polishing, alone, could not have. Although Micro-CT provided images sufficient for basic taxonomic assignment, it could not reveal certain minute structures of taxonomic significance, such as setae. This precluded lower level taxonomic placement of some specimens. In instances where natural fractures exposed the interior of an insect's body, ESEM scans revealed infinitesimally small parts of the exquisitely preserved internal anatomy to a greater level of detail than the Micro-CT scans. However, since ESEM can only resolve exposed structures, it cannot be used to study the internal anatomy of intact insects.

Even our preliminary survey of the new amber insects enormously increases knowledge of Upper Hell Creek insects, particularly Diptera. The Diptera are the third largest order of insects today, and that alone makes them ecologically important. Moreover, dipterans are eminent pollinators and decomposers, integral to many food chains, and one of the most significant vectors of disease among terrestrial animals (Labandiera, 2005). And, larval dipterans (many of them aquatic) likely played a pivotal role in the Type I ecologies of the Hell Creek ecosystem (Olson, 1966; DePalma, 2010). Until now, the insect component of the Hell Creek Formation was known only poorly, through trace fossils such as insect-mediated leaf damage and burrows, and from a few poorly preserved and indeterminate body fossils (Labandeira et al., 2002). Our discovery of amber insects remedies this, showing that the Hell Creek ecosystem supported a dipteran community that was both diverse and thriving. The dipteran and zygopteran amber insects we have discovered fill an important gap in the catalog of Maastrichtian insect diversity and in our understanding of Hell Creek paleoecology.

CONCLUSION

Although insects play an important role in the ecology of virtually all terrestrial and freshwater aquatic biomes, our knowledge of Hell Creek insects previously has been limited mainly to trace fossils. However, newly discovered fossil insects in Hell Creek amber now reveal a once thriving insect community, including dipterans and zygopteran Odonata. Moreover, preliminary assessment suggests that the Hell Creek dipteran fauna was quite diverse. Since modern dipteran communities play an important ecological role worldwide, the same can be expected of Hell Creek dipterans. Further study of Hell Creek amber insects, now underway, promises to refine our understanding of Upper Cretaceous dipterans, dipteran evolution, and insect paleoecology. And, our collection, preparation and imaging methods can serve as a model for future amber insect research.

ACKNOWLEDGEMENTS

Gratitude and appreciation go to the following people and institutions: Terry, Randolph , and Ruth Smith, Dr. Robert DePalma, Mindy Cox, Peter and Neal Larson and the Black Hills Institute of Geological Research, Walter Stein and family, Dr. Fred Cichocki, Andrew Tyson, Robert Feeney, Petra Tuns, Fallon Cohen, Nancy Ambrose, the Palm Beach Museum of Natural History, The Graves Museum of Archaeology and Natural History, Dr. Larry Martin, Dr. David Burnham, Dr. Paul Selden, Dr. Michael Engel, the University of Kansas Department of Geology, the University of Kansas Department of Biology, Dr. Manuel Dierick, Kim Holrah, Dr. Emmanuel Paul, Dr. Anton Oleinik, Dr. Edward Petuch, Bill Lindsey and Family, Sam Marty and family, Dr. Philip Bjork, Dr. David Grimaldi, Dr. Kenneth Carpenter, Dr. Robert Bakker, and John Gurche.

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