**Ant queen:** the egg-laying caste of an ant colony; also called a gyne

**Paleosol:** fossilized soil

nests have the greatest chance of burial in lake deposits. Species that spend most of their time searching and mating on the ground are far less commonly encountered as imprint fossils. In contrast to amber fossils, imprint fossils generally preserve larger ant species. Imprint fossils are found by splitting stones, and as a consequence, small specimens are easily overlooked. It is perhaps not surprising then that most ant imprints are dominated by species with large queens, such as *Oecophylla* in Bembridge deposits and the giant *Titanomyrma* in Messel and Green River deposits (up to 60 mm long!).

## Ichnofossils

In addition to body fossils, the presence of ants can be recorded through ichnofossils (or trace fossils), i.e., the traces of their activity in paleosols such as burrows and nests. In the case of recent taxa, the nest architecture can be very distinctive (107), but such architectures are not found in Mesozoic ichnofossils. The identification of ant nests from the Mesozoic is particularly important because they potentially predate the earliest occurrence of body fossils. Unless body fossils are found associated within such nests, however, it remains virtually impossible to identify the kind of ant that built the nest and even to determine whether the tracemaker was an ant, because different groups of animals may have evolved similar burrowing techniques (100). For instance, distinguishing between traces of social insects such as ants and termites can be particularly difficult (38). The oldest trace fossils that have been suggested to be ant ichnofossils are from the Late Jurassic of Colorado (47, 48), but this remains highly controversial and was later dismissed by other authors (10, 38). The oldest nest that seems attributable to ants is from the Late Cretaceous of Utah (92).

## New Imaging Techniques

Traditional light microscopy has long been the only technique available to study fossil insects preserved as imprints or amber inclusions. It is still largely in use because it allows for the observation of most external characters. But critical structures can be inaccessible owing to the position of the specimen or, in the case of amber, they can be hidden by turbidity, debris, bubbles, or other inclusions between the amber surface and the insect. Imaging techniques such as X-ray computed tomography (CT) or microtomography (μ-CT) are now increasingly used in amber studies, which provide a three-dimensional virtual reconstruction of the fossils (17, 45, 51). Propagation phase-contrast X-ray synchrotron imaging (PPC-SRμCT), which has been developed specifically for amber inclusions (60, 105), allows for high-resolution reconstructions as well as virtual dissections (**Supplemental Figure 5**), thus providing access to all external and internal features (80). The increasing use of PPC-SRμCT might help reduce the gap between fossils, especially controversial Cretaceous fossils, and extant species of ants.

Supplemental Material

# THE PROBLEM OF RECOGNIZING TRUE ANTS IN THE EARLY FOSSIL RECORD

A critical discussion of ant fossils first needs to establish what synapomorphies are used to define the family Formicidae. For examination of fossils these are largely going to be morphological, although, as seen below, behavioral synapomorphies can be inferred from fossils as well. There are generally four widely accepted morphological synapomorphies that define modern ants (considered here as ants from the Tertiary to recent periods). These are an elongated scape, geniculate antennae, petiole, and metapleural gland. The elbowed (geniculate) shape of the ant