White Paper

Detailed imaging of exceptionally preserved microfossils to track events in early animal evolution

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New laboratory techniques allow the extraction of exceptionally delicate microfossils from samples of mudrock, yielding new insights into the fine-scale features of extinct animals. However, highly sensitive imaging techniques are required to capture the full range of preserved details, which are critical for accurate palaeobiological interpretation.

Introduction

Over the past few decades, palaeontologists have intensively studied the fossil record of spores, pollen and marine plankton to gain insights into evolutionary patterns, ancient environments, and the distribution of petroleum reservoirs. Special laboratory techniques have been developed to extract these microscopic fossils from rocks, which by virtue of their carbonaceous ("organic walled") composition can be isolated using a variety of inorganic acids.

Recently, however, it has become clear that a variety of larger and more delicate carbonaceous fossils can be extracted using a modified laboratory procedure (Butterfield and Harvey, 2012). These "small carbonaceous fossils" (or SCFs) commonly include the cuticular remains of animals, and are particularly well preserved in rocks of Cambrian age (i.e., half a billion years old). Therefore, SCFs offer a new window onto the earliest evolution of arthropods, worms and other cuticle-bearing animal groups, often revealing fine-scale details that are not preserved elsewhere in the fossil record. However, the challenge is to observe and image the SCFs in sufficient detail to resolve pivotal features of their anatomy for reconstructing their place in the tree of life, and their significance for early ecosystems. Although scanning electron microscopy is useful to an extent, high-resolution transmitted light microscopy is necessary to reveal fine details of the external, internal and superimposed parts of the fossils. This has been particularly true for the description of some recently discovered SCFs which include the earliest records of shrimp-like crustaceans and microscopic loriciferan worms – two groups which record milestones in the diversification of animal life and the establishment of marine ecosystems.

Methods

Small carbonaceous fossils (SCFs) were extracted from mudrock samples of Cambrian age from subsurface Saskatchewan, Canada, using a laboratory procedure described by Butterfield and Harvey (2012). Individual microfossils were mounted onto glass microscope slides using a small hand-held eye-dropper, and fixed using an epoxy resin of similar refractive index to the glass slide and cover-slip. Microfossils were observed and imaged using a Zeiss Axio Imager M2m at increasingly high magnification (x 2.5, 10, 20, 40 and 100 objectives), with the use of differential interference contrast (DIC, aka Normarski) optics at higher magnification. DIC is crucial for resolving the very smallest structures in the fossils, such as tiny hairs (setules) less than 1 µm in diameter. Images were produced using automated Z-stack photography to obtain fully-focussed images from multiple focal planes.

Results

The best-preserved SCFs retain enough fine-scale anatomy for a detailed assessment of their biological affinities, ecological role and evolutionary significance. For example, some fragments of arthropodan cuticle can be shown to preserve structures that are unique to crustaceans (Harvey et al. 2012).

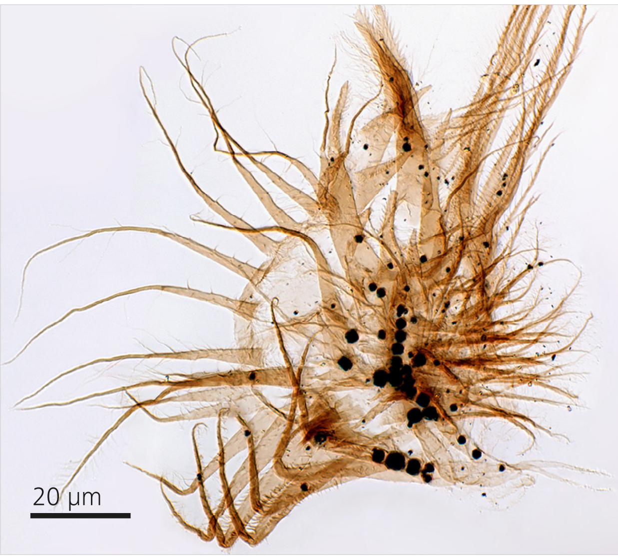

Figure 1 *A fragment of crustacean filtering limb preserved as a small carbonaceous fossil (SCF) from the Cambrian period of Saskatchewan, Canada. The fossil consists of a flattened carbonaceous film derived from the cuticle of the animal, obscured in places by opaque crystals of iron sulphide (pyrite).*

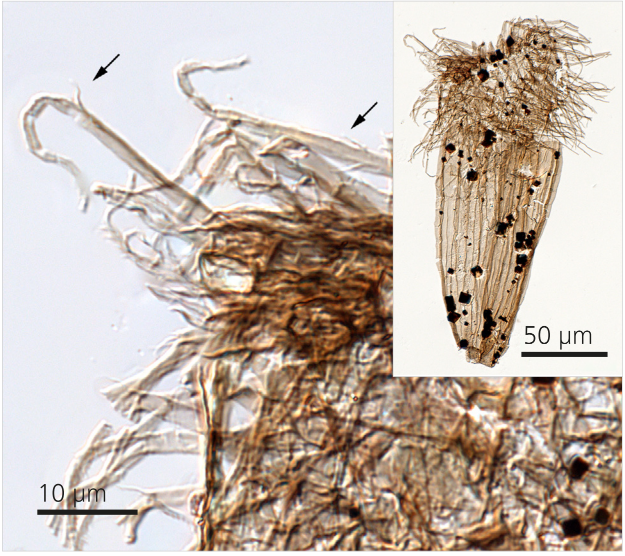

Figure 2 *An exceptionally well-preserved fossil loriciferan worm, extracted using acid from a sample of Cambrian-age mudrock from Saskatchewan, Canada. The fine bristle-like appendages of the head are preserved in exquisite detail, including the sub-micron-scale sensory setules (arrows).*

In particular, it is the arrangement and spacing of fine hairs (setules) arising from larger hairs (setae) that diagnose crustacean-type filtering appendages (Figure 1). In life, these would have been borne on the legs and mouthparts of shrimp-like animals, and used to capture micron-scale food particles from the water. Such intricate structures were previously thought to have evolved much later in the history of arthropods, so their discovery in Cambrian rocks points to a cryptic early radiation of crustaceans.

Other SCFs are derived from another cuticle-bearing animal group, the loriciferan worms (Harvey and Butterfield 2017). Loriciferans are a little-known group of exclusively microscopic animals, only discovered in modern marine sediments in the 1980s, and previously unknown in the fossil record. Most of the loriciferan fossils consist only of the body, but one specimen preserves an intact head (Figure 2).

Because some other groups of worms have similar body-plans, a detailed examination of the very smallest features is required to test between different hypotheses of affinity. However, the number and arrangement of the fine head bristles (scalids), combined with the presence of sub-micron-scale scalid adaptations for sensory functions (Figure 2, arrows), demonstrate that the fossils represent true loriciferans. The confirmed presence of loriciferans in the Cambrian period (around 500 million years ago) provides a crucial calibration point for molecular-based estimates of the timeline of animal evolution. The extremely small size of the fossil loriciferans (< 300 µm) proves the long history of a specialized animal "meiofauna" living among grains of sand on the sea-bed. Clearly, the fine-scale preservation seen in small carbonaceous fossils has much to tell us about the tempo and mode of early animal evolution.

References:

Butterfield, N.J., and Harvey, T.H.P., 2012, Small carbonaceous fossils (SCFs): A new measure of early Paleozoic paleobiology: Geology, v. 40, p. 71–74, doi: 10.1130/G32580.1.

Harvey, T.H.P., and Butterfield, N.J., 2017, Exceptionally preserved Cambrian loriciferans and the early animal invasion of the meiobenthos: Nature Ecology & Evolution, v. 1, p. 0022, doi: 10.1038/s41559-016-0022.

Harvey, T.H.P., Vélez, M.I., and Butterfield, N.J., 2012, Exceptionally preserved crustaceans from western Canada reveal a cryptic Cambrian radiation: Proceedings of the National Academy of Sciences, doi: 10.1073/pnas.1115244109.

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