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# Chemical clues to the earliest animal fossils

Steroid biomarkers show that the Ediacaran fossil *Dickinsonia* was an animal

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The Ediacara biota are a diverse assemblage of macroscopic body forms that appear in the sedimentary rock record between 570 and 541 million years ago. First recognized in Namibia and Australia, these remarkable organisms have since been found in Russia, China, Canada, Great Britain, and other regions. Although they immediately preceded the rapid appearance and diversification of animals in the Cambrian (541 to 485 million years ago), their position within the tree of life has long been a puzzle. Some Ediacaran fossils appear segmented, but most lack obvious characters such as appendages, a mouth, or a gut that might link them to animal clades. On page xxx, Bobrovskiy *et al.* use biomarkers to confidently assign at least one Ediacaran group, *Dickinsonia* and related taxa, to the Metazoa (animals) (1).

The Ediacara biota were first described at a time when paleontologists relied upon overall similarities in form to establish phylogenetic affinities. Various Ediacaran groups were allied with later animal clades based on such similarities. Thus, *Dickinsonia* and other apparently segmented taxa were considered annelids, *Rangaea* a pennatulacean cnidarian, and *Parvancorina* a likely arthropod (2). In 1992, Seilacher instead proposed that most of these organisms were part of a single clade that formed a separate, independent kingdom of complex multicellular organisms, the Vendozoa (3). After this, scientists considered a range of novel solutions, and the fossils were allied with a wide array of clades.

More recent systematic inquiry has focused on identifying discrete morphological characters. There has been growing consensus that the Ediacaran biota does not represent a single clade, but a diversity of taxa, mostly of Metazoa (4, 5). Based on a detailed analysis of the features of *Dickinsonia* fossils, Gold *et al.* have suggested that they were animals with bilateral symmetry, like most animals today (6). Thus, we can identify dis-

crete groups among the fossils, but how they relate to Cambrian and later clades has remained unresolved. Bobrovskiy *et al.* now resolve this controversy for *Dickinsonia* and *Andiva*, quilted forms with a medial suture, based on biomarkers associated with the fossils.

Almost all eukaryotes produce and use sterols, a class of lipids that perform vital membrane behavior and cell signaling functions. Following death, these sterols are transformed into more stable sterane hydrocarbons, which may be recovered from sediments provided that they have not been highly altered by subsequent metamorphism. Sterols and steranes can be particularly useful as biomarkers, because some aspects of sterol structure are specific to particular source organisms (7). For example, Love *et al.* identified 24-isopropylcholestane, which is prevalent in several sequences of rocks between 660 million and about 535 million years ago, as a steroid characteristic of demosponges, (8).

Bobrovskiy *et al.* use an approach designed to identify diagnostic hydrocarbon biomarkers associated with individual fossils preserved in remarkably unaltered, fine-grained sediments of the White Sea region in Russia. In earlier work, they isolated an organic biofilm associated with circular impressions known as Beltanelliformis, analyzed the entrained hydrocarbons, and assigned a colonial cyanobacterial origin (9). For the current study, the authors sampled thin layers of organic material associated with fossils of *Dickinsonia* and *Andiva* from the same sequence of rocks for biomarkers. They also analyzed samples of adjacent layers of sediment to control for more broadly distributed biomarkers.

The surrounding sediments contained diverse steranes, most of which comprised compounds with 29 carbon atoms (stigmasteroids) that are diagnostic of green algae. In contrast, the steroids in the *Dickinsonia* fossil biofilms were almost exclusively composed of compounds with 27 carbon atoms (cholesteroids), a signature of all animal phyla other than sponges and a few mollusk taxa. The steroid assemblages from the smaller *Andiva* fossils were confounded by

background signals and harder to interpret.

The cholesterol predominance from *Dickinsonia* allowed Bobrovskiy *et al.* to refute alternative hypotheses of their affinities to lichens or large protists. Nevertheless, the White Sea *Dickinsonia* organic remains are enigmatic in another, biologically striking way. The sterols of living organisms are produced with a single three-dimensional configuration, or stereochemistry, but the steroids preserved in sedimentary rocks are typically mixtures that include more reduced and thermodynamically stable forms, as a result of chemical transformations during burial and subsequent heating. In contrast, the *Dickinsonia* cholesteroloids mostly have the same 5 $\beta$ (H) stereochemistry. The only known pathway to this steroid, informally termed coprostanane, is via the steroid coprostanol, which is produced in the gut of higher mammals (11–13). Coprostanane is thought to be unstable on geological time scales (10). Bobrovskiy *et al.* attribute the presence of these unusual steroids to reduction of *Dickinsonia* cholesterol by bacteria during the original decomposition of the animal. Yet, coprostananes are absent in much younger, exceptionally preserved animal fossils, where the dominant steranes are 5 $\alpha$ (H)-cholestanes (14). The association of unusual steroids associated with *Dickinsonia* suggests that it may have had a distinct metabolic physiology.

Molecular clock evidence suggests that animals originated before 720 million years ago, although the pattern of their divergence during the Cryogenian (720 million to 635 million years ago) and Ediacaran (635 million to 541 million years ago) remains unresolved (15). As molecular clock estimates and morphological characters from fossils offer limited resolution, our best hope for unraveling the early history of animals and the affinities of the Ediacara Biota lies with identifying biomarkers that allow us to differentiate specific metazoan clades, particularly among the bilaterians. Further refining the phylogenetic signals from biomarkers may also help to resolve the early history of animals during the Cryogenian and early Ediacaran. Moreover, the fossil-specific biomarker approach taken by Bobrovskiy *et al.*

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promises to yield many new insights into the fossilization processes leading to soft-tissue preservation across the animal kingdom and throughout geological time.

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