

Protein and chitin preservation in polymeric sheets in Miocene *Ecphora gardnerae* shells from Maryland, USA

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The shells of muricoid gastropod *Ecphora gardnerae* from the Miocene St. Marys Formation of Maryland, USA, have been shown to contain polymeric sheets within the calcareous matrix. These membranes were found to have amino acids and complex sugar compounds. To explore these biomolecular features further, four new *E. gardnerae* shells were collected and analyzed for proteins and sugar compounds. Using direct analysis in real time (DART) mass spectrometry, we were able to show that the membranes contain chitin. With mass spectrometry-based paleoproteomic techniques utilizing a hydroxylamine extraction or sample preparation by easy extraction and digestion (SPEED), we found preserved peptides from all four shells including peptides that are specific to Gastropoda or Mollusca. The membranes derive from the calcitic layer of the shell and are likely analogous to chitin membranes used by other extant mollusks to control nucleation, growth, and physical properties in the calcareous layers, but future research in gastropod biology will help to address the function of these membranes.

Key words: Gastropoda, Muricidae, *Ecphora*, paleoproteomics, DART-MS, chitin, Miocene, USA.

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