SUPPLEMENTAL TEXT: DETAILED METHODS

Samples and preparation

Pinnids are ideal for observing the effects of fossil preservation on ultrastructure and interprismatic organic compounds. *Pinna* shells are composed of an outer prismatic and an inner nacreous layer. The prismatic layer is formed by calcite crystals (Gilbert et al., 2011) surrounded by an organic matrix, or envelope – the interprismatic matrix (IM; Dauphin et al., 2010; Dauphin et al., 2003). The inner nacre layer consists of aragonite crystals, with characteristic iridescence often preserved in fossils. Iridescence is generated by the lamellar periodic structure of alternating organic sheets (Addadi et al., 2006) and aragonite tablet layers (Wise, 1970), with thickness comparable to visible light wavelengths. The prismatic and nacre layers are often separated in *Pinna* fossils.

*Pinna* fossils date back to the Mississippian Period (∼345 Ma), and the genus includes 15 extant species (Cash, 1882; Gilbert et al., 2017; Lemer et al., 2014; Rosewater, 1961; Schultz and Huber, 2013; Turner and Rosewater, 1958; Wheelton, 1905). Sampling only within *Pinna* allows for a direct comparison between closely related taxa, minimizing potential effects of evolutionary change in processes or patterns of biomineralization (Jope, 1969). Pinnids are not uncommon in warm, nearshore to offshore environments, and tend to form thickets (Butler and Brewster, 1979; Idris et al., 2008). Consequently, pinnids are reasonably abundant in both the modern and fossil records, minimizing biases related to rarity (Gilbert et al., 2017; Lemer et al., 2014; Schultz and Huber, 2013). For additional information on basic pinnid biology and evolution, see (Gilbert et al., 2017; Lemer et al., 2014; Schultz and Huber, 2013).

Two specimens of *Pinna* were analyzed here: **Pn1** is a modern specimen of *Pinna nobilis*, collected live on the coast of Mallorca, Spain, in 1991. The specimen (MCZ-371544) is accessioned in the Malacology Collection at the Museum of Comparative Biology, Harvard University. **Ps6** is a specimen of *Pinna* sp. from the Late Cretaceous Owl Creek Formation (Maastrichtian Stage ∼ 66 Ma), collected in 2010 from the type locality in Tippah Co, MS. The specimen is accessioned (AMNH-99982) in the Division of Invertebrate Paleontology at the American Museum of Natural History. Precise locality data may be provided by these repositories upon request.

Both specimens were washed in ethanol, air-dried, embedded to prevent breaking when cutting. Then a ∼1cm² sample in the area of thickest nacre (near the umbo) was cut from the shell with a diamond saw. This sample was embedded in epoxy, polished, and coated with a conductive layer of Pt for PEEM analysis following the methods of (De Stasio et al., 2003; DeVol et al., 2014; Gilbert et al., 2000; Gilbert et al., 2017). Shell mounts were oriented with shell layers perpendicular to the polished surface within ±5° to maximize comparability, and to expose a shell cross-section with both nacre and prismatic layers, including organic envelopes. Quality of preservation was assessed through analysis of shell mineralogy, ultrastructure, and crystal orientation of both the nacreous and prismatic regions of prepared shells. Organic
compounds were analyzed from the interprismatic organic envelope separating the nacreous and prismatic layers, and between calcite prisms (white bars in Fig. 1).

**Spectroscopy**

We used the methods of Gilbert et al. (2017) to analyze modern and Late Cretaceous *Pinna* shell ultrastructure and interprismatic organics using PhotoEmission Electron spectroMicroscopy (PEEM). This method enables acquisition of both X-ray Absorption Near-Edge Structure (XANES) spectroscopy (Stöhr, 2013) and Polarization-dependent Imaging Contrast (PIC) mapping (DeVol et al., 2014; Gilbert, 2012; Gilbert et al., 2011; Metzler et al., 2007; Metzler et al., 2008; Pokroy et al., 2015). PEEM analysis utilizes a tunable source of soft x-ray photons for XANES spectroscopy and an elliptically polarizing undulator (EPU) insertion for PIC mapping. PEEM works by collecting electrons photo-emitted under x-ray illumination, magnifying the electron image, and converting it to a visible light image that is collected by a computer in real time. As the illuminating x-ray energy is scanned, stacks of images are saved in which each pixel contains the complete spectrum across an absorption edge (carbon or oxygen K-edge here). Similarly, in PIC mapping stacks of images are saved as the linear polarization angle is scanned by the EPU. PEEM is thus used either as a spectromicroscope to identify compounds or minerals or as a PIC mapper to identify crystal orientations. In both modes the spatial resolution is 20 nm at best, 60 nm here, with a field of view of 60 µm. Individual analysis methods are briefly described below; for additional, detailed methodological detail regarding sample acquisition and preparation, PEEM analysis, and XANES spectroscopy, see (Gilbert et al., 2017).

**PIC mapping**

Polarization-dependent Imaging Contrast mapping (PIC mapping) was used to observe the preservation of nacre and prismatic layer ultrastructure in modern and fossil *Pinna* shells. Each PIC map was composed of a stack of 19 images acquired with the photon energy set at the oxygen K-edge to observe the main polarization-dependent peak in carbonates (534 eV)(DeVol et al., 2014; Gilbert et al., 2017; Gilbert et al., 2011; Metzler et al., 2007; Metzler et al., 2008). Images were acquired by rotating the linear polarization angle between 0° and 90° over 5° steps; images were stacked using default parameters in the Polarization Analysis Package from Gilbert Group Macros (GG–Macros, 2017) and run in Igor Pro Carbon®. Note that the beam illuminates the sample surface from the right, at 30° grazing incidence; thus, the polarization plane is rotated from the image plane by 60° around the vertical (in the laboratory and the image), from the left. Color in PIC maps represents the in-plane and off-plane angles formed by the crystallographic c-axis and the polarization plane. The c-axis projected onto the polarization plane is termed c’-axis, and the angle it forms with the vertical in the polarization plane is termed c’-angle (DeVol et al., 2014; Gilbert et al., 2011; Pokroy et al., 2015). A vertical c’-axis (0°) is shown as cyan; a horizontal c’-axis (±90°) is shown as red; intermediate c’-axis orientations follow the color legend in Figure 1. The c-axis off-plane angle is displayed as brightness, with black indicating 90° off-plane (directly into the X-ray beam) and full-brightness-colors in-plane (Gilbert et al., 2017). Black may also represent non-crystallographic material, such as organic compounds visible surrounding individual crystals (Fig. 1), or missing crystals.

**XANES spectroscopy**
XANES oxygen K-edge spectroscopy was used to detect the mineral preservation of aragonite nacre and calcite prisms in modern and fossil *Pinna* shells. For oxygen spectra the x-ray photon energy was scanned between 525 – 555 eV, with a 0.1 eV energy step size between 530 – 545 eV for fine structural resolution, and a 0.5 eV step size in the featureless region. 181 images (10^6 pixels each) were captured with 55 x 55 μm size. Spectra were then extracted from 31 x 31 pixel square microscopic regions (55 nm each), representing an area of 1.705 μm^2. The extracted spectra were normalized by dividing by an Io background acquired from freshly deposited, uncontaminated thick Pt. Stacking of images, extraction and normalization of spectra were done using GG-Macros (GG-Macros 2017).

As observed by (DeVol et al., 2014), aragonite and calcite have different oxygen K-edge spectra (aragonite displays six total peaks and calcite only four). In both minerals the first peak at 534 eV indicates the O_{1s} → π* transition associated with the carbonate π*C–O bond. This peak is most sensitive to the crystal orientation direction, and is the one used for PIC mapping. The second peak is a polarization-independent peak associated with the O–Ca bond, and the remaining peaks represent the carbonate C–O bond with O_{1s} → σ* character (σ* peaks). Thus, aragonite displays five characteristic σ* peaks and calcite only three.

We utilized XANES carbon K-edge spectroscopy to observe the state of biochemical bonds within the interprismatic organic matrix of modern and fossil *Pinna* shells. For carbon spectra the photon energy was scanned between 275 – 325 eV, with 0.1 eV and 0.5 eV step sizes following (Boyce et al., 2010). Many authors have previously assigned each carbon peak to a specific bond, e.g. (Benzerara et al., 2006; Boese et al., 1997; Boyce et al., 2010; Brandes et al., 2004; Kaznacheyev et al., 2002; Myneni, 2002; Solomon et al., 2012). Carbon spectra were extracted from microscopic areas along the white segments in Figure 1, and normalized to an uncontaminated Pt Io again using GG-Macros (GG–Macros, 2017).

**REFERENCES**


Schultz, P. W., and Huber, M., 2013, Revision of the worldwide recent Pinnidae and some remarks on fossil European Pinnidae, Öhringen, Germany, ConchBooks.


Figure S1. Specimens used in this study: modern *Pinna nobilis* (MCZ-371544); (A) interior of both valves showing nacre in the anterior half of each shell; (B) anterior end of the left valve in (A). The sample for PEEM analysis was taken from the area of the thickest nacre just posterior to the anterior adductor muscle scar (box outline). Late Cretaceous *Pinna* sp. (AMNH-99982); (C) interior showing only nacre; (D) exterior showing darker prismatic layers and brighter exposed nacre in the top right quarter. The sample for PEEM analysis was taken from the box outline.