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Fossil Corals With Various Degrees of Preservation Can Retain Information About Biomineralization-Related Organic Material

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Scleractinian corals typically form a robust calcium carbonate skeleton beneath their living tissue. This skeleton, through its trace element composition and isotope ratios, may record environmental conditions of water surrounding the coral animal. While bulk unrecrystallized aragonite coral skeletons can be used to reconstruct past ocean conditions, corals that have undergone significant diagenesis have altered geochemical signatures and are typically assumed to retain insufficient meaningful information for bulk or macrostructural analysis. However, partially recrystallized skeletons may retain organic molecular components of the skeletal organic matrix (SOM), which is secreted by the animal and directs aspects of the biomineralization process. Some SOM proteins can be retained in fossil corals and can potentially provide past oceanographic, ecological, and indirect genetic information. Here, we describe a dataset of scleractinian coral skeletons, aged from modern to Cretaceous plus a Carboniferous rugosan, characterized for their crystallography, trace element composition, and amino acid compositions. We show that some specimens that are partially recrystallized to calcite yield potentially useful biochemical information whereas complete recrystalization or silicification leads to significant alteration or loss of the SOM fraction. Our analysis is informative to biochemical-paleoceanographers as it suggests that previously discounted partially recrystallized coral skeletons may indeed still be useful at the microstructural level.

Keywords: amino acids, trace elements, X-ray diffraction, mineralogy, scleractinians

INTRODUCTION

Fossil stony corals (phylum Cnidaria, class Anthozoa) are valuable archives for paleoclimatic studies. Elemental and isotopic compositions of their aragonite skeleton can reflect environmental and chemical conditions of seawater in which they grew (e.g., Cohen and McConnaughey, 2003), allowing reconstruction of past seawater conditions (e.g., Watanabe et al., 2001; Gothmann et al., 2015). Proteins, polysaccharides, and lipids comprise the skeletal organic matrix (SOM), which is secreted by cells of the calicoblastic tissue layer of the animal (Puverel et al., 2005; Mass et al., 2014; reviewed by Drake et al. (2020a). Of these matrix components, proteins are the best studied; their

preservation in the mineral can lead to inferences of function and provide indirect genetic information in the absence of preserved DNA (e.g., Schroeter et al., 2017). Recent analyses have shown that intact endogenous peptides remain in invertebrate skeletons for 1000s–100,000s of years (Sakalauskaite et al., 2019; Drake L. et al., 2020b; Sakalauskaite et al., 2020). Across various taxa, intact peptides may retain amino acids longer in fossil specimens than if those biomolecules exist individually as free amino acids (Bada et al., 1999), allowing for detection of ecological and ocean chemistry signatures including the occurrence of photosymbiosis in fossil scleractinian corals (Muscatine et al., 2005; Tornabene et al., 2017) and past local nitrogen cycling regimes (Wang et al., 2015).

Nevertheless, the utility of either component of a fossil coral, inorganic or organic, is limited by its preservation (Nothdurft et al., 2007; Rabier et al., 2008). Fossil corals can undergo submarine and/or subaerial post-depositional alterations including dissolution of primary aragonite, precipitation of secondary aragonite cements and/or high-Mg calcite, and recrystallization from aragonite to calcite, with subsequent alteration of the original aragonitic geochemical signature (Brand and Veizer, 1980; Sayani et al., 2011; Griffiths et al., 2013) and potential degradation of proteins with loss of amino acids (Tomiak et al., 2013; Tomiak et al., 2016; Drake et al., 2020b). All primary aragonite corals with minimal macro- and micro-structural inorganic degradation are considered equally, but partially recrystallized corals are often wholly excluded from bulk analysis (e.g., Gothmann et al., 2015), or their recrystallized portions are removed prior to further analysis (e.g., Tornabene et al.). The organic fraction of coral skeleton is typically ~1% or less (Wainwright, 1963), providing insufficient material for microanalysis if it is significantly degraded, particularly if peptide sequences are the target (Peled et al., 2020). While geochemical signatures may be altered in partially recrystallized samples (McGregor and Gagan, 2003; Allison et al., 2007; Sayani et al., 2011), it is not yet clear whether amino acid data from those samples remain useful.

Methods for determining mineralogy, trace element composition, and amino acid characteristics of coral skeletons are well-established but are not frequently used together. Crystallographic determination of calcium carbonate minerals can be performed non-destructively using x-ray diffraction (XRD) on ground skeleton powders and can be conducted quantitatively (Chung, 1974). Geochemical analysis of element/Ca in such powders by mass spectrometry requires small sample sizes (<100 μg) and is destructive, yielding relative abundance information for traditionally measured elements including Li, B, Mg, Sr, Cd, Ba, U, Na, Mn, Fe that are then standardized to the Ca signal (Yu et al., 2005; Misra et al., 2014; Guillermic et al., 2020). Amino acid composition and racemization analysis is a destructive method and requires a larger amount of skeleton material (typically 1–10 s mg), yielding data about a subset of the 20 common naturally occurring amino acids (Kaufman and Manley, 1998). Amino acid racemization is the reversible process wherein one enantiomer converts to the other over time and is affected by environmental conditions such as temperature (Neuberger, 1948). As most organisms on Earth produce and use the L enantiomer of amino acids (Bai et al., 2009; reviewed by Genchi (2017), the initial conversion is generally considered to be from the L to D form; D/L ratios of certain amino acids can thus be applied as a relative or specific dating tool for fossils from the late Pleistocene to the present (Bada, 1985; Kaufman, 2003; Demarchi, 2020). Use of all three tools for the same specimens can therefore clarify the preservation of not just the abiotic component of fossil corals, but the biomolecular component as well, while using a relatively small amount of material.

Here we describe our analysis of coral skeletons ranging in age from modern to putatively Cretaceous, plus a Carboniferous rugosan coral. We analyzed the mineralogy, geochemistry, and amino acid characteristics for these corals and show that even partially recrystallized fossil coral skeletons likely retain important biochemical information. This work provides a framework for biochemists and paleoceanographers to consider expanding the pool of potential specimens for analysis.

METHODS

Samples

Fossils specimens were loaned from the Natural History Museum of Los Angeles County (NHMLAC) Invertebrate Paleontology Department, American Museum of Natural History (AMNH) Paleontology Division, Yale Peabody Museum (Peabody) Invertebrate Paleontology Division, and Harvard Museum of Comparative Zoology (MCZ) Invertebrate Paleontology Collection (**Table 1**) (Zoology, 2020). One modern coral was loaned from the NHMLAC Marine Biodiversity Center while another was provided from a private research collection. Provenance of fossil corals is reliant on collection location information on file at the loaning museums and in some cases age assignments are questionable based on the scientific literature (**Table 1**). For instance, we can with some confidence assign the Pleistocene *Montastraea cavernosa* and *Orbicella annularis* corals to stage 5e of the Key Largo formation (Hendy et al., 2020); however, we cannot absolutely rule out that it was actually derived from stage 4 (Multer et al., 2002; Muhs et al., 2011). Elemental, mineralogical, amino acid, and proteomic data for two fossil *O. annularis* specimens and one *M. cavernosa* have been previously reported (Drake et al., 2020b).

Cleaning

All specimens were slabbed on a water saw to use <~10% of the borrowed coral head. Fragments were cleaned of organic material in equal parts 3% bleach and 50% hydrogen peroxide, milled or ground, and cleaned three further times in bleach/hydrogen peroxide for 30 min (milled powder) or overnight (ground powder) each cleaning followed by copious rinsing using modified methods of (Stoll et al., 2001). Several checks of the final product suggest that only endogenous intracrystalline SOM remained (Drake et al., 2020b). Samples for XRD were milled using a #2 carbide bur while samples for amino acid analysis were ground to <125 um on an agate mortar and pestle and sieved. Cleaned coral powder analyzed for amino acid parameters was

TABLE 1 | Modern and fossil coral specimen descriptions including data provided by the lender, verification of stated ages based on stratigraphic information confirmed in the literature, and mineralogy (A = aragonite, C = calcite) averaged between samples milled within Vs. between corallites or at different layers within the sample. Specimens loaned by the AMNH, NHMLAC, Peabody and MCZ have been returned to the lenders; specimens from the private research collection remain in the possession of the authors at this time.

Order	Accepted Species Name	Species Name in Collection + # Code Here	Source Location	Lender Stated Aged	Age Valid Based on Stratigraphic Information?	Age Based on Scientific Literature	Age Validation or Invalidation References	Museum	Cabinet	Drawer	Collection Date	Collector or Donation Collection	Catalog or Specimen #	Acquisition #	Average %A/C
Scleractinia	*Pocillopora damicornis*	*Pocillopora damicornis* (modern)	Oluwalu, HI (Olowalu, Maui, HI), 3 feet	modern	yes	Modern	—	LANHM	Stony corals full	4	—	Freddie Curtis	11140	A8693.66	100/0
Scleractinia	*Orbicella annularis*	*Orbicella annularis* (modern)	Coral Gardens, south of southern tip of Ambergris Island, Belize	modern	Yes	modern	—	Lisa Greer	—	—	—	L. Greer	—	—	100/0
Scleractinia	*Porites panamensis*	*Porites californica*	"Puritan", F-30,Marques Bay, Carmen Island, Gulf of California	Pliocene	yes	Upper Pliocene	Durham 1950 GSA Memoir 43	AMNH	244	1	—	—	AMNH-FI-113436 (old #F-30)	—	100/0
Scleractinia	*Orbicella annularis*	*Montastraea annularis 3*	East side of Key Largo, Dade County, FL	Pleistocene	yes	125138 ka, Pleistocene	Fruitjer et al. (2000) GSA Bulletin; Multer et al. (2002) Facies	LANHM	Pleistocene Florida	—	12/15/75	E. Wilson	LACMIP 5111.3	5111	100/0
Scleractinia	*Orbicella limbata*	*Orbicella limbata*	San Domingo	—	—	no information to support age	—	MCZ	—	—	—	—	MCZ:IP: 196571	—	100/0
Scleractinia	*Porites nodifera*	*Porites clavaria*	1/2 mile west of Port Limon, Costa Rica	—	—	Probaly Pliocene in Rio Banano formation	Taylor 1975 LSU dissertation	MCZ	—	—	1895	Robert T. Hill	MCZ:IP: 196568	—	100/0
Scleractinia	*Stylophora affinis*	*Stylophora affinis*	San Domingo	Miocene	?	no information to support age	—	MCZ	—	—	—	William More Gabb	MCZ:IP: 196570	—	100/0
Scleractinia	*Porites maigensis*	*Porites maigensis*	Burdigal; Maigen bei Eggenburg, Niederosterreich (Lower Austria)	Miocene	Yes	Miocene	Nebelsick 1989 Faces	AMNH	243	15	—	Kuhn	AMNH-FI-113437 (old #13)	—	96.5/3.5
Scleractinia	*Orbicella annularis*	*Montastraea annularis 4*	East side of Key Largo, Dade County, FL	Pleistocene	yes	125-138 ka, Pleistocene	Fruitjer et al. 2000; Multer et al. (2002)	LANHM	Pleistocene Florida	—	12/15/75	E. Wilson	LACMIP 5111.4	5111	93.5/6.5
Scleractinia	*Montastraea cavernosa*	*Orbicella cavernosa*	San Domingo	—	—	no information to support age	—	MCZ	—	—	—	William More Gabb	MCZ:IP: 196576	—	86.5/13.5
Scleractinia	*Porites sp.*	*Porites sp.*	Sab. Inf.; Anvers, France	Upper Eocene	no	Oligocene to Pliocene	Lankester 1865 Geological Magazine	AMNH	243	15	—	—	AMNH-FI-2980-1	—	85/15
Scleractinia	*Orbicella annularis*	*Montastraea annularis 6*	East side of Key Largo, Dade County, FL	Pleistocene	yes	125-138 ka, Pleistocene	Fruitjer et al. 2000 GSA Bulletin; Multer et al. (2002) Facies	LANHM	Pleistocene Florida	—	12/15/75	E. Wilson	LACMIP 5111.6	5111	85/15
Scleractinia	*Montastraea cavernosa*	*Montastraea cavernosa 1*	East side of Key Largo, Dade County, FL	Pleistocene	yes	125-138 ka, Pleistocene	Fruitjer et al. 2000 GSA Bulletin; Multer et al. (2002) Facies	LANHM	Pleistocene Florida	—	12/15/75	E. Wilson	LACMIP 5111.7	5111	77.5/22.5

(Continued on following page)

TABLE 1 | (*Continued*) Modern and fossil coral specimen descriptions including data provided by the lender, verification of stated ages based on stratigraphic information confirmed in the literature, and mineralogy (A = aragonite, C = calcite) averaged between samples milled within Vs. between corallites or at different layers within the sample. Specimens loaned by the AMNH, NHMLAC, Peabody and MCZ have been returned to the lenders; specimens from the private research collection remain in the possession of the authors at this time.

Order	Accepted Species Name	Species Name in Collection + # Code Here	Source Location	Lender Stated Aged	Age Valid Based on Stratigraphic Information?	Age Based on Scientific Literature	Age Validation or Invalidation References	Museum	Cabinet	Drawer	Collection Date	Collector or Donation Collection	Catalog or Specimen #	Acquisition #	Average %A/C
Scleractinia	*No accepted name*	*Favia distans*	San Domingo	Miocene	?	no information to support age	—	MCZ	—	—	—	William More Gabb	MCZ:IP: 196574	—	53/47
Scleractinia	*Stylophora conferta*	*Stylophora conferta*	Bowden Beds, Bowden, Jamaica, WI	Miocene	?	may be Pliocene	Budd 2000 Coral Reefs; Woodring 1928 Carnegie Institute Publication	AMNH	242	5	—	F.C. Nicholas	AMNH-FI-11474-1	—	35/65
Scleractinia	*Orbicella annularis*	*Montastraea annularis 2*	East side of Key Largo, Dade County, FL	Pleistocene	yes	125-138 ka, Pleistocene	Fruitjer et al. (2000) GSA Bulletin; Multer et al. (2002) Facies	LANHM	Pleistocene Florida	—	12/15/75	E. Wilson	LACMIP 5111.2	—	0/100
Scleractinia	*Favia sp*	*Favia sp. 1*	Nuevitas, Cuba	Late Cretaceous	probably	Upper Cretaceous	Albear 1947 AAPG Bulletin	Peabody	—	—	—	Braun-Schuchert Collection	539715	4207	0/100
Scleractinia	*Favia sp*	*Favia sp. 2*	St. Trinita di Mantechi Magiore (actually Trinita di Montecchio Maggiore)	Pliocene	?	could also be Oligocene	Budd and Bossellini 2016 J Systematic Paleontology	Peabody	—	—	—	Braun-Schuchert Collection	539716	4207	0/100
Rugosa	*Zaphrenthis dalii*	*Zaphrentis dalli*	Burlington Limestone, Burlington, IA	—	—	Mississippian, Carboniferous	Lauden 1937 AAPG Bulletin	AMNH	242	6	—	James Hall	AMNH-FI-6758-1	—	0/5 (95 % quartz)

always handled in a glove bag or laminar flow hood to avoid biological contamination.

X-Ray Diffraction

Sub-samples, ~5–20 mg, of cleaned coral powder from inside vs. between corallites, newer vs. older material, or on two randomly chosen spots per slab were analyzed for mineral content by XRD on a Panalytical X'Pert Pro X-ray Powder Diffractometer. Powders were run on a zero diffraction background plate. Spectra were analyzed in X'Pert Hi-Score software and relative amounts of aragonite and calcite were determined by the Reference Intensity Ratio method (Chung, 1974) using 01-072–1652 calcite and 00-041–1475 aragonite references for all samples. An internal reference standard was not applied as powders were also intended for further mass spectrometry analysis. Sample preparation by milling could induce recrystallization from aragonite to calcite (Gill et al., 1995; Foster et al., 2008; Waite and Swart, 2015); we therefore also performed XRD analyses on ground and cleaned powders from a subset of samples with mixed mineralogy, yielding similar results between the two preparation methods (**Supplementary Figure S1**). This suggests that preparation in our study did not impact our mineralogy data.

Elemental Analysis

Sub-samples used for XRD analysis were then analyzed for trace elemental composition. Powders were cleaned following (Barker et al., 2003) including a clay removal step, an oxidative step and a final dilute acid leach in 0.001 N HCl. Samples were then dissolved in 1N HCl and analyzed for Ca concentration and trace elements (e.g., X/Ca ratios). Ca concentrations were analyzed using an ICP-AES ® Ultima 2 HORIBA and trace elements using a Thermo Scientific ® Element XR (HR-ICP-MS) at Ifremer (Plouzané, France). Samples were diluted to fixed calcium concentrations (30 ppm Ca) using 0.1 M HNO_3 and 0.3 M HF matching multi-element standards Ca concentration to avoid any matrix effects (Misra et al., 2014; Guillermic et al., 2020). External reproducibility and analytical uncertainty were determined on the consistency standard Cam-Wuellestorfi (courtesy of the University of Cambridge) (Misra et al., 2014).

Amino Acid Content and Racemization

Proteins in ground skeleton powder were hydrolyzed to amino acids following methods of Penkman et al. (2008) with modifications. Briefly, ~20–30 mg cleaned skeleton powder was dissolved in 2 M HCl for 1 h at room temperature (10 ml acid/mg powder). The sample was then dried under a stream of N_2 (Ultra High Purity) and stored at -30°C until hydrolysis. Residues were hydrolyzed to amino acids in 7 M HCl (20 ml acid/mg starting material) at 110°C for 24 h, allowed to cool, then dried first under an N_2 stream and then in a Speed Vac at room temperature. Samples terminated after the leaching phase were analyzed directly for free amino acids (FAA). Samples carried through to full hydrolysis in concentrated acid were analyzed for total hydrolyzable amino acids (THAA).

All samples were prepared in duplicate and analyzed at the Northern Arizona University Amino Acid Geochronology Laboratory using standard methods with modifications optimized for microfossils (Kaufman and Manley, 1998; Kaufman, 2000; Kaufman, 2003). Rehydrated samples were spiked with L-homo-arginine as an internal standard and then injected into an HPLC fitted with a reverse-phase C18-packed column. "Blank" samples were included. D and L enantiomers of Asx (aspartic acid plus asparagine), Glx (glutamic acid plus glutamine), Ser (serine), Ala (alanine), Val (valine), Phe (phenylalanine), Leu (leucine), and Ile (isoleucine) were quantified for each sample and we calculated the average relative proportion of each total amino acid (D plus L) within that pool.

Scanning Electron Microscopy

Thin sections 30 μm thick were prepared with ¼-μm diamond polishing. These were gold-coated and then analyzed on a Tescan Vega-3 SMU variable-pressure scanning electron microscope.

Statistics

Amino acid data were log-transformed and analyzed by principal component analysis in Past4.01 with 1,000 bootstrap replicates (Hammer et al., 2001). Wilcoxon rank sum tests and Kruskal-Wallis H tests of amino acid data were performed in R Studio (R Studio Team, 2019). Statistical comparisons for the trace elements between mineralogies were made using unpaired t-tests (GraphPad Prism version 7.03, GraphPad Software, San Diego, CA United States).

RESULTS

Elemental Ratios

All samples except the rugosan, *Zaphrenthis dalli*, were calcium carbonate although some had recrystallized partially or fully to calcite (**Table 1**; **Supplementary Figure S2**). *Z. dalli* was almost completely silicified and was therefore not analyzed for elemental composition but was processed for amino acid analysis to represent an extreme outlier in terms of the effects of diagenesis on amino acid properties of hexacoral exoskeletons. We compared the B/Ca, Sr/Ca, and Mg/Ca values of all corals to the range known from modern corals (**Figure 1**, Supplementary Material, **Supplementary Table S1**). Samples that are >99% aragonite present B/Ca, Mg/Ca, Sr/Ca, and Ba/Ca in the range of modern corals (95% confidence interval), supporting that they remain primary aragonite. This was also observed in the Li/Ca and Mn/Ca compositions except for *M. annularis* 4 and *O. limbata* presenting higher Li/Ca and Mn/Ca. Samples partially recrystallized to calcite (between 7 and 50%) also fell in the range of modern corals for Mg/Ca, Sr/Ca, Li/Ca, and/Ba/Ca (95% confidence interval); this is also true for B/Ca except for *Porites sp., S. conferta,* and *M. cavernosa* 1. Samples completely recrystallized to calcite (>99%) present significantly lower B/Ca, Sr/Ca and higher Mg/Ca in comparison to primary aragonitic samples and modern corals.

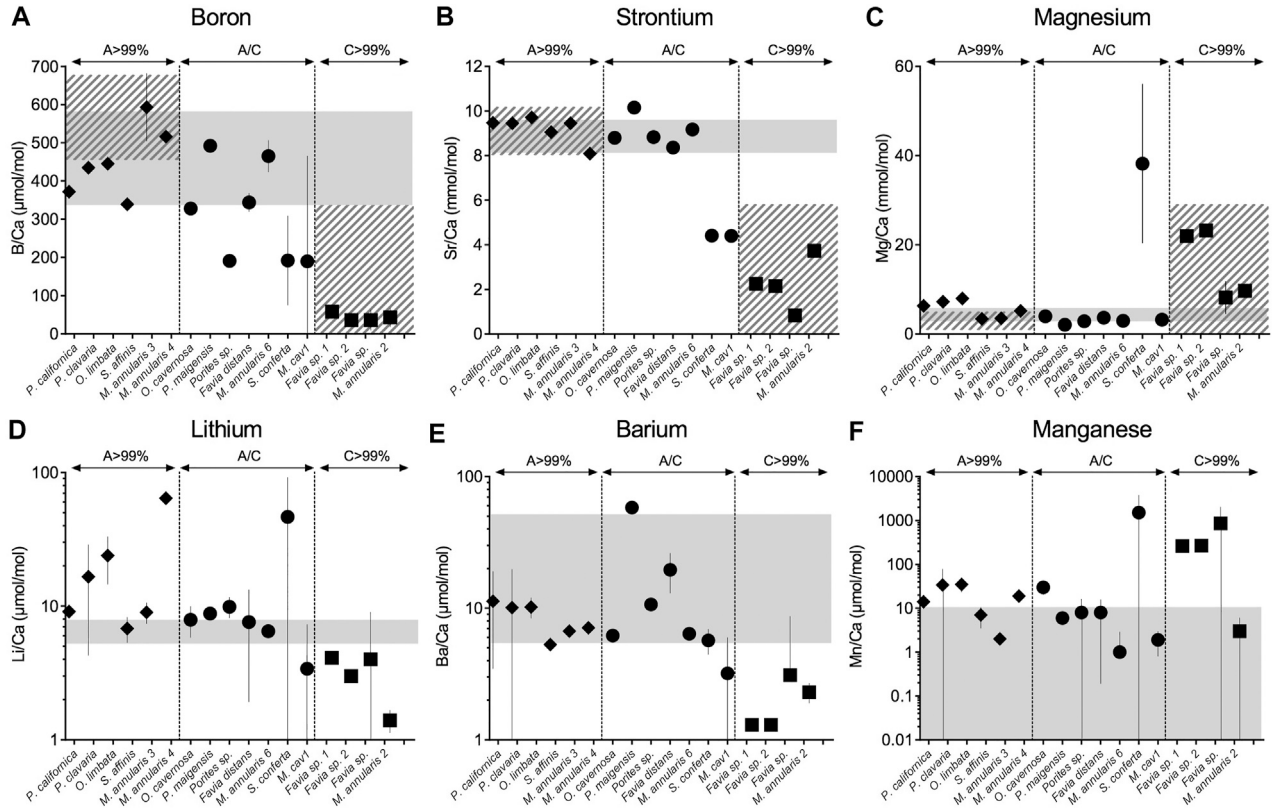

FIGURE 1 | Boron **(A)**, strontium **(B)**, magnesium **(C)**, lithium **(D)**, barium **(E)**, and manganese **(F)** relative abundance in fossil coral skeleton by individual specimen **(A–C)**. Solid grey bars indicate the range of element/Ca ratios from modern corals (see Supplementary Material). Hatched grey bars represent the range of boron, strontium and magnesium in published fossil corals for aragonite (>99%) and calcite (>99%) (de Villiers et al., 1994; Quinn and Sampson, 2002; Swart et al., 2002; Allison et al., 2007; Hendy et al., 2007; Nothdurft and Webb, 2009; Griffiths et al., 2013; Hathorne et al., 2013; Gothmann et al., 2015). In line with previous studies, fully recrystallized skeletons from aragonite to calcite show disruption of their elemental compositions. Except for *Porites* sp. and *S. conferta,* mixed mineralogies (up to 50% aragonite/calcite), present boron, strontium and magnesium within the range of aragonitic modern samples, samples recrystallized to calcite (>99%) present lower B/Ca, lower Sr/Ca and higher Mg/Ca relative to modern corals (unpaired *t*-test, $p < 0.001$).

Amino Acids

Next we took a targeted approach to the amino acid data from all fossils, including *Z. dalli*, and some modern coral skeletons used in previous coral skeletal proteomics analyses (Drake et al., 2020b) using principal component analysis (PCA). We examined the amino acid relative composition (% THAA and FAA Asx, Glx), fraction of THAA that is FAA, total pmol amino acids per mass of skeleton, racemization (D/L THAA and D/L FAA Asx, Glx, and Ser) and THAA and FAA L-Ser/L-Asx across the samples. As expected, D/L THAA and FAA Asx were generally reflective of coral stratigraphic ages, with higher D/L ratios for most of the older corals. We observed a pattern in which 1) some specimens partially recrystallized to calcite clustered in the general direction of unmodified modern and fossil specimens from which SOM proteins have successfully been sequenced, and 2) several fully primary aragonite specimens that were not a part of this broad cluster despite exhibiting trace element composition within the range of modern aragonitic specimens (**Figure 2**; **Supplementary Table S2**). Further, we observed that all corals fully recrystallized to calcite except one, or silicified, showed completely unique amino acid characteristic patterns. Combined, PCs 1 and 2 explain 83% of the variance within the PCA. Going forward, we now classify each fossil specimen as "Good candidate-aragonite," "Poor candidate-aragonite," "Good candidate-other," "Poor candidate-other," or "Silicified," and we separately group the modern specimens for which a skeletal proteome has been sequenced.

To examine the driving patterns of these broad clusters, we specifically looked for differences between the corals according to mineralogy for % THAA Asx, THAA L-Ser/L-Asx, and total pmol peptide amino acids per mass of skeleton. For these three parameters, coral skeletal proteins are known to be biased toward the acidic residues although relative abundances of all amino acids vary across taxa (Young, 1971; Mass et al., 2012; Tomiak et al., 2016), these values may reflect contamination by modern amino acids (Kaufman, 2006), and a lower proportion of FAA in the THAA pool may reflect the maintenance of primary peptide sequence information, respectively. All fossil specimens considered to be "good candidates", regardless of mineralogy, displayed similar % THAA Asx to modern specimens from which skeletal proteomes have been successfully sequenced, whereas "poor candidates" exhibited significantly lower %

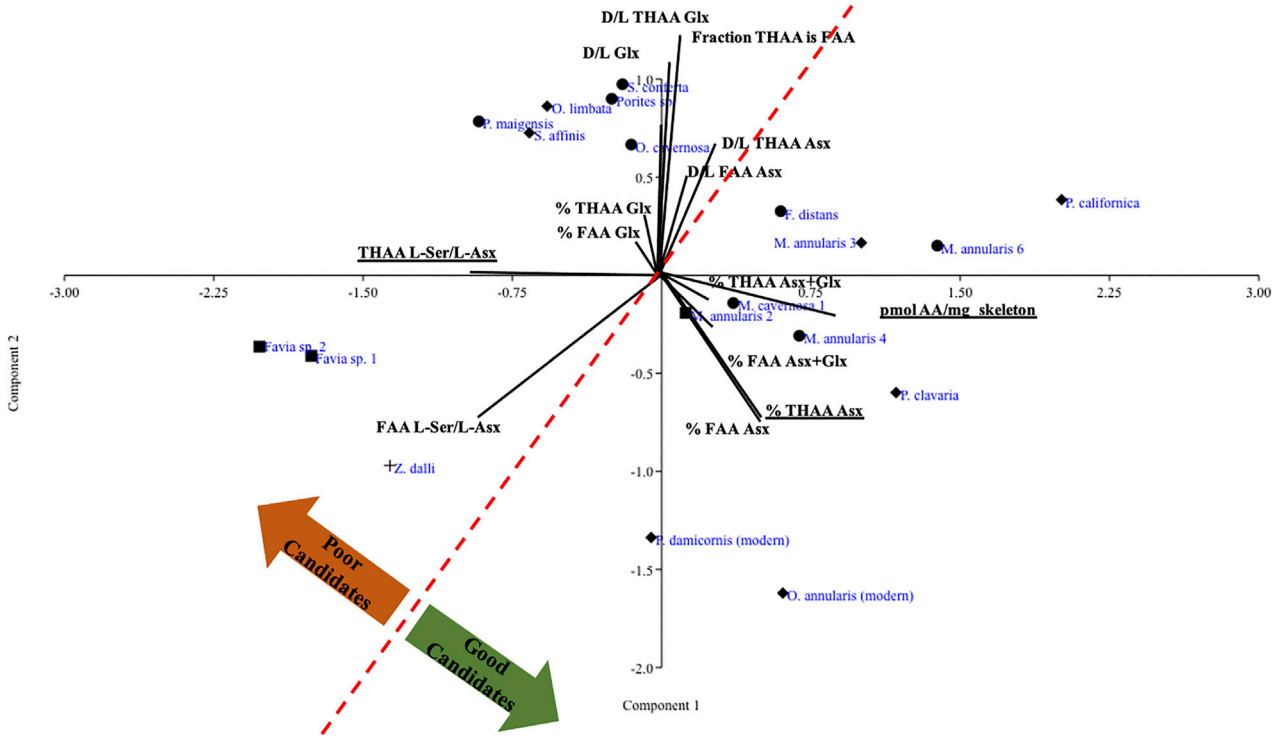

FIGURE 2 | PCA of log transformed amino acid relative composition and racemization data for all fossil corals plus representative modern corals. Specimens that are >99% aragonite are noted by diamonds, specimens that are a blend of aragonite and calcite are noted by circles, and specimens fully recrystallized to calcite are noted by squares, following the same symbol coding as in **Figure 1**; the silicified specimen is noted by a plus sign.

THAA Asx ($p < 0.05$; **Figure 3A**; **Supplementary Table S3**). Similarly, "good candidates" and modern specimens did not differ in their THAA L-Ser/L-Asx whereas "poor candidates" exhibited significantly higher values than aragonitic or mixed mineralogy "good candidates" ($p < 0.05$; **Figure 3A**; **Supplementary Table S3**). We also note a clear trend in pmol peptide-associated amino acids per mg skeleton to be higher in the "good candidates", although our statistical power for this measure was not high enough to detect significant differences.

DISCUSSION

Past paleoceanographic reconstruction studies using fossil corals have safely limited their specimen choices to those that contain <1% non-aragonite material (e.g., Allison et al., 2007; Gothmann et al., 2015). This is warranted as, for example, inclusion of 1% high-Mg calcite leads to a 1°C temperature reconstruction from geochemical data (Allison et al., 2007). However, based on data presented here, we propose that selecting corals with <50% recrystallization to calcite that have trace element ratios and amino acid composition in the range of modern coral skeletons will allow the expansion of specimens for biomineral organic matter analysis.

Mineral Preservation, Diagenesis and Elemental Composition

XRD data show clear evidence of diagenetic effects, with some samples partially or completely recrystallized to calcite, or in the case of *Z. dalli*, silicified. The elemental compositions of the 100% aragonite and 100% calcite samples are consistent with diagenetic effects and associated mineralogies; however, samples recrystallized up to 50% calcite present B/Ca, Sr/Ca and Mg/Ca signatures within the range of modern aragonitic corals and the 100% aragonite fossil samples (**Figure 3**). There appears to be a transition to more block-like crystal morphologies with increasing amounts of calcite (**Supplementary Figure S3**). Samples completely recrystallized to calcite present elemental composition outside the range of modern corals but in line with inorganic precipitation experiments and thermodynamic redistribution of the trace elements (McGregor and Gagan, 2003; Allison et al., 2007; Griffiths et al., 2013; Gothmann et al., 2015 among others).

The discrimination of an element substituting for Ca^{2+} in the crystal lattice is predicted by its size and the thermodynamic equilibrium of the reactions (Brand and Veizer, 1980; Menadakis et al., 2009). If this element is larger than Ca^{2+}, it is predicted to be discriminated against in tighter rhombohedral calcite compared to orthorhombic aragonite. This is observed from inorganic

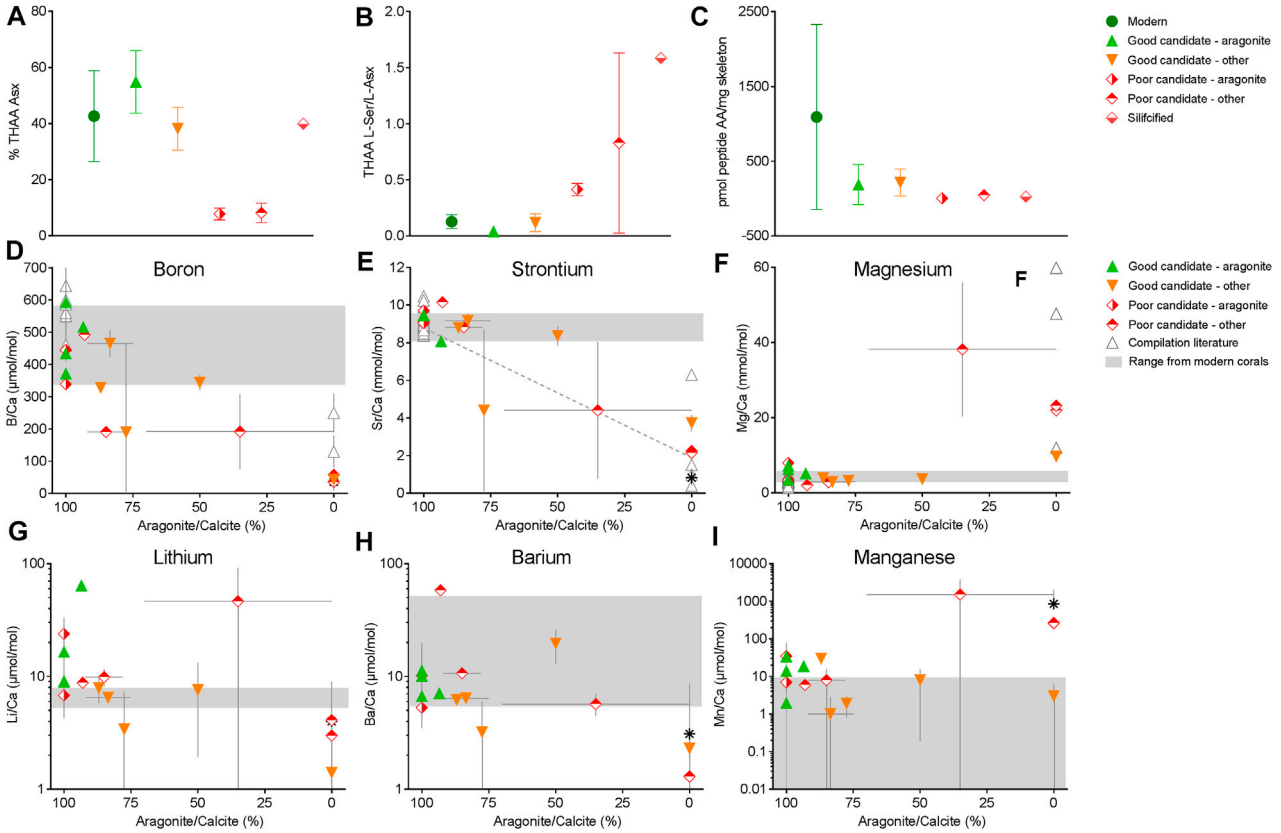

FIGURE 3 | Amino acids % THAA Asx **(A)**, THAA L-Ser/L-Asx **(B)**, pmol peptide-AA/mg skeleton **(C)**, boron **(D)**, strontium **(E)**, magnesium **(F)**, lithium **(G)**, barium **(H)**, and manganese **(I)** abundance of fossil coral skeleton by % aragonite **(D–F)**. In **(D–F)**, Solid grey bars indicate the range of element/Ca ratios from modern corals (see Supplementary Material), **(E)** dotted line is the linear regression between Sr/Ca and % aragonite from McGregor and Gagan, (2003). With few exceptions, those results suggest that some recrystallized skeletons can be suitable for peptide analyses and that trace element data are useful indicators of potential good and poor candidates for organic matter analyses. In this study, most "poor candidates" showed disruption of elemental ratios compared to modern aragonitic samples (e.g., lower B/Ca, lower Sr/Ca and higher Mg/Ca relative to modern corals).

precipitation experiments for Sr^{2+}, Ba^{2+}, Li^{+} (e.g., higher concentration in aragonite than calcite), and Mg^{2+} and Mn^{2+} (e.g., lower concentration in aragonite than calcite) (Shen et al., 1991; Gabitov et al., 2011; DeCarlo et al., 2015; Mavromatis et al., 2018; Gabitov et al., 2019). For boron, inorganic precipitations also present higher concentrations in aragonite than calcite (Mavromatis et al., 2015). Across studies examining diagenetic effects on fossil corals on geochemical patterns, subaerial diagenesis (e.g., dissolution and precipitation of secondary calcite from meteoric water) has been shown to cause a decrease in Sr/Ca and increase in Mg/Ca. Submarine diagenesis, including precipitation of secondary aragonite presents higher Sr/Ca (Sayani et al., 2011) but lower Mg/Ca, and/or precipitation of high-Mg calcite presents a decrease in Sr/Ca (Allison et al., 2007) and an increase in Mg/Ca, in line with inorganic precipitation.

Trace element composition of all samples fully recrystallized to calcite are in line with dominant subaerial diagenesis in an open system as we observe elemental ratios consistent with inorganic calcite precipitation (e.g., low Sr/Ca, Ba/Ca, B/Ca, Li/Ca, and high Mg/Ca). This suggests that all should be excluded from further analysis, but we do note that one fully recrystallized sample (*M. annularis 2*) is classified as a potential good candidate based on amino acid analysis. In contrast, partially recrystallized samples' trace element and amino acid data suggest that some useful organic matter may remain as described below. Although we do not have the original geochemical signal of the fossil samples, some partially recrystallized to calcite (up to 50% for *Favia distans*) seem to retain the aragonitic signature based on the range of modern corals (**Figures 1**, **3**). This can potentially be attributed to simultaneous dissolution/precipitation (e.g., closed system) from subaerial diagenesis which is thought to retain the primary signature (Sayani et al., 2011). Li/Ca and Mn/Ca ratios are more variable than the other elemental ratios explored in this study. Specifically, the >99% aragonite corals *O. limbata* and *M. annularis* 4 present Li/Ca and Mn/Ca ratios, potentially reflecting the presence of secondary aragonite precipitated from a reducing diagenetic fluid (Gothmann et al., 2015). In all cases, the disruptions of the Li/Ca and Mn/Ca ratios do not appear correlated with the preservation of the SOM, since *O. limbata* is considered a poor candidate and *M. annularis* 4 a

good candidate for amino acid analyses. Further investigations of the isotopic composition (for example $\delta^{18}O$) would be useful to deconvolve the diagenesis patterns and their link with amino acid preservation.

Amino Acid Preservation and Degradation

Inorganic and organic alteration of biominerals, including both protein fragmentation and amino acid loss and increases in crystal size, respectively, occur simultaneously and continuously (Hare, 1980; Tuross et al., 1989). Retention of the organic phase implies that the inorganic component has remained stable, with organic molecules only available for microbial degradation and loss upon alteration of the inorganic phase (Collins et al., 2002). However, organic molecules can become altered within intact mineral, with higher rates observed with increased temperatures, increased absolute and varying amounts of water, marine vs. freshwater conditions, and presence of oxygen and/or sugars (Hare, 1962; Hare, 1974; 1980; Painter, 2003; McCobb et al., 2004), all of which are associated with aerial localization or microbial degradation of the specimen (McGregor and Gagan, 2003; Sayani et al., 2011). Specifically in invertebrates, alteration of the inorganic phase may also allow the intrusion of exogenous amino acids, particularly the abundant metabolic by-product Ser (review by (Mitterer, 1993). Four of seven fossil coral specimens studied here were partially (up to 50%) recrystallized to calcite, yet retained more Asx (THAA fraction) and peptide-associated amino acids and exhibited lower L-Ser/L-Asx (THAA fraction) than other calcite-recrystallized specimens and also fell within the 95% confidence interval of modern corals' B/Ca, Sr/Ca and Mg/Ca (**Figures 2**, **3**), suggesting that the recrystallization in these "good candidate" <99% aragonite specimens likely occurred quickly in a sub-aerial locale, minimizing both degradation and loss of proteinaceous matter and modification of trace element content. B/Ca, Sr/Ca and Mg/Ca analyses may therefore be useful in revealing a larger pool of potential fossil coral candidates including samples partially recrystallized to calcite for organic matter analyses. However, geochemical metrics alone do have limitations, with two of five samples that have fully aragonitic mineralogical and elemental signatures, plus two partially recrystallized samples with aragonitic trace element composition, displaying likely highly degraded proteins. This indicates that the processes that lead to geochemical alteration cannot be assumed to equally alter organic matter. Further, major mineral alterations such as silicification may lead to changes to amino acid content similar to conversion to >50% calcite, as exhibited by high L-Ser/L-Asx (THAA fraction) and low pmol amino acids per mass of skeleton of the 95% silicified rugosan coral, *Z. dalli*, collected from the Burlington formation which is lower to middle Mississippean in age (240–250 mya). There are numerous records of the genus *Zaphrenthis* from the Burlington (Bassler, 1950) Examinations of hypercalcifying non-scleractinian hexacorals such as rugose and tabulate corals, particularly of varying degrees of preservation, will clarify the degree of organic matter preservation in these important fossils as well.

Coral skeletons are not homogenous and the solubility of the different inorganic phases may impact mineral organic matter alteration. Rapid accretion deposits (RADs) display disorganized crystal orientations, presence of amorphous calcium carbonate, higher Sr/Ca and Mg/Ca, higher skeletal organic matter content, and larger defect sizes compared with thickening deposits (TDs) (e.g., Cuif and Dauphin, 1998; Meibom et al., 2006; Meibom et al., 2008; Brahmi et al., 2012; Rollion-Bard and Blamart, 2015; Mass et al., 2017), likely making them more soluble. Incorporation of specific highly acidic proteins such as the coral acid rich proteins 1 and coral acid rich protein 4, which are abundant in Asp, is also apparent in RADs (Mass et al., 2014). Therefore, coral skeletons exposed to environmental conditions that encourage inorganic phase alterations would likely display those changes preferentially in the RADs yielding localized element/Ca composition alteration, protein fragments and amino acids loss, loss of specific Asp-containing proteins in particular, and Ser intrusion. However, the altered trace element signal may not be detectable by the analysis of bulk skeleton conducted here, as the modern species-specific variation in trace elements between RADs and TDs falls within the modern intra-specific range in bulk values (Cuif and Dauphin, 1998; **Figure 1**). Hence, geochemical analyses may suggest that the bulk skeleton is unaltered or minimally altered (**Figures 1**, **Figures 3D–F**), whereas microstructural changes (not tested here) and consequent degradation and loss of organic matter may be observed (**Figures 3A–C**). Future studies that focus on the organic content of these microstructures will further reveal the effects of localized biomineral degradation on SOM preservation.

CONCLUSION

As the biological components of fossil bio-carbonates are increasingly used to reconstruct past ocean chemical conditions and ecological processes (Muscatine et al., 2005; Wang et al., 2015; Tornabene et al., 2017), it is important that biomolecules be in an optimal state of preservation. In the present study, combined XRD and elemental ratios were used to assess the preservation and bulk diagenesis of fossil coral samples. Percent THAA Asx, THAA L-Ser/L-Asx and the peptide AA/mg skeleton were then used to assess the potential "good candidates" for organic matter analyses in fossil corals presenting various degrees of recrystallisation. Our results support the exclusion of fully calcite-recrystallized and silicified samples in studies of fossil organic matter. However, some samples partially recrystallized to calcite (up to 50%) were characterized as potential "good candidates" for organic matter analyses.

The present work shows that geochemical analysis is a good first step toward detecting organic matter diagenesis of fossil biominerals. However, this alone does not capture the entire story, not ruling out fully primary aragonite specimens that appear to have experienced localized organic matter degradation and loss while rejecting partially recrystallized samples that appeared to retain useful biological information. We are not the first to advocate for a multidisciplinary approach when choosing biominerals that have gone through some degree of degradation and/or preservation (Tuross et al., 1989). We

suggest that standard amino acid analysis, including the calculation of peptide-associated amino acid content from both THAA and FAA analyses, be added to geochemists' and paleoceanographers' methodological repertoire to carefully expand the pool of bio-carbonates used to study biologically-associated oceanographic processes.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

JD and MG designed the study, and collected and analyzed data. Geochemical method development and analyses were supported as part of projects led by RE. DJ provided guidance on interpreting stated fossil coral ages. All authors contributed to writing the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/feart.2021.643864/full#supplementary-material

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