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Stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of organic matrix from coral skeleton

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The evolutionary success of reef-building corals in nutrient-poor tropical waters is attributed to endosymbiotic dinoflagellates. The algae release photosynthetic products to the coral animal cells, augment nutrient flux, and enhance the rate of coral calcification. Natural abundance of stable isotopes ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) provides answers to modern and paleobiological questions about the effect of photosymbiosis on sources of carbon and oxygen in coral skeletal calcium carbonate. Here we compare 17 species of symbiotic and nonsymbiotic corals to determine whether evidence for photosymbiosis appears in stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of an organic skeletal compartment, the coral skeletal organic matrix (OM). Mean OM $\delta^{13}\text{C}$ in symbiotic and nonsymbiotic corals was similar (-26.08% vs. -24.31%), but mean OM $\delta^{15}\text{N}$ was significantly depleted in ^{15}N in the former (4.09‰) relative to the latter (12.28‰), indicating an effect of the algae on OM synthesis and revealing OM $\delta^{15}\text{N}$ as a proxy for photosymbiosis. To answer an important paleobiological question about the origin of photosymbiosis in reef-building corals, we applied this proxy test to a fossil coral (*Pachythecalis major*) from the Triassic (240 million years ago) in which OM is preserved. Mean OM $\delta^{15}\text{N}$ was 4.66‰, suggesting that *P. major* was photosymbiotic. The results show that symbiotic algae augment coral calcification by contributing to the synthesis of skeletal OM and that they may have done so as early as the Triassic.

symbiosis | calcification | paleobiology

Productivity and growth of scleractinian corals in shallow, nutrient-poor tropical marine waters is significantly enhanced by endosymbiotic dinoflagellates. The algae release products of photosynthesis to animal cells, take up, retain, and recycle inorganic nutrients, and augment the rate of skeletal calcification because it can be two to three times faster in the light than in the dark (1, 2). Natural abundance of stable isotopes ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) has answered paleobiological and modern questions about the effect of photosymbiosis on sources of carbon and oxygen in coral skeletal calcium carbonate (3, 4). Similarly, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ have provided information on how coral algae and animal cells use carbon and nitrogen (5–11). Here we investigate natural abundance of stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) in another coral skeletal compartment, the skeletal organic matrix (OM).

The scleractinian coral skeleton is a two-phase composite structure consisting of fiber-like crystals of aragonitic calcium carbonate intimately associated with an intrinsic OM (12). The OM is generally <0.1% of the total skeleton by weight (13) but is believed to initiate nucleation of calcium carbonate and provide a framework for crystallographic orientation and species-specific architecture (14–16). Inhibition of matrix synthesis by emetin or cycloheximide brings coral calcification to a halt without affecting photosynthesis or respiration (17).

Decalcification of coral skeleton yields water-soluble and -insoluble fractions, which include glycoproteins and glycosaminoglycans (13, 18–25). Matrix glycoprotein contains sulfated

polysaccharide and protein rich in acidic amino acids, especially aspartate and glutamate (13). Matrix monosaccharides and amino acids vary qualitatively and quantitatively depending on species. As such they are useful in taxonomy (21, 22, 26) and discrimination between modern symbiotic and nonsymbiotic corals (22, 27). The first sequence of a coral OM protein has been described (28).

The morphological relationship between organic and mineral phases in coral unit crystals is poorly understood. OM is synthesized by cells of the calciblastic epithelium and then secreted into the adjacent subepithelial space in which it initiates and controls calcification (17, 24, 29–31). Electron microscopy of thin sections of the coral tissue–skeleton interface reveals a 3- μm -thick reticulum of acid polysaccharide-rich fibrils in this space (24, 29). When corals are exposed to a matrix precursor such as [^{14}C]aspartate, the amino acid is incorporated rapidly into animal tissue protein and appears in matrix proteins within 20 min (17).

There is a metabolic link between symbiont photosynthesis and OM synthesis as coral algae fix $^{14}\text{CO}_2$ and release ^{14}C -labeled products to coral animal cells. After decalcification of the skeleton, ^{14}C can be detected in OM (32–34).

Recognizing the fundamental role of skeletal OM in coral calcification, we analyzed $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in algae, animal tissue, and OM of 17 species of modern scleractinian corals. We compared symbiotic and nonsymbiotic forms to determine whether activities of the algae influence the natural abundance of OM stable isotopes. The data reveal a highly significant difference between symbiotic and nonsymbiotic corals with respect to OM $\delta^{15}\text{N}$ but not with respect to OM $\delta^{13}\text{C}$, establishing OM $\delta^{15}\text{N}$ as a potential proxy for photosymbiosis. We wondered whether such a proxy could be used to obtain retrospective information in fossil corals. In a seminal paper, Stanley and Swart (35) used skeletal mineral data (carbonate $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) as a proxy for photosymbiosis in Late Triassic corals. The remarkable discovery of aragonite crystals and skeletal OM in a Triassic coral, *Pachythecalis major* (36, 37), enabled us to pursue this question by using OM $\delta^{15}\text{N}$. Accordingly, we analyzed the extant OM ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) and skeletal carbonate ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) in *P. major*. The resulting data support the contention that *P. major* was photosymbiotic.

Methods

We analyzed eight symbiotic and nine nonsymbiotic modern species of scleractinian corals including *Oculina* spp. The latter

Abbreviation: OM, organic matrix.

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Table 1. Corals used in this study

Collection site	Species	Collection depth, m
Modern symbiotic		
New Caledonia	<i>Acropora</i> sp.	4; f
	<i>Fungia danai</i> (Edwards and Haime, 1851)	4; f
	<i>Merulina</i> sp.	4; f
	<i>Pavona decussata</i> (Dana, 1846)	4; f
	<i>Pocillopora damicornis</i> (Linnaeus, 1758)	4; f
Red Sea	<i>Stylophora pistillata</i> (Esper, 1797)	1, 5, 10, 15, 20, 30; f
Hawaii	<i>Fungia scutaria</i> (Verrill, 1864)	1; f
	<i>Pocillopora damicornis</i> (Linnaeus, 1758)	1, 10; f
North Carolina	<i>Oculina arbuscula</i> (symbiotic)	1; ds
	<i>Oculina arbuscula</i> (aposymbiotic)	6; ds
Florida	<i>Oculina varicosa</i> (aposymbiotic)	100; ds
Monaco	<i>Stylophora</i> sp.	aq; ds
	<i>Acropora</i> sp.	aq; ds
Modern nonsymbiotic		
Celtic Sea	<i>Caryophyllia ambrosia</i> (Alcock, 1898)	unk; ds
New Caledonia	<i>Dendrophyllia alcocki</i> (Wells, 1954)	412; ds
	<i>Enallopsammia</i> sp.	435; ds
	<i>Paracyathus</i> sp.	425; ds
	<i>Solenosmilia variabilis</i> (Duncan, 1873)	unk; ds
	<i>Stephanocyathus spiniger</i> (Marenzeller, 1888)	250; ds
Great Barrier Reef	<i>Tubastrea coccinea</i> (Lesson, 1829)	12–15; f
	<i>Tubastrea micrantha</i> (Ehrenberg, 1834)	12–15; f
Hawaii	<i>Tubastrea coccinea</i> (Lesson, 1829)	4; f
New Caledonia	<i>Tubastrea micrantha</i> (Ehrenberg, 1834)	2; f
Indian Ocean	<i>Madrepora oculata</i> (Linnaeus, 1758)	unk; ds
Mediterranean Sea	<i>Madrepora oculata</i> (Linnaeus, 1758)	unk; ds
Fossil		
Turkey	<i>Pachytheclis major</i> Cuif 1975	n/a

aq, aquarium; ds, dried skeleton; f, freshly collected; n/a, not applicable; unk, unknown.

are facultative symbioses containing algae in shallow water and lacking them (aposymbiotic) in shaded habitats and deep water (Table 1). The corals were collected fresh from the sea or aquaria or obtained as dry “museum” skeletons. Replicate colonies of a few species were included. Algae and animal tissue from freshly collected corals were analyzed also.

Freshly collected corals were processed according to skeletal architecture (perforate or imperforate). Animal tissue was removed from imperforate skeletons with a jet of sea water (Waterpik, Teledyne, Ft. Collins, CO) and from perforate skeletons by crushing to a coarse sand, shaking the slurry in 100 ml of filtered seawater, and decanting the supernatant containing the algae and animal tissue (32). In both cases, the resulting tissue homogenates were centrifuged to separate algae from animal tissue (38). Tissue fractions were acidified with 0.1 M HCl to dissolve any residual skeletal calcium carbonate and remove seawater bicarbonate, lyophilized, and stored at -20°C . Tissue-free skeletons, including dry museum specimens, were soaked in sodium hypochlorite (commercial bleach) overnight, rinsed with distilled water, and dried in an oven at 50°C . They then were crushed to a coarse sand in a ceramic mortar and pestle, treated with NaOH (2 M, 80°C , 15 min) to remove any residual extrinsic organic material such as animal tissue and endolithic organisms (see below), rinsed in distilled water, oven-dried (50°C), and then reduced to a fine powder in a motor-driven agate mill (Retsch Type RMO, Bioblock Scientific, Haan, Germany). Approximately 15–50 g of skeletal powder was decalcified by dropwise addition of 2 M HCl over several hours until the powder dissolved and the evolution of bubbles ceased. The resulting slightly turbid solution, containing aggregate matrix (i.e., soluble and insoluble fractions) was neutralized with 0.1 M NaOH and then

dialyzed (Spectra/Por, Spectrum Laboratories, Houston; molecular-mass cutoff, 3.5 kDa) for 24 h at 5°C against distilled water (four changes of 6 liters with stirring). The dialyzed material was lyophilized and weighed. Recoveries ranged from 0.01% to 1.5% by weight (data not included here). Our OM weights were generally higher than those reported previously (13). Because different extraction methods and different operational definitions of OM (i.e., soluble vs. aggregate) make comparison with previous studies difficult, we initiated a separate investigation to determine OM weights by thermogravimetry. Preliminary results indicate that OM weights averaged 1.6% for the coral *Stylophora pistillata*, suggesting that recovery may depend on extraction protocol and that percent OM weights may now be expected to exceed previous estimates (Puverel, S., Houlbreque, F., Tambutte, E., Zoccola, D., Payan, P., Tambutte, S., and D.A., unpublished data).

Depending on age, location within a colony, and species, scleractinian skeletons may harbor endolithic filamentous algae and fungi (39). Following the convention used in all previous studies of OM, skeletons containing endolithic filamentous algae, identified by their green color, were discarded.

Three specimens of the Triassic coral *P. major* (Upper Triassic/Lower Norian) were collected from the Alakir Valley in Turkey. The largest (specimen 1), a conical white calyx (50 mm high; distal diameter, 40 mm; proximal diameter, 25 mm) encased in gray micrite, was processed at the University of California, Los Angeles. The calyx walls were 5–10 mm thick. The specimen was cut transversely with a diamond saw into 1-cm-thick slabs. Calyx wall material was removed from each slab with a diamond drill (Dremel, Racine, WI) and yielded 49.17 g of finely powdered skeleton. A few milligrams were saved for carbonate $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ analyses. To avoid destruc-

tion of OM in the finely powdered skeleton, we omitted treatment with hot NaOH. The powder was dissolved in 2 M HCl as described above. The solution was adjusted to pH 7.0 with 0.1 M NaOH, dialyzed, and lyophilized, yielding 0.59 g of tan powder (1.19% of skeleton weight). Approximately 23 g of the micrite was also powdered. It behaved like a carbonate-rich sediment, dissolving in 2 M HCl with concomitant evolution of bubbles. The solution was neutralized, dialyzed, and lyophilized, yielding 0.4 g of residue (1.7% of total weight). Specimens 2 and 3 were processed in Orsay and treated as described above except that after decalcification, the solution was centrifuged ($3000 \times g$, 10 min) to remove particulate material. The supernatant was desalted on a Sephadex G-25 column (Amersham Pharmacia) and purified further by ultrafiltration (Pall-Filtron cells) using molecular-mass cutoffs of 3.0 kDa for sample 2 and 0.5 kDa for sample 3 and then lyophilized.

Stable isotopes were measured in the departments of Geology (University of Texas) and Earth Sciences (University of California, Santa Cruz). At the University of Texas, dried samples of algae, tissue, and OM were pulverized and treated with 10% HCl to remove any residual CaCO_3 . Samples were analyzed by combustion at 850°C in quartz tubes (40) so that both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were obtained from the same sample. Carbon is reported relative to the Pee Dee Belemnite standard, and nitrogen isotopic compositions were normalized by using the N-1 standard ($+1.36\text{‰}$) and are reported relative to atmospheric nitrogen. Both isotopic compositions were reproducible to better than 0.3‰ . At the University of California, Santa Cruz, isotopes of algae, tissue, and OM were analyzed with a Carlo Erba 2500 elemental analyzer attached to a VG Optima gas-source mass spectrometer. Sample powder (4–40 mg) was flash-combusted by O_2 in a He stream, and sample gases were converted to CO_2 and N_2 on heated catalyst columns. CO_2 and N_2 then were separated on a chromatographic column before injection into the mass spectrometer. The SD of replicate analyses of an internal laboratory gelatin standard was $<0.2\text{‰}$ for both carbon and nitrogen values. Isotopes of *P. major* skeletal carbonate ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) were analyzed with a VG Micromass Autocarb coupled to the mass spectrometer. Each sample was reacted in a bath of 100% phosphoric acid at 90°C . Average precision, as determined for replicate analyses of calcite (NBS-19) and CM (a lab standard), was $<0.1\text{‰}$ for both $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$. Laboratory bias was not a concern, because both laboratories consistently discerned, with a high level of significance, the difference between OM $\delta^{15}\text{N}$ in symbiotic vs. nonsymbiotic corals. Stable isotope data are expressed relative to standards using the conventional per mil (‰) notation.

Stable isotope data were evaluated statistically by using MINITAB 13 (Minitab, State College, PA). Means were compared by using one-way ANOVA. When more than two means were compared, we used Tukey's procedure for pairwise comparison. The family error rate was 0.05. Individual error rates are reported with the results.

Results

Fig. 1 shows the range of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in modern symbiotic and nonsymbiotic species. In the symbiotic group there is a modest 8‰ range in $\delta^{13}\text{C}$ in algae (-9‰ to -16.8‰) and animal tissue (-12.8‰ to -20.8‰) and a substantial 23‰ range (-12.7‰ to -35.8‰) in the skeletal OM. The mean $\delta^{13}\text{C}$ values for algae, animal, and OM compartments (-13.8‰ , -16.5‰ , and -24.3‰ , respectively; Table 2) indicate progressive depletion of ^{13}C from producer (algae) to consumer (animal tissue and OM) compartments. OM $\delta^{13}\text{C}$ is depleted by almost 8‰ relative to animal tissue, a feature that supports the argument that OM is not contaminated by animal tissue. Similarly, the range of

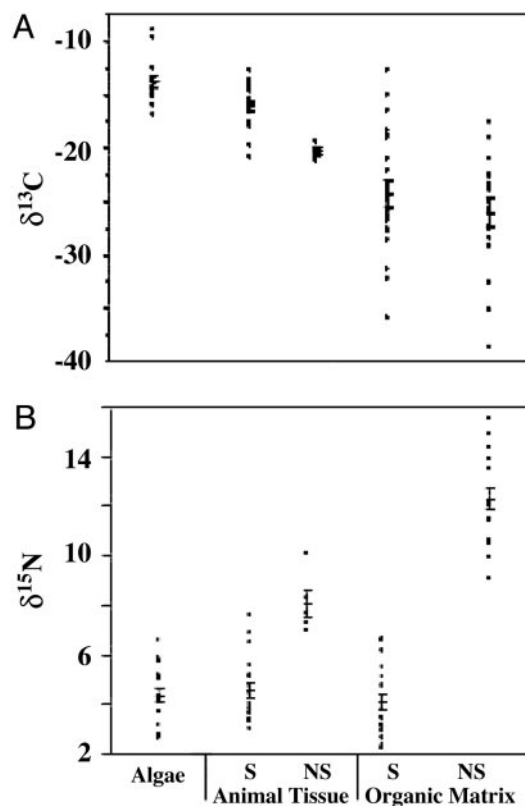


Fig. 1. Range of $\delta^{13}\text{C}$ (A) and $\delta^{15}\text{N}$ (B) values in algae, animal tissue, and OM in symbiotic (S) and nonsymbiotic (NS) corals. Shown are mean $\text{‰} \pm$ SEM (horizontal bars).

$\delta^{13}\text{C}$ values is modest in nonsymbiotic animal tissue (-19.4‰ to -21.2‰) and substantial in OM (-17.6‰ to -38.5‰) (Fig. 1A). Animal tissue and OM are both depleted in ^{13}C , the latter by $\approx 6\text{‰}$ relative to animal tissue. Mean OM $\delta^{13}\text{C}$ in symbiotic and nonsymbiotic corals (Table 2) are not significantly different [ANOVA: $F_{(1, 38)} = 0.98$; $P > 0.33$].

$\delta^{15}\text{N}$ in modern symbiotic corals ranges in algae from 2.6‰ to 6.6‰ , in animal tissue from 3.0‰ to 7.6‰ , and in OM from 2.2‰ to 6.7‰ (Fig. 1B). The mean OM $\delta^{15}\text{N}$ is $4.09\text{‰} \pm 1.51\text{‰}$ (Table 2). $\delta^{15}\text{N}$ in nonsymbiotic species ranges from 7.0‰ to 10.1‰ in animal tissue and 9.9‰ to 15.5‰ in OM (Fig. 1B), revealing a progressive enrichment of ^{15}N in animal tissue and OM (Table 2). Nonsymbiotic $\delta^{15}\text{N}$ OM is enriched by 4.4‰ over nonsymbiotic animal tissue to $12.28\text{‰} \pm 1.81\text{‰}$. In contrast, mean $\delta^{15}\text{N}$ values in algae, animal tissue, and OM in symbiotic corals are similar. There is no trend in $\delta^{15}\text{N}$ that would indicate trophic enrichment. In this respect, OM $\delta^{15}\text{N}$ in symbiotic corals seems uncoupled from conditions that affect $\delta^{13}\text{C}$. The most striking observation is the significant difference in mean OM $\delta^{15}\text{N}$ between symbiotic and nonsymbiotic corals [ANOVA: $F_{(1, 39)} = 247.7$; $P \ll 0.001$; Figs. 1B and 2 and Table 2]. The latter are more enriched than the former by an average of 8.73‰ . These data indicate that the algae have a significant effect on the magnitude of coral OM $\delta^{15}\text{N}$, rendering it potentially useful as a proxy for photosymbiosis in coral paleobiological research.

Some species exhibit unusual $\delta^{15}\text{N}$ values. The mean *Fungia scutaria* OM $\delta^{15}\text{N}$ ($6.4\text{‰} \pm 0.3\text{‰}$) is significantly higher than that of other symbiotic corals [$3.5\text{‰} \pm 1\text{‰}$; ANOVA: $F_{(1, 22)} = 43.02$; $P < 0.001$]. The reason is obscure but may be related to the fact that *F. scutaria* is a large, solitary polyp; the other symbiotic species are all colonial.

Table 2. Mean stable isotope values (‰ ± SD of the mean) (n) for coral organic fractions

Isotope	Organic fraction	Symbiotic	Nonsymbiotic	<i>P. major</i>
$\delta^{13}\text{C}$	Algae	-13.84 ± 2.02 (17)	n/a	
	Animal	-16.53 ± 2.22 (17)	-20.30 ± 0.83 (5)	
	Organic matrix	-24.31 ± 5.60 (23)	-26.08 ± 5.52 (17)	-23.23 ± 2.60 (3)
$\delta^{15}\text{N}$	Algae	4.35 ± 1.23 (18)	n/a	
	Animal	4.56 ± 1.40 (17)	8.08 ± 1.23 (5)	
	Organic matrix	4.09 ± 1.51 (24)	12.28 ± 1.81 (17)	4.66 ± 2.72 (3)

Data for *Oculina* spp. are included with nonsymbiotic groups (see *Results* and *Discussion*). n/a, not applicable.

The mean OM $\delta^{15}\text{N}$ for both symbiotic and aposymbiotic *Oculina* spp. is significantly higher still ($11.4\text{‰} \pm 2.2\text{‰}$) and is not significantly different from the mean OM $\delta^{15}\text{N}$ for nonsymbiotic corals ($12.28\text{‰} \pm 1.81\text{‰}$; ANOVA: $F_{(1, 15)} = 0.927$; $P > 0.35$; Fig. 2). OM $\delta^{15}\text{N}$ in *Oculina* spp. is clearly aligned with the nonsymbiotic group.

To determine whether OM $\delta^{15}\text{N}$ is a useful proxy for photosymbiosis, we analyzed stable isotopes of the Triassic coral *P. major*. OM $\delta^{13}\text{C}$ in the three specimens of *P. major* were -23.3‰ , -25.8‰ , and -20.6‰ . The average (-23.23‰) is not significantly different from OM $\delta^{13}\text{C}$ for modern symbiotic or nonsymbiotic scleractinians (individual error rate = 0.0196; Table 2), confirming that this isotope is not a useful organic marker for photosymbiosis. $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ in skeletal carbonate (3.5‰ and -3.5‰ , respectively) falls within the range of data obtained by Stanley and Swart (35) for this species (see *Discussion*). The $\delta^{13}\text{C}$ of the micrite organic fraction was -28.9‰ .

OM $\delta^{15}\text{N}$ in *P. major* specimens 1–3 are 8.5‰ , 3.0‰ , and 2.47‰ , respectively. The mean value ($4.65\text{‰} \pm 1.92\text{‰}$) is not significantly different from that of modern symbiotic corals and is significantly different from that of nonsymbiotic corals (individual error rate = 0.0105; Fig. 2 and Table 2). Because $\delta^{15}\text{N}$ of the organic residue recovered after dissolution of micrite was -0.2‰ , contamination of OM by micrite constituents is not a concern. We conclude from these data that *P. major* was very likely photosymbiotic.

Discussion

Gautret *et al.* (22) first observed significant biochemical differences in OM isolated from modern symbiotic and nonsymbiotic coral skeletons. Here we show that these coral ecotypes also differ significantly with respect to OM $\delta^{15}\text{N}$ and that OM $\delta^{15}\text{N}$ is a useful proxy for photosymbiosis in paleobiological research on corals.

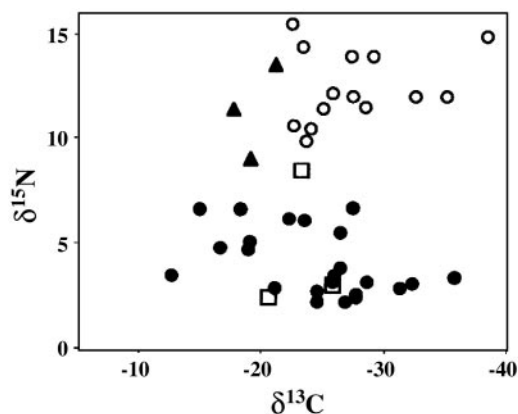


Fig. 2. Paired $\delta^{13}\text{C}/\delta^{15}\text{N}$ values (‰) for OM. ●, symbiotic; ○, nonsymbiotic; ▲, *Oculina* spp.; □ *P. major*.

Differences in absolute value of $\delta^{15}\text{N}$ depends on coral nutritional lifestyle and $\delta^{15}\text{N}$ of source nitrogen. Nonsymbiotic corals exhibit a strict heterotrophic nutritional lifestyle. They feed mainly on particulate organic nitrogen sources already enriched in ^{15}N . After assimilation of these sources, the coral animal tissue $\delta^{15}\text{N}$ is enriched further. In this study it averages 8.08‰ (Table 2). In contrast, symbiotic corals exhibit both phototrophic and heterotrophic nutritional lifestyles. $\delta^{15}\text{N}$ of the algae, animal, and matrix compartments is depleted in ^{15}N and averages 4.33‰ (Table 2). This value is near the low end of the general range of $\delta^{15}\text{N}$ values (-3‰ to 20‰) previously reported for marine organisms (41) and might be explained by the phototrophic uptake and assimilation of ^{15}N -depleted dissolved inorganic ammonium by the symbiotic algae. Phototrophy may well predominate over heterotrophy in symbiotic corals, because even at the low ambient concentrations associated with coral reefs, ammonium uptake is sufficient to supply the daily demand of a symbiotic coral for new nitrogen (42). The similarity of $\delta^{15}\text{N}$ in symbiotic coral compartments might be the result of nutrient recycling, which could bring ammonium assimilation products to some equilibrium $\delta^{15}\text{N}$ value in algae and animal tissue, including OM precursors (1, 10, 11, 43). It is interesting to note that in several symbiotic corals from the Great Barrier Reef, OM $\delta^{15}\text{N}$ is less enriched in ^{15}N than algae or animal tissue and averages $\approx 1.3\text{‰}$ (11). Such a trend might result from synthesis of OM proteins unique to those species. Finally, in some Caribbean corals, particularly *Montastrea cavernosa* living below 10 m, $\delta^{15}\text{N}$ of algae and animal tissue is $<1.00\text{‰}$ (8). To this observation we add the recent discovery of cyanobacteria living in apparent stable endosymbiosis in animal cells of *M. cavernosa* (44). These cyanobacteria are capable of fixing dissolved atmospheric nitrogen and releasing products of nitrogen fixation to algae and animal cells. Because $\delta^{15}\text{N}$ of atmospheric nitrogen is $\approx 0.00\text{‰}$, we would expect $\delta^{15}\text{N}$ of ammonium nitrogen, the first stable product of nitrogen fixation, to be relatively depleted in ^{15}N but with room for enrichment during assimilation by the algae. This scenario provides an alternative explanation as to why $\delta^{15}\text{N}$ in symbiotic corals is depleted in ^{15}N but awaits confirmation that symbiotic corals generally contain cyanobacteria and nonsymbiotic corals do not.

In contrast to OM $\delta^{15}\text{N}$, OM $\delta^{13}\text{C}$ is not a useful proxy for photosymbiosis, but the values of $\delta^{13}\text{C}$ in algae, animal tissue, and OM do evoke an interesting hypothesis. Symbiotic algae ($\delta^{13}\text{C} = -13.84\text{‰}$; Table 2) are customarily enriched in ^{13}C (7, 45, 46). Ninety percent or more of this carbon is translocated to the host animal cells (1). The animal cells also acquire carbon by feeding on demersal zooplankton, the $\delta^{13}\text{C}$ of which averages approximately -19‰ (47). The combination of translocation, holozoic feeding, and concomitant isotopic fractionation results in animal tissue depleted in ^{13}C by $1\text{--}3\text{‰}$, consistent with producer–consumer trophic dynamics (48). Here, the mean for animal tissue is -16.5‰ (Table 2), but there is a further depletion of ^{13}C in OM in both symbiotic and nonsymbiotic corals of $\approx 7\text{--}8\text{‰}$. This depletion is 3-fold larger

than that usually attributed to trophic dynamics. We hypothesize that fractionation of carbon isotopes is substantial during synthesis of OM in animal calicoblast cells. The resulting large isotopic difference between animal cells and OM is maintained because newly synthesized OM is not retained by the calicoblast cells but secreted immediately and mineralized rapidly. That is, animal tissue $\delta^{13}\text{C}$ is not “diluted” by retention of a pool of isotopically lighter OM precursors. This hypothesis is supported by the data of Allemand *et al.* (17), who used [^{14}C]aspartate to show that synthesis of OM and concomitant CaCO_3 precipitation is rapid, occurring within 20 min of introduction of the labeled precursor. There also may be intrinsic biochemical differences between animal tissue and OM, which include preferential incorporation of ^{13}C -depleted (or $^{-}$ -enriched) amino acids into OM (16).

The interpretation that *P. major* was very likely photosymbiotic is consistent with the findings of Stanley and Swart (35), who used a method based on skeletal carbonate $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ to determine whether fossil corals, including *P. major*, may have contained photosynthetic endosymbionts (3). They compared modern symbiotic and nonsymbiotic scleractinians with Triassic and Jurassic fossils. Data from 13 Triassic species were similar to modern symbiotic corals with respect to carbonate $\delta^{18}\text{O}$ and enriched with respect to carbonate $\delta^{13}\text{C}$. The authors concluded that Late Triassic corals were very likely symbiotic. Jurassic corals were clearly aligned with nonsymbiotic corals.

The alignment of *Oculina* spp. with nonsymbiotic corals with respect to OM $\delta^{15}\text{N}$ is not surprising. These species exemplify temperature- and light-dependent facultative symbioses in temperate, often turbid, warm-water habitats along the coast of North Carolina and Florida (49, 50). Algae are present in *Oculina arbuscula* in shallow water (1 m) but in densities (cells per cm^2) often an order of magnitude less than that normally found in tropical symbiotic corals. Given the lower annual rates of insolation, frequent episodes of increased turbidity at the latitude and depth of collection, and greater dependence on heterotrophic nutrition (50), the flux of organic nitrogen between algae, animal tissue, and OM in shallow-water *O. arbuscula* would seem to be insufficient to generate a symbiosis-specific OM $\delta^{15}\text{N}$ signature. *O. arbuscula* from 6 m, collected from dark habitats (e.g., under ledges), and *Oculina varicosa* from 100 m are both aposymbiotic and depend largely on heterotrophic nutrition. As such, they also behave as nonsymbiotic corals.

As an extraction product, skeletal OM is vulnerable to contamination by fragments of animal tissue and by endoliths. This potential problem has not yet been addressed critically in scleractinian corals. We treated pieces of intact skeleton with sodium hypochlorite, and coarsely ground skeleton with NaOH , to remove any residual animal tissue fragments. This step is especially crucial to the decontamination of perforate skeletons. The finding that the skeletal OM in both symbiotic and nonsymbiotic corals has $\delta^{13}\text{C}$ values very different (Table 2) from those of the algae and animal tissue argues strongly against the interpretation that OM represents “trapped” remnants of host tissue (51). Corals also differ in the extent of visible contamination by endolithic algae. Although green skeletons can be discarded easily, white skeletons may still contain chlorophyll-free algal filaments and colorless fungal hyphae. We carried out one trial experiment in which a large piece of green skeleton (*F. scutaria*) was processed by our method to determine whether the OM stable-isotope signature differed from similarly processed *F. scutaria* white skeleton. OM $\delta^{13}\text{C}$ from the single large sample of green skeleton (-22.2‰) fell within the range observed for white skeleton ($-23.0\text{‰} \pm 6.38\text{‰}$; $n = 3$). OM $\delta^{15}\text{N}$ from the green skeleton (6.2‰) was similar to that for OM $\delta^{15}\text{N}$ from white skeleton ($6.46\text{‰} \pm 0.32\text{‰}$; $n = 3$), suggesting in a preliminary way that endoliths (including fungi) were removed

by the extraction protocol. Because sample sizes were small, this interpretation is still subject to confirmation. A less likely alternative interpretation is that endoliths have survived the extraction protocol, have isotope signatures intrinsically similar to those of OM, and also differ significantly in abundance in symbiotic and nonsymbiotic corals.

The effects of diagenesis on *P. major* OM might be expected to include, at a minimum, selective loss of amino acids (52) and thereby raise questions about the meaning of the OM $\delta^{15}\text{N}$ signal. However, Cuif (36) described the remarkable status of preservation of *P. major* aragonite crystals and the extant OM associated with these crystals. The proportion of acidic, alkaline, basic, aromatic, and alcoholic amino acids in the extant OM and the pattern of sulfated polysaccharides in *P. major* wall structures is comparable to those of modern coral OM (19, 53). The difference in OM $\delta^{15}\text{N}$ for the three specimens of *P. major* (8.5‰ , 3.01‰ , and 2.47‰) could have resulted from different extraction protocols. Sample 1 consisted of aggregate (insoluble and soluble) OM, because it was obtained without centrifugation and dialyzed by using 3.5-kDa cutoff membranes. Samples 2 and 3 consisted only of soluble OM having been subjected to centrifugation and passage through ultrafiltration cells with molecular mass cutoffs of 3.0 and 0.5 kDa, respectively. Classically, centrifugation is used to separate insoluble OM from the soluble OM (15). Insoluble OM represents $\approx 66\%$ of the total OM in corals (D.A., unpublished data) and is viewed as a structural framework only (14). By omitting centrifugation, sample 1 very likely includes more insoluble OM than samples 2 and 3. On the other hand, by using ultrafiltration pore sizes with molecular mass cutoffs of 3 and 0.5 kDa, more of the low molecular mass constituents are retained in the soluble OM, including any small peptides that may occur in fossil specimens. These observations suggest that more attention should be given to low molecular mass soluble fractions in studies of coral OM. The function of soluble OM in corals has never been demonstrated experimentally. By inference from data on molluscan shell soluble OM (54), it is hypothesized to be one of mineral nucleation and growth modulation.

With respect to scleractinian evolution, *P. major* skeletal microstructure is comparable to that of Permian plerophyllids (37), the major difference between the two families being the mineralogy. Whereas in all Paleozoic corals, plerophyllid skeletons were calcitic, the evolution of biomineralization in *P. major* has led to production of modern aragonitic units. This divergence suggests that photosymbiosis may have played a role in coral skeletogenesis after the complete decay of Rugosa during the Upper Permian.

Coral skeletons have been proven to be good proxy physiological and environmental recorders. However, all the data come from analysis of the mineral fraction (55). Here we demonstrate that OM $\delta^{15}\text{N}$ is a good proxy recorder of photosymbiosis, providing paleobiological information reaching back to the Paleozoic. The difference between symbiotic and nonsymbiotic OM $\delta^{15}\text{N}$ demonstrates unambiguously an effect of photosymbiosis on OM synthesis (22, 27). It now seems that symbiotic algae may control calcification by both modification of physicochemical parameters within the coral polyps (56, 57) and augmenting the synthesis of OM (17).

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