Identification and Characterization of *Marginopora vertebralis* in Tonga
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Abstract

*Tongan specimens of the foraminifer Marginopora vertebralis are morphologically characterized and compared to published images of the species in order to document and describe this understudied population. Despite being well-studied in the Great Barrier Reef, and to a lesser extent described in localities throughout the Pacific, *M. vertebralis* in Tonga has been largely ignored in literature describing the variability and distribution of the species. To solve this deficiency, we present images and descriptions of several key aspects of test morphology. We find that Tongan specimens clearly conform to defining aspects of the species, while also exhibiting several variations that have not been previously documented. Tongan specimens display a unique red coloration of the test, unusually frequent and biconcave microspheric individuals, and a transitional phase preceding reproductive chamber growth in plicated specimens. Preliminary findings and potential applications of compositional analysis via EDS are discussed, as is the future potential and implications of these finding for use of *M. vertebralis* as an ecological assessment tool.*

Introduction

Shallow seafloor sediments around islands in the nation of Tonga have been observed to contain large numbers of white discs, the nature and origin of which has been largely unknown to local residents and scientific agencies. These discs are identified as the calcite tests of the foraminifer species *Marginopora vertebralis*. *M. vertebralis* is a benthic species found in and around reef ecosystems throughout the Pacific (Ross, 1974; Lee et al., 2016; Langer and Hottinger, 2000). It is among the largest of modern species, characterized by a biconcave discoidal test that grows up to 3cm in diameter over lifespans as long as 3 years (Ross, 1972). Tests develop in two categories: asexually produced megalospheric individuals containing an embryonic apparatus at their center, and sexually produced microspheric individuals (Fig. 2.3)(Ross, 1972). *M. vertebralis* harbors photosynthetic dinoflagellate algae intracellularly, giving healthy organisms visible coloration (Fig. 7.1)(Ross, 1972). These endosymbionts, similar to the zooxanthellae found in coral (Lee et al., 1997) enable the large size and success of *M. vertebralis*
in the high-sunlight, low-nutrient environments of tropical reef ecosystems (Hallock, 1985; Hallock et al., 2003). LBFs have also been observed to undergo bleaching responses to many of the same stressors, and have been studied as possible early-warning systems for bleaching events (Hallock, 2006).

Their relatively direct calcification mechanisms (Bentov, Brownlee, and Erez, 2009) and sensitivity to environmental fluxes have also led Marginopora vertebralis and other large benthic forams (LBFs) to be used extensively as models of calcifying activity in invertebrates (Elderfield, Bertram, and Erez, 1997; Bentov and Erez, 2005) and of the impacts of pollutants (Reymond, Uthicke, and Pandolfi, 2011), eutrophication (Reymond et al., 2013; ) and climate change (Vogel and Uthicke, 2012; Prazeres, Roberts, and Pandolfi, 2017) on calcareous organisms.

Positive identification of these forams is necessitated by the species-specific nature of aspects of their biology such as calcification strategies (Kuile & Erez, 1987) and endosymbiont species (Lee et al., 1997). Furthermore, the field of foram taxonomy, and specifically that of *M. vertebralis*, has a long history of conflicting and confusing species definitions (Lee et al., 2016). Taxonomical disputes have inspired repeated re-definition of the boundaries based on morphological and phylogenetic analysis (e.g. Gudmundsson, 1994; Holzmann et al., 2001), heightening the importance of thorough documentation to accommodate any future changes in the species definition.

This study aims to positively identify and characterize specimens from the *M. vertebralis* community in Tonga. This serves as a preliminary step for future scientific work, including the potential to develop useful monitoring and assessment tools from this widespread resource. We document morphological traits of Tongan specimens, and compare with traits well-described by others (e.g. Ross, 1972; Holzmann et al., 2001; Lee et al., 2004; Lee et al., 2016). Furthermore, we describe preliminary results of compositional probing of foram tests using scanning electron microscopy (SEM), and propose a number of venues for future research and potential applications of *M. vertebralis* as a tool for assessing and monitoring reef ecosystems in Tonga and elsewhere.
Methods

Sample Collection

Foram specimens for this study were collected by hand in shallow seagrass habitats near ‘Atata Island and Sopu reef in Tonga (Fig. 1). Individuals were selected for size, color, and physical variation in order to best sample the various types of test present in the community. Specimens were kept in test tubes of sea water or containers with both sand and seawater for a short time (<48 hours) before being washed with seawater and preserved in a test tube with either 70% ethanol or a DNA preservative solution (70g ammonium sulfate/100ml, 25mM sodium citrate, 10mM EDTA, pH 5.2) for long-term storage and transport. Upon returning to New Zealand, the samples were stored in a refrigerator at 2°C for approximately 2 months before analysis was resumed. Examination of the samples took place over another 2 months, during which specimens were kept at room temperature in their original test tubes and extracted as needed for examination and imagining.

Preparation for Microscopy

A number of the larger, in-tact specimens collected in 'Atata and Sopu were used as archetypes to document the appearance and variation of morphological traits and aid in confirming classification as *M. vertebralis*. Early specimens were examined and imaged on a Nikon SMZ800 binocular microscope with a Nikon DS-Fi1 camera attachment, while another batch of specimens was prepared for use with the SEM. These tests were placed overnight in a bath of 50% hydrogen peroxide to dissolve organic matter, then rinsed thoroughly with tap water and brushed with a fine nylon artist brush to remove debris, then dried overnight at 60°C. The bleached, dried specimens were mounted in various orientations using either Sealey's Ultraclear Araldite epoxy adhesive or Sealey's Knead-it Aqua polymer repair compound. Some specimens were ground and polished on a 160-grit lap wheel followed by 1200 and 2000-grit sandpaper and cleaned ultrasonically in 70% ethanol. Polished tests were re-dried at 60°C for several days to eliminate any water absorbed during the polishing process. All mounted specimens were stored in sealed containers with anhydrous silica beads until they could be carbon-coated and viewed on the SEM. Electron images were taken on a Jeol JSM-IT300 microscope, and compositional
readings were taken via energy dispersive X-ray spectroscopy (EDS) with an Oxford Instruments X-Max® 50 detector and AZtecEnergy EDS software. Contrast and lighting were enhanced on some images using Adobe Lightroom 7.

**Imaging for Identification**
Several specimens were prepared specifically to obtain images similar to SEM figures in published works describing *M. vertebralis* in order to confirm the species identity, and highlight any recurring differences between *M. vertebralis* in Tonga and populations elsewhere. Electron micrographs, light micrographs, and drawn diagrams from Gudmundsson (1994), Holzmann et al. (2001), Lee et al. (2017), and Ross (1972) were collected via screenshot and used for comparison.

**Compositional Testing**
Some preliminary compositional probing of specimens was performed with EDS during imaging. Spectra for these analyses were acquired within three rectangular sample areas drawn on flat, horizontal surfaces in the area of interest. Compositional data excluded carbon in the sample because it is indistinguishable from the carbon coating applied to the test. Peaks deemed likely to be erroneous by the EDS software based on their low magnitude and high standard deviation were not considered.

**Results**

**Embryonic apparatus**
A roughly circular internally-lobed chamber identified as the embryonic apparatus was observed at the center of many specimens. The centers of many other tests were missing, while others were covered in secondary calcification or too cloudy to see internal detail. Specimens with a visible embryonic apparatus (Fig. 2.1) show three distinct lobes around a closed internal chamber (proloculus), matching description and diagrams of *M. vertebralis* embryos (Fig. 2.5)(Gudmundsson, 1994; Ross, 1972). Several dozen small particles found in one of the sample
test tubes were also discovered to be *M. vertebralis* embryos, similar to those at the center of developed tests but lacking any surrounding structure. These free-floating embryos (Fig. 2.2) are more transparent and clearly show three distinct lobes and a closed teardrop-shaped proloculus. Fewer microspheric individuals were found among the collected specimens, those found (Fig. 2.3) exhibited the initial planispiral growth pattern described by Gudmundsson (1994) (Fig. 2.4).

**Apertural face:**
The outside edge along the perimeter of the test (referred to as the apertural face) exhibits a slightly convex shape with many evenly-spaced elliptical, circular, and irregularly-shaped pores opening into the interior of the test (Fig. 3.1). These “lipped” apertures have a smooth, raised periphery and appear slightly sunken in the surrounding surface of rough tendril-like calcite (Fig. 4.1). One row of circular, closely-spaced marginal apertures is present along each edge of the apertural face where it junctions with the disc face. These marginal apertures are oriented either predominantly (Fig. 3.1) or partially (Fig. 6.1) towards the disc face. Notably absent from some Tongan specimens (Fig. 3.1), but not others (Fig. 6.1), are the curved ridges along the edge of the apertural face between each of the marginal apertures (Fig. 3.2). These ridges are also absent in specimens of *M. vertebralis* var. *rossi* described by Lee et al. (2016) (Fig 3.3). Apertures on reproductive chambers (Figs. 4.2, 9) appeared more irregular in shape and frequently abutted or melded with one another, but were otherwise similar to non-reproductive specimens.

**Cross section:**
Specimens broken in half and viewed from the side exhibited two distinct areas within the internal skeleton: a marginal portion of constant depth running parallel to the disc face, and a medial portion that increased in width as the test grew outward. The marginal skeleton comprises the marginal chamberlets visible from the disc face (Figs. 7.2, 7.3), as well annular canals running around the circumference of the test, connecting each marginal chamberlet with the one after it via canals called “stolons.” The medial skeleton displays a wedge shape described
by Ross (1972) and contains irregular pockets (medial chamberlets) organized within successive “waves” created by the progressive addition of new apertural faces with each new annular ring (Fig. 5.1).

In some images (Figs. 5.2, 5.5) the marginal chamberlets appear to only have one stolon connecting to the annular canal, while others have 2 (Figs. 5.4, 7.3).

**Disc face**

The disc face of living specimens was generally observed to be bluish-grey around the perimeter, yellowish-grey towards the center, and vibrant red or sometimes cream colored at the center (Figs. 7.1, 7.2). Preliminary EDS analysis of the disc face indicated trace levels (1.6% wt) of iron in the calcite at the center of two specimens exhibiting red coloration.

The disc face is covered by a thin calcite plate with slightly domed scale-like formations overlying roughly rectangular marginal chamberlets (Figs. 6.1, 7.3). Figure 7.3 shows a disc face ground at a shallow angle to display the intact face plate, the underlying chamberlets, and the internal structure of medial chamberlets. The face plate was most evident in SEM imagery, as it typically appears transparent and therefore largely invisible in light microscopy, as shown in Figure 7.2. Marginal chamberlets exhibited the openings of two stolons connecting them to the marginal canals, visible from above in Figure 7.3 and in cross section in Figure 5.2.

**Plication**

Many larger specimens displayed plication (Figs. 8, 9): extreme waviness and branching leading to concave structures around the perimeter of the test. Small-scale morphological features (i.e. apertures, marginal chamberlets) of plicated regions appeared largely similar to those in non-plicated tests. Images of plication from other publications (Figs 8.2, 8.3) show structures of similar appearance and proportions, but at a smaller scale than was observed in Tongan specimens. Medial skeletons of plicated regions were frequently observed to be less orderly and contain large cavities similar to reproductive chambers (Fig. 9.3).
Preliminary EDS analysis of plicated specimens indicated that plicated regions in several tests had a higher incidence of trace elements such as potassium, sodium, and phosphorus in the calcite skeleton and reproductive chambers than calcite from the un-plicated areas.

**Reproductive chambers**

Annular rings that were partially or fully hollow were observed around the perimeter of some larger plicated and non-plicated specimens (Figs. 9, 10). These match the description of reproductive chambers formed in *M. vertebralis* preceding asexual reproduction (Fig. 10.3). In many specimens (e.g. Fig. 9.1) these chambers showed a high degree of degradation and breakage of the apertural face, leaving only the side walls of the chamber attached to the test (Fig. 9.2).

Some specimens appear to transition gradually into reproductive chambers through a progressively hollower medial skeleton. These ‘transitioning’ layers have a disorganized internal appearance, with large cavities interspersed among medial chamberlets that are often larger and more irregularly shaped than those in the interior of the test (Fig. 9.3) Other specimens (Fig. 10.1) have an abrupt transition between growth types, more consistent with images in other publications (Fig. 10.3). Plicated tests generally exhibited the gradual transition, while flat tests were generally abrupt. Marginal chamberlets were still observed on the outside of reproductive regions despite the absence of a medial skeleton or annular canals. These retained a similar external appearance but had a more fan-shaped profile than those surrounding the medial skeleton (Fig. 10.2). Internal views of the chambers revealed buttress-like structures along the interior walls (Fig. 9.2) and thin calcite columns connecting the previous and succeeding apertural surfaces in partially hollow areas (Fig. 9.3).

**Mutations**

Several specimens were observed to have mutations of varying severity, four of which are documented in Figure 11. Two specimens had mutations near the middle of the test (Figs. 11.1, 11.2) with morphology characteristic of the apertural face, including reproductive chamber
growth (Fig. 11.2). Other specimens exhibited leaf-like growths (Figs. 11.3, 11.4) with all of the anatomical features of a typical test other than orientation. Preliminary EDS analysis of the specimens in figures 11.2 and 11.3 revealed no substantial compositional differences in the calcite of mutated areas versus the rest of the test, nor between tests with mutations and those without.

Discussion

Identification
Comparative morphology confirms that the specimens collected in Tonga are Marginopora vertebralis. Tongan specimens closely match the morphologic hallmarks of the species, exhibiting similar appearance of the embryonic apparatus, peripheral apertures, and chamber construction. These features have been used at length in foram taxonomy because of their distinctive nature (Ross, 1972; Gudmundsson, 1994; Lee, Burnham, and Cevasco, 2004; Lee et al., 2016). Despite close similarities, the Tongan M. vertebralis community is neither identical to other populations nor homogenous within Tonga. Several notable and potentially new observations are discussed below.

Characterization of morphology

Embryonic apparatus.
The embryonic apparatus of megalospheric Tongan specimens (Fig. 2) has a distinct flexostyle, a lobe that is a characteristic feature described by Ross (1972) and Gudmundsson (1994) as one of the few reliable ways to distinguish M. vertebralis from similar genera Amphisorus and Sorites. This feature is clearest in embryos without further development (Fig. 2.2). The presence of these within a sample of hand-selected specimens might be explained by their tendency to settle on parent tests shortly after spawning (Ross, 1972) allowing for their accidental inclusion.
Megalospheric individuals were more common among small samples, reflecting observations that asexual reproduction is the dominant form seen in forams (Ross, 1972). However, microspheric individuals were by no means uncommon, and appeared to equal or exceed megalospheric specimens among the larger tests collected. This is a substantial difference from populations in the GBR, where microspheric tests have been described as very scarce (Ross, 1972; Lee et al., 2016). Test morphology also differed somewhat from these descriptions. Young specimens closely matched descriptions from Gudmundsson (1994) being flatter and planispiral in initial growth (Figs. 2.3, 2.4), while older microspheric specimens (e.g. Fig. 7.2) were observed to have biconcave tests similar to megalospheric specimens. This observation again conflicts with Lee et al. (2016), who state that microspheric individuals in the GBR remain planar for the duration of their lives, maintaining “uniform thickness from the embryonic apparatus through the reproductive chambers,” while only megalospheric individuals develop biconcave tests. If these conflicting observations are both true, they represent another significant difference between Tongan and GBR populations.

Apertural face:
The apertural faces of Tongan specimens all exhibited the lipped apertures and rough calcite face typical of *M. vertebralis*. Interestingly, several of the specimens selected specifically to showcase their apertural face (Figs. 1.1, 2.1, 2.2) closely match the description of *M. vertebralis* variant *rossi* from the GBR (Lee et al. 2016). These specimens shared the more irregularly-shaped and less ordered medial apertures (Figs. 4.1, 4.3, 4.4), as well as marginal apertures that were similarly oblique (Fig. 3.1) and not bounded by the ridge structures common in ‘typical’ *M. vertebralis* (Fig. 3.2). The overall test shapes of these specimens also matched descriptions of var. *rossi*, being abnormally flat and shallowly biconcave, and reaching a large size (~2.5cm) with no sign of plication (Lee et al., 2016). The conclusion that these specimens are in fact var. *rossi* would be surprising, as previous work on the variant has only described populations in the Herron Island channel at a depth of ~30m (Reymond et al., 2013; Lee et al., 2016), far deeper than the shallow seagrass habitats where the Tongan specimens originated. This discrepancy in habitats would be especially interesting because var. *rossi* is considered to be an ecomorphotype, having variations
dictated by environmental, rather than genetic differences (Lee et al, 2016). More purposeful specimen collection and preparation is necessary to confirm if var. rossi is present in Tonga. Most of the other specimens imaged on the apertural face (e.g. Fig. 6.1) more closely matched descriptions of ‘normal’ M. vertebralis apertures (Figs. 3.2, 6.2) (Ross, 1972; Gudmundsson 1994; Lee et al., 2016).

Cross sections
Cross sectional images of Tongan specimens largely match those in the literature (Fig. 5). The well-defined annular canals seen in Tongan specimens (Fig, 5.1) are a distinct feature used to identify M. vertebralis because they are not present in otherwise similar genera (Gudmundsson, 1994).

One interesting note is that there appears to be some variation within both Tongan and Australian populations in the number of stolons (tubes) connecting marginal chamberlets and the underlying annular canals. Some specimens (Fig. 5.2, 5.5) appear to exhibit unidirectional stolons to the proceeding canal only, while others (Figs. 5.4, 7.2) exhibit connections to both previous and proceeding canals. Although it is possible that the appearance of a solitary stolon is actually created by the test fracturing around (rather than through) the stolon on one side, such consistency of this effect seems unlikely. This observation conflicts with clear descriptions of marginal chamber construction (Ross, 1972; Gudmundsson, 1994), and would require further imaging of these and other specimens to confirm.

Disc face:
Light images of the disc face (Fig. 7) showcase the natural coloration of the unbleached test, the most notable aspect of which is the vibrant red center present in many Tongan specimens (Figs. 7.1, 7.2). Although test coloration in M. vertebralis caused by the presence of endosymbionts has been documented before, specimens from the GBR are described as “yellow-brownish green” with a cream-colored edge and lighter center (Ross, 1972; Lee et al, 2016). No record has been made of a vibrant red center in M. vertebralis, and no images could be located depicting it on the web. This suggests that this trait, and possibly the endosymbionts responsible for it, are unique
to Tonga. It is also possible that the red coloration is not related to symbionts; it was noted during preparation for the SEM that the red centers of some tests were more resistant to bleaching by peroxide than the other colors in the test, potentially indicating the red is associated with the calcite skeleton rather than the cytoplasm. This theory might help explain the presence of iron in the calcite at the center of two tests that originally displayed red coloration.

If related to algal symbionts, this vibrant coloration makes *M. vertebralis* a promising candidate for monitoring reef-bleaching conditions. Forams with symbionts exhibit bleaching responses to similar stressors (Hallock et al., 2006), so large, brightly colored tests could enable differentiation between bleached and healthy specimens from afar, allowing expedited assessment of communities for early-warning of bleaching events. Confirming the uniqueness and determining the cause of this coloration is a clear venue for future research, and may be an important step towards developing one of the most promising applications of this species.

**Plication:**

Plication observed in Tongan specimens appeared similar to documented cases (Figs. 7.2, 7.3). Plication has been observed in *M. vertebralis* from many localities (Smout, 1963; Ross, 1972; Lee et al, 2016), and was common among the larger (>1.5cm diameter) Tongan specimens. The causes of plication are not well understood. Phylogenetic analysis of plicated specimens has determined genetic differences between plicated and non-PLICATED variants to be insubstantial (Lee et al, 2016), suggesting that this variation may be an ecomorphological trait caused by environmental conditions. One proposed culprit is shallow, high-energy habitats, the antithesis of the deep, low-energy habitats thought to be responsible for the consistently flat tests of *M. vertebralis* var. *rossi* (Lee et al, 2016). This theory may lose some ground in light of the possibility discussed above that var. *rossi* may be present in shallow conditions in Tonga.

One potential new lead is our preliminary finding that plicated regions in several tests from Tonga had a higher incidence of trace elements, suggesting a decrease in calcification selectivity associated with plication. Future research to confirm and describe compositional differences associated with plication is a promising path towards determining the cause of it. If a
consistent link to one or several environmental variables can be established, it would enable *M. vertebralis* to be used to expedite data collection about that variable. A single minimally trained surveyor with a snorkel could, by estimating the proportion and degree of plicated specimens within an area, determine the extent of a given influence on it.

The same potential exists with mutated tests (Fig. 11), which have already been linked with heavy metals pollution in other benthic forams (Yanko, 1994) and could be an equally visible indicator of environmental conditions if a consistent cause is identified for *M. vertebralis*.

Reproductive chambers

Description of the nature and function of reproductive chambers in *M. vertebralis* is comparatively scarce in the literature, with only Ross (1972) providing comprehensive imagery, and even then not describing reproductive chambers in association with plication. Given what is known, Tongan specimens (Figs 9, 10) appear to conform to what is normal for the species, having fully hollow rings with more porous, delicate exteriors than the rest of the test (Ross, 1972). One notable feature of Tongan specimens is an apparently gradual onset of reproductive chamber growth in plicated individuals, manifested by several partially hollow annular rings preceding the fully hollow reproductive chambers (Fig. 9.3). This contrasts with the non-plicated specimens and figures from the literature (Figs. 10.3, 10.4), which appear to switch from medial skeleton to reproductive chamber across a single apertural face. Whether these transitioning stages have the capability to produce offspring is unknown, but the presence of structural features would likely hinder the breakage necessary to release the developed offspring (Ross, 1972). The correlation between gradual onset and plication is purely observational at this stage, and future research is needed to confirm the association and determine the cause or function of this growth type.

Summary

The evidence gathered in this study shows *Marginopora vertebralis* is abundant in Tonga, and is morphologically variable both within Tonga and compared to populations elsewhere. While many of these variations (e.g. reduced appearance of ridges along the apertural face, the
possible presence of a single stolon per marginal chamberlet) are mainly curiosities, others, if confirmed, are significant additions to the described variation in *M. vertebralis*. The most important findings of this study are that 1) *M. vertebralis* var. *rossi*, a variant previously only described in deep channels of the GBR (Lee et al., 2016), appears to be present in shallow regions of Tonga. 2) Microspheric individuals appear more abundant in Tonga than elsewhere, and capable of greater biconcave test growth and development than those described elsewhere. 3) Collected specimens exhibit a bright red coloration of the test which may be unique to Tonga and the cause of which is unknown. If related to symbionts, this color may greatly facilitate the use of *M. vertebralis* in monitoring coral health. 4) Plicated specimens are abundant in Tonga, and preliminary EDS analysis indicates an increase in trace elements in the calcite of plicated regions of skeleton. This provides a new potential lead in determining the cause of this widely observed phenomenon, and highlights the potential for EDS in future studies of foram biology. 5) Plicated individuals exhibit a gradual skeletal transition into reproduction chamber growth, having several low-density annular rings with unique morphology. Determining the cause or purpose of this trait may also be relevant to understanding plication in general.

By describing the *M. vertebralis* population in Tonga and characterizing several new forms present among these specimens, this study builds on the work of Ross (1972), Gudmundsson (1994) and Lee et al. (2016) in documenting the variability within the species and providing several new questions for future research. In a broader sense, this study lays ground work for promising applications of *M. vertebralis* in Tonga, proposing ways in which its large size, physical variation, and vibrant coloration may someday be tools to aid scientific research in the area.

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References


Figure 1. Sample collection sites in Tonga. Area A and subset (a) show collection area in ‘Atata, coordinates 21°03’11”S 175°15’25”W. Area B and subset b show collection area near Sopu reef on Tongatopu, coordinates 21°07’35”S 175°12’22”W.
Figure 2. Embryonic apparatus in Tongan specimens 1) Megalospheric individual with a partially clouded embryonic apparatus 2) Embryo found in one of the samples showing internal structure with proloculus (P), flexostyle (F) and vorhorf (V). Note the distinct flexostyle similar to that shown in image 5 3) Side-by-side comparison of a microspheric (left) and megalospheric (right) specimen. Note the planispiral growth in the microspheric individual. 4) Diagram from Gudmundsson (1994) of microspheric embryo appearance in *M. vertebralis*. 5) Diagram from Gudmundsson (1994) of megalospheric embryo appearance in *M. vertebralis*. 
**Figure 3.** Low magnification views of the apertural face 1) A non-reproductive specimen from Tonga. Note the smaller marginal pores along either edge (Black arrows). 2) Image from Holzmann et al. (2001) of *M. vertebralis* from Guam. Note the prominence of the marginal apertures and the ridges along the edge of the apertural face (white arrows) absent from the specimen in image 1. 3) Image from Lee et al (2016) of *M. vertebralis* var. *rossi* from the GBR. Note the similar appearance of the marginal pores to those in image 1.
Figure 4. Detailed apertural face images 1) Non-reproductive specimen from Tonga. Note the similarity of aperture shape to images 3 and 4. 2) Reproductive specimen from Tonga. Note the more irregular and melded apertures. 3) Image from Lee et al. (2016) showing apertural face of *M. vertebralis* var. *rossi* from the GBR. 4) Magnified view of the same specimen. 5) Image from Lee, Burnham, and Cevasco (2004) of *M. vertebralis* from the GBR. Note the more circular apertures compared to Tongan specimens.
**Figure 5.** Cross sectional view of flat tests. 1) Low magnification view of a specimen from Tonga. Note the annular canals (Ac) between annular rings similar to image 3, and the wave-like appearance created by the successive creation of new apertural faces (Dashed white lines) during test growth. 2) Higher magnification of the same specimen. Arrows point to annular canals, circles indicate stolons connecting surface chamberlets to annular canals. 3) Image from Lee et al. (2016) of cross section of *M. vertebralis* from the GBR. 4) Image from Lee et al. (2016) of Cross section of resin cast of *M. vertebralis* from the GBR. Note double stolons from each chamberlets compared to single connections in images 3 and 5. 5) Image from Lee et al. (2016) of *M. vertebralis* from the GBR.
**Figure 6.** Juncture of the disc face and apertural face. 1) Specimen from Tonga. Note that the final row of chamberlets (black arrows) forms ridges along the marginal apertures (white arrows) similar to those visible in Figure 3.2. 2) Image from Holzmann et al. (2001) of *M. vertebalis* from Guam. Note the medial apertures (dashed arrows) are more circular and organized into vertical rows than those of the Tongan specimen.

**Figure 7.** Disc face images of specimens from Tonga. 1) Photograph of recently collected specimens from Tonga. Note the red coloration at the center. 2) Light micrograph showing the coloration stemming from intracellular symbionts, visible through the transparent surface layer of calcite. Note that the individual here is microspheric. 3) Electron micrograph of a disc face with a shallow cut exhibiting the overlying surface plate (lower right, rendered opaque by the SEM), the marginal chamberlets (center and lower left) with visible stolons (St) connecting to annular canals, and the underlying skeleton (upper right). Note the two stolons per chamberlet compared to the single stolons visible in Fig. 5.1.
Figure 8. Edge view of plicated tests. (1) Specimen from Tonga. (2 & 3) Images from Lee et al. (2016) of plicated M. vertebralis from the GBR. Note the similarities in morphology and proportions of the three specimens despite the discrepancy in size.
Figure 9. Reproductive chambers in plicated tests from Tonga. 1) Low magnification of the apertural and disc faces of an complete, unmounted test. Note the fragile outermost chamber has been broken, either during transport or during the organism’s lifetime. 2) Magnified view of the same specimen, showing the internal structure of the reproductive chamber and the apertural face of the preceding chamber. Note the buttress-like structures along the inside wall (arrow). 3) Cutaway of another specimen showing the gradual, “messy” transition into fully hollow reproductive chambers (upper bracket). Note the thin pillar structures (arrow) connecting present in the partially hollow skeleton (lower bracket).
Figure 10. Reproductive chambers in non-plicated tests. 1) Low magnification of a wavy specimen from Tonga showing accumulated reproductive chambers. Note the fully hollow nature of chambers and the abrupt transition from medial skeleton growth. 2) Magnified view of the same specimen, showing the relationship between the successive apertural faces separating chambers and the marginal chamberlets on the external shell. 3) Image from Ross (1972) showing typical appearance of reproductive chambers (no scale given). Note the change in test width not seen in image 1. 4) Image from Lee et al. (2016) of M. vertebralis from the GBR showing an abrupt transition into reproductive chamber growth.
Figure 11. Various mutations observed in Tongan specimens. 1) Center growth exhibiting morphology typical of the apertural face. 2) Center growth resembling reproductive chambers. 3) Near-center growth of a new test fragment with marginal and medial skeleton morphology mimicking the base test. 4) Severely mutated individual with out-of-axis growths mimicking the morphology of the base test.