Morphometrics Data Collection Protocol

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Camera Setup

1. Extend tripod legs
    a. Place 2 legs on the edge of table
    b. Set tripod height 10-15 cm from table edge
2. Release pin for camera plate
3. Screw camera (Canon G16) into plate
4. Replace camera and plate on tripod
5. Point camera directly downwards
6. Put bubble level on camera screen
7. Camera Settings: Program > Macro > Manual focus (press ^ on wheel and cycle down to focus)
    a. Adjust exposure for lighting

Corallite Placement & Measurement

1. When starting with new sample, take picture of bag and sample # as a placekeeper
2. Place frag on bed of aluminum foil to make corallite parallel
    a. Make sure the angle of the shot does not expose layers of septa below
        i. Avoided by taking a direct overhead shot of corallite
3. Select 5 corallites for measurements
    a. Guidelines for selection:
        i. Cannot be near margin of coral
        ii. Cannot be near broken edge of coral
        iii. No new corallites
        iv. Must have neighbors

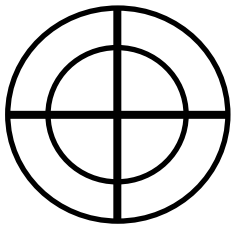

Figure 1.

4. Use dental pick to help center corallite in frame
    a. Dental pick must be in each picture as a scale
        i. Tick=1mm (from start of one line to the end of 1 line)
5. Corallite Height (CH) & Theca Height (TH) must be measured using the calipers
    a. Take 4 measurements per metric (1 per quadrant- see Figure 1)
6. Corallite density (CS) can be measured with calipers or on Image J (depending on quality of photo and/or fragment curvature
    a. CS is measured from the center of one corallite to the next for all of the polyps neighboring
        i. In excel, Create separate spreadsheet for CS because of varying #s of neighbors/corallite

Image Analysis

Photoshop Editing

1. Download pictures onto the desktop of the computer with Photoshop
2. Create a Subfolder within this folder labeled BW Corrections
3. In Photoshop click File→Automate→Batch
    a. Play→ Black&White
    b. Source→ Folder created on desktop with pictures
    c. Destination→ BW Corrections
    d. Click Ok
4. Press Enter for every photo in batch
5. Move folder to common drive

Image J Analysis

1. File→ Open Image
2. + zooms in on image, - zooms out, place cursor where you would like to zoom
3. Zoom in to caliper
    a. Click the line tool and create a line from the end of one tick to the beginning of another
        i. Includes small black line
    b. Click Analyze→Set Scale
        i. Known Distance: 1
        ii. Unit of length: mm
4. Zoom into corallite
5. Draw a line for your measurement
    a. Click Analyze→Measure
    b. A results box will pop up
        i. Length (the last column) is your measurement in mm
    c. For CD and CW refer to Figures 2 & 3
    d. L1S, L4S, and T1C, will be measured using Figure 1 above and pictures below as references
        i. 1 measurement per Quadrant
6. For Excel and data entry organization, refer to template spreadsheet

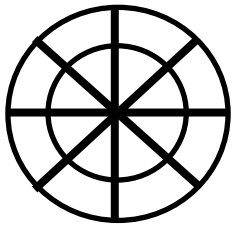

Figure 2. CD

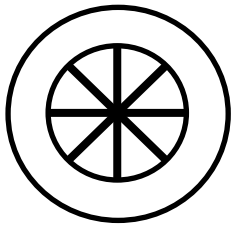

Figure 3. CW

Statistical Analysis

1. For plot generation and pairwise statistical analyses, follow the instructions in the R scripts "Morphometrics_figures.R" and "Morphotype_figures.R"
2. For multivariate statistics in Primer v7, follow the instructions in the Primer user manual by Clarke and Gorley and in the PERMANOVA+ manual by Anderson et al. These manuals describe the statistical tests used in Studivan et al. 2018 in comprehensive detail.
3. For comparison of morphological variation to genotypic variation, follow the appropriate sections (GenAlEx, Structure, Structure Harvester, CLUMPP, Distruct) of the "microsat_analysis_README" in the "Mcav-microsats" repository.