**Calice Morphology Protocol**

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Desired morphometrics:

* Calice diameter
* Calice depth
* Calice spacing
* Calice density

Materials

* Bleach solution
* Drying oven
* DSLR camera
* Digital Vernier calipers (+/- 0.01mm)

Methods

1. Bleach skeletons to remove organic tissues (could use 4% sodium hypochlorite solution)
2. Dry skeletons overnight
3. Randomly select 3-10 calices
4. Use Vernier calipers to measure the following:
   1. Calice depth
   2. Septa width and length (can be done with photo)
   3. Calice width and length (can be done with photo)
5. Photograph skeleton under magnification, with scale for reference
6. Use image j or another tool (Coral Point Count V3.5) to measure
   1. Calice area
   2. Calice circumference
   3. Collumnella Area
   4. Calice spacing: mean distance to nearest 3 other calices
   5. Calice density: Number of calices present within a randomly placed square

From Matias’ proposal: “To quantify variation in morphological traits across light gradients, I will collect (after one year) one of the fragments from each environment (two per colony one each from shallow and one from deep). I will bleach skeletal fragments, and characterize calyx morphology from 10 haphazardly - selected corallites per fragment, by a configuration of 48 landmarks to reconstruct space shape from 92 dimensions (48 landmarks x 2 – 4) (Savriama, Y. et al. BMC Evol. Biol. 11, 280 (2011)). The decomposition of shape variation into symmetric and asymmetric components will be done via a full Procrustes fit and Principal Component Analysis to compare shape changes. I will take pictures of each corallite at 2× and 4× objectives using a high-resolution digital camera attached to a stereoscopic microscope. Landmark acquisition and statistical analyses will be done from corallite photographs using the ImageJ, TpsDig2, MorphoJ, and R software.”

References

Todd et al. 2004c

Ow & Todd 2010

Notes: We should essentially replicate Ow & Todd 2010 Table 1 and Fig 2 methods.