**Materials and Methods**

*Geologic setting*

* Collection site
  + Coastal cliffs and river valleys northwest of Whanganui City, North Island, New Zealand during Jan. 2014?
    - from Liow et al. 2017
  + Cheilostome bryozoan-encrusted bivalves
    - from Liow et al. 2017
  + Targeted TST
    - Carter & Naish 1998
  + Shells are abundant
    - Abbott et al 2005
  + Gillespie et al. 1998 discussed TST areas and areas that have bryozoans
* Geologic Setting
  + Pleistocene (2.3 mya) of Wanganui Basin
    - Liow et al. 2017; cites Carter & Naish 1998. Naish et al. 1998, Abbott et al. 2005 <NEED TO READ>
  + Wanganui Basin is filled by several kilometers of siliciclastic sediments, comprising sandstones, siltstone, mudstones, locally carbonate-rich shell beds and volcanic ash layers, forming a cyclic depositional sequence record spanning the last *ca* 2 Myr with a well-established, high-resolution chronostratigraphy
    - Liow et al. 2016; cites Carter & Naish 1998; Abbott et al. 2005; Naish et al. 2005; Proust et al. 2005
  + Collected from shellbeds with transgressive systems tracts (TST)
    - Liow et al. 2016
    - Carter & Naish 1998
  + Nukumaru Limestone (NKLS)
    - Oldest;
    - Abbott et al. 2005
    - MIS 79 is top of limestone (Naish et al 2005)
  + Nukumaru Brown Sand (NKBS)
    - Oxygen isotope stage 73, 75 and 77 (Abbott et al. 2005)
  + Tewkesbury formation
    - Oxygen isotope stages 67, 69, 71 (Abbott et al. 2005)
  + Waipuru
    - Cyclothem 15, 68-70 isotope stage (Abbott et al. 1998)
  + Upper Kai-Iwi
    - Basin cycle 23-34 (Naish et al 2005)
    - Sequence 38 (Naish et al 2005)
    - MIS 17 (Naish et al 2005)
    - TST (Naish et al 2005)
    - Oxygen isotope stage 17; basin sequence 40; sequence/cyclothem 7 (Proust et al. 2005)
    - MIS 17 starts around 712 kya (wiki; check)
  + SHCSBSB
    - HST (Naish et al 2005)
    - MIS 13-12 (Naish et al 2005)
    - Oxygen isotope stage 11; basin sequence 43; sequence/cyclothem 10 (Proust et al. 2005)
  + Tainui
    - TST (Naish et al 2005)
    - MIS 13-12 (Naish et al 2005)
      * Also have as older than SHCSBSB
    - Oxygen isotope stage 13; basin sequence 42; sequence/cyclothem 9 (Proust et al. 2005)
      * Has older than SHCSBSB
    - MIS 13 starts around 524 kya; MIS 12 starts around 478 kya (wiki; check)
  + Modern

Basin cylces 11-12 correspond to MIS 78-57 (abbott 2005)

*Steginoporella magnifica processing*

* shell substrates were cleaned using one or a combination of the following techniques depending on fragility: tapping to remove sediment, gentle washing under running water, scrubbing with a soft toothbrush and washing in an ultrasonic bath.
  + Liow et al. 2016
* Specimens were collected, cleaned, and stored. <*probably need more detail here*>
* Specimens were given a specimenID and imageID. The specimenID is a number, whose range corresponds with a geologic site, and description of the facet of encrustation (CC = concave, CV = convex). The imageID is a number in sequential order always starting from 1, followed by the AV, magnification, and backscatter. The final code may look like: ###\_CC/CV\_1/n\_15v\_x30\_BSE.
* The images are saved as .tif files. Every image has an associated .txt file with metadata, which was used for cross referencing and checking.
* The .tif files were turned into .jpg files for later processing.
* The scale for x30 magnification is 0.606 pixels per 1 μm

*Pipeline*

* Piped together two machine learning modules developed within the Voje lab (Porto & Voje 2020; Di Martino et al. 2022) to create “Steginator” (<https://github.com/agporto/Steginator>), which MAB forked to her own repository for use (<https://github.com/megbalk/Steginator-magnifica>).
  + This pipeline connects the identification of zooids (deepBryo) of *Steginoporella magnifica* with the automatic landmarking by ML-Morph. (see Figure LM for landmarks).
* MAB and Porto ran lab SEM images (created by MHR and Sara) of *Steginoporella magnifica* (see above) based on predetermined filtering (see below).

*Image selection*

* Images were filtered to include only those taken at 30 magnification.
* Images of only *Steginoporella magnifica* were examined; other species were identified and removed
* Each image was examined by MAB to look for erroneous landmarking. Examples of “errors” are: broken or incomplete zooids, which alters linear measurements; distortion in the images since specimens were curved (concave or convex); inaccuracy in landmarking due to debris in the image or misidentification of a landmark.
  + MAB believes that we are likely underestimating variability within a colony. This is because zooids positioned orthogonal to vertical were often mis-landmarked and because it seems the ML defaults/assumes a round bottom for the zooid and so MAB found zooid bottoms often mis-landmarked and so excluded them from the study.
  + Also removed “wonky” zooids, where the zooids are severely deformed
* Minimum 5 zooids per colony for image/colony to be used

*Analyses*

* Created separate GitHub repository, magnifica (<https://github.com/megbalk/magnifica>)
* Used R for all analyses and the following packages <*fill out later*>

Trait Extraction

* Use outputMetadata.R to combine output.csv from ML processing (Steingator-magnifica) with metadata file (“image\_merge\_txt\_usingfileName\_DONE\_17Apr2023.csv”)
  + Output is “meta.images.Jun2023.csv”
* Use exploratoryAnalysis.R read in “meta.images.Jun2023.csv” and calculate traits
  + Output is “traits.csv”
* Calculated the following traits based on landmarks (Figure linear):
  + Zooid height (zh) from 4 to 12
    - Similar to LZ in Voje et al. 2019
  + Median process width at the base (mpw.b) from 5 to 6
  + Cryptocyst width at midline (cw.m) from 10 to 11
  + Cryptocyst width at distal end (cw.d) from 8 to 7
  + Operculum width at midline (ow.m) from 19 to 0
    - Similar to WO in Voje et al. 2019
* Converted lengths from pixels to μm
* Log10 transformed all linear measurements

Sensitivity Analyses

* Checked for normality of traits
* Examined number of colonies per formation
* Examined number of zooids per colony

P & G Matrix estimation

* gp.R file reads in “traits.csv”
* scale data using discriminant analysis (dat\_lg\_N)
* create P matrix (phen.var) as a covariate matrix
  + standardize by trait means <NEED TO DO THIS>
* Create G matrix
  + estimate priors for G and estimate G using MCMC glmm
    - used same priors for individual Gs and global G
  + checked that samping from correct space in distribution
  + retrieve G from posteriors <*I do not know what this means*>

P and G correlation within each formation across traits (i.e., matrices)

* Random skewers

Change in G across formations

* Multivariate time series analysis

Got d18O and age values from DiMartino & Liow 2021 (<https://royalsocietypublishing.org/doi/full/10.1098/rspb.2020.2047#d1e1096>) supplementary material (<https://datadryad.org/stash/dataset/doi:10.5061/dryad.dz08kprw9>)

* Dimartino took all values inbeween bottom and top of formation and took median of 018 values then sd of those values too
* Ranges for NKBS, Waipuru, and Upper Kai-Iwi are not that different and if anything warmer...

**Supplemental**

A close-up of a plant

Description automatically generatedA close up of a plant

Description automatically generated

**References**

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**Figures**

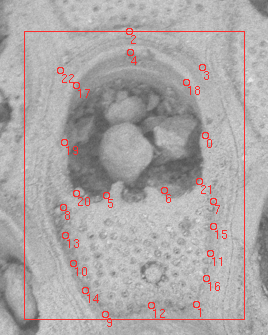


Figure LM Picture of a Steginoporella magnifica zooid and landmarks 0-22 used for later extraction of linear measauremens.

A close-up of a cell

Description automatically generated with low confidence

Figure linear: picture of all the possible linear traits to extract; only extracted a few for this analysis; <NEED TO CHANGE IMAGE>