**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
* Checked for quality of ML measurements compared to my own
  + 10 images from each formation
  + At least 5 colonies (i.e., all images of the same colony)
  + Placed landmarks
  + Measured self, measured using code
  + Did this three times

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**

Porto, A. and Voje, K.L., 2020. ML‐morph: A fast, accurate and general approach for automated detection and landmarking of biological structures in images. *Methods in Ecology and Evolution*, *11*(4), 500-512.

Di Martino, E., Berning, B., Gordon, D.P., Kuklinski, P., Liow, L.H., Ramsfjell, M.H., Ribeiro, H.L., Smith, A.M., Taylor, P.D., Voje, K.L. and Waeschenbach, A., 2022. DeepBryo: a web app for AI-assisted morphometric characterization of cheilostome bryozoans. *bioRxiv*, pp.2022-11.

Voje, K.L., Di Martino, E. and Porto, A., 2020. Revisiting a landmark study system: no evidence for a punctuated mode of evolution in Metrarabdotos. *The American Naturalist*, *195*(5), pp.899-917.

Liow, L.H., Di Martino, E., Krzeminska, M., Ramsfjell, M., Rust, S., Taylor, P.D. and Voje, K.L., 2017. Relative size predicts competitive outcome through 2 million years. *Ecology letters*, *20*(8), pp.981-988.

Liow, L.H., Di Martino, E., Voje, K.L., Rust, S. and Taylor, P.D., 2016. Interspecific interactions through 2 million years: are competitive outcomes predictable?. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1837), p.20160981.

Carter, R.M. and Naish, T.R., 1998. A review of Wanganui Basin, New Zealand: global reference section for shallow marine, Plio–Pleistocene (2.5–0 Ma) cyclostratigraphy. *Sedimentary Geology*, *122*(1-4), pp.37-52.

Abbott, S.T., Naish, T.R., Carter, R.M. and Pillans, B.J., 2005. Sequence stratigraphy of the Nukumaruan Stratotype (Pliocene‐Pleistocene, c. 2.08–1.63 Ma), Wanganui Basin, New Zealand. *Journal of the Royal Society of New Zealand*, *35*(1-2), pp.123-150.

Naish, T.R., Field, B.D., Zhu, H., Melhuish, A., Carter, R.M., Abbott, S.T., Edwards, S., Alloway, B.V., Wilson, G.S., Niessen, F. and Barker, A., 2005. Integrated outcrop, drill core, borehole and seismic stratigraphic architecture of a cyclothemic, shallow‐marine depositional system, Wanganui Basin, New Zealand. *Journal of the royal society of new zealand*, *35*(1-2), pp.91-122.

Proust, J.N., Lamarche, G., Nodder, S. and Kamp, P.J., 2005. Sedimentary architecture of a Plio-Pleistocene proto-back-arc basin: Wanganui Basin, New Zealand. *Sedimentary Geology*, *181*(3-4), pp.107-145.

Naish, T.R., Abbott, S.T., Alloway, V., Beu, A.G., Carter, R.M., Edwards, A.R., Journeaux, T.D., Kamp, P.J., Pillans, B.J., Saul, G. and Woolfe, K.J., 1998. Astronomical calibration of a southern hemisphere Plio-Pleistocene reference section, Wanganui Basin, New Zealand. *Quaternary Science Reviews*, *17*(8), pp.695-710.

Gillespie, J.L., Nelson, C.S. and Nodder, S.D., 1998. Post-glacial sea-level control and sequence stratigraphy of carbonate–terrigenous sediments, Wanganui shelf, New Zealand. *Sedimentary Geology*, *122*(1-4), pp.245-266.

**Figures**

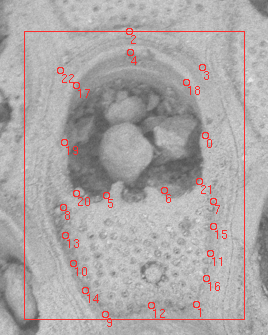


Figure LM Picture of a Steginoporella magnifica zooid and landmarks 0-22 used for later extraction of linear measauremens.

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>