**Role of nitrogen-fixing cyanobacteria in growth and toxicity of culturally significant *Cycas***

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Roslyn Gleadow (PI) and Georgia Lloyd (PhD student)

**Original project aims**

This project aims to document the contribution that cyanobacteria make to the nitrogen economy of *Cycas*, adding value to study of cycad toxicity and the place of cycads in Australian Indigenous cultures. This complements a parallel project conducted by PhD student Georgia Lloyd on the chemical and social ecology of *Cycas.*

Our specific aims for this project were to:

1) Measure $d^{15}N$ and foliar toxin levels in the widespread *Cycas media* grown in pots and supplied with high or low levels of nitrogen fertiliser.
2) Assess the reliance on fixed nitrogen in naturally occurring plants by measuring the $d^{15}N$ of *Cycas angulata* plants and soil collected from our field sites in the Gulf of Carpentaria, Northern Territory.
3) Determine where the cyanobacteria are located in the roots and estimate their abundance in six Australian *Cycas* species through anatomical and morphological studies.

**Progress to date**

We are making good progress with the project. There have been three changes to original proposal. First, the honours student who was going to do the anatomical studies decided not to do honours. As a result we redirected some of the funds (as discussed at the time) to employ Kira Maher for who completed an honours project on cassava toxicity in my group in 2023. She is working flexibly, on average one day per week on this project. Second, we were unable to source all six Queensland species of *Cycas* because the nursery that grows them was completely wiped out by floods. Instead we have focussed on the two Queensland species available: *Cycas opholitica* and *Cycas megacarpa* and included *C. media,* the species used in the nutrient trial.

Specific progress again the milestones are:

1) *Cycas media nutrient experiment (G. Lloyd, assisted by K. Maher)*
    a. We have measure $d^{15}N$ and foliar toxin levels in the widespread *Cycas media* grown in pots and supplied with high or low levels of nitrogen fertiliser. These indicate that there is greater reliance N nitrogen fixation in the low-fertiliser treatment, although there is a lot of variability. I am seeking advice from Prof Chris Greening, an expert in unusual microbial associations based at Monash University, to help in interpreting the data
    b. We have made a good start on measuring the toxin levels in the leaves. There have been a whole string of equipment and calibration problems. The project is now on track and due to be completed early in 2024.

2) *Field studies in the NT (G. Lloyd)*
    a. We have ground the *Cycas angulata* leaves collected in the field to ANU ready to be sent to ANU for isotope analysis (Figure 1).
    b. We have done a full nutrient analysis of the soil collected from our field sites in the Gulf of Carpentaria, Northern Territory. We have not yet measured the $d^{15}N$.

3) *Anatomical and morphological studies for C. ophiolitica, C. megacarpa and C. media (K. Maher)*
    a. The mass and number of the coralloid roots has been determined for both species at two different ages (Figure 2)
    b. Sections of a subsample of coralloid roots of both species and both ages have been made. These are now ready to be analysed in ImageJ. The aim is to calculate the amount of root volume dedicated to house cyanobacteria from the area colonised and the size and number of these specialised roots.

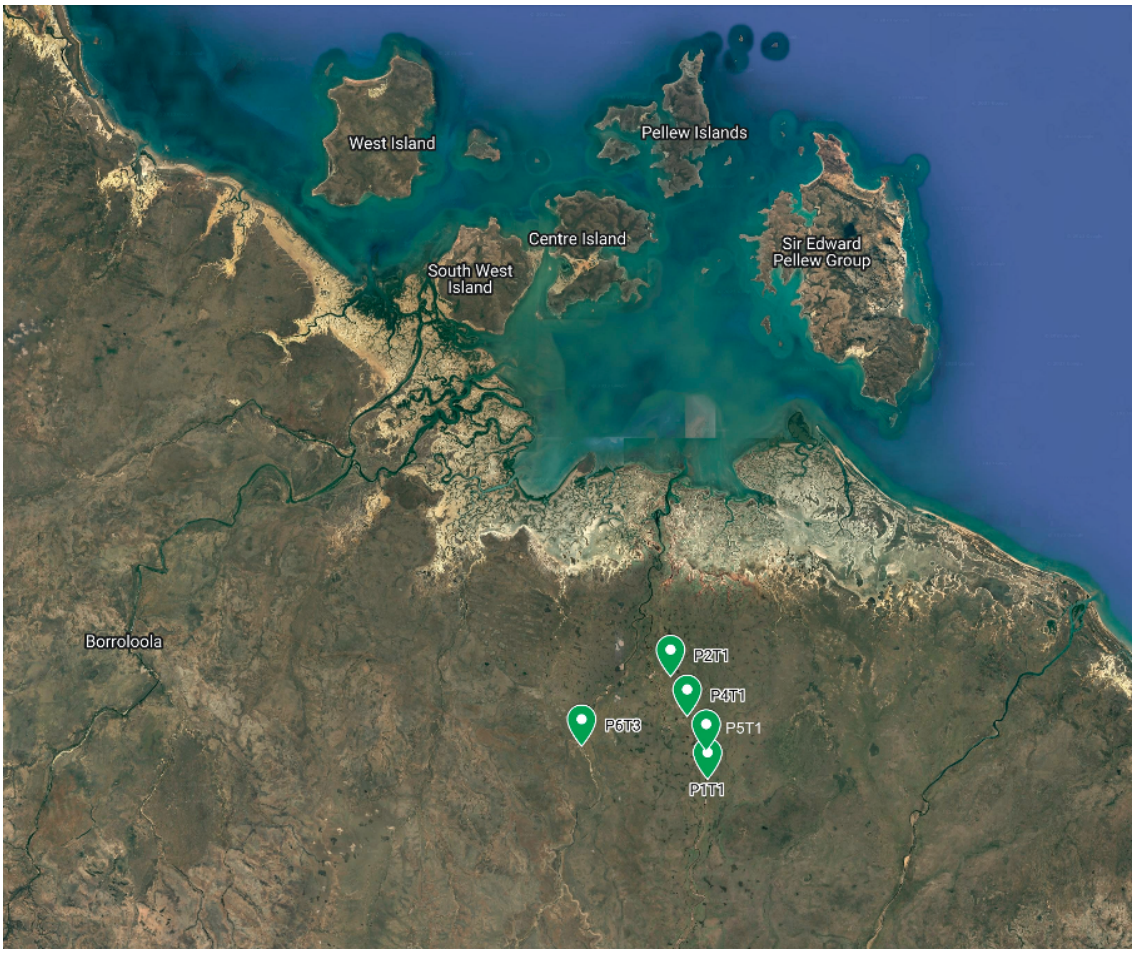
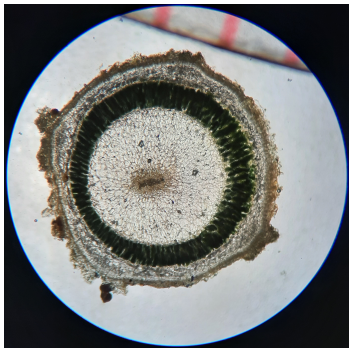



**Figure 1.** *Cycas angulata* sampling sites on Manangoora Pastoral Lease, Gulf of Carpentaria, N.T. in relation to the town of Borroloola (left).

*Fig. 2a. Cycas megacarpa*
coralloid roots
Plant age: 1 year



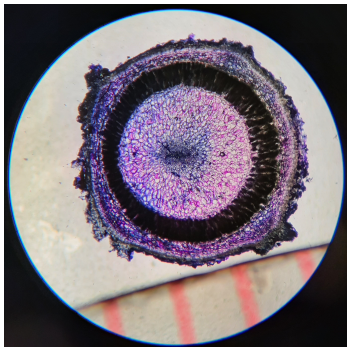


*Fig. 2b. Cycas megacarpa* coralloid root section: before and after staining with Toluidine blue. Magnification= x40

**Figure 2.** – example of coralloid roots of one of the Queensland Cycas species, and an example section through the root showing the thick band of cyanogbacteria.