RESOLVING THE TAXONOMY AND CONSERVATION STATUS OF POTENTIALLY NEW SPECIES OF *TECTICORNIA* (CHENOPODIACEAE) IN THE PILBARA

The genus *Tecticornia*, commonly known as samphires, is a unique group of salt-loving plants that form the dominant vegetation in saline habitats, where they play a critical role at the dynamic interface between water and land, actively cycling nutrients and providing a stabilising influence on sediment movement. Despite their significance in the landscape, there is a general lack of understanding of the group because samphires are extremely difficult to identify. Indeed, they are considered one of the most taxonomically challenging plant groups in Australia due to their reduced morphology (they have no true leaves and tiny, nondescript flowers) and minute but taxonomically informative seed. An understanding of the taxonomy of this important genus, which occupies areas vulnerable to threats from development activity and climate change (due to changes in sea level and hydrological regimes), is essential for informed environmental impact assessment processes. Supporting critical research on this largely neglected plant group also aligns with the need for environmental offsets to address residual impacts and potentially confers a 'Nature Positive' outcome if impacts can be mitigated and remediated.

Even though samphires are found across the Australian continent, an understanding of their true diversity is lacking. Several species complexes exhibit considerable variation in morphology, and it is unclear if they represent a few widely variable species or multiple cryptic species. Previous surveys suggest that several *Tecticornia* from the Pilbara region may represent unrecognised new species; however, the taxonomic status of these potential novelties cannot be resolved without first understanding how they can be uniquely distinguished, both morphologically and genetically, from all other known species in the genus. Polyploids (plants with multiple sets of chromosomes) are also common in the genus and may further confound our understanding of morphological variation within and between populations. It is unclear, for example, if plants with higher numbers of chromosomes are correspondingly larger in size or exhibit variation in seed morphology, as observed in other groups.

Research to address this uncertainty will provide environmental and nature resource management regulators with greater confidence when assessing project developments that impact samphire habitats. Furthermore, this research also provides greater certainty (de-risking) to development proponents and project operators, especially in respect to the mitigation of impacts to samphire species and the communities they form, and the application of regulatory frameworks designed to limit and offset impacts.

New tools to solve an old problem.

This multi-faceted project will use modern methods to assess morphological and genetic variation among samphires in the Pilbara and across the genus. Field collections from saline habitats in the Pilbara will provide material for study, which will be used in conjunction with the nearly 10,500 specimens of *Tecticornia* currently lodged in herbaria across Australia (https://avh.ala.org.au/occurrences/search?taxa=Tecticornia#tab_mapView). Sampling will include the collection of voucher specimens, as well as material for DNA extraction (for molecular custom bait capture sequencing) and flow cytometry (to confirm polyploidy). High resolution images will be taken in the field and laboratory to aid taxonomic assessment and provide illustrations for online fact sheets and an electronic key. While these outputs will be focused on resolving the status of Pilbara samphires these results will also have application across the continent.

Phylogenetic analyses of the molecular data from this study integrated with sequences already available for *Tecticornia*, will clarify patterns of variation and confirm the total number of genetically unique taxa (basic taxonomic units) present across the genus in the Pilbara. These data, along with information from the flow cytometry study, will complement morphological research and inform taxonomic decision-making, enabling novel entities to be formally described and their conservation status assessed. Molecular sequence data will also be submitted to open access databanks to ensure this invaluable resource will be available to future users.

This project will develop laboratory processes that may eventually allow end-users to send sterile samples for DNA sequencing to confirm the identification of sampled plants (if the species has been previously sequenced). This will enable the identity of samphires to be determined at any time of the year, rather than having to rely on the presence of mature seed, which is only seasonally available and currently necessary for accurate taxonomic identification. Such an innovation in samphire identification would deliver significant operational efficiencies to the environmental consulting industry and their proponents, as it would unburden constraints on timing associated with the need to collect samphire material when it is fruiting. This innovation would also confer greater confidence in decision making by environmental regulators and biodiversity managers, as it would offer a step-change in the accuracy and proficiency with which samphires could be confidently and reliably identified without relying on specialist knowledge.

Using Artificial Intelligence to automate the identification of Tecticornia.

Specimen identification using Artificial Intelligence (AI) is in the early stages of development and may prove useful in automating identification. This project will explore the potential of this emerging technology to provide high-throughput identification without the need for a taxonomic expert or molecular sequencing. The ability for AI to discriminate species will depend on the development of an adequate 'training library' to facilitate the recognition of each taxon. High-resolution imagery of voucher specimens linked to molecular data will be used to train the AI. Refinement and testing of the AI's accuracy and precision will be ongoing as more data are accumulated.

Project goals and timeline.

A three-year study is proposed to complete this multi-faceted project, reflecting the complexity of the focus group. Specific aims are to:

- undertake field work to photograph and collect voucher specimens for taxonomic study, DNA sequencing, flow cytometry, and AI analyses;
- refine DNA barcoding techniques to analyse genetic variation across the genus to clarify the genetic distinction of taxa;
- utilise flow cytometry to determine the extent of polyploidy (number of multiple chromosome sets) and its potential influence on morphological variation;
- assess molecular data and preserved herbarium specimens to help resolve the taxonomy of potentially new species;
- publish scientific papers describing new taxa with comprehensive descriptions and conservation assessments;
- produce an electronic identification key and online fact sheets that can be printed as a field guide;
- explore the potential of automated, high-throughput identification techniques using commercial DNA barcoding and AI assessment of herbarium specimens.

Field work for this project will be focused initially on the Pilbara, both its coastal and inland samphire habitats, and adjoining northwest bioregions. Over time the field research will expand to cover all samphire habitats in Western Australia. The research effort will also engage with jurisdictional herbarium across the nation to access their holding of samphire specimens.

Activity	**Process**	**Time**	**Result**
Field surveys and analysis of herbarium specimens	Collect samples from coastal habitats and inland salt lakes; visit various Australian Herbaria	Year 1 – Year 2	Acquire samples for taxonomic study, flow cytometry, and molecular analyses
Molecular study	Bait capture sequencing and analyses	Year 1 – Year 2	DNA data available for phylogenetic analyses
Cytology study	Flow cytometry analyses	Year 1 – Year 2	Detection of polyploids and correlation with morphological data
Taxonomic study	Specimen analyses; collect morphological data; obtain images	Year 1 – Year 3	Baseline data for delimiting new taxa; produce fact sheets and descriptions
Information Dissemination	Progress reports; publications; online data; conference presentations	Year 1 – Year 3	Publication of results in various forms

Staffing

Due to the project's scope, staff with wide-ranging and specialised skills will be required. A post-doctoral researcher will undertake the DNA bait capture data analyses and flow cytometry study, refine the AI identification systems, and co-author taxonomic descriptions of newly recognised species. A technician will provide field support, co-ordinate digitisation of specimens, photograph plants and seeds, and assist with morphologic research including field work, data acquisition and electronic key development.

Supervision and project expertise will be provided by Drs Kelly Shepherd and Anže Žerdoner Čalasan. Dr Shepherd (https://www.researchgate.net/profile/Kelly-Shepherd-2/research) is a Senior Research Scientist at the Western Australian Herbarium and current expert on Australian samphires. She has published a wide range of systematic, molecular, and taxonomic papers on the group (https://www.researchgate.net/profile/Kelly-Shepherd-2/research) and is the most appropriate person to support the taxonomic component of this project.

Dr Žerdoner Čalasan (https://www.researchgate.net/profile/Anze-Zerdoner-Calasan) has expertise in bait capture sequencing, flow cytometry and molecular analyses, and has attended an AI identification workshop (https://www.researchgate.net/profile/Anze-Zerdoner-Calasan) and will provide support for the molecular analyses.

The project will be managed by Professor Stephen van Leeuwen from the Indigenous Stewardship, Biodiversity and Environment Group (https://staffportal.curtin.edu.au/staff/profile/view/stephen-van-leeuwen-c22dacec/) within the School of Molecular and Life Sciences at Curtin University.

Budget

Funding will support a post-doctoral researcher, technical officer, project management, field work and herbarium visits, specimen lodgement costs, flow cytometry, DNA sequencing, and lab consumables.