Phylogenetic analysis of *Tecticornia* using the external transcribed spacer (ETS) nuclear marker: insights into the evolution of C_4 photosynthesis

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BACKGROUND

Tecticornia is a genus of succulent halophytes in the family Chenopodiaceae. The genus currently contains two C_4 species and 39 C_3 species. Many species are endemic to Western Australia, where they play a key ecological role in salt-affected areas (Fig. 1). Photosynthesis is carried out in fused leaves and stems, known as vegetative articles (Fig. 2). High salt tolerance and unique C_4 anatomy (Kranz-Tecticornoid) make it a valuable genus for studying the steps in the evolution of C_4 photosynthesis.

Previous phylogenetic studies, using the internal transcribed spacer (ITS) nuclear marker, and chloroplastic markers, found difficulties resolving relationships among the species.

To study the steps of C_4 evolution in this genus, the C_3 species most closely related to the C_4 species must be identified. These species will be used in further molecular and anatomical studies.

Figure 1. *Tecticornia* plants on a salt lake (Cowcowing Lakes, Western Australia)

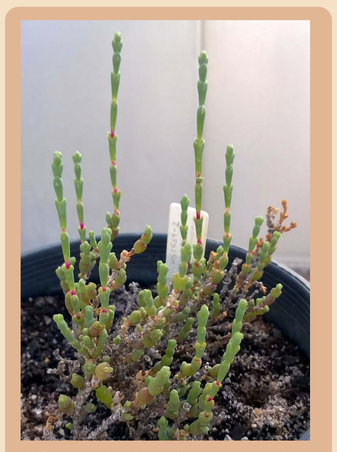


Figure 2. *Tecticornia indica subsp. bidens* (a C_4 species).

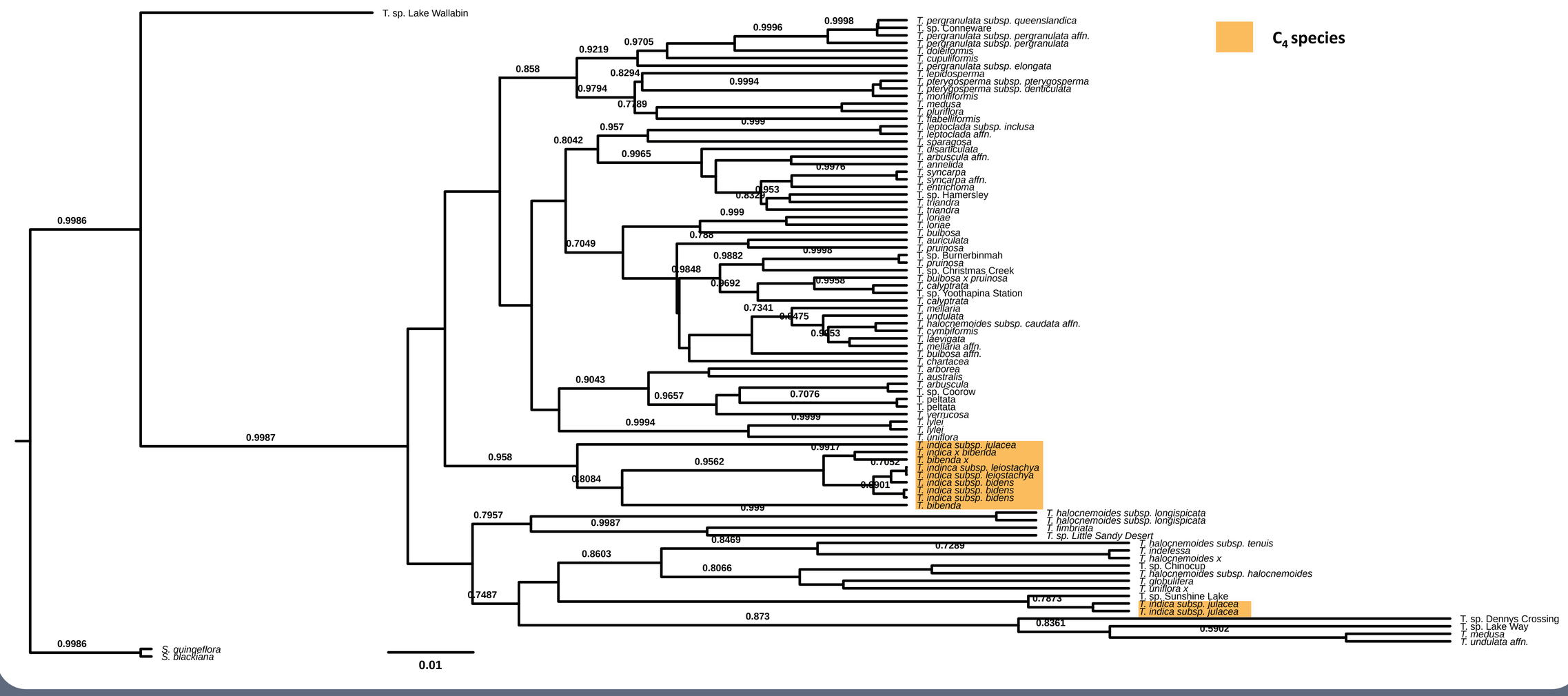
AIMS

- To resolve the phylogeny of *Tecticornia* using the external transcribed spacer (ETS) nuclear marker.
- To determine the number of origins of C_4 photosynthesis in *Tecticornia*.
- To identify C_3 species most closely related to C_4 species.

RESULTS

Sequences were obtained from 68 herbarium specimens, including hybrids, subspecies, and putative new species. Combining ETS and ITS sequence information produced a phylogeny with greater resolution than previous phylogenetic studies. (Fig. 3).

Paralogous ETS sequences were amplified in many species, including all C_4 species. This produced conflicting topology in early ITS and ETS trees, and some species are represented only by the ITS sequence. The presence of paralogues in *Tecticornia* species is possibly due to recent hybridisation events.



CONCLUSIONS AND FURTHER WORK

- Combining ITS and ETS marker sequences has resolved individual clades of species, but the backbone of the phylogeny remains unresolved.
- The C_4 species *T. indica* is not monophyletic. The phylogeny supports two origins of C_4 photosynthesis in *Tecticornia*.
- Strategies to obtain missing orthologue sequences are underway.

ACKNOWLEDGEMENTS

Thank you to Silvia Wienken, Michael Pirie, Max Lauterbach, Katharina Bohley, Denise Schmidt, and Uschi Martine (Johannes Gutenberg-Universitat Mainz). This work was completed with support from an Australian Research Council Discovery Project Grant (ML), an Australian Postgraduate Award (ND), a UWA Top-up Scholarship (ND), and an R.N. Robertson Travelling Fellowship from the Australian Society of Plant Scientists (ND).