

Phylogenetic analysis of *Tecticornia* using the external transcribed spacer (ETS) nuclear marker: insights into the evolution of C₄ photosynthesis

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Background

Tecticornia is a genus of succulent halophytes in the family Chenopodiaceae. The genus currently contains two C₄ species and 39 C₃ 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 C₄ anatomy (Kranz-Tecticornoid) make it a valuable genus for studying the steps in the evolution of C₄ 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 C₄ evolution in this genus, the C₃ species most closely related to the C₄ 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 C₄ species).

Aims

- To resolve the phylogeny of *Tecticornia* using the external transcribed spacer (ETS) nuclear marker.
- To determine the number of origins of C₄ photosynthesis in *Tecticornia*.
- To identify C₃ species most closely related to C₄ species.

Results

Figure 3. . Phylogeny of *Tecticornia* constructed using ETS and internal transcribed spacer (ITS) sequences. The tree was obtained through Bayesian inference. Bayesian posterior probabilities (> 0.7) appear above the branches. C₄ species are highlighted in orange. Supported clades are in bold.

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 C₄ 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 C₄ species *T. indica* is not monophyletic. The phylogeny supports two origins of C₄ photosynthesis in *Tecticornia*.
- Strategies to obtain missing orthologue sequences are underway.

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