Preliminary ITS Phylogeny of the Australian Genus *Ptilotus* (Amaranthaceae)

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Introduction

Ptilotus is a genus in the Amaranth family with around 100 species, only one of which, *Ptilotus conicus*, has a distribution that extends outside of Australia, to Timor and adjacent islands. ***Ptilotus* is the 12th largest plant genus native to Australia**, and has its center of diversity in arid western Eremean region of Australia (CAVP, 1993; Bean, 2008). Like much of the flora found in this region, *Ptilotus* species have xeromorphic adaptations to this harsh climate; some have pubescent stems and/or leaves, succulent stems and/or leaves, and one species, *Ptilotus aphyllus,* is leafless at maturity. Most *Ptilotus* species are herbaceous perennials or annuals, but some species may be shrubs or subshrubs. Many species have conspicuous, colorful, long-lasting inflorescences, a feature which makes them potentially valuable in floriculture (see Fig. 1 and 2; Lee et al., 2008). This study provides the first well-sampled phylogeny of the genus and will significantly enhance a monographic treatment in preparation for the ***Flora of Australia*** project.

Fig. 1. *Ptilotus rotundifolius* with colorful inflorescences and in a typical habitat for many *Ptilotus* species in Western Australia. Photo: Emil Thoma

Fig. 2. *Ptilotus trichocephalus* with long, hair-like tepals and a sprawling habit common to many *Ptilotus* species. Photo: Robert Davis

Materials and Methods

Taxon Sampling

- Herbarium specimens or dried leaf samples of 75 taxa in the genus *Ptilotus* and one outgroup taxon, *Aerva javanica*, were provided by the Western Australian Herbarium.

Extraction, Amplification and Sequencing

- Following the manufacturers protocol, DNA was extracted using the DNeasy Mini Plant Kit (Qiagen, Valencia, California, U.S.A.).
- The internal transcribed spacer (ITS) region was amplified by PCR using the forward primer ITS5A (5'-CCTTATCATTTAGAGGAAGGAG-3') and the reverse primer 26S-25R (5'-TATGCTTAAARTCAGCGGGT-3').
- Sequences were manually edited using Geneious 6.0 software (Biomatters, available from http://www.geneious.com) and aligned using the default settings of the webPRANK multiple sequence aligner (Löytynoja and Goldman, 2010).

Phylogenetic Reconstruction

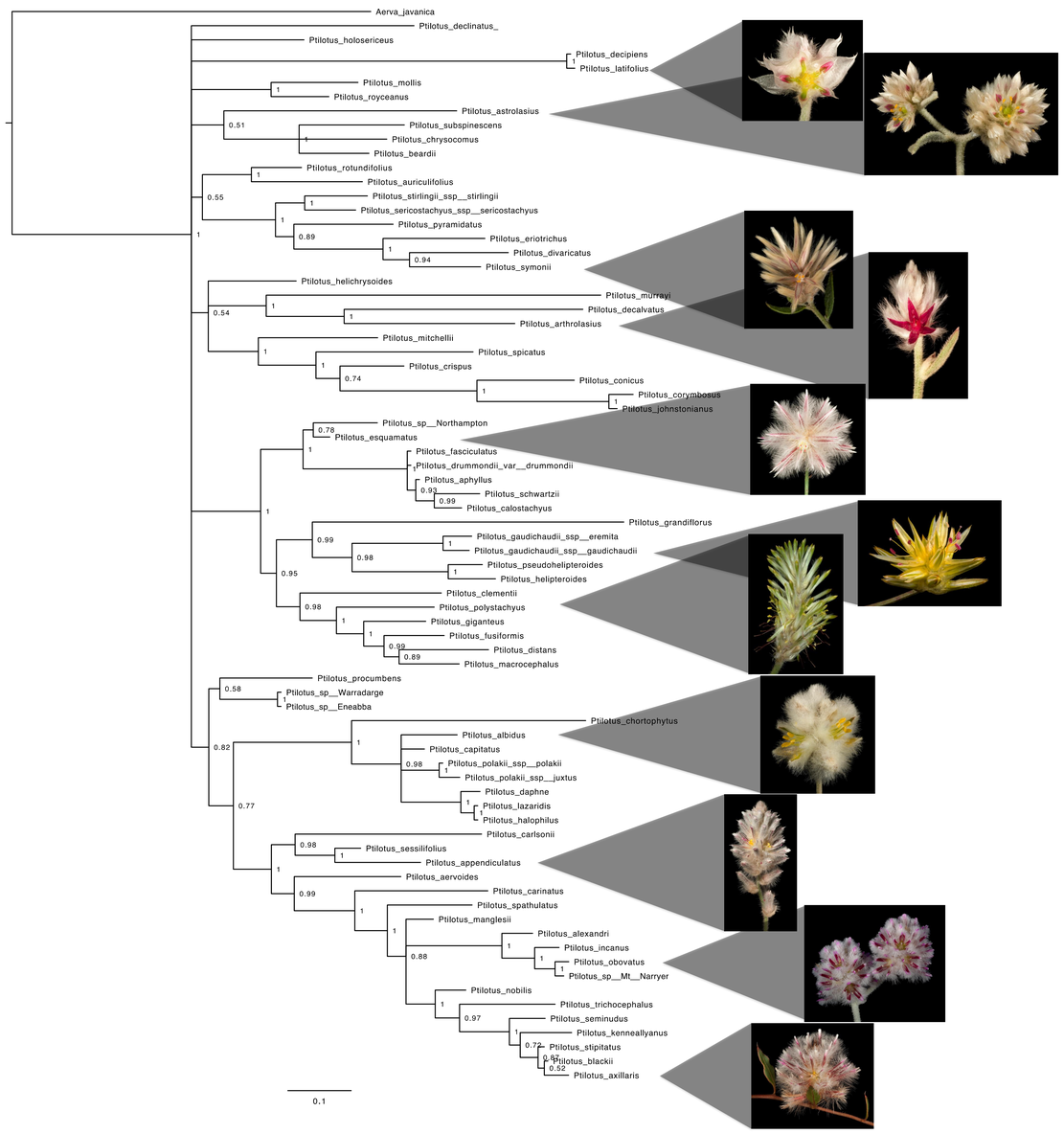
- MrBayes 3.2.1 was used on the Cyber infrastructure for Phylogenetic Research portal (http://www.phylo.org/; Miller et al., 2010)
- The best nucleotide substitution model was determined to be GTR+I+G by JModelTest (http://code.google.com/p/jmodeltest2).
- The analysis was run for 10,000,000 generations with trees sampled every 1,000 generations.
- Adequate convergence and mixing was determined using Tracer v1.5 (http://tree.bio.ed.ac.uk/software/tracer).
- A 50% majority rule consensus tree was visualized using Figtree v1.4.0 (http://tree.bio.ed.ac.uk/software/figtree).

Test of Substitutional Saturation

- Using the program DAMBE, a test of substitutional saturation was preformed on the sequence alignment and phylogenetic tree, and a nucleotide substitution saturation plot was generated to test the phylogenetic utility of ITS within the genus (Xia et al., 2003).

Photo: *Ptilotus* near Onslow, WA

Fig. 3. A rooted 50% majority rule consensus tree using Bayesian inference. Node labels are posterior probabilities, and branch lengths are substitutions per site. Photos: Kevin Thiele



Results and Discussion

ITS Phylogeny

This preliminary phylogeny of the ITS region shows a basal polytomy (Fig. 2). Relationships between taxa within large clades tend to be well supported. Two possible explanations and solutions for the lack of support along the backbone of the tree are:

1. It is a genuine picture of the phylogeny for ITS, perhaps reflecting a rapid cladogenesis within the genus (this has been found to be the case with other arid Australian genera). Molecular markers with equal or more variation need to be added to resolve basal nodes.
2. Substitutional saturation of the ITS region resulting in a loss of signal at depth. Molecular marks with less variation than ITS need to be added to resolve the backbone.

Test of Substitutional Saturation

- For the ITS sequences, I_{ss} (0.131–0.162) << $I_{ss.c}$ (0.700–0.712), and the saturation plot shows a linear increase in transitions and transversions with increased genetic distance, transitions being more common than transversions (Fig. 4).
- This result indicates that there is little or no saturation in the ITS

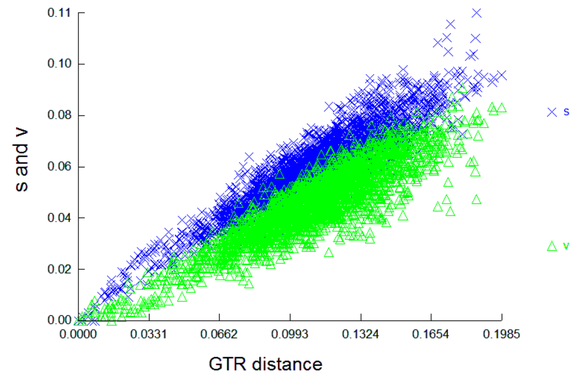


Fig. 4. Output from the program DAMBE, plotting the proportion of transitions (s) and transversions (v) against sequence divergence using GTR distance.

Future Work

- Field work is planned in Western Australia and the Northern Territory for August 2013 to collect specimens of rare and/or isolated taxa not included in the current phylogeny.
- DNA from herbarium specimens of taxa from the states of South Australia, the Northern Territory and Queensland will be extracted to cover the remaining taxa.
- Additional molecular markers from both the nuclear and chloroplast genomes will be sequenced in an attempt improve the phylogenetic picture of *Ptilotus*. Markers will be targeted to have similar levels of variation as ITS, so as to help resolve the phylogenetic backbone.
- This phylogeny will enable further studies of the character evolution and biogeography of the genus.

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