Fall 2014

A Molecular Framework Phylogeny for *Ptilotus* (Amaranthaceae)

Timothy A. Hammer
Old Dominion University

A MOLECULAR FRAMEWORK PHYLOGENY FOR *PTILOTUS* (AMARANTHACEAE)

by

Timothy A. Hammer
B.S. May 2012, Old Dominion University

A Thesis Submitted to the Faculty of
Old Dominion University in Partial Fulfillment of the
Requirements for the Degree of

MASTER OF SCIENCE

BIOLOGY

OLD DOMINION UNIVERSITY
December 2014

Approved by:

_____
Lytton Musselman (Director)

_____
Tatyana Lobova (Member)

_____
Kevin Thiele (Member)

ABSTRACT

A MOLECULAR FRAMEWORK PHYLOGENY FOR *PTILOTUS* (AMARANTHACEAE)

Timothy A. Hammer
Old Dominion University, 2014
Director: Dr. Lytton Musselman

Ptilotus (Amaranthaceae) is an Australian genus with over 100 species, most of which occur in arid Western Australia. *Ptilotus* has been a taxonomically difficult genus; despite rigorous morphological studies into the genus over many years, previous workers have found it difficult to delimit infrageneric groups due to inconsistent morphological variation. With the goal to establish a phylogenetic framework for the genus, 100 taxa were sampled, including 87 *Ptilotus* spp., and the ITS nrDNA and *matK* cpDNA were sequenced. The phylogeny was reconstructed using Bayesian, maximum likelihood and maximum parsimony analyses on separate and concatenated datasets. Morphological characters were assessed and compared to clades on the phylogeny to identify synapomorphies and aid in the construction of an infrageneric classification. A diversification rate analysis was used to identify rate shifts in speciation across the phylogeny. Four major clades of the monophyletic *Ptilotus* were resolved, three small clades together comprising 27% of sampled taxa and a large, diverse clade comprising the remaining 73%. Four floral synapomorphies were identified as uniquely occurring within the latter, although none were common to all taxa in the clade. The diversification rate analysis identified a probable rate shift at the base of *Ptilotus*, indicating that the genus may have undergone a rapid diversification early in its evolution. This rapid

diversification provides a plausible explanation for the lack of consistent variation in morphology among the major clades.

This thesis is dedicated to Dr. Timothy J. Motley for inspiring me to study plant systematics.

ACKNOWLEDGMENTS

The author would like to especially thank Dr. Timothy Motley, Dr. Kevin Thiele and Robert Davis, without whom this project could not have been possible. The contributions of herbarium loans and/or plant materials are greatly appreciated from the following herbaria: the Western Austalian Herbarium (PERTH), the Australian National Herbarium (CANBR), the Northern Territory Herbarium (Alice Springs), and the Queensland Herbarium (BRI). The author would also like to acknowledge the following individuals for their help during the course of the project: Suman Neupane, Sushil Paudyal, Hunter Shanks, Peter Jobson, Dr. Lytton Musselman, and Dr. Tatyana Lobova.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Ptilotus R.Br. (Amaranthaceae) comprises *c.* 152 named taxa and 109 recognized species, all except one species restricted to Australia (*P. conicus* R.Br. extends to Timor and adjacent islands). The center of species diversity is in arid and semi-arid Western Australia (Bean, 2008), with smaller numbers of species found in open habitats in mesic temperate areas and in the wet-dry tropics. No species are found in alpine communities or heavily shaded forests. *Ptilotus* includes rare and threatened species and is an ecologically important floristic component in arid Australia (Davis & al. 2014).

Ptilotus are annual or perennial herbs or shrubs with terminal or axillary, spicate inflorescences. Many have large, colorful inflorescences of pink or white flowers which persist on the plant for a prolonged period of time, making the genus of interest in floriculture (Lee & al., 2007; Able & al., 2014). Some species are visually dominant across large areas, particularly after rains. The flowers are bisexual with 5 tepals and up to 5 stamens; in many taxa, one or more stamens are reduced to staminodes. Stamens are united at the base, forming a staminal cup. Some taxa bear attenuate lobes on the staminal cup, referred to as pseudostaminodes. Tepals, bracts and bracteoles of many species bear prominent, dense hairs, from which the genus name is derived (from the Greek *ptilotos*, "feathered" or "winged"). As with many other amaranths, the dried perianth persists around the mature fruit, the two acting together as a diaspore with the tepal hairs perhaps aiding in wind dispersal.

Brown (1810) described two genera, *Trichinium* and *Ptilotus*, differing mainly in perianth indumentum (Bentham, 1870), the former having perianth segments glabrous apart from basal hairs in a few taxa and the latter having dorsal hairs on the perianth. Almost since their recognition the two genera were regarded as very close, with Poiret (1817) and Sprengel (1825) uniting them (the former under *Ptilotus*, the latter under *Trichinium*). Moquin-Tandon (1849) and Bentham (1870), by contrast, retained them as distinct, though with the latter remarking that they "might perhaps be really better considered as sections of one genus" (*l.c.* p. 217). Black (1948) united them under *Ptilotus* (following Poiret's choice of genus name), and echoed Bentham in commenting that they "are too closely related to be kept apart" (*l.c.* p. 324); no subsequent authors have recommended the reinstatement of *Trichinium*.

Despite considerable taxonomic effort in the genus, particularly during the last four decades of the 20th century by Benl (Bean, 2008), *Ptilotus* remains problematic. Lack of consistent morphological differences and difficulties in delimiting major groups of taxa has made the formulation of a robust infrageneric classification for *Ptilotus* difficult. A number of authors erected infrageneric classifications, either of the combined genera under *Ptilotus* or of the separate genera. These ranged from simple subdivision of the genus into sections representing the original genera (sects *Ptilotus* and *Trichinium*, e.g. Black 1948) to the more detailed subdivision by Bentham (1870) of *Trichinium* into nine series, these later transferred to *Ptilotus* by Schinz (1934). None, however, have become well established. Benl (1971), in the most complete treatment of the genus to that time, chose to not recognize infrageneric groups, and subsequent authors have followed him in this. Benl (1990) subsequently reduced the monotypic genus *Dipteranthemum*

F.Muell. to a subgenus of *Ptilotus*, following a reinterpretation of the characters upon which Mueller had erected it.

Only one prior molecular study of *Ptilotus* has been published, a phenetic (neighbor-joining) analysis of 14 *Ptilotus* species and one outgroup taxon (*Gomphrena celosioides* Mart.) using ITS, to investigate the relationships between two species, *P. nobilis* F.Muell. and *P. exaltatus* Nees (Lee & al., 2007). This study led to *P. exaltatus* being synonymised under *P. nobilis*, but provided no insights into the phylogeny of the genus as a whole.

Phylogenetic studies of Amaranthaceae (e.g., Sage & al., 2007; Müller & Borsch, 2008) have led to the recognition of a strongly supported monophyletic aervoid clade comprising *Ptilotus*, *Aerva* Forssk. (12 spp.) and the monotypic *Nothosaerva brachiata* (L.) Wight. While *Ptilotus* is almost entirely Australian, *Aerva* and *Nothosaerva* have an African and south Asian distribution (with the widespread *A. javanica* naturalized in Australia). The three genera are similar in overall (vegetative) morphology, but differ significantly in their floral morphology. All species of *Ptilotus* have bisexual flowers with capitate stigmas and indehiscent fruits; some species have pseudostaminodes. Species of *Aerva* are dioecious (*A. javanica*), variously polygamous (*A. lanata*, *A. leucura* and *A. sanguinolenta*) or bisexual (*A. microphylla* and *A. revoluta*), have bilobed stigmas, dehiscent (capsular) fruits, and pseudostaminodes. *Nothosaerva brachiata* has bisexual flowers with 1 or 2 fertile stamens, lacks pseudostaminodes, and has a capitate stigma and dehiscent (capsular) fruit (Townsend, 1993).

The aervoid clade is sister to the achyranthoid and gomphrenoid clades (Müller & Borsch, 2008). While limited by sparse taxon sampling, Sage & al. (2007) provide an

indication that *Aerva* may be paraphyletic with respect to *Ptilotus* and/or *Nothosaerva*, with a clade comprising *A. leucura* Moq. and *A. sanguinolenta* (L.) Blume sister to *Ptilotus*, while *A. javanica* (Burm. f.) Juss. ex Schult. is sister to *N. brachiata*. A phylogenetic analysis of *Aerva* by Thiv & al. (2006), sampling all but two taxa and based on nuclear and chloroplast markers, identified two major clades within *Aerva*.

The present study provides the first robust molecular phylogenetic framework for *Ptilotus*, based on nrDNA ITS and cpDNA *matK*. Taxon sampling represents the diversity of morphology within the genus. The phylogeny is used to test the monophyly of *Ptilotus*, clarify phylogenetic relationships within the genus, assess congruence of robust clades with morphological variation, and identify phylogenetically informative characters that may later aid in the first robust infrageneric classification of *Ptilotus*.

CHAPTER II

MATERIALS AND METHODS

Plant material

Silica-dried leaf material and herbarium voucher specimens were obtained for 100 *Ptilotus* taxa, including 87 currently recognized species (over 70% of species in the genus) and 6 infraspecies. Taxa were sampled to cover all major informally recognized groups and to span as much morphological and geographic variation as possible (Fig. 1), including all taxa that have distinctive and/or unusual morphologies (e.g. *P. crosslandii* (F.Muell.) Benl. and *P. royceanus* Benl). Taxa not included in general have clear affinities with one or more included taxa. Of the 100 accessions, 95 were new sequences, and 80 taxa are sequenced here for the first time. Taxa representing both *Ptilotus* and *Trichinium* were sampled, including both species described under *Ptilotus* and all six species described under *Trichinium* by Brown (type species have not been designated for either genus). Outgroup taxa comprised *Amaranthus caudatus* L. (ITS1, 5.8s, ITS2 and *matK* sequences obtained from GenBank), *Achyranthes aspera* L., *Gomphrena kanisii* J.Palmer, *Gomphrena flaccida* R.Br., *Aerva javanica* (sequenced for this study), *Aerva artemisioides* and *Aerva lanata* (L.) Juss. ex Schult. (ITS1, ITS2 and *matK* sequences obtained from GenBank; Thiv & al., 2006). Newly collected specimens were done under license of colleagues at the Western Australian Herbarium and the Northern Territory herbarium. Plant material was imported into the USA using the import permit P587-07116-011. A list of voucher specimens is provided in the appendix.

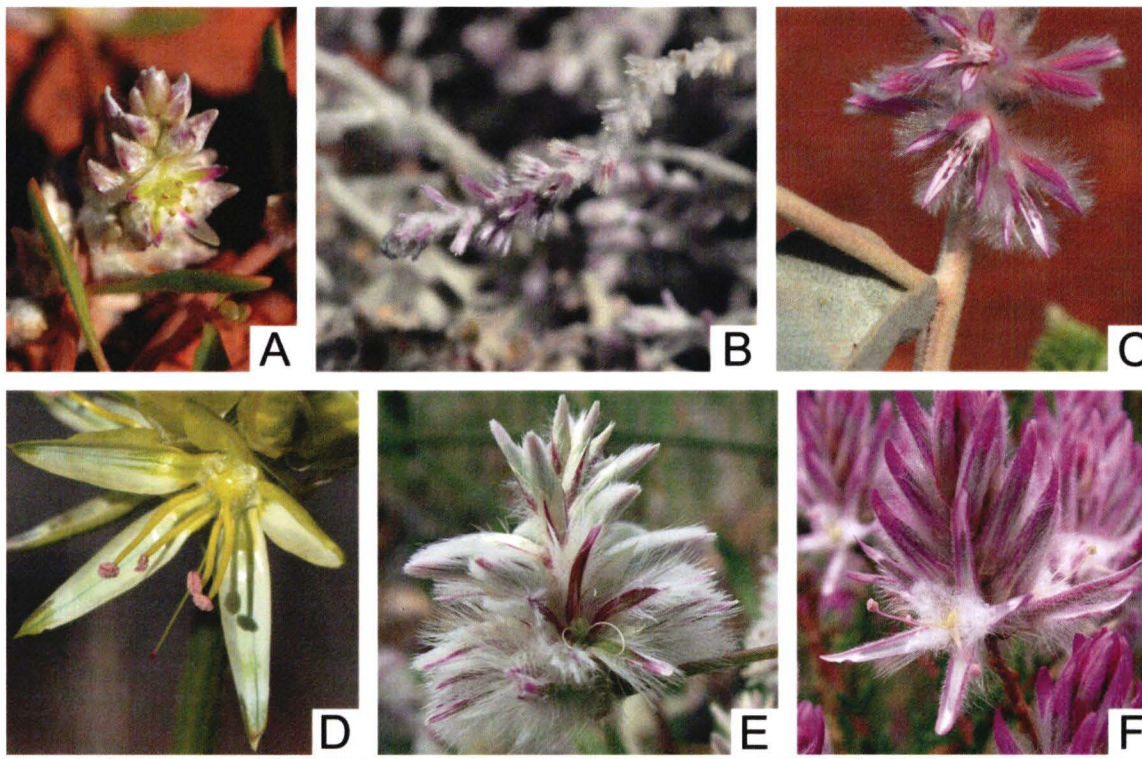


Fig. 1. A representation of the morphological diversity found within *Ptilotus*: **A,** *P. chamaecladus*; **B,** *P. royceanus*; **C,** *P. rotundifolius*; **D,** P. *gaudichaudii ssp. gaudichaudii*; **E,** *P. sericostachyus*; **F,** *P. polakii*; photos by Timothy Hammer (A–D) and Robert Davis (E–F).

DNA extraction

Plant material was pulverized for 1 minute in a mini-bead beater. DNA was extracted using DNeasy Mini Plant Kits (Qiagen, Valencia, California, USA) following the manufacturers protocol. To aid in cell lysis of herbarium material, an additional step supplemented the protocol after the addition of buffer AP1 with the addition of 30 µl of proteinase-K and 30 µl of β-mercaptoethanol followed by incubation of the pulverized plant material at 42°C for 12-24 hours.

Amplification and sequencing

Amplification of ITS nrDNA (ITS1, 5.8S, ITS2) was performed using ITS5A (forward) and 26S-25R (reverse) primers (Motley & al., 2005). Primers used to amplify

matK cpDNA were *matK*-AF (Thiv & al., 2006) and *matK*1R (Sang & al., 1997). Each 25 µl PCR reaction was performed on an ABI 2720 thermal cycler using 12.5 µl of GoTaq Green Master Mix (Promega, Madison, Wisconsin, USA), 1 µl of each 10 µM primer, 1.25 µl dimethylsulfoxide, 0.25 µl bovine serum albumin, 8 µl water and 1 µl of DNA template. ITS reactions were run using an initial 3 min denaturation at 95°C followed by 40 cycles of 94°C denaturation for 45 s, 60°C annealing for 1 min, decreasing by 0.2°C each cycle, and 72°C extension for 50 s, followed by a final 7-min extension at 72°C. Amplification of *matK* used an initial 1 min denaturation at 94°C followed by 33 cycles of 94°C denaturation for 1 min, 53°C for 1 min and 72°C for 2 min, followed by a final 7-min extension at 72°C. Presence and size of amplified DNA was determined by gel electrophoresis of the PCR products, which were purified using the AMPure PCR purification protocol (Agencourt, Beverly, Massachusetts, USA). Sanger sequencing was performed by Macrogen (Seoul, Korea) on an ABI 3730XL.

Phylogenetic analyses

Sequence chromatograms were manually edited to obtain a consensus sequence using Geneious 6.0 (Kearse & al., 2012). Consensus sequences were aligned using default settings of the webPRANK multiple sequence aligner (Löytynoja & Goldman, 2010); aligned sequences were inspected and manually adjusted as necessary in Geneious. The nucleotide substitution models for ITS and *matK* datasets were determined by jModelTest v2.1.4 (Darriba & al., 2012).

Bayesian inference (BI) was performed using MrBayes v.3.2.1 (Ronquist & al., 2012). Analyses were run for 15 million Markov Chain Monte Carlo (MCMC) generations with trees sampled every 1000 generations. Completion was determined by

the average standard deviation of split frequencies falling below 0.01. Trace files generated by the Bayesian MCMC runs were analyzed using Tracer v.1.6 to determine adequate convergence and mixing (Rambaut & Drummond, 2007). The initial 25% of topologies were discarded before reaching likelihood stationarity; 50% majority rule consensus trees were constructed to summarize the remaining topologies. Maximum likelihood (ML) analyses were implemented on GARLI v2.01 (Zwickl, 2006) with 8 independent search replicates to obtain the best tree, and bootstrap support obtained with 100 bootstrap replicates. BI and ML searches were conducted on the Cyberinfrastructure for Phylogenetic Research (CIPRES) portal (Miller & al., 2010). Maximum parsimony (MP) analyses were conducted using PAUP* v4.0b10 (Swofford, 2002) with 1000 random addition sequences, one tree held at each step and tree bisection-reconnection (TBR) branch swapping. Bootstrap analyses (Felsenstein, 1985) were conducted with 1000 replicates.

Summation of the bootstrap values was calculated using SumTrees v3.3.1 in the DendroPy v.3.12.0 python package (Sukumaran & Holder, 2010). TreeGraph 2 was used display the final trees with posterior probabilities and bootstrap support values (Stöver & Müller, 2010). All trees were rooted at *Amaranthus caudatus*.

Congruence tests

The ITS and *matK* gene trees were assessed for congruence, to determine whether the sequences could be appropriately concatenated, using two methods, the Mantel test employed on the zt software (Bonnet & Van de Peer, 2002) and the I_{cong} statistic (de Vienne & al., 2007). So-called "rogue taxa" (taxa with highly uncertain and labile positions in the concatenated phylogeny) were identified using the RogueNaRok software

(Aberer & al., 2013). The ML bootstrap trees for both datasets were used in the RogueNaRok analysis, and the leaf stability index used to identify unstable leaves across all bootstrap trees (Thorley & Wilkinson, 1999). All rogue taxa were resequenced and the alignments checked to eliminate the possibility of their incongruence being the result of sequence or alignment error.

Rates of species diversification

Time-calibrated phylogenetic trees were constructed for the concatenated dataset with the BEAST (Bayesian Evolutionary Analysis Sampling Trees) v2.0 software package, using 50 million MCMC generations sampled every 5000 generations (Bouckaert & al., 2014). The Speciation Birth-Death sampling model was used as the tree prior (Gernhard, 2008), and lognormal relaxed (uncorrelated) was used as the clock model. The MCMC runs in BEAST were examined in Tracer v1.6 for adequate convergence (Rambaut & Drummond, 2007). The output trees from BEAST were used in BAMM (Bayesian Analysis of Macroevolutionary Mixtures) using the speciation/extinction model to reconstruct the posterior density of evolutionary rates across the phylogeny (Rabosky, 2014a). BAMM uses reversible jump MCMC to explore branch-specific rates in speciation and extinction, which are allowed to vary through time and among lineages (Rabosky & al., 2014b). To account for incomplete taxon sampling, 70% taxon coverage was assumed. BAMM was run for 10 million MCMC generations. BAMMtools (Rabosky & al., 2014c) was used to set the priors for BAMM, assess MCMC convergence and visualize the output.

Morphological analysis

Herbarium specimens of sampled taxa were used to analyze discrete floral and vegetative characters. A character state matrix of potentially informative characters was obtained from an unpublished interactive key to the Western Australian (WA) taxa in the genus constructed by Robert Davis; this was supplemented with characters coded from loaned specimens from CANB and BRI for non-WA taxa. Character states were manually mapped onto the phylogeny to identify those that may aid in delimiting robust infrageneric groups in *Ptilotus*.

CHAPTER III

RESULTS

The aligned ITS region comprised 817 base pairs (bp), and the aligned *matK* region comprised 861 bp; the aligned combined ITS+*matK* dataset was thus 1678 bp. Bayesian and Akaike information criteria (BIC and AIC) in jModelTest agreed that the best nucleotide substitution model for ITS was GTR+I+G, and the best model for *matK* was GTR+G. These models were used in the MrBayes and BEAST analyses. The ITS dataset had 310 parsimony informative characters and a consistency index (CI) of 0.34, while the *matK* dataset had 145 parsimony informative characters and a CI of 0.62. The combined dataset had a CI of 0.40 and 455 parsimony informative characters (Table 1).

Table 1. Summary Statistics from ITS, *matK* and combined datasets for *Ptilotus* and outgroup species analyzed in this study.

	ITS	***matK***	**Combined ITS+*matK***
Unaligned length (bp)	428-707	532-759	950-1444
Aligned length (bp)	817	861	1678
Variable characters (%)	49.45	30.78	39.87
Parsimony-informative characters	310	145	455
Tree length (MP)	4076	448	2461
Consistency index	0.34	0.62	0.40
Homoplasy index	0.82	0.27	0.57
Retention index	0.08	0.85	0.69
ML score (ln*L*)	-10943.28	-4084.95	-15661.68

Congruence analyses

The two gene trees differed in their topology, but many clades were resolved in both trees. Both the mantel test and the I_{cong} statistic showed that the two gene trees were more congruent than would be expected by chance (Mantel p = 0.001; I_{cong} p = 5e-25); accordingly, the concatenated dataset was used for all further analyses. The RogueNaRok analysis identified five taxa (*P. auriculifolius*, *P. decipiens*, *P. declinatus*, *P. holosericeus*, and *P. halophilus*) considered unstable using the leaf stability index (Fig. 2). These taxa jump between clades or subclades in the two gene trees or rest on unresolved polytomies. Incongruences between the individual gene trees and the concatenated tree are discussed in detail below. The topology of trees generated from the concatenated dataset with and without the rogue taxa are identical, and the rogue taxa in the concatenated tree were placed in positions that are considered congruent with current understandings of relationships; accordingly, the rogue taxa were included in the final analysis.

Phylogenetic relationships

All three analysis methods (BI, ML and MP) gave largely congruent results; the BI 50% majority rule consensus tree of the concatenated dataset is given in Fig. 3 with support values from each analysis on the branches, and key morphological features mapped to taxa. Support values reported are Bayesian posterior probabilities (PP), ML bootstrap support (ML-BS) and MP bootstrap support (MP-BS) respectively (PP/ML-BS/MP-BS).

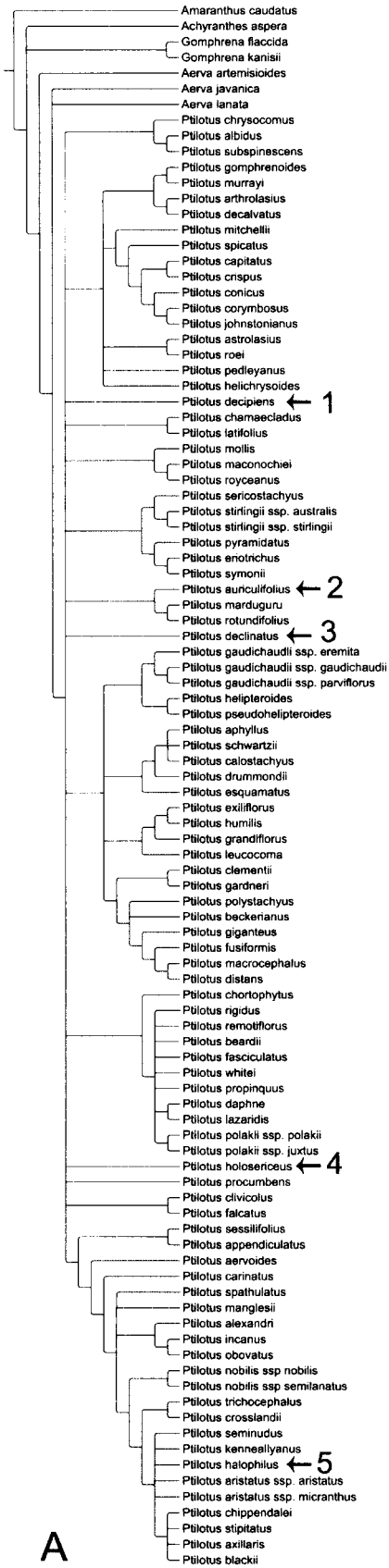
The outgroup taxa *Achyranthes aspera* and *Gomphrena* (represented by a clade comprising *G. flaccida* and *G. kanisii*) form an unresolved basal node, with the aervoids

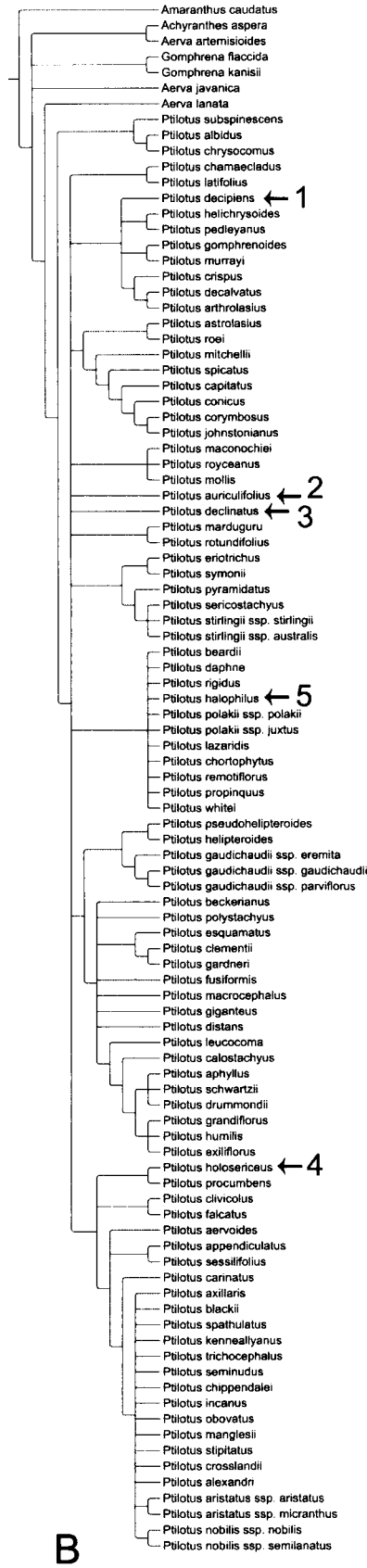
(*Aerva* spp. + *Ptilotus* spp.) monophyletic though with weak support in most analyses (0.65/51/100). A more strongly supported (0.99/84/55) clade comprises all the aervoids except *A. artemisioides*.

The monophyly of *Ptilotus* is strongly supported (1.00/99/100). Four major clades are recovered within *Ptilotus*, referred to here informally as A, B, C and D. Most species in the three basal clades (A, B and C) and *Aerva* share the plesiomorphic character states of 5 fertile stamens, straight styles that are centrally fixed on the ovaries, and unbranched hairs on the exterior of the tepals (Figs. 5–6). By contrast, most species in the species-rich Clade D share the apomorphies of reduction of some fertile stamens to staminodes, eccentrically fixed style, which may or may not be curved or sigmoidal in shape, and exterior tepal hairs that are verticillate (with whorled branches at each septum; Figs. 5–6). Some taxa in clade D have one or more of the plesiomorphic conditions.

Note that a distinction has been made at times between verticillate and subverticillate hairs, the latter having very short projections rather than distinct branches at the trichome septae. However, the length of the septal branches grades continuously from short to long, and for the purpose of this study the differences in size of the projections is not regarded as significant compared with the presence or absence of branches.

Fig. 2. 50% majority rule Bayesian cladograms for the separate ITS (A) and *matK* (B) datasets, showing nodes with only ≥ 0.95 posterior probability and/or ≥ 70% bootstrap support. Rogue taxa, identified by the RogueNaRok analysis, are numbered and identified with an arrow.


Amaranthus caudatus
Achyranthes aspera
Gomphrena flaccida
Gomphrena kanisii
Aerva artemisioides
Aerva javanica
Aerva lanata
Ptilotus chrysocomus
Ptilotus albidus
Ptilotus subspinescens
Ptilotus gomphrenoides
Ptilotus murrayi
Ptilotus arthrolasius
Ptilotus decalvatus
Ptilotus mitchellii
Ptilotus spicatus
Ptilotus capitatus
Ptilotus crispus
Ptilotus conicus
Ptilotus corymbosus
Ptilotus johnstonianus
Ptilotus astrolasius
Ptilotus roei
Ptilotus pedleyanus
Ptilotus helichrysoides
Ptilotus decipiens ← 1
Ptilotus chamaecladus
Ptilotus latifolius
Ptilotus mollis
Ptilotus maconochiei
Ptilotus royceanus
Ptilotus sericostachyus
Ptilotus stirlingii ssp. australis
Ptilotus stirlingii ssp. stirlingii
Ptilotus pyramidatus
Ptilotus eriotrichus
Ptilotus symonii
Ptilotus auriculifolius ← 2
Ptilotus marduguru
Ptilotus rotundifolius
Ptilotus declinatus ← 3
Ptilotus gaudichaudii ssp. eremita
Ptilotus gaudichaudii ssp. gaudichaudii
Ptilotus gaudichaudii ssp. parviflorus
Ptilotus helipteroides
Ptilotus pseudohelipteroides
Ptilotus aphyllus
Ptilotus schwartzii
Ptilotus calostachyus
Ptilotus drummondii
Ptilotus esquamatus
Ptilotus exiliflorus
Ptilotus humilis
Ptilotus grandiflorus
Ptilotus leucocoma
Ptilotus clementii
Ptilotus gardneri
Ptilotus polystachyus
Ptilotus beckerianus
Ptilotus giganteus
Ptilotus fusiformis
Ptilotus macrocephalus
Ptilotus distans
Ptilotus chortophytus
Ptilotus rigidus
Ptilotus remotiflorus
Ptilotus beardii
Ptilotus fasciculatus
Ptilotus whitei
Ptilotus propinquus
Ptilotus daphne
Ptilotus lazaridis
Ptilotus polakii ssp. polakii
Ptilotus polakii ssp. juxtus
Ptilotus holosericeus ← 4
Ptilotus procumbens
Ptilotus clivicolus
Ptilotus falcatus
Ptilotus sessilifolius
Ptilotus appendiculatus
Ptilotus aervoides
Ptilotus carinatus
Ptilotus spathulatus
Ptilotus manglesii
Ptilotus alexandri
Ptilotus incanus
Ptilotus obovatus
Ptilotus nobilis ssp nobilis
Ptilotus nobilis ssp semilanatus
Ptilotus trichocephalus
Ptilotus crosslandii
Ptilotus seminudus
Ptilotus kenneallyanus
Ptilotus halophilus ← 5
Ptilotus aristatus ssp. aristatus
Ptilotus aristatus ssp. micranthus
Ptilotus chippendalei
Ptilotus stipitatus
Ptilotus axillaris
Ptilotus blackii
A


Amaranthus caudatus
Achyranthes aspera
Aerva artemisioides
Gomphrena flaccida
Gomphrena kanisii
Aerva javanica
Aerva lanata
Ptilotus subspinescens
Ptilotus albidus
Ptilotus chrysocomus
Ptilotus chamaecladus
Ptilotus latifolius
Ptilotus decipiens ← 1
Ptilotus helichrysoides
Ptilotus pedleyanus
Ptilotus gomphrenoides
Ptilotus murrayi
Ptilotus crispus
Ptilotus decalvatus
Ptilotus arthrolasius
Ptilotus astrolasius
Ptilotus roei
Ptilotus mitchellii
Ptilotus spicatus
Ptilotus capitatus
Ptilotus conicus
Ptilotus corymbosus
Ptilotus johnstonianus
Ptilotus maconochiei
Ptilotus royceanus
Ptilotus mollis
Ptilotus auriculifolius ← 2
Ptilotus declinatus ← 3
Ptilotus marduguru
Ptilotus rotundifolius
Ptilotus eriotrichus
Ptilotus symonii
Ptilotus pyramidatus
Ptilotus sericostachyus
Ptilotus stirlingii ssp. stirlingii
Ptilotus stirlingii ssp. australis
Ptilotus beardii
Ptilotus daphne
Ptilotus rigidus
Ptilotus halophilus ← 5
Ptilotus polakii ssp. polakii
Ptilotus polakii ssp. juxtus
Ptilotus lazaridis
Ptilotus chortophytus
Ptilotus remotiflorus
Ptilotus propinquus
Ptilotus whitei
Ptilotus pseudohelipteroides
Ptilotus helipteroides
Ptilotus gaudichaudii ssp. eremita
Ptilotus gaudichaudii ssp. gaudichaudii
Ptilotus gaudichaudii ssp. parviflorus
Ptilotus beckerianus
Ptilotus polystachyus
Ptilotus esquamatus
Ptilotus clementii
Ptilotus gardneri
Ptilotus fusiformis
Ptilotus macrocephalus
Ptilotus giganteus
Ptilotus distans
Ptilotus leucocoma
Ptilotus calostachyus
Ptilotus aphyllus
Ptilotus schwartzii
Ptilotus drummondii
Ptilotus grandiflorus
Ptilotus humilis
Ptilotus exiliflorus
Ptilotus holosericeus ← 4
Ptilotus procumbens
Ptilotus clivicolus
Ptilotus falcatus
Ptilotus aervoides
Ptilotus appendiculatus
Ptilotus sessilifolius
Ptilotus carinatus
Ptilotus axillaris
Ptilotus blackii
Ptilotus spathulatus
Ptilotus kenneallyanus
Ptilotus trichocephalus
Ptilotus seminudus
Ptilotus chippendalei
Ptilotus incanus
Ptilotus obovatus
Ptilotus manglesii
Ptilotus stipitatus
Ptilotus crosslandii
Ptilotus alexandri
Ptilotus aristatus ssp. aristatus
Ptilotus aristatus ssp. micranthus
Ptilotus nobilis ssp. nobilis
Ptilotus nobilis ssp. semilanatus
B

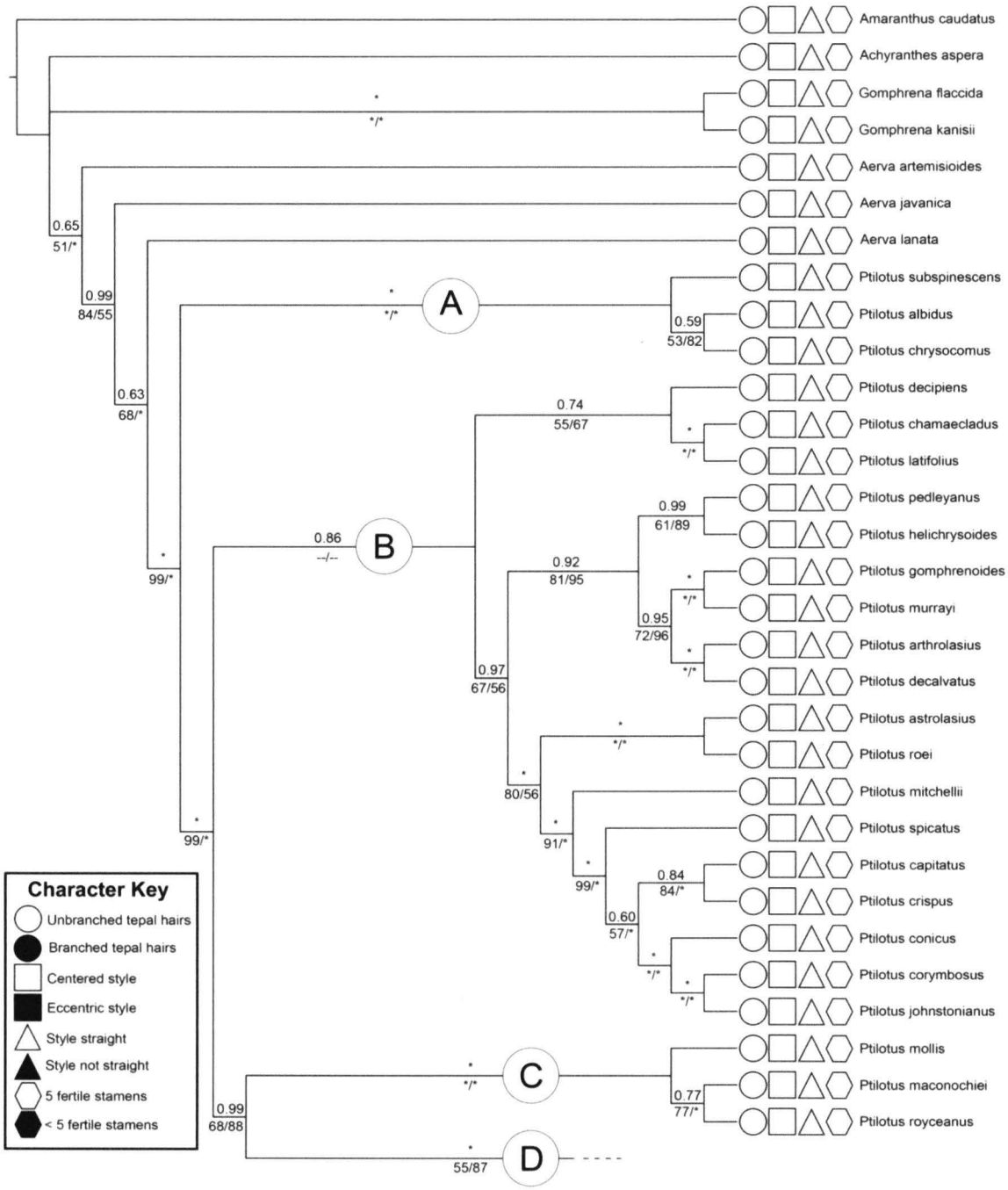


Fig. 3. 50% majority rule Bayesian consensus tree with major clades (A, B, C, and D) labeled. Clade D is continued on **Fig. 4**. Bayesian posterior probabilities are above the branches, and ML and MP bootstrap percentages are below the branches respectively. An asterisk (*) indicates 100% support, and dashes (--) indicate < 50% bootstrap support. Symbols on the leaf tips denote character states.

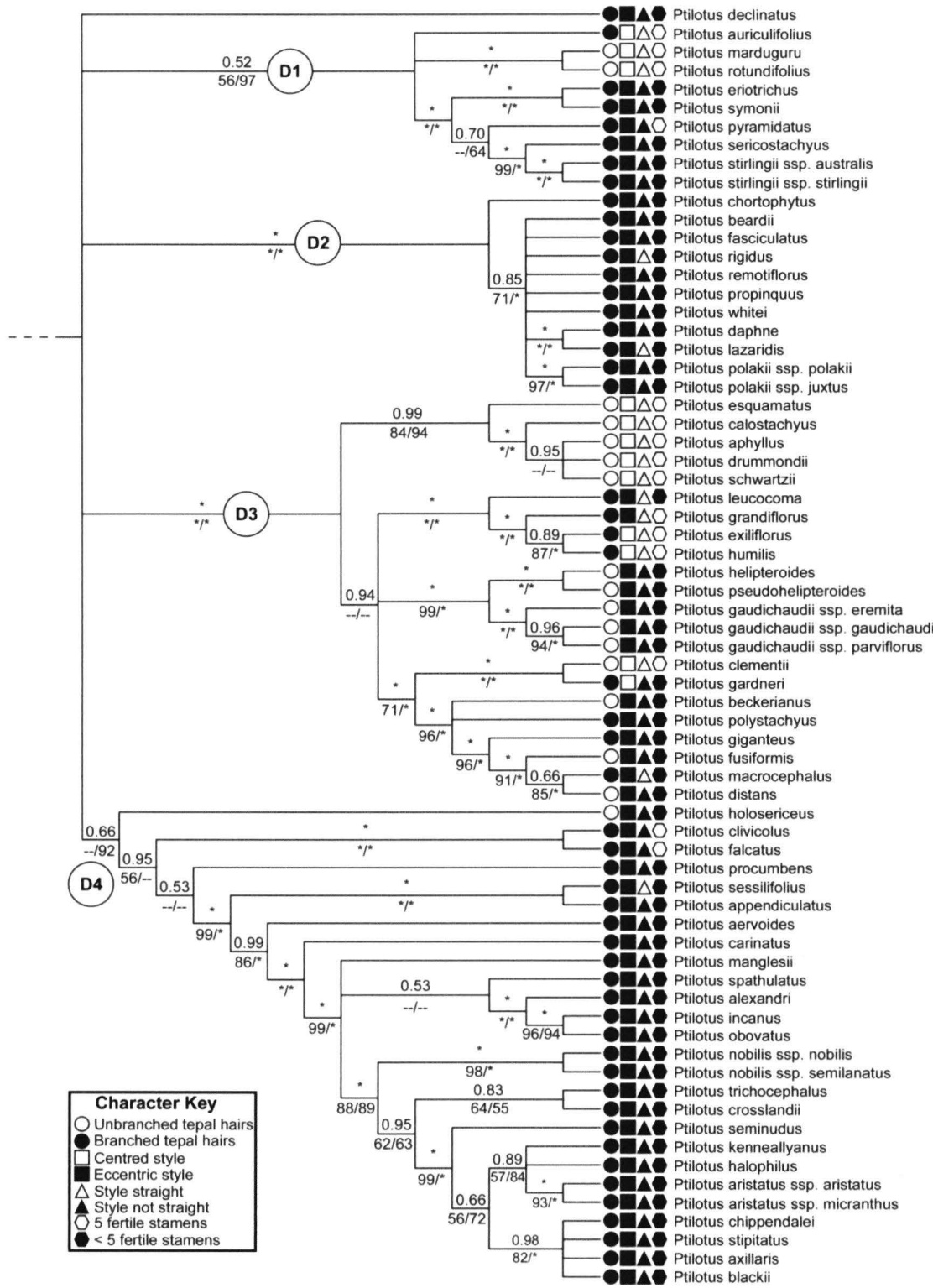


Fig. 4. 50% majority rule Bayesian consensus tree of Clade D continued from **Fig. 3** with the subclades (D1-D4) labeled.

Clade A comprises *Ptilotus albidus* (C.A.Gardner) Benl, *P. chrysocomus* R.W.Davis and *P. subspinescens* R.W.Davis and has strong support (1.00/100/100). All three species are shrubs with glabrous stems and tepal hairs that exceed the tepal apex, and all are restricted to rocky slopes or breakaways in the Eremaean Province. These morphological features are uncommon in the genus. Relationships between the species within this clade are not well resolved.

Fig. 5. A representation of the diversity of style position and shape in *Ptilotus*: **A,** *P. astrolasius;* **B,** *P. drummondii*; **C,** *P. chamaecladus*; **D,** *P. distans*; **E,** *P. seminudus*: **F,** *P. chortophytus*; photos by Robert Davis.

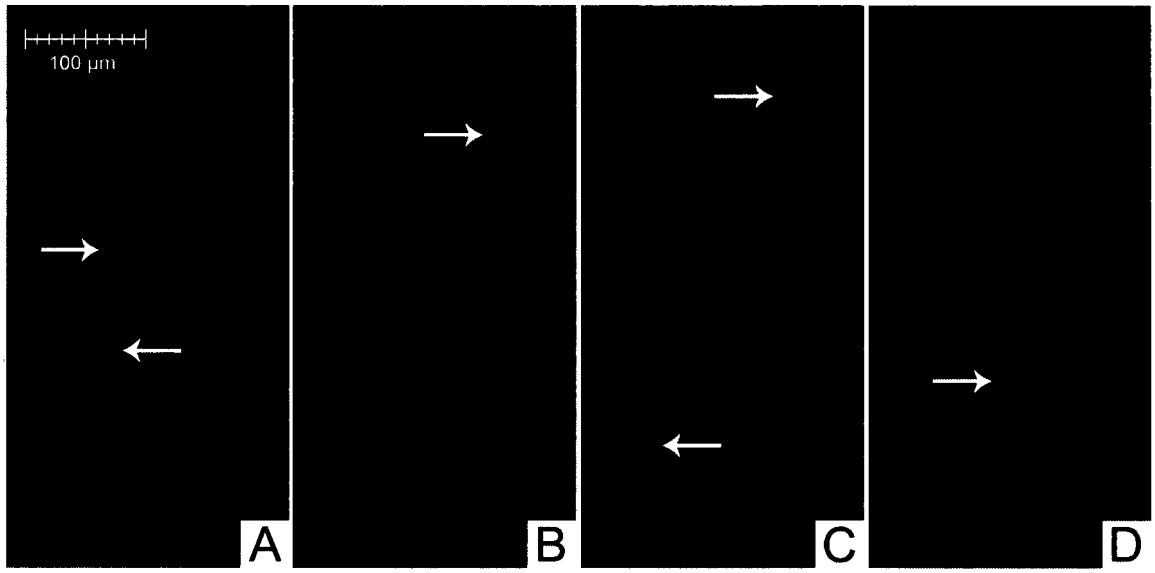


Fig. 6. Images of tepals hairs, showing unbranching hairs in **A,** *P. albidus* and **B,** *P. conicus* and varying sizes of branching hairs in **C,** *P. symonii* and **D,** *P. trichocephalus*. Arrows point to septa. Photos by Robert Davis.

Clade B is not supported (0.86/--/--), but comprises two supported subclades (informally, B1 and B2). Subclade B1 comprises *P. decipiens* (Benth.) C.A.Gardner, *P. chamaecladus* Diels, and *P. latifolius* R.Br. and is not well supported (0.74/55/67), but *Ptilotus latifolius* and *P. chamaecladus* are strongly supported as sister taxa (1.00/100/100); these taxa are also morphologically very similar, except that the latter shares with *P. decipiens* bracteoles that are longer than the tepals. Subclade B2 is supported in the Bayesian analysis (0.97/67/56). Relationships between species within this subclade are likewise generally well resolved in the Bayesian analysis. Of the 15 species in this subclade, 10 occur in the monsoon-tropical Kimberley region of Western Australia and are less xeromophic in morphology than most species in other clades; some (e.g. *P. conicus*) resemble species of *Aerva* in growth habit. *Ptilotus helichrysoides* (F.Muell.) F.Muell., a highly xeromorphic species found on rocky screes in the Eremean

Province of Western Australia, has been considered by previous taxonomists to have no close affinity to any other species (e.g. Bentham, 1870). The placement of *P. helichrysoides* as sister to *P. pedleyanus* Benl & H.Eichler is congruent with morphology: both species have strongly revolute leaf margins, which apart from the slightly revolute leaf margins in *P. eriotrichus* (Ewart & J.White) P.S.Short is unique in *Ptilotus*.

Clades C and D are strongly supported as sister in the Bayesian analysis (0.99/68/88). Clade C is well supported in all analyses (1.00/100/100) and includes three species, *P. mollis* Benl, *P. maconochiei* Benl and *P. royceanus*. All are restricted to rocky slopes and cliffs and have a dense, persistent indumentum on the stems and leaves, 5 fertile stamens, a straight style placed centrally on the ovary, and unbranched hairs on the exterior of the tepals. *Ptilotus royceanus* has interrupted inflorescences, an unusual feature in the genus, while *P. mollis* and *P. maconochiei* have compact spikes. Benl (1979) remarked on the similarity of these three species, though noting the unusual inflorescence of *P. royceanus* (note that this character is not present in populations found in Queensland; Bean, 2008). *Ptilotus dissitiflorus* (F.Muell.) F.Muell. (not sequenced for this study) also has an interrupted inflorescence; however, other morphological features (an eccentrically placed style and 3 or 4 fertile stamens) indicate that this is likely to be related to species in Clade D.

Clade D includes 68 of the 93 *Ptilotus* taxa represented in this study and is strongly supported in the Bayesian analysis (1.00/55/87). At its base is a polytomy of four subclades (D1, D2, D3 and D4) and *P. declinatus* Nees.

Subclade D1 has low support except in the MP analysis (0.52/56/97), and comprises two well-supported (1.00/100/100) monophyletic groups and *P. auriculifolius*

(A.Cunn. ex Moq.) F.Muell. The very high support (1.00/100/100) for the sister taxa *P. marduguru* Benl and *P. rotundifolius* (F.Muell.) F.Muell. confirms a suggestion by Benl (1980) that these two taxa are closely related. *Ptilotus auriculifolius* has verticillate hairs, along with the other species in D1, while *P. marduguru* and *P. rotundifolius* have unbranched hairs. Within the subclade, *P. pyramidatus* (Moq.) F.Muell. has the plesiomorphic condition of 5 fertile stamens, while *P. symonii* Benl and *P. eriotrichus* have 3 fertile stamens, and *P. sericostachyus* (Nees) F.Muell. and *P. stirlingii* (Lindl.) F.Muell. have 2 fertile stamens.

Subclade D2 is well supported in all analyses (1.00/100/100) and includes 11 sampled taxa. The relationships within the clade are not well resolved except for the high support for the sister relationships of *P. daphne* Lally and *P. lazaridis* Benl (1.00/100/100) and *P. polakii* F.Muell. ssp. *polakii* Lally and *P. polakii* ssp. *juxtus* Lally (1.00/97/100). All members of subclade D2 are shrubs, with the exception of the basally positioned perennial herb *P. chortophytus* (Diels) Schinz. These species also all share the apomorphic condition of 2 fertile stamens, verticillate hairs and eccentrically placed styles. *Ptilotus rigidus* Lally and *P. lazaridis* have straight styles, with the remainder having curved or sigmoidal styles. A polytomy in the clade is seen in both of the separate analyses for ITS and *matK*, indicative of little phylogenetic signal due to low variation in nucleotide sequences. In the highly variable ITS marker, the most distant pair (*P. daphne* and *P. fasciculatus* W.Fitzg.) have 26 bp differences, with differences of 10-20 bp common in this subclade. By comparison, the maximum sequence difference in the well-resolved clade comprising *P. eriotrichus*, *P. symonii*, *P. pyramidatus*, *P. sericostachyus* and *P. stirlingii* is 61 bp (commonly in the range 40-60 bp).

Subclade D3 comprises 22 sampled taxa and is highly supported in all analyses (1.00/100/100). It is distinctive in that most species have the plesiomorphic conditions of 5 fertile stamens, straight styles centrally fixed on the ovary, and unbranched tepal hairs (although some species vary in some or all of these character states). A monophyletic group comprising *P. esquamatus* (Benth.) F.Muell., *P. calostachyus* F.Muell., *P. aphyllus* Benl, *P. drummondii* (Moq.) F.Muell., and *P. schwartzii* (F.Muell.) Tate is strongly supported (0.99/84/94). These species are all similar in floral characteristics, the latter four also being very similar in growth form. Sister to this group is a larger clade of 17 taxa (0.94/--/--), most of which are well supported in all analyses. Within this subclade, *Ptilotus esquamatus, P. grandiflorus* F.Muell., *P. humilis* (Nees) F.Muell., *P. exiliflorus* R.W.Davis and *P. leucocoma* (Moq.) F.Muell. share a prostrate growth form. These species differ from *P. esquamatus* in having verticillate hairs on the tepals.

The largest subclade (D4) encompasses 26 taxa. *Ptilotus holosericeus* (Moq.) F.Muell. is well-supported at the base of D4 only in the MP analysis (0.66/--/92). *Ptilotus falcatus* R.W.Davis & T.Hammer and *P. clivicola* R.W.Davis & T.Hammer differ morphologically from the remainder of the species in D4 in having 5 fertile stamens, while within the subclade 2 or 3 fertile stamens are common (Davis & al., 2014). These plesiomorphies support a basal relationship to the remainder of the subclade. The remaining taxa in this subclade form a well-supported monophyletic group in all analyses (1.00/99/100). Nearly all species have curved or sigmoidal styles, and all of the species have an eccentric style with the exception of *P. aervoides* (F.Muell.) F.Muell. *Ptilotus holosericeus* is the only member of the subclade to have unbranched rather than verticillate hairs on the tepals, but the lack of support at the base of the subclade may

indicate a more distant relationship with the other species in this subclade. *Ptilotus obovatus*, one of the most polymorphic and widespread members of the genus, is sister to *P. incanus* (R.Br.) Poir. (1.00/96/94), a morphologically similar species. The morphologically unusual *P. crosslandii* is sister to *P. trichocephalus* Benl (0.83/64/55), supporting an observation by Benl that these species are morphologically close and confirming the synonymy of the monotypic *Dipteranthemum* within *Ptilotus* (Benl, 1990). In addition to having unusually long outer tepals, both species are annual herbs, have a prostrate growth form, 3 fertile stamens, a curved, eccentric style, and verticillate hairs. *Ptilotus seminudus* (J.M.Black) J.M.Black, *P. kenneallyanus* Benl, *P. halophilus* R.W.Davis, *P. aristatus* Benl sspp., *P. chippendalei* Benl, *P. stipitatus* Benl, *P. axillaris* (Benth.) F.Muell. and *P. blackii* Benl form a highly supported monophyletic group (1.00/99/100).

Gene tree incongruence

While the tests performed comparing the topology of the gene trees support congruence greater than expected by chance, there are nonetheless notable differences between the gene trees, though these differences are likely due to the lack of resolution in these trees. There is an incongruence in the relationship between the outgroup taxa, with the ITS tree having a monophyletic clade of *Aerva* + *Ptilotus*, while the *matK* tree places *A. artemisioides* as sister to *Achyranthes aspera* and *A. javanica* on a polytomy which includes a clade of *Gomphrena*. The rogue taxon *P. holosericeus* occupies a position on the basal polytomy in *Ptilotus* on the ITS tree; however in the *matK* and combined trees, *P. holosericeus* is placed at the base of clade D4 (and strongly sister to *P. procumbens* in the *matK* tree). This placement reflects the morphology of this species and its previous

placement within *Trichinium* by Moquin-Tandon (1849). *Ptilotus decipiens* is placed at the basal polytomy of *Ptilotus*, and outside of a clade including *P. chamaecladus* and *P. latifolius* in the ITS tree, while it is well-supported in its relationship as sister to the three clades that include *P. helichrysoides*, *P. gomphrenoides* and *P. crispus* in the *matK* tree respectively.

In the ITS tree, *P. auriculifolius* is resolved in a clade with *P. marduguru* and *P. rotundifolius*, while *P. declinatus* rests on the polytomy sister to this clade. In the *matK* tree, both *P. auriculifolius* and *P. declinatus* rest on the polytomy. Morphology supports the placement of these taxa within clade D, especially *P. declinatus*, which has verticillate hairs, a curved and eccentrically placed style, and 4 fertile stamens. A less striking incongruence occurs in subclade D3 with the placement of *P. esquamatus*. It is placed at the base of *P. clementii* and *P. gardneri* in the *matK* tree and at the base of the *P. drummondii*, *P. aphyllus*, *P. schwartzii* and *P. calostachyus* clade in the ITS tree; its morphology supports a close relationship with *P. drummondii* (Benl, unpubl.). *Ptilotus halophilus* is placed within clade D2 in the *matK* tree and within clade D4 in the ITS tree. Given the strong support for the placement of *P. halophilus* in the ITS and combined trees and the strong affinity to *P. seminudus* (Davis, 2004), the *matK* sequence may be anomalous.

Rates of species diversification

Figure 7 shows the smallest set of distinct configurations of diversification rate shifts across the phylogeny, which can account for 95% of the posterior distribution in the BAMM analysis. The most frequently sampled configuration (f = 0.67) in the posterior shows a shift in the rate of speciation occurring at the base of *Ptilotus*. The

second-most frequently sampled configuration (f = 0.15) shows a rate shift occurring at the node connecting clades B and C+D, suggesting that clade A may have diversified under a different rate regime than the rest of *Ptilotus*, though the macroevolutionary cohort matrix (the pairwise probability that any two leaves of the tree share a common macroevolutionary rate dynamic; Fig. 8) indicates that most species of *Ptilotus* have a high probability of sharing the same rate dynamic. Three of the shift configurations show a rate shift within subclade D4, which may reflect an increase in speciation rate within this subclade.

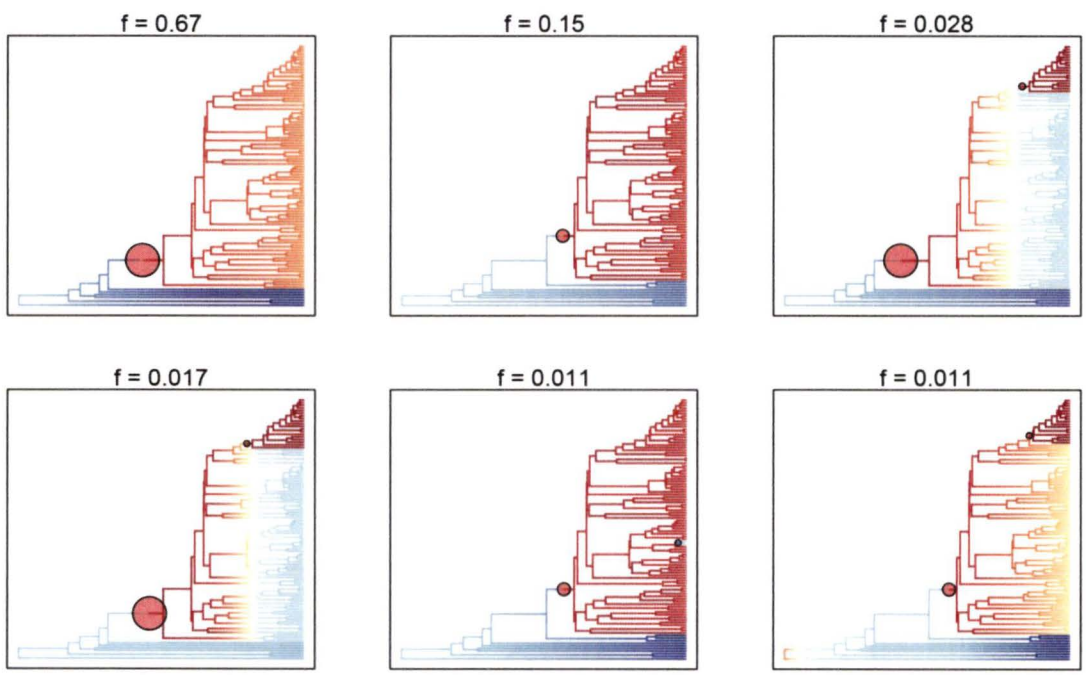


Fig. 7. Credible set (95%) of rate shift configurations, showing the six most likely rate shifts in the phylogeny. Red circles denote locations of rate shifts for each distinct shift configuration; circle size is proportional to the overall marginal probability of a shift on the branch.

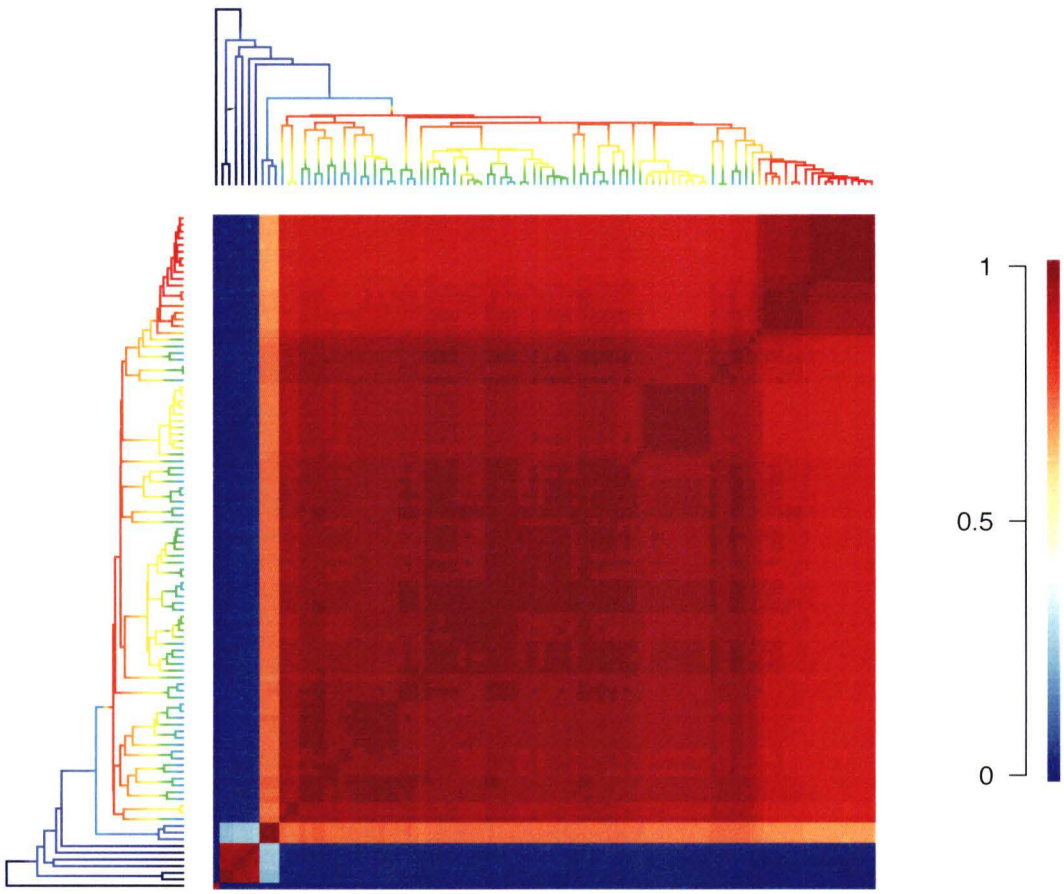


Fig. 8. Macroevolutionary cohort matrix, which shows the pace of diversification on the phylogeny (blue = slower; red = faster). The center matrix indicates the probability that any given taxon shares a rate shift with another given taxon, where dark red is the highest probability (p = 1.0) and dark blue is the lowest probability (p = 0.0).

CHAPTER IV

DISCUSSION

Our analyses provide strong support for a monophyletic *Ptilotus*, probably nested within a paraphyletic *Aerva* consistent with the findings of Sage & al. (2007). This is in contrast to Thiv & al. (2006), who considered *Aerva* to be monophyletic; however, their tree was rooted on two species of *Ptilotus* only, thus was unable to test for monophyly of *Aerva*. A rigorous test for monophyly or paraphyly of *Aerva* awaits sequence data for the critical genus *Nothosaerva*, and will be the subject of a subsequent paper.

Infrageneric Classifications

This study presents the first rigorous framework phylogeny for *Ptilotus* and confirms the monophyly of the genus. Four clades of *Ptilotus* are evident. Although taxonomists working on *Ptilotus* have found it difficult to find characters that can consistently define infrageneric groups, our analyses show an overall pattern of character evolution: taxa in clades A, B and C share the plesiomorphies of five fertile stamens, unbranched tepal hairs, and straight styles centrally fixed on the ovary; and most species in clade D have reduced numbers of fertile stamens, branched tepal hairs, and curved or sigmoidal styles that are eccentric on the ovary. Clade D, however, is morphologically diverse, with some members retaining (or regaining) the plesiomorphic condition in these characters. This diversity in clade D has likely confounded previous attempts to produce a stable and robust infrageneric classification.

In the current phylogeny, the original species of *Ptilotus* described by Brown (1810; *P. conicus* and *P. corymbosus*) occur in clade B, and the original six species of

Trichinium in clade D. The expanded circumscription of these genera by Bentham (1870), however, is incongruent with the phylogeny: most of Bentham's *Trichinium* species fall in clade D (except *P. arthrolasius*, *P. roei* and *P. helichrysoides*), and his *Ptilotus* species fall into clade B (except *P. humilis* and *P. grandiflorus*), though these species had been moved between the genera by other authors (e.g., von Mueller, 1874). All series erected by Bentham (1870) within *Trichinium* (and later transferred to *Ptilotus*) are paraphyletic, as are sect. *Ptilotus* of Black (1948) and subgenus *Ptilotus* of Benl (1990).

Despite Benl's multi-decade (1958–1994) work on the taxonomy of *Ptilotus*, he failed to establish a coherent framework for an infrageneric classification in the genus. This is understandable in the light of the current phylogeny, as no consistent and readily observable synapomorphies are available to define clades or subclades. Given this, I prefer to recognize clades in the genus informally rather than to erect a formal infrageneric classification that would have little utility outside the context of the phylogeny itself.

Diversification of Ptilotus

Likely rate shifts identified on the phylogeny indicate that *Ptilotus* diversified rapidly after its divergence from *Aerva*, perhaps explaining the lack of clear morphological synapomorphies defining clades. Given the African-south Asian distributions of *Aerva* and *Nothosaerva*, it is likely that the common ancestor of *Aerva* and *Ptilotus* was distributed outside Australia, with the ancestor of *Ptilotus* possibly reaching Australia from the north sometime between the Miocene (*c.* 23 Ma) and late Pliocene (*c.* 2.5 Ma). The close proximity of the continents during this time allowed for

dispersal between the once separated Australian and Asian floras. The continents reached their closest proximity by around the middle Pleistocene (*c.* 781–126 ka), Australia only being separated from the lesser Sunda islands and the connected Asian landmass by a relatively short distance (Barlow, 1996). An increasingly arid environment in Australia commenced at 25–10 Ma, with severe aridity developing at 5–2 Ma (Bowler, 1982; Hill & al., 1999; Byrne & al., 2008). This time period corresponds to the dates of the rapid diversification of many genera now dominant in arid Australia (Crisp & al., 2004). Xeromorphic adaptations evident in *Ptilotus* include a dense indumentum (often of dendritic hairs) on the stem and/or leaves of many species. Since this character is present in every clade of *Ptilotus* and is also present in *Aerva* (Miller, 1996), it is likely that *Ptilotus* was pre-adapted to arid conditions and diversified rapidly into the expanding arid biome. Other widespread Australian lineages that may likewise have been pre-adapted to arid conditions in Australia include *Banksia* (Proteacaeae; Mast & Givnish, 2002) and many genera in Chenopodiaceae (Shepherd & al., 2004).

Rapid diversification in *Ptilotus* may have been facilitated by a paleopolyploidy event in a common ancestor of *Ptilotus*. Species in the genus have some of the highest chromosome numbers recorded to date in Amaranthaceae (Townsend, 1993). A cytological study of 13 *Ptilotus* species (Stewart & Barlow, 1976) found that all species had a base chromosome count of n = 27, with the exception of tetraploid individuals of the widespread and morphologically diverse species *P. obovatus* (n = 54). *Aerva javanica* has a base chromosome count of n =16 (with n = 32 tetraploids also documented; Soliman, 2006). The study by Stewart and Barlow (1976) did not encompass the taxonomic diversity found within *Ptilotus*, sampled species occupy only

two clades (B and D), and few chromosome counts are available for other aervoids. Palaeopolyploidy has been implicated in rapid radiations within the Poaceae, Solanaceae, Fabaceae and Brassicaceae (Soltis & al., 2009). Further sampling and chromosome counts to determine whether a paleopolyploidy event is indicated at the time of the rapid diversification of *Ptilotus* are warranted.

The largest clade in the phylogeny, clade D, has the greatest morphological diversity and the least basal resolution, with its four subclades joined at an unresolved polytomy. Diversification in this clade may be linked to the emergence of novel phenotypic characters adaptive for seed dispersal (branched tepal hairs; see Townsend, 1993) and a possible shift in pollination syndrome (reduced numbers of fertile stamens, eccentric ovary and curved or sigmoid style).

Reduction in fertile stamens to < 5 is a common feature of many species within clade D, though a few subclades retain 5 fertile stamens. The reduction of fertile stamens reaches its extreme in *P. alexandri*, with only one fertile stamen and four staminodes (Benl, 1974). It is unclear if staminodes have any function in *Ptilotus* as they are not showy and do not appear to provide a reward for visiting pollinators. Some *Ptilotus* species with reduced fertile stamen number lack staminodes, and these may be nonfunctional. Nonfunctional staminodes may interfere with pollination, and thus may eventually be lost in a lineage (Walker-Larsen & Harder, 2000). Varying number and arrangement of stamens, varying placement and curvature of the style, and varying tepal colors (also quite common), suggest that differing pollination mechanisms may be employed within clade D. There are no published studies on pollination mechanisms in *Ptilotus*.

Many *Ptilotus* species grow in areas of Australia with highly weathered soils, which are low in exchangeable phosphorus (Orian & Milewski, 2007; Lambers & al, 2010). Studies of *P. polystachyus* (subclade D3), one of the most widespread *Ptilotus* species (Bean, 2008) and a common species on nutrient-deficient, sandy soils, show that it is a P hyper-accumulator and remarkably tolerant of vacuolar P concentrations that would be toxic to most plants (Ryan & al., 2009; Suriyagoda & al., 2012). P hyperaccumulation and toxicity tolerance has also been observed in other species in clade D including *P. macrocephalus, P. aervoides* and *P. nobilis* (Islam & al., 1999). These species are also some of the most widespread in the genus (Bean, 2008). Many native Australian plants that grow in oligotrophic, P-deficient soils lack an ability to down-regulate P uptake, but unlike *Ptilotus* suffer from P-toxicity when P availability is high (Shane & al., 2004). Some resistance to P toxicity has also been found in the Australian taxon *Grevillea crithmifolia* (Proteaceae; Shane & Lambers, 2006). The phylogeny presented here provides an important framework for assessing the evolution of this novel feature in the genus and its close relatives, and for exploring the significance of this trait in a major adaptive radiation in arid Australia.

CHAPTER V

CONCLUSIONS

This study presents the first molecular phylogenetic framework for *Ptilotus*, a large genus in Amaranthaceae important in arid and semi-arid Australia. The monophyly of *Ptilotus* is highly supported, but the genus is likely nested within a paraphyletic *Aerva*. Diversification rate analysis suggests that *Ptilotus* may have diversified rapidly in response to increasingly arid conditions in Australia. Pre-adaptation to arid conditions and a paleopolyploidy event may have facilitated its radiation. Rapid cladogenesis may account for the lack of consistent character state differences within the genus, which has hindered previous attempts to construct a robust infrageneric classification. Within the most diverse clade, floral character state changes and traits that enable increased P uptake and storage are evident, suggesting adaptations that may have facilitated its diversification and success in a harsh but widespread environment.

LITERATURE CITED

Aberer, A.J., Krompass, D. & Stamatakis, A. 2013. Pruning rogue taxa improves phylogenetic accuracy: an efficient algorithm and webservice. *Syst. Biol.* 62(1): 162–166. http://dx.doi.org/10.1093/sysbio/sys078

Able, A.J., Smyth, H. & Joyce, D. 2014. Postharvest physiology and volatile production by flowers of *Ptilotus nobilis*. *Postharvest Biol. Tech.* 88: 61–71. http://dx.doi.org/10.1016/j.postharv bio.2013.10.002

Barlow, B.A. 1994. Phytogeography of the Australian region. Pp. 5-6 in: Groves, R.H. (ed.), *Australian Vegetation*, ed. 2. New York: Cambridge University Press.

Bean, A.R. 2008. A synopsis of *Ptilotus* (Amaranthaceae) in eastern Australia. *Telopea* 12: 227–250.

Benl, G. 1971. Ein bestimmungsschüssel für die gattung *Ptilotus* R.Br. (Amaranthaceae). *Mitt. Bot. Staatssamml. Münch.* 9: 135–176.

Benl, G. 1979. Two new taxa of *Ptilotus* (Amaranthaceae). *J. Adel. Bot. Gard.* 1: 201-204.

Benl, G. 1980. Five new taxa of Ptilotus (Amaranthaceae) from Western Australia. *Nuytsia* 3: 157–172.

Benl, G. 1990. Further taxonomic studies in Australian Amaranthaceae. *Mitt. Bot. Staatssamml. Münch.* 29: 495–502.

Benl, G. (unpubl.). *Ptilotus*. Flora of Australia 5. Manuscript. ABRS Canberra.

Bentham, G. 1870. *Flora australiensis: a description of the plants of the Australian territory*. Pp. 217–245. London: L. Reeve & Co.

Black, J.M. 1924. Amaranthaceae. Pp. 209–216 in: *Flora of South Australia*, vol. 2. Adelaide.

Black, J.M. 1948. Amaranthaceae. Pp. 323–332 in: *Flora of South Australia*, vol. 2. Adelaide.

Bonnet, E. & Van de Peer, Y. 2002. Zt: a software tool for simple and partial Mantel test. *J. Stat. Softw.* 7(10): 1–12.

Borsch, T. 1998. Pollen types in the Amaranthaceae: morphology and evolutionary significance. *Grana 37*: 129–142.

Bouckaert R., Heled, J., Kühnert, D., Vaughan, T.G., Wu, C.H., Xie, D., Suchard, M.A., Rambaut, A. & Drummond A.J. 2014. BEAST2: A software platform for Bayesian evolutionary analysis. *PLoS Comput. Biol.* 10(4): e1003537. http://dx.doi.org/10.1371/journal.pcbi.1003537

Brown, R. 1810. *Prodromus Florae Novae Hollandiae et Insulae Van Diemen*. Pp. 414–415. London: Richard Taylor & Son.

Byrne, M., Yeates, K., Joseph, L., Kearney, M., Bowler, J., Williams, M.A.J., Cooper, S., Donnellan, S.C., Keogh, J.S., Leys, R., Melville, J., Murphy, D.J., Porch, N. & Wyrwoll, K-H. 2008. Birth of a biome: insights into the assembly and maintenance of the Australian arid zone biota. *Mol. Ecol.*17: 4398–4417. http://dx.doi.org/10.1111/j.1365-294X.2008.03899.x

Crisp, M.D. & Cook, L.G. 2013. How was the Australian flora assembled over the last 65 million years? A molecular phylogenetic perspective. *Annu. Rev. Ecol. Evol. Syst.* 44: 303–324. http:// dx.doi.org/10.1146/annurev-ecolsys-110512-135910

Darriba, D., Taboada, G.L., Doallo, R. & Posadam D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: 772. http:// dx.doi.org/10.1038/nmeth.2109

Davis, R.W. 2004. Two new species of *Ptilotus* (Amaranthaceae) from Western Australia. *Nuytsia* 15: 221–226.

Davis, R.W. 2007. A new rare and geographically restricted Ptilotus (Amaranthaceae) from the Pilbara bioregion of Western Australia. *Nuytsia* 16: 265–268.

Davis, R.W., Hammer, T.A. & Thiele, K.R. 2014. Two new and rare species of *Ptilotus* (Amaranthaceae) from the Eneabba sand plains, Western Australia. *Nuytsia* 24: 123–129.

De Vienne, D.M., Giraud, T. & Martin, O.C. 2007. Congruence index for testing topological similarity between trees. *Bioinformatics* 23: 3119–3124.

Farris, J.S., Kallersjo, M., Kluge, A.G. & Bult, C. 1995. Constructing a significance test for incongruence. *Syst. Biol.* 44: 570–572.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.

Gernhard, T. 2008. The conditioned reconstructed process. *J. Theor. Biol.* 253: 769–778.

Hopper, S.D. 2009. OCBIL theory: towards an integrated understanding of the evolution, ecology and conservation of biodiversity on old, climatically buffered, infertile landscapes. *Plant Soil* 322: 49–86. http://dx.doi.org/10.1007/s11104-009-0068-0

Kadereit, G., Borsch, T., Weising, K. & Freitag, H. 2003. Phylogeny of Amaranthaceae and Chenopodiaceae and the evolution of C_4 photosynthesis. *Int. J. Plant Sci.* 164: 959–986.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P. & Drummond, A. 2012. Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28: 1647–1649. http://dx.doi.org/10.1093/bioinformatics/bts199

Lambers, H., Brundrett, M.C., Raven, J.A. & Hopper S.D. 2010. Plant mineral nutrition in ancient landscapes: high plant species diversity on infertile soils is linked to functional diversity for nutritional strategies. *Plant Soil* 334: 11–31. http://dx.doi.org/10.1007/s11104-010-0444-9

Lee, K.K., Harrison, D.K., Johnston, M.E. & Williams, R.R. 2007. Molecular taxonomic clarification of *Ptilotus exaltatus* and *Ptilotus nobilis* (Amaranthaceae). *Aust. Syst. Bot.* 20: 72–81.

Löytynoja, A. & Goldman, N. 2010. WebPRANK: a phylogeny-aware multiple sequence aligner with interactive alignment browser. *B. M. C. Bioinformatics* 11: 579.

Mast, A.R. & Givnish, T.J. 2002. Historical biogeography and the origin of stomatal distributions in *Banksia* and *Dryandra* (Proteaceae) based on their cpDNA phylogeny. *Amer. J. Bot.* 89: 1311–123. http://dx.doi.org/10.3732/ajb.89.8.1311

Miller, A.G. 1996. Amaranthaceae. Pp. 295–299 in: Miller, A.G. & Cope, T.A. (eds.), *Flora of Arabian Peninsula and Socotra,* vol. 1. Edinburgh: Edinburgh University Press.

Miller, M.A., Pfeiffer, W. & Schwartz, T. 2010. *Creating the CIPRES Science Gateway for inference of large phylogenetic trees*. Proceedings of the Gateway Computing Environments Workshop (GCE). Pp. 1–8. New Orleans, LA.

Moquin-Tandon, A. 1849. *Prodromus systematis naturalis regni vegetabilis*, vol 13. Pp. 281–298. Paris: Sumptibus Sociorum Treuttel et Würtz.

Motley, T.J., Wurdack, K.J. & Delprete, P.G. 2005. Molecular systematics of the Catesbaeeae-Chiococceae complex (Rubiaceae): Flower and fruit evolution and biogeographic implications. *Amer. J. Bot.* 92: 316–329.

Müller, K. & Borsch, T. 2008. Phylogenetics of Amaranthaceae based on *matk/trnk* sequence data: Evidence from parsimony, likelihood, and Bayesian analyses. *Ann. Missouri Bot. Gard.* 92: 66–102.

Orian, G.H. & Milewski, A.V. 2007. Ecology of Australia: the effects of nutrient-poor soils and intense fires. *Biol. Rev.* 82: 393–423. http://dx.doi.org/10.1111/j.1469-185X.2007.00017.x

Poiret, J.L.M. 1817. Ptilotus. Pp 619-620 in: Lamarck, J.B., *Encyclopédie méthodique: botanique*, vol. 4. Paris, Agasse. http://dx.doi.org/10.5962/bhl.title.49178

Rabosky, D.L. 2014a. Automatic detection of key innovations, rate shifts, and diversity-dependence on phylogenetic trees. *PLoS ONE.* http://dx.doi.org/10.1371/journal.pone.0089543

Rabosky, D.L., Donnellan, S.C., Grundler, M. & Lovette, I.J. 2014b. Analysis and visualization of complex macroevolutionary dynamics: an example from Australian scincid lizards. *Syst. Biol.* 63: 610–627. http://dx.doi.org/10.1093/sysbio/syu025

Rabosky, D.L., Grundler, M., Anderson, C., Title, P., Shi, J.J., Brown, J. W., Huang, H. & Larson, J.G. 2014c. BAMMtools: an R package for the analysis of evolutionary dynamics on phylogenetic trees. *Methods Ecol. Evol.* http://dx.doi.org/10.1111/2041-210X.12199

Rambaut, A. & Drummond, A.J. 2007. Tracer v1.6, Available from http://beast.bio.ed.ac.uk/Tracer

Ronquist, F., Teslenko, M., van der Mark, P. Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* 61. http://dx.doi.org/10.1093/sysbio/sys029

Ryan, M.H., Ehrenberg, S., Bennett, R.G. & Tibbett, M. 2009. Putting the P in *Ptilotus*: a phosphorus-accumulating herb native to Australia. *Ann. Bot.* 103: 901–911. http://dx.doi.org/110.1093/aob/mcp021

Sage, R.F., Sage, T.L., Pearcy, R.W. & Borsch, T. 2007. The taxonomic distribution of C_4 photosynthesis in Amaranthaceae sensu stricto. *Amer. J. Bot.* 94: 1992–2003.

Sang, T., Crawford, D.J. & Stuessy, T.F. 1997. Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). *Amer. J. Bot. 84*: 1120–1136.

Schinz, H. 1934. Amaranthaceae. Pp. 55–58 in: Engler, A. & Prantl, K. (eds.), *Die natürlichen Pflanzenfamilien: nebst ihren Gattungen und wichtigeren Arten insbesondere den Nutzpflanzen*, vol. 16c. Leipzig: Engelmann.

Shane, M.W. & Lambers, H. 2006. Systemic suppression of cluster-root formation and net P-uptake rates in *Grevillea crithmifolia* at elevated P supply: a proteacean with resistance for developing symptoms of 'P toxicity'. *J. Exp. Bot.* 57: 413–423. http://dx.doi.org/10.1093/jxb/erj004

Shane, M.W., McCully, M.E. & Lambers, H. 2004. Tissue and cellular phosphorus storage during development of phosphorus toxicity in *Hakea prostrata* (Proteaceae). *J. Exp. Bot.* 55: 1033–1044. http://dx.doi.org/ 10.1093/jxb/erh111

Shepherd, K.A., Waycott, M. & Calladine, A. 2004. Radiation of the Australian Salicornioideae (Chenopodiaceae) – based on evidence from nuclear and chloroplast DNA sequences. *Amer. J. Bot.* 91: 1387–1397. http://dx.doi.org/10.3732/ajb.91.9.1387

Soliman, M.A. 2006. Cytogenetical studies on *Aerva javanica* (Amaranthaceae). *Fl. Medit.* 16: 333–339.

Soltis, D.E., Albert, V.A., Leebens-Mack, J., Bell, C.D., Paterson, A.H., Zheng, C, Sankoff, D., dePamphilis, C.W., Wall, P.K., & Soltis, P.S. 2009. Polyploidy and angiosperm diversification. *Amer. J. Bot.* 96(1): 336–348. http://dx.doi.org/10.3732/ajb.0800079

Sprengel, C. 1825. Amaranthaceae. Pp 816-817 in: Linnaei, C. Systema Vegetabilium (ed. 16), vol. 1. Gottingae: Sumtibus Librariae Dieterichianae.

Stewart, D. A. & Barlow, B. A. 1976. Infraspecific polyploidy and gynodioecism in *Ptilotus obovatus* (Amaranthaceae). *Aust. J. Bot.* 24: 237–248.

Stöver, B.C. & Müller, K.F. 2010. TreeGraph 2: Combining and visualizing evidence from different phylogenetic analyses. *B. M. C. Bioinformatics* 11: 7.

Sukumaran, J. & Holder, M.T. 2010. DendroPy: A Python library for phylogenetic computing. *Bioinformatics* 26: 1569–1571.

Suriyagoda, L.D.B, Lambers, H., Renton, M. & Ryan, M.H. 2012. Growth, carboxylate exudates and nutrient dynamics in three herbaceous perennial plant species under low, moderate and high phosphorus supply. *Plant Soil* 358: 105–117. http://dx.doi.org/10.1007/s11104-012-1311-7

Swofford, D.L. 2002. PAUP*: Phylogenetic analysis using parsimony, version 4.0 Beta. Sunderland, Massachusetts: Sinauer.

Thiv, M., Thulin, M., Kilian, N. & Linder, H.P. 2006. Eritreo-arabian affinities of the Socotran flora as revealed from the molecular phylogeny of *Aerva* (Amaranthaceae). *Syst. Bot.* 31: 560–570.

Thorley, J. & Wilkinson, M. 1999. Testing the phylogenetic stability of early tetrapods. J. Theor. Biol. 200: 343—344.

Walker-Larsen, J. & Harder, L.D. 2000. The evolution of staminodes in angiosperms: patterns of stamen reduction, loss, and functional re-invention. *Amer. J. Bot.* 87: 1367–1384.

Zwickl, D.J. 2006. *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion.* Dissertation, University of Texas at Austin.

APPENDIX

LIST OF SPECIMENS USED

GenBank Assessions

Taxon	ITS sequences	*matK* sequences
Aerva artemisioides	AJ829477 (ITS 1) & AJ829504 (ITS 2)	AJ833649
Aerva lanata	AJ829493 (ITS 1) & AJ829520 (ITS 2)	AJ833663
Amaranthus caudatus	AF210907 (ITS1, 5.8s, ITS2)	AY514809

Original Sequences

Taxon	Material loan source	Herbarium Barcode No.	Collector & Coll. No.
Achyranthes aspera	PERTH	PERTH 08600309	R. Barrett 8308
Aerva javanica	PERTH	PERTH 08335990	L. Hunt LH 20
Gomphrena flaccida	PERTH	PERTH 08185530	P. Courtney 203
Gomphrena kanisii	PERTH	PERTH 07787863	G. Byrne 2773
Ptilotus aervoides	PERTH	PERTH 08523746	R. Davis, T. Hammer, B. Anderson RD 12267
Ptilotus albidus	PERTH	PERTH 07892152	K. Thiele 3289
Ptilotus alexandri	PERTH	PERTH 07457774	C. Page, DHI 78
Ptilotus aphyllus	PERTH	PERTH 07714386	R. Davis 2007
Ptilotus appendiculatus	PERTH	PERTH 08321272	R. Davis 11812
Ptilotus aristatus ssp. aristatus	CANB	AD 138137	R. Bates 58592
Ptilotus aristatus ssp. micranthus	CANB	CANB 387899.1	G. Leach & M.B. Leach 1851
Ptilotus arthrolasius	PERTH	PERTH 08321264	R. Davis 11821
Ptilotus astrolasius	PERTH	PERTH 08321531	R. Davis 11795
Ptilotus auriculifolius	PERTH	PERTH 08321329	R. Davis 11815
Ptilotus axillaris	PERTH	PERTH 08321485	R. Davis 11793

Appendix Continued

Taxon	Material loan source	Herbarium Barcode No.	Collector & Coll. No.
Ptilotus beardii	PERTH	PERTH 07908903	R. Davis 11343
Ptilotus beckerianus	CANB	CBG 8910367.1	F.E. Davies 1432
Ptilotus blackii	PERTH	PERTH 08326959	R. Davis 11966
Ptilotus calostachyus	PERTH	PERTH 08321302	R. Davis 11816
Ptilotus capitatus	PERTH	PERTH 07498292	G. Byrne 1642
Ptilotus carinatus	PERTH	PERTH 07298110	D. J. Edinger 5125
Ptilotus chamaecladus	PERTH	PERTH 08552142	R. Davis, T. Hammer, B. Anderson RD 12296
Ptilotus chippendalei	CANB	AD 97347196	R.J. Chinnock 572
Ptilotus chortophytus	PERTH	PERTH 07153724	R. Davis 10923
Ptilotus chrysocomus	PERTH	PERTH 07254113	J. Williams s.n.
Ptilotus clementii	PERTH	PERTH 08321310	R. Davis 11817
Ptilotus clivicola	PERTH	PERTH 08486433	C. Payne s.n.
Ptilotus conicus	PERTH	PERTH 08042977	R.L. & M.D. Barret RLB 3134
Ptilotus corymbosus	PERTH	PERTH 08110166	G. Byrne 3400
Ptilotus crispus	PERTH	PERTH 07977492	R.L. & M.D. Barrett RLP 3156
Ptilotus crosslandii	PERTH	PERTH 08523622	R. Davis, T. Hammer, B. Anderson RD12286
Ptilotus daphne	PERTH	PERTH 08599750	N. Gibson 6505
Ptilotus decalvatus	PERTH	PERTH 08355444	P. Chukowry BES PC 039
Ptilotus decipiens	PERTH	PERTH 05619289	D.J. Edinger 1870
Ptilotus declinatus	PERTH	PERTH 08599742	R. Davis & P. Jobson RD 12004
Ptilotus distans	PERTH	PERTH 06606806	RLB 2600
Ptilotus drummondii	PERTH	PERTH 07758987	G. Byrne 2040
Ptilotus eriotrichus	PERTH	PERTH 08051054	I. Fordyce MU 55-37
Ptilotus esquamatus	PERTH	PERTH 07725434	R. Davis 11292
Ptilotus exiliflorus	PERTH	PERTH 08016658	J. Firth 692
Ptilotus falcatus	PERTH	PERTH 08341117	R. Warner & S. Werner WWF 12-14
Ptilotus fasciculatus	PERTH	PERTH 07292767	J. Richardson s.n.
Ptilotus fusiformis	PERTH	PERTH 08321337	R. Davis & R. Butcher RD11819
Ptilotus gardneri	PERTH	PERTH 06001262	R.W. Purdie 4895
Ptilotus gaudichaudii ssp. eremita	PERTH	PERTH 08406839	R. Davis 12114

Appendix Continued

Taxon	Material loan source	Herbarium Barcode No.	Collector & Coll. No.
Ptilotus gaudichaudii ssp. gaudichaudii	PERTH	PERTH 08552096	R. Davis, T. Hammer, B. Anderson RD 12283
Ptilotus gaudichaudii ssp. parviflorus	CANB	CANB 796574.1	R.W. Purdie 7847
Ptilotus giganteus	PERTH	PERTH 06702384	K.F. Kenneally, K 11831
Ptilotus gomphrenoides	PERTH	PERTH 08552088	R. Davis, T. Hammer, B. Anderson RD 12280
Ptilotus grandiflorus	PERTH	PERTH 07757751	MET 22546
Ptilotus halophilus	PERTH	PERTH 08051038	I. Fordyce KL 25-1
Ptilotus helichrysoides	PERTH	PERTH 08322716	J. Jackson, A. Brown, & B. Moyle DLP 128
Ptilotus helipteroides	PERTH	PERTH 08523770	R. Davis, T. Hammer, B. Anderson RD 12274
Ptilotus holosericeus	PERTH	PERTH 08326533	R. Davis 11829
Ptilotus humilis	PERTH	PERTH 08523649	R. Davis 12361
Ptilotus incanus	PERTH	Not available	P.J. Hurter WP15
Ptilotus johnstonianus	PERTH	PERTH 08144850	H. Dauncey H337
Ptilotus kenneallyanus	PERTH	PERTH 07564015	G. Byrne 1484
Ptilotus latifolius	PERTH	PERTH 08321639	R. Davis 11791
Ptilotus lazaridis	PERTH	PERTH 08406812	P.J. Hurter s.n.
Ptilotus leucocoma	CANB	CANB 796532.1	R.W. Purdie 7832
Ptilotus maconochiei	BRI	AQ 747412	D.T. Kelman 141
Ptilotus macrocephalus	PERTH	PERTH 08321558	R. Davis 11787
Ptilotus manglesii	PERTH	PERTH 08599734	R. Davis & P. Jobson RD 12005
Ptilotus marduguru	PERTH	PERTH 07332831	Sweedman LSJ 6574
Ptilotus mitchellii	PERTH	PERTH 07759495	E. Thoma ET 1335
Ptilotus mollis	PERTH	PERTH 08362602	J. Bull ONS JSF 338.02
Ptilotus murrayi	PERTH	PERTH 08599947	K. Thiele 4583
Ptilotus nobilis ssp. nobilis	PERTH	PERTH 08552045	R. Davis, T. Hammer, B. Anderson RD 12295
Ptilotus nobilis ssp. semilanatus	CANB	CANB 796514.1	R.W. Purdie 7814
Ptilotus obovatus	PERTH	PERTH 08423754	R. Davis, T. Hammer, B. Anderson RD 12268
Ptilotus pedleyanus	BRI	AQ 758110	J. Silcock JLS680
Ptilotus polakii ssp. juxtus	PERTH	PERTH 08321604	R. Davis 11797

Appendix Continued

Taxon	Material loan source	Herbarium Barcode No.	Collector & Coll. No.
Ptilotus polakii ssp. polakii	PERTH	PERTH 08321418	R. Davis 11788
Ptilotus polystachyus	PERTH	PERTH 08523738	R. Davis 12276
Ptilotus procumbens	PERTH	PERTH 07392400	P. Armstrong PA 06/860
Ptilotus propinquus	CANB	AD 99430039	H.P. Vonow 2184
Ptilotus pseudohelipteroides	PERTH	PERTH 06592813	G. Byrne 307
Ptilotus pyramidatus	PERTH	PERTH 08273065	C. Tauss 4102
Ptilotus remotiflorus	BRI	AQ 833077	A.J. Emmott AJE-B689A
Ptilotus rigidus	PERTH	PERTH 08529922	J. Jackson 290
Ptilotus roei	PERTH	PERTH 08523789	R. Davis, T. Hammer, B. Anderson RD 12277
Ptilotus rotundifolius	PERTH	PERTH 08552134	R. Davis, T. Hammer, B. Anderson RD 12288
Ptilotus royceanus	NT	D 0263951	P. Jobson 10779
Ptilotus schwartzii	PERTH	PERTH 08523592	R. Davis, T. Hammer, B. Anderson RD 12273
Ptilotus seminudus	PERTH	PERTH 07897685	G. Cockerton & N. McQuoid LCH 15892
Ptilotus sericostachyus	PERTH	PERTH 07576552	R. Davis 11298
Ptilotus sessilifolius	PERTH	PERTH 07360258	R. J. Cranfield 21749
Ptilotus spathulatus	PERTH	PERTH 08375682	W.A. Thompson & J. Allen 1445
Ptilotus spicatus	PERTH	PERTH 08313954	G. Byrne 3052
Ptilotus stipitatus	PERTH	PERTH 07714491	R. Davis 11184
Ptilotus stirlingii ssp. australis	PERTH	PERTH 07256361	R. Davis 10956
Ptilotus stirlingii ssp. stirlingii	PERTH	PERTH 08408181	R. Davis s.n.
Ptilotus subspinescens	PERTH	PERTH 06467334	E. Thoma & A. Joder 145
Ptilotus symonii	PERTH	PERTH 08149720	K. Thiele 3722
Ptilotus trichocephalus	PERTH	PERTH 07375115	R. Davis 10988
Ptilotus whitei	NT	D 0229930	P. Jobson 10452

VITA

TIMOTHY A. HAMMER
Department of Biological Sciences, Old Dominion University, 110 Mills Godwin Building/45th St., Norfolk, Virginia 23529-0266

EDUCATION:

Old Dominion University
M.S., Biology — December 2014
B.S., Biology – *Summa cum laude* — May 2012
ECPI University
B.S., Computer Information Science – *Magna cum laude* — May 2009

RELEVANT WORK EXPERIENCE:

Graduate Research Assistant — 05/13 – Present
Conducted independent research in the molecular plant systematics lab for the completion of graduate research at Old Dominion University.
Graduate Teaching Assistant — 08/12 – 05/13
Taught the environmental science and human biology laboratories under the instruction of Dr. Tatyana Lobova at Old Dominion University.

PUBLICATIONS:

Davis, R. W., **Hammer, T.**, and Thiele, K.R. (2014). Two new and rare species of *Ptilotus* (Amaranthaceae) from the Eneabba sand plains, Western Australia. *Nuytsia* 24: 123–129.

PROFESSIONAL PRESENTATIONS:

Botany 2014, Botanical Society of America; Boise, ID — July 2014
Oral presentation
Title: A molecular framework phylogeny for *Ptilotus* R.Br. (Amaranthaceae): implications for classifications within the Aervoids
Association of Southeastern Biologists 2014; Spartanburg, SC — April 2014
Oral presentation
Title: A molecular framework phylogeny for *Ptilotus* R.Br. (Amaranthaceae)
Botany 2013, Botanical Society of America; New Orleans, LA — July 2013
Poster Presentation
Title: Preliminary phylogeny of the Australian genus *Ptilotus* (Amaranthaceae)