Mulling over the mulla mullas: taxonomy and evolution of the Australian genus *Ptilotus* and relatives in the aervoid clade (Amaranthaceae)

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BSc, MSc

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Nomina si nescis, perit & cognitio rerum.

If you do not know the names, the knowledge of the things also dies.

Carl Linnaeus in *Philosophia Botanica* (1751: 158)

THESIS DECLARATION

I, Timothy Andrew Hammer, certify that:

This thesis has been substantially accomplished during enrolment in the degree.

This thesis does not contain material which has been submitted for the award of any other degree or diploma in my name, in any university or other tertiary institution.
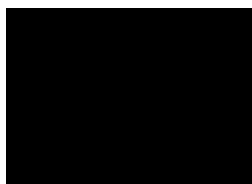
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ABSTRACT

Ptilotus (commonly mulla mullas) is one of the most species-rich genera in the plant family Amaranthaceae *sensu stricto*, and the most species-rich genus of the family within Australia, with over 120 species. All species of *Ptilotus* occur in Australia, mostly in arid and semi-arid regions. Based on previous studies, *Ptilotus* has been placed within the 'aervoid clade' with *Aerva*, *Kelita*, *Nothosaerva* and *Omegandra*. The phylogenetic relationships among the aervoid genera have not been resolved, and therefore the taxonomic hypotheses of the monophyly of these genera remain untested. Furthermore, unclear morphological boundaries of these genera and within some widespread *Ptilotus* species complexes have led to precarious taxonomic decisions. The unresolved phylogenetic relationships among the genera and among the major clades of *Ptilotus* have hindered investigations into the origins and drivers of the patterns of diversification within the aervoids.

In this thesis, I filled these gaps in our knowledge with five studies presented in three parts, focused on (1) reconstructing a well-supported phylogenetic backbone for the aervoids and the major clades within *Ptilotus* and establishing a stable generic taxonomy, (2) resolving the taxonomy of two widespread species complexes within *Ptilotus* by integrating analyses of ecological and morphological data, and (3) testing hypotheses of the chronological, geographic and climatic origins of the aervoids and *Ptilotus* and elucidating potential drivers for the hyper-diversity of the aervoids within *Ptilotus* in arid and semi-arid Australia.

The first study included in Part 1 investigated *A. artemisioides*, which has an atypical floral morphology that is unique within the family. Molecular data placed this species outside of the aervoids. Therefore, the genus *Wadithamnus* was erected to accommodate this species. The relationships between aervoid genera and the major clades of *Ptilotus* were investigated in the second study of Part 1 by using a phylogenomic approach to reconstruct a well-supported phylogeny for 36 aervoid species based on 69 gene sequences derived from assembled whole-plastid genomes. This was then used to constrain relationships on a larger phylogeny based on Sanger-derived sequences for 135 taxa. This newly resolved phylogeny of the aervoids established *Aerva* as polyphyletic, which was resolved by reinstating *Ouret* and erecting the new genus *Paraerva*. *Kelita* was found to be deeply nested within *Ptilotus* and is formally synonymised. Morphological characters were identified to support the new taxonomic framework.

Two studies within Part 2 revisit previous taxonomic decisions within the *P. nobilis* and *P. macrocephalus* species groups. These studies recognise new species taxonomies on the basis of clarified morphological differences, including the reinstatement of species and the description of new species. The morphology was supported by geospatial analyses, which showed the species to be segregated based on edaphic and climatic differences. These two studies showed how geospatial analyses of large-scale ecological data can be easily integrated with traditional taxonomic investigations, thereby supporting taxonomic decisions and yielding insights into the ecology and evolution of the taxa. Analyses of the *P. macrocephalus* complex identified climatic niche shifts as possibly important in the speciation and radiation of *Ptilotus* in Australia.

In Part 3 of the thesis, the chronological and geographic origins of the aervoids and *Ptilotus* were tested by reconstructing a dated phylogeny for the Amaranthaceae-Chenopodiaceae alliance. Climatic niche evolution was investigated by inferring the climatic origins of major clades and subsequent niche shifts by reconstructing ancestral states of climatic variables on the aervoid phylogeny, visualised using a phyloecospace approach. The study revealed that the aervoids are likely to have originated in Oligocene

Africa-Asia and *Ptilotus* arrived in northern Australia via dispersal in the Early Miocene. The diversification of *Ptilotus* was rapid, with all major clades arising in the western Eremaean Region by the time of further aridification in the Middle Miocene. Multiple and independent niche shifts were identified for species groups within clades of *Ptilotus* into both monsoonal northern and temperate southern Australia. The arrival of the ancestor of *Ptilotus*, pre-adapted to aridity and prior to the onset of severe aridification in Australia, enabled the lineage to have an ecological advantage and to fill niche spaces that were opening up throughout the aridifying continent, explaining why the aervoids are unusually hyper-diverse in arid and semi-arid Australia.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

AUTHORSHIP DECLARATION: CO-AUTHORED PUBLICATIONS

This thesis contains work that has been published, accepted for publication or prepared for publication. Below are the authorship declarations that are required as part of this thesis with the bibliographic details of these works, their locations within the thesis, contribution to the work and signatures of the co-authors.

Details of the work:
Hammer TA, Davis RW, Thiele KR (2017) *Wadithamnus*, a new monotypic genus in Amaranthaceae. *Phytotaxa* 295, 173–184. https://doi.org/10.11646/phytotaxa.295.2.5
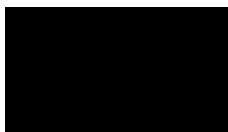
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Student contribution to work:
80%

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(Amaranthaceae): evidence from whole plastid genomes and morphology. *Taxon*.

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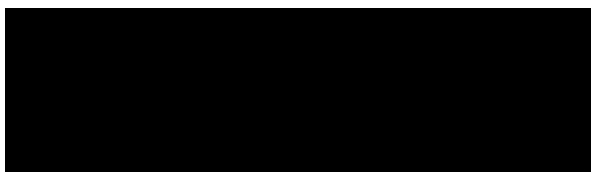
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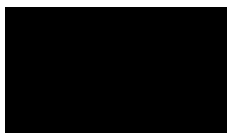
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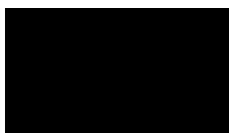
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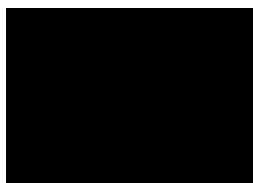
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I, Michael Renton, certify that the student statements regarding their contribution to each of the works listed above are correct.

Coordinating supervisor signature:

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THESIS OVERVIEW

This is a 'thesis by publication' written in accordance with the guidelines of the Graduate Research School, The University of Western Australia.

Thesis Layout

Chapters 1 and 7 are presented as a general introduction and general discussion, respectively, and were not written for publication. The body of this thesis comprised of three parts (Parts 1–3), which consist of a total of five chapters. Chapters 2–6 are written as stand-alone publications with a relevant introduction for the study undertaken. Because of the nature of writing multiple publications on the same group of organisms, there is a bit of overlap in the material introduced for each chapter and with the general introduction.

Chapter 1: General Introduction

PART 1

Chapter 2: *Wadithamnus*, a new monotypic genus in Amaranthaceae
- Chapter 2 was published in the journal *Phytotaxa* on 8 February 2017.

Chapter 3: Resolving the intergeneric relationships of the aervoid clade and the backbone of *Ptilotus* (Amaranthaceae): evidence from whole plastid genomes and morphology
- The manuscript was peer-reviewed and accepted for publication in the journal *Taxon* on 5 February 2019.

PART 2

Chapter 4: The noble and the exalted: A multidisciplinary approach to resolving a taxonomic controversy within *Ptilotus* (Amaranthaceae)
- Chapter 4 was published in *Australian Systematic Botany* on 17 July 2018.

Chapter 5: Of a different feather: two new species of featherheads from *Ptilotus macrocephalus* (Amaranthaceae) complex
- Chapter 5 was published in *Australian Systematic Botany* on 8 May 2019.

PART 3

Chapter 6: Out of the arid: origin of the genus *Ptilotus* (Amaranthaceae) and evidence for multiple radiations into seasonally wet Australia
- Chapter 6 is written for publication but has yet to be submitted to a journal.

Chapter 7: General Discussion

Explanation of the Text

Though some chapters have been published, all chapters are provided in the thesis in a manuscript format for visual consistency. The chapters have retained the citation style and of the journal they were published in. A link (DOI) to the online published version is provided at the beginning of each manuscript. Supplemental materials for each chapter are available at https://www.taxonomytim.com/thesis. Acknowledgements and literature cited are provided at the end of each chapter for the respective manuscript.

Nomenclatural authorities are provided after the first mention in the text of each chapter for all scientific names (written in italics), provided as the International Plant Names Index standard abbreviation—the only exception being Chapter 2, which follows the style of *Phytotaxa*. All herbarium codes follow Index Herbariorum.

All Chapters of the thesis, with the exception Chapters 2 and 3, have the formatting and citation style of *Australian Systematic Botany*. The text and citation styles of Chapters 2 and 3 are formatted consistent with the journals in which they were published—*Phytotaxa* and *Taxon*, respectively. Appendices 3.1–3.3 for Chapter 3 are at the end of the chapter. The text is written with Australian English spelling throughout.

Appendices

Appendices A and B are presented at the end of the thesis. These appendices show relevant research on *Ptilotus* completed during the course of my PhD candidature. They are **not** presented for examination. Appendix A includes a manuscript written for publication on the physiology of phosphorus hyperaccumulation within *Ptilotus*. Appendix B includes seven publications that I authored during my PhD candidature as part of the endeavour to resolve the taxonomic issues within *Ptilotus*, describe newly discovered species, and complete a comprehensive identification key to the species of *Ptilotus* in Western Australia.

GENERAL INTRODUCTION

Thesis justification

The flora of Australia has fascinated botanists since European exploration of the continent (George 1999, Benson and Eldershaw 2007) and has shaped the identity of modern Australia and how Australians relate to their environment (Smith 2011, Ryan 2013). How this distinctive flora assembled over evolutionary time has been of much research interest (e.g. Crisp *et al.* 2004, Byrne *et al.* 2008, Byrne *et al.* 2011, Crisp and Cook 2013, Crayn *et al.* 2015).

Many Australian plant lineages evolved in relative isolation following the breakup of Gondwana at c. 40–30 Ma and the continent's subsequent northward drift (Crisp *et al.* 2004, Hopper and Gioia 2004, Byrne *et al.* 2011, Crisp and Cook 2013). Other Australian plant lineages have arrived more recently (c. 32–25 Ma) from Asia, as Australia moved within dispersal range of many taxa, which allowed for the exchange of flora between the continents (Crayn *et al.* 2015). Both Gondwanan and immigrant plant lineages would have had to adapt to a continent undergoing severe climatic changes. The punctuated aridification of Australia commenced at the Eocene-Oligocene boundary (c. 32 Ma), which was correlated with a change in ocean currents initiated by the separation of Australia and Antarctica (Martin 2006). The aridification began to intensify in the Middle Miocene (Lawver and Gahagan 2003, Martin 2006). Today, two-thirds of Australia is arid or semi-arid, with the infertile landscape dominated by open, xeric grasslands, shrublands and woodlands (Stafford Smith and Morton 1990, Martin 2006). For those lineages with high species diversity in arid Australia, the aridification of the continent would have been an important factor driving their evolutionary history (Byrne *et al.* 2008).

One such diverse arid group of plants is the genus *Ptilotus* (Amaranthaceae), commonly known as 'mulla mullas', which has over 120 species that are endemic to Australia (one species also occurs in the southern islands of Indonesia). Most diversity within the genus is in arid and semi-arid Australia, particularly in Western Australia. *Ptilotus* accounts for approximately one-eighth of the species diversity within Amaranthaceae *s.str.* (i.e. excluding Chenopodiaceae Vent.; Hernández-Ledesma *et al.* 2015) and is the most species-rich member of the family in Australia, followed by *Gomphrena* L. (c. 33 species; Palmer 1998).

Ptilotus has been placed in the informally-named 'aervoid clade', with *Aerva* Forssk., *Kelita* A.R.Bean, *Nothosaerva* Wight and *Omegandra* G.J.Leach & C.C.Towns., following a family-wide phylogenetic study by Müller and Borsch (2005). *Aerva* (c. 12 spp.) and *Nothosaerva* (1 sp.) are widely dispersed across Africa and southern Asia, while the monotypic *Kelita* and *Omegandra* are endemic to eastern and northern Australia, respectively. *Ptilotus* is the only genus of the aervoids with species native to arid Australia. It has considerably more taxonomic and morphological diversity than any other genus in the family in Australia. There is no resolved phylogeny of the aervoids, and it is not known how *Ptilotus* relates to the other genera. Much work has been done in recent years to resolve other large sections of Amaranthaceae, e.g. the gomphrenoids (Sánchez-del Pino *et al.* 2009), but the relationships of the aervoid genera are poorly understood.

Resolution of phylogenetic and taxonomic relationships among the aervoid genera and recently divergent species groups within *Ptilotus* will enable rigorous hypothesis testing of the evolution of the clade and the role of ecological drivers, such as aridity, in the diversification of *Ptilotus* within Australia.

Introduction to morphology

Aerva was included within Amaranthaceae by Jussieu (1789: 88) in the original circumscription of the family, and all other aervoid genera have been confidently placed within the family based on morphology. The aervoids are shrubs or annual or perennial herbs with simple leaves, which are usually alternate but may be opposite or subopposite in a few species. Stems and leaves of plants can vary from glabrous to covered in a densely tomentose or woolly indumentum. The flowering unit is a single flower with two subtending scarious bracteoles (see Acosta *et al.* 2009). This may be a reduction of a cymose florescence with three or more flowers that is present in some genera of Amaranthaceae (see Chapter 2). In the aervoids, the flower and the bracteoles are subtended by a single scarious bract and are arranged along the axis of a spiciform or paniculate (in some *Aerva*) inflorescence, which are placed in the leaf axils or terminating stems. The size, shape, colour and indumentum of the bract and bracteoles are important diagnostic characters for some *Ptilotus* species.

The perianth of Amaranthaceae comprises a single whorl with segments that have been referred to in the past as 'sepals' (or calyx), 'perianth segments' or 'tepals'. The papery perianth of *Ptilotus* is petaloid in appearance in some species, being colourful and attractive (Fig. 1). Early observations of the Amaranthaceae and related families considered these perianth segments to be sepals (e.g. Jussieu 1789, Poiret 1817, Sprengel 1825, Payer 1857, Mueller 1868). Not convinced that the perianth whorl in Amaranthaceae was derived from the calyx, or preferring a more neutral term, other authors that have referred to them as 'perianth segments' (e.g. Brown, 1810, Bentham 1870, Black 1948, Townsend 1985, Benl 1986) or 'tepals' (e.g. Benl 1971, Miller 1993, Townsend 1993). Studies on the evolution and ontogeny of the perianth within the Caryophyllales (i.e. Amaranthaceae and related families) have determined that there has been a reduction from two whorls to one by the loss of the corolla (e.g. Ronse De Craene 2013, Ronse De Craene and Brockington 2013). Early work conducted for this thesis (e.g. Chapter 2) followed other Australian authors in referring to the perianth segments as 'tepals' (e.g. Benl 1994, Palmer 1998, Bean 2008, Lally 2008, Davis *et al.* 2014), but more recently completed work for this thesis followed Vrijdaghs *et al.* (2014) and Borsch *et al.* (2018), who have persuasively argued for a change back to 'sepals'.

The calyx of most aervoids is pentamerous (tetramerous in *Nothosaerva* and *Omegandra*) and has three inner and two outer sepals (two each in *Nothosaerva* and *Omegandra*), with the outer sepals enclosing the inner sepals in bud. The inner and outer sepals may be nearly identical or conspicuously different in morphology. In many species, the outer and inner surfaces of the sepals are covered in hairs, which are multicellular and may be unbranched ('nodose' or 'septate') or have whorls of branches at the septa ('verticillate') (Hammer *et al.* 2015). Hairs on the outer surface, especially within *Ptilotus* can be long and spreading, and in some species are longer than the sepal apex (e.g. Fig. 1A). The sepal inner surface can be glabrous or mostly glabrous (e.g. Fig. 1B), with some scattered straight hairs or have a basal tuft of densely woolly hairs (Fig. 1A, E). Indumentum type and hair type and position on the sepal surface are often diagnostic for *Ptilotus* species. The sepals can be white, green, yellow, red or various shades of pink (Fig. 1).
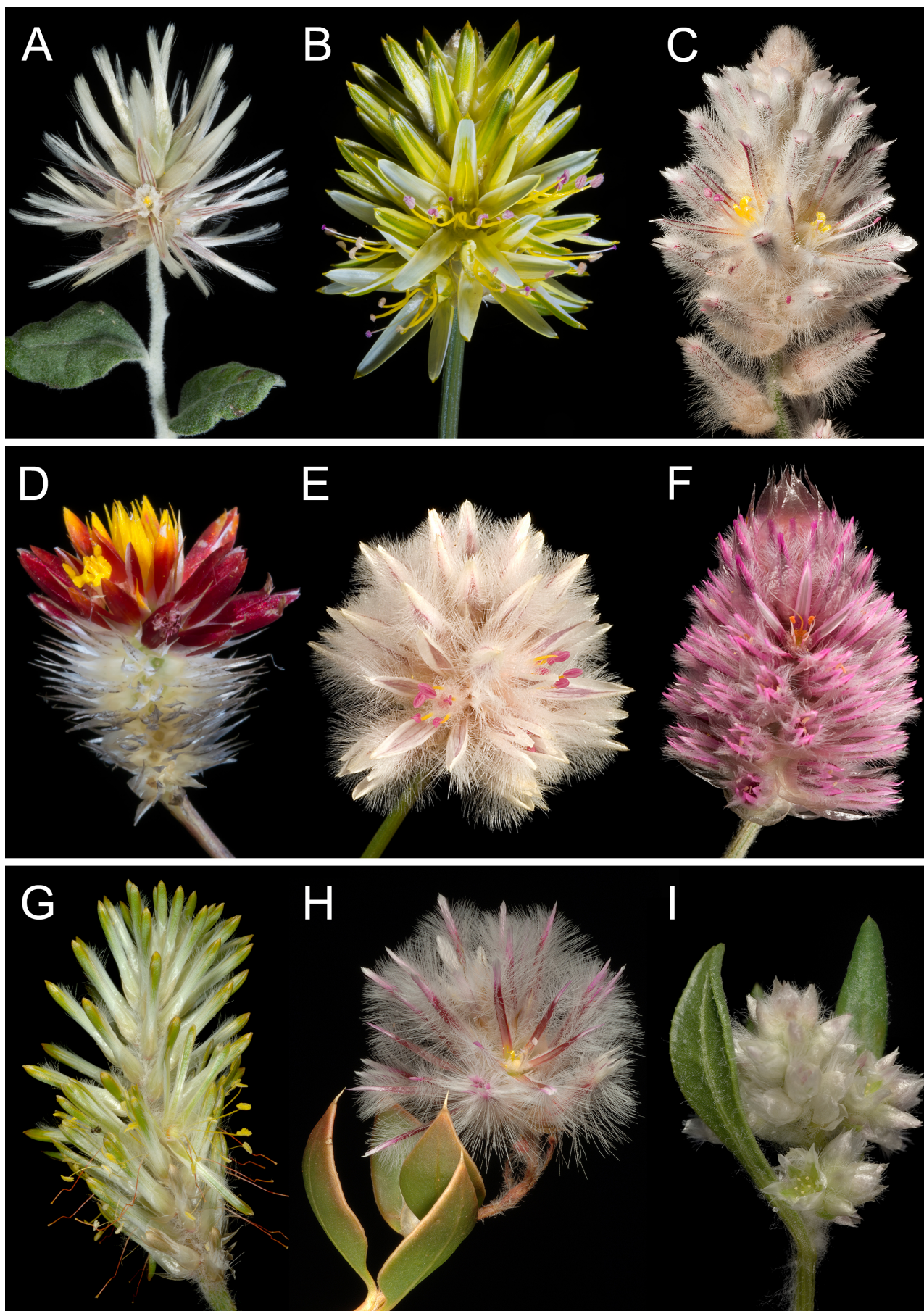
Fig. 1. Floral diversity within *Ptilotus*: **A.** *P. eriotrichus* (*T.Hammer* et al. *TH 79*), **B.** *P. gaudichaudii* (*T.Hammer* et al. *TH 83*), **C.** *P. appendiculatus* (*K.R.Thiele 4596*), **D.** *P. conicus* (*K.R.Thiele 5482*), **E.** *P. schwartzii* (*K.R.Thiele 3396*), **F.** *P. helipteroides* (*K.R.Thiele 3505*), **G.** *P. polystachyus* (*K.R.Thiele 3651*), **H.** *P. axillaris* (*K.R.Thiele 3622*), **I.** *P. villosiflorus* (*K.R.Thiele 3564*). Voucher specimens are indicated in parenthesis and housed at PERTH. Photographs by Kevin Thiele.

The androecium in aervoids typically consists of five stamens that rest on a thin, membranous staminal cup and are placed opposite the sepals (e.g. Fig. 1E, I). The length of the staminal cup is variable, being very short in some species to long and tube-like (e.g. up to c. 11 mm long in *P. auriculifolius* (Moq.) F.Muell.). Species of *Aerva* and several species of *Ptilotus* have small appendages or lobes on the staminal cup (referred to as 'pseudostaminodes' by some authors). In many species of *Ptilotus*, there is a reduction in fertile stamen number, from 4 to 1 (see discussion in Hammer *et al.* 2015), with fertile stamens variably replaced by conspicuous or inconspicuous, filamentous or flattened staminodes. Fertile stamen number is very consistent within species, with the exception of *Ptilotus manglesii*, which has 3–5 fertile stamens. Several species of *Ptilotus* are gynodioecious (e.g. *P. obovatus* (Gaudich.) F.Muell.; Stewart and Barlow 1976), a dimorphic sexual system occurring in fewer that 1% of plant species in which individuals are either hermaphrodite or female (male-sterile) (Godin and Demyanova 2013). A few species of *Aerva* (e.g. *A. javanica* (Burm.f.) Juss. ex Schult.) and one species of *Ptilotus* (i.e. *P. crispus* Benl) are dioecious, with female plants lacking a developed androecium and male plants lacking a developed style and stigma.

The gynoecium of aervoid species consists of a single superior ovary with a pendulous ovule. The style is straight and centred on the ovary apex in *Aerva* and many species of *Ptilotus*, but in some species of *Ptilotus* the style is curved or sigmoidal and excentrically fixed to the ovary apex (see Hammer *et al.* 2015). The ovary and style can be glabrous or have hairs. The stigma can be bilobed (e.g. *Aerva*) or unlobed (e.g. *Ptilotus*). The fruit is typically an indehiscent utricle with a membranous pericarp that contains a single seed and remains within the persistent calyx, which seems to influence seed dormancy in some species (Williams *et al.* 1989). Several species of *Ptilotus* (e.g. *P. chamaecladus* Diels) have a fruit that hardens considerably, possibly being a further mechanism of seed dormancy (see Appendix B6). The fruit within its persistent, papery and often hairy, calyx form a propagule that is likely to be wind dispersed (Hammer *et al.* 2015).

Taxonomic history of *Aerva*

On an expedition to *Arabia felix* (i.e. Yemen) as part of an 18th century Danish initiative to study the Middle East, the naturalist and student of Linnaeus, Peter Forsskål was tasked with collecting plants, animals and minerals and recording their local Arabic names (Provençal 2010: 5). In *Flora Aegyptiaco-Arabica*, he described the plants he encountered on the journey through Egypt and to Arabia, including the new genus *Aerva*, erected for the species *A. tomentosa* Forssk. (Forsskål 1775: 171). Forsskål named the genus from the Yemeni Arabic name اروا, which he Latinised to 'Ærua' (properly transliterated as 'Arwā; Provençal 2010: 20). As the letter 'v' is used in place of 'u' in classical Latin, the name was printed 'ÆRVA' in the protologue, and many subsequent authors have followed this spelling. The genus *Arthraerua* (Kuntze) Schinz is an etymological descendent of the name and has preserved the originally intended name 'Aerua'. Some early publications (e.g. Jussieu 1789, 1803) used '*Aerua*', but this spelling is largely superseded.

Some species of *Aerva* have complex nomenclatural histories, in part due to relatively unusual morphologies leading to the tenuous placement of species within genera, and in part due to morphological variability leading to the description of the same species more than once. Decades before Forsskål described *Aerva tomentosa*, Linnaeus included it in *Species Plantarum* as *Celosia lanata* L. (Linnaeus 1753: 205). Burman also named it, as both *Iresine javanica* Burm.f. and *Iresine persica* Burm.f. (Burman 1768). The former name was later recombined, as *Illecebrum javanicum* (Burm.f.) L. by Linnaeus (1774) and as *Achyranthes javanica* (Burm.f.) Pers. by Persoon (1805). Two more of Linnaeus's species, *Achyranthes lanata* L. and *A. sanguinolenta* L. (Linnaeus 1753: 204, 1762: 294), were also later recombined, as *Illecebrum lanatum* (L.) L. and *I. sanguinolentum* (L.) L. (Linnaeus 1771: 344). *Illecebrum*

javanicum and *I. lanatum* were recognised by Jussieu (1803: 131) as belonging to the genus *Aerva*, but were only later formally combined within *Aerva*, as *A. javanica* and *A. lanata* (L.) Juss. ex Schult. respectively, by Schultes (1819: 564–565). *Aerva lanata* was also collected and described by Forsskål, but he included it as *Achyranthes villosa* Forssk. and overlooked its similarity to *Aerva tomentosa* (Forsskål 1775: 48). *Illecebrum sanguinolentum* was later combined as *Aerva sanguinolenta* (L.) Blume by Blume (1825: 547). During the latter half of the 19ᵗʰ century, a concept for *Aerva* began to emerge with more species being added to the genus, including *A. microphylla* Moq. and *A. leucura* Moq. (Moquin-Tandon 1849: 301–302), *A. congesta* Balf.f. ex Baker (Baker 1877: 267) and *A. glabrata* Hook.f. (Hooker 1885: 728).

Adding to the complexity, the genus *Ouret* Adans. was described shortly after Linnaeus's *Species Plantarum*, based on Linnaeus's *Achyranthes lanata* and *Celosia lanata* (Adanson 1763: 268, 586). The name *Ouret* was left unused for a century until Kuntze revived it with the Latinised spelling *Uretia* in his controversial 1891 publication *Revisio generum plantarum* (Kuntze 1891). Perhaps thinking that *Ouret* (as '*Uretia*') had priority due to being erected 12 years prior to *Aerva*, Kuntze made new combinations for all known *Aerva* species under *Uretia*, including *U. lanata* (L.) Kuntze, *U. persica* (Burm.f.) Kuntze and *U. sanguinolenta* (L.) Kuntze. The unprecedented number of nomenclatural changes in Kuntze's *Revisio* (1,074 replacement genera and c. 30,000 new combinations; see Nicolson 1991) led to a swift response from the botanical community in a proposal for the conservation of numerous names he had synonymised (Ascherson 1892), including the conservation of *Aerva* over *Ouret* ('*Uretia*') (Ascherson 1892: 333). Prior to a ruling on this proposal, Hiern (1900: 883) recognised three species of *Ouret* in his publication on the flora of Africa, citing the '*Uretia*' names of Kuntze as synonyms. The conservation of *Aerva* over *Ouret* was proposed a second time by Harms (1904: 17) before being ratified the next year by the Second International Botanical Congress in Vienna, Austria (Briquet 1906: 241).

Further species continued to be added to *Aerva*, including species endemic in Madagascar (e.g. *A. coriacea* Schinz and *A. triangularifolia* Cavaco; Schinz 1931, Cavaco 1952) and southern Arabia (*A. artemisioides* Vierh. & O.Schwartz; Schwartz 1939: 43). The taxonomy of *Aerva* from the mid-20ᵗʰ century to the present day has been mostly limited to the synonymisation of taxa (e.g. Townsend 1974) and flora treatments (e.g. Townsend 1985, Miller 1996). No comprehensive treatment of *Aerva* has been published, and much taxonomic and nomenclatural confusion continues within the genus.

The monotypic genera

Another of Linnaeus's species that challenged early authors was *Achyranthes brachiata* L. (Linnaeus 1767: 50). This was combined by Linnaeus into *Illecebrum* as *I. brachiatum* (L.) L. (Linnaeus 1771: 213), who also described *I. bengalense* L. (Linnaeus 1771: 213); both species are now regarded as conspecific. *Illecebrum bengalense* was combined into *Achyranthes* (as *A. bengalensis* (L.) Lam.) by Lamarck (1785: 549), while *I. brachiatum* was included in *Aerva* (as *A. brachiata* (L.) Mart.) by Martius (1825: 83). The species was recognised as distinct by Wight, who first erected the genus *Pseudanthus* Wight (Wight 1852: 3) for it, subsequently erecting *Nothosaerva* (Wight 1853: 1) when he realised that *Pseudanthus* was illegitimate due to the earlier homonym *Pseudanthus* Sieber ex Spreng. *Nothosaerva* is derived from the Greek *nóthos* (νόθος, 'false') and the genus name *Aerva*. Among other characters, Wight (1852) described the species as having four sepals, two stamens and a short style with an unlobed stigma, easily differentiating it from the known species of *Aerva*.

Two recently named genera from Australia have been assumed to be related to *Aerva*, *Nothosaerva* and *Ptilotus*, based on their morphological similarity. In 1993, specimens of an unusual species collected in near-coastal Northern Territory and Queensland were sent to German botanist, and *Ptilotus* expert,

Gerhard Benl to verify that it was not a species of *Ptilotus*. Benl confirmed this, identified the specimens as possibly *Aerva* and confirmed that they were related to other aervoid genera. A combination of characters precluded its membership in any known genus of Amaranthaceae, leading to the description of the monotypic *Omegandra* (Leach *et al.* 1993). *Omegandra* was so named for the resemblance of its staminal cup to the Greek letter omega (Ω), differing in this from all other aervoid genera (in which the staminal cup fully encircles the ovary). Leach *et al.* (1993) interpreted the androecium to comprise five stamens (only two of which are fertile), and the perianth to comprise four sepals (five in most other aervoids). While not discussed by Leach *et al.* (1993), *Nothosaerva* also has two fertile stamens and four sepals.

A second Australian monotypic genus, *Kelita* Bean, was described by Bean (2010) based on specimens from eastern Queensland. Bean justified separating *Kelita* from the other Australian genera (*Omegandra* and *Ptilotus*) on the basis of multiple characters including uncinate (hooked) sepal apices and a hard and indehiscent fruit.

Taxonomic history of *Ptilotus*

Ptilotus was described by Robert Brown (1810) based on two species, *P. conicus* R.Br. (Fig. 1D) and *P. corymbosus* R.Br. collected in the Gulf of Carpentaria by Brown onboard the *Investigator* as part of an expedition to survey the coast of Australia and nearby islands from 1802 to 1805. Also described by Brown (1810) was the genus *Trichinium* R.Br., which included six species. Three of these were collected on the same expedition as *Ptilotus*, while the others were collected on separate expeditions to Tasmania and the south-eastern and north-western coasts of Australia. Brown regarded that the two genera were close (noting in the protologue for *Ptilotus* "*Affinitate proximus Trichinio*"; Brown 1810: 415). Brown segregated the genera on the basis that *Trichinium* had an indumentum of long, spreading hairs on the outer surfaces of the calyx that was absent in *Ptilotus*.

The first taxonomic revision of *Ptilotus* and *Trichinium* was by Poiret (1816: 619), who united the two as separate sections of the genus *Ptilotus*, citing the remark by Brown about their close similarity. Poiret (1816) went on to elucidate the differences of sect. *Trichinium* and sect. *Ptilotus*, principally the shape, size and indumentum of the calyx. He noted that species from sect. *Ptilotus* had a glabrous perianth except for basal hairs, while species from sect. *Trichinium* were much hairier. It is unclear why Poiret chose to unite the two genera under the name *Ptilotus* rather than *Trichinium*: both genera were published together (hence neither has priority), but he chose to include the larger genus within the smaller one. This was reversed by Sprengel (1825: 816–817), who included the two species of *Ptilotus* under *Trichinium*. Neither Poiret (1816) nor Sprengel (1825) included any new species in their treatments. Species were added to both *Trichinium*, e.g. *T. obovatum* Gaudich. and *T. polystachyum* Gaudich. by Gaudichaud (1829: 445) and *T. nobile* Lindl. by Lindley (in Mitchell 1838: 22), and *Ptilotus*, e.g. *P. declinatus* Nees and *P. exaltatus* Nees by Nees von Esenbeck (1845) and *P. erubescens* Schltdl. by Schlechtendal (1847).

Differing opinions persisted as to whether *Trichinium* and *Ptilotus* should be treated separately or as one genus. Moquin-Tandon (1849) kept the genera separate in his revision of Amaranthaceae in de Candolle's *Prodromus Systematis Naturalis Regni Vegetabilis*, recognising 49 species in *Trichinium* and five species in *Ptilotus*. He included *P. conicus*, *P. corymbosus*, *P. lanatus* A.Cunn. ex Moq. and *P. exaltatus* under *Ptilotus*, based on their glabrous sepals (Moquin-Tandon 1849: 281–282). In the case of *P. exaltatus* the sepals are typically hairy, but this is not clear on the type material (*L.Preiss 1367* at LD). Ferdinand von Mueller treated them as separate genera in early volumes of *Fragmenta Phytographiæ Australiæ* (e.g. Mueller 1864: 89–90), but subsequently (Mueller 1868) followed Poiret (1816) in uniting

the genera under the name *Ptilotus*. Mueller also synonymised the monotypic genera *Goniotriche* Turcz. and *Hemisteirus* F.Muell. (see Appendix B3) within *Ptilotus*.

Bentham (1870), however, again separated the genera, including ten species in *Ptilotus* and 47 in *Trichinium*. He established the first infrageneric classification for *Trichinium*, recognising nine series, but confessing "[i]n the subdivision of the genus I have been unable to establish any natural well-characterised sections" (Bentham 1870: 217–218). He considered that some characters previously considered important, such as staminal cup lobes, divided species that were otherwise almost identical, basing his classification mostly on the nature and position of different kinds of hairs. With respect to the separation of *Ptilotus* and *Trichinium*, he commented that they may be better treated as sections under a single genus, with *Trichinium* the preferred name "as being that which belongs to four-fifths of the species, and entails therefore the least change, besides that it is the most familiar of the two from the number of species that have been cultivated or figured" (Bentham 1870: 217). He considered, however, that both names were etymologically contradictory and unsuitable for the unified genus.

Brown (1810) provided no etymology for either *Ptilotus* or *Trichinium*, with subsequent authors inferring contrasting derivations. Bentham (1870: 217), for example, believed that *Ptilotus* indicated "having featherless wings". Black (1924: 214) and Benl (1986: 321), by contrast, regarded that the name derived from the Greek "*ptilotos*" ("winged" or "feathered"), the latter adding "referring to the hairy flowers". Greek-English lexicons translate the noun *ptílon* (πτίλον) to "(soft) feather" or "down" and the adjective *ptilōtós* (πτιλωτός) to "feathered" or "winged", especially referring to soft, downy feathers (Liddel and Scott 1901: 1340). Bentham (1870), in apparently misinterpreting the meaning of *Ptilotus*, was perhaps trying to reconcile the name with Brown's protologue descriptions. The origin of the name *Trichinium* is somewhat less ambiguous. Black (1924) considered the genus name to derive from the Greek adjective *tríchinos* (τρίχῐνος) ("of hair"; Liddel and Scott 1901: 1581), while Bentham (1870: 217) derived it from the Greek neuter noun (second declension) *tríchinon* (τρίχινον) "a garment of hair" (Liddel and Scott 1901: 1581) with the diminutive suffix -*ion* (-ιον) in Greek (-*ium* in Latin; Stern 1973: 306).

The two genera were again combined (under *Ptilotus*) by Mueller (1882: 28–29), followed by Schinz (1893: 110) and Maiden and Betche (1916: 72–73). Black (1924) briefly separated *Trichinium* and *Ptilotus* in the first edition of his *Flora of South Australia* by Black (1924), only to reverse this and unite them again under *Ptilotus* for the second edition (Black 1948). He justified his choice of *Ptilotus* for the unified genus (following Poiret 1816) using Art. 56 of the *ICN* adopted by the Fifth International Botanical Congress, Cambridge in 1930: "If the names or epithets are of the same date, the author who unites the groups has the right of choosing one of them. The author who first adopts one of them, definitely treating another as a synonym or referring it to a subordinate group, must be followed" (Rendle 1934). No subsequent authors have recognised *Trichinium* as separate from *Ptilotus*.

In the latter half of the 20th century, the taxonomy of *Ptilotus* benefited significantly from the work of Benl, who summarised the histories and morphologies of accepted species, clarified nomenclature, described new species, wrote Flora treatments (including a draft treatment for the *Flora of Australia*), and provided an identification key for the 76 species (and numerous infraspecies) known to him (e.g. Benl 1958, 1970, 1971, 1974, 1979, 1980, 1986, 1988, 1994).

Benl (1990) included the monotypic genus *Dipteranthemum* F.Muell. ex Oliv. within *Ptilotus* as subgenus *Dipteranthemum* (with the sole species *Ptilotus crosslandii* (F.Muell. ex Oliv.) Benl). The name refers to the two elongated and wing-like outer sepals in this species. In the same publication he described *P.*

trichocephalus Benl, which he regarded as closely related and morphologically similar to *P. crosslandii*, but oddly did not also include this in subgenus *Dipteranthemum*.

Recent publications by Australian botanists have applied appropriate critical analyses to Benl's taxonomic concepts (e.g. Bean 2008, Albrecht and Lally 2010), resolved species complexes (e.g. Lally 2008, 2009) and described several new species (e.g. Davis 2004, 2007, 2009, Davis *et al.* 2014).

Early molecular phylogenies

With the advent and wide application of molecular phylogenetics, earlier taxonomic classifications based on morphology have been tested using independent molecular characters, and monophyletic groups have been used to delimit genera and other taxa. Early molecular phylogenies of Amaranthaceae (e.g. Kadereit *et al.* 2003, Müller and Borsch 2005, Sage *et al.* 2007) included samples of *Aerva*, *Nothosaerva* and *Ptilotus,* and resolved relationships with respect to major clades within the family. Müller and Borsch (2005) found that previous infra-familial classifications were inconsistent with their phylogeny (discussed in Chapter 3) and established an informal classification based on well-supported clades. They included *Aerva*, *Nothosaerva* and *Ptilotus* in a clade referred to as the 'aervoids'. Focused as they were on resolving broad-scale relationships within the family, these analyses included only one or a few species of many genera, meaning that many intergeneric relationships could not be properly tested.

The first phylogeny that sampled more broadly within the aervoids was a biogeographic study of *Aerva* by Thiv *et al.* (2006). This included sequences of nuclear and plastid markers for ten of the 12 species accepted in the genus, some (e.g. *A. lanata*) with multiple accessions. Two species of *Ptilotus* (*P. manglesii* (Lindl.) F.Muell. and *P. drummondii* (Moq.) F.Muell.), were included as outgroups. The multiple accessions of *A. lanata* did not resolve as monophyletic, but rather were recovered as polyphyletic and sister to other species in geographic proximity (e.g. *A. leucura* in Africa or *A. sanguinolenta* in Asia), indicating that morphological boundaries of these species may need to be revised. While Thiv *et al.* (2006) concluded that *Aerva* was monophyletic, their limited choice of outgroups precluded effectively testing the relationships of *Aerva* and *Ptilotus*. Sage *et al.* (2007) included three species of *Aerva*, *Nothosaerva brachiata* (L.) Wight and two species of *Ptilotus* in a phylogeny of Amaranthaceae and found that *Aerva* was paraphyletic with respect to *Ptilotus*, rendering the biogeographic and evolutionary conclusions of Thiv *et al.* (2006) doubtful.

Molecular studies within *Ptilotus*

Lee *et al.* (2007) used ITS nrDNA sequences to test species relationships of the green-flowered *P. nobilis* (Lindl.) F.Muell. and pink-flowered *P. exaltatus* in a phenetic (neighbour-joining) analysis. They sampled 14 species of *Ptilotus*, including multiple accessions of *P. nobilis* and *P. exaltatus*. While these two species formed a group on their phenetic tree (inaccurately referred to as a phylogeny), the samples were found to be intermixed within this cluster. Failing to find consistent morphological characters to separate the species, they concluded that they were merely different colour-forms of the same species. The two species were formally combined (under *P. nobilis*) by Bean (2008) citing Lee *et al.* (2007).

The first detailed molecular phylogeny of *Ptilotus* was reconstructed as part of my MSc research (Hammer *et al.* 2015). ITS nrDNA and *matK* cpDNA markers of 87 *Ptilotus* species were newly sequenced, covering c. 70% of the known species and most of the morphological diversity in the genus. Seven outgroup taxa were used, including three species of *Aerva* (representing the aervoid clade) and *Amaranthus caudatus* L., *Achyranthes aspera* L. and two Australian species of *Gomphrena* (representing the amaranthoid, achyranthoid and gomphrenoid clades of Amaranthaceae, respectively). This analysis

provided a rigorous test of the monophyly of *Ptilotus*, allowed an assessment of relationships between species originally described in *Trichinium* with those originally described in *Ptilotus*, and provided an assessment of the monophyly of the subgenera of Benl (1990). Four main clades were resolved within *Ptilotus* (informally named clades A–D). The last and largest of these (clade D), with 63 species, was unresolved, with four subclades (D1–D4) placed in a polytomy with *Ptilotus declinatus* Nees. Clades A and C were the smallest with three species each, and Clade B was represented by 18 species. Though all of Brown's original species of *Ptilotus* and *Trichinium* fell within Clades B and D, respectively, the expanded circumscription of these genera by Bentham (1870) was incongruent with the phylogeny, with species of each genus falling into the other clade. Bentham's series of *Trichinium*, and *P.* subg. *Ptilotus* of Benl (1990), were found to be paraphyletic; *P.* subg. *Dipteranthemum* was found to be nested deeply within Subclade D4.

Though no morphological synapomorphies for the clades within *Ptilotus* could be found, four floral characters (branched sepal hairs, styles excentrically place on the ovary apex, curved or sigmoidal styles, and fewer than five fertile stamens) were identified as occurring exclusively within the larger Clade D. While not all species in Clade D had all or any of these character states, all species sampled for the phylogeny within Clades A–C were found to have unbranched sepal hairs, straight styles that were centred on the ovary apex, and five fertile stamens. Hammer *et al.* (2015) indicated that the innovations in floral morphology seen in Clade D may have contributed to its relatively high species diversity and the ecological success of some of its species, which include the most widespread and abundant members of the genus.

The sister of *Ptilotus* has been resolved on the previous phylogenies of Amaranthaceae *s.str.* as the African and southern Asian genus *Aerva*, raising important questions about the historical biogeography of the clade, and especially why it is that the Australian genus is so diverse in arid Australia. A time-calibrated and comprehensive phylogeny of the aervoids is needed to investigate the geographic and chronological origins in this group. A study of the evolution of climatic niches within the aervoids will indicate the environmental origin and subsequent diversification within major clades of the aervoids and *Ptilotus* and reveal if environmental adaptations may have been important in driving the diversification of the lineage.

Phosphorus physiology within *Ptilotus*

In addition to studies on its systematics and taxonomy, *Ptilotus* has been the subject of previous studies on phosphorus physiology (e.g. Islam *et al.* 1999, Ryan *et al.* 2009), which have indicated a presumably novel ability of the species tested to both grow well in low phosphorus (P) soils and to hyperaccumulate P when levels are high. In glasshouse trials, *Ptilotus nobilis* and *P. polystachyus* (Gaudich.) F.Muell. were able to accumulate P to concentrations that are toxic in other plants, including co-occurring native species in other genera, without suffering from P toxicity (Islam *et al.* 1999, Ryan *et al.* 2009). This ability was posited to be a novel trait of *Ptilotus*. I carried out a study as part of my PhD to assess P-hyperaccumulation across multiple clades of *Ptilotus*, to see how widespread the ability is and to compare the response to other Australian amaranths and non-amaranths. This study found that P-hyperaccumulation and tolerance to high P concentration were widespread within *Ptilotus*. Other amaranths studied hyperaccumulate at high P but show effects of P toxicity at the levels tested. Other aervoids outside *Ptilotus* could not be tested for this study, so the extent of combined hyperaccumulation and tolerance to high P concentrations could not be assessed outside the genus. The study did not become a central part of the story of this thesis on the evolutionary success of *Ptilotus*; a paper on the physiology of phosphorus within *Ptilotus* is included as Appendix A, which is intended as a supplement to this thesis, rather than a chapter for examination.

Thesis aim and objectives

The principal aim of this thesis is to advance knowledge of the evolution of the diverse, yet under-studied, genus *Ptilotus* by testing previous taxonomic hypotheses within the aervoids, resolving phylogenetic relationships between major clades in the group, and investigating what may have driven the diversity in arid and semi-arid Australia.

Part 1. Previous molecular studies that included the aervoids demonstrate the need for phylogenetic clarification of relationships with and among *Aerva*, *Nothosaerva* and *Ptilotus* in order to inform a stable generic taxonomy based on monophyletic groups that accurately reflect evolutionary relationships. These studies (e.g. Sage *et al.* 2007) suggest that *Aerva* may be paraphyletic with respect to the other genera. The Australian genera *Kelita* and *Omegandra*, which have been considered related to the aervoids based on morphology, have yet to be sequenced to test their relationships. Consequently, the first objective in Part 1 of this thesis is to resolve the aervoid phylogeny and to produce an updated taxonomy that is consistent with evolutionary relationships and supported by diagnosable morphological characters. Part 1 includes two published chapters that accomplish this objective. The first chapter elucidates the relationships of the aberrant species *A. artemisioides* to the other aervoids. The second chapter uses full chloroplast genomes and Sanger sequences with a near-comprehensive sampling to resolve the backbone of the aervoids and of *Ptilotus*, and establishes a new taxonomic framework for the aervoids. The resolved phylogeny is then used in Part 3 to investigate the historical biogeography and ecological drivers of diversification for the aervoids.

Part 2. The taxonomy of certain species groups within *Ptilotus* has been considered problematic by some authors due to unclear morphological boundaries, which in one case has led to the synonymisation of a widespread and iconic species (in the *P. nobilis* species group) and in another case has resulted in a failure to recognise species as distinct (in the *P. macrocephalus* species group). The second objective of the thesis is to revisit and critically re-evaluate these previous taxonomic decisions by clarifying the morphology of the species concerned, assessing the relative importance of commonly-used characters, and incorporating large-scale ecological analyses to support the revised taxonomy of these groups in an integrative taxonomic approach. The chapters of Part 2 are two published papers, which resolve these respective species complexes, and show how aggregated, georeferenced herbarium collections can be employed to rigorously test hypothesis of the ecological segregation of metapopulation lineages to support recognition of separate species. These studies also indicate that ecological factors (i.e. climate and soil) have played an important role in driving speciation in recently divergent lineages within *Ptilotus*.

Part 3. Understanding the evolution of the aervoids and *Ptilotus* offers insights into the evolution of Amaranthaceae *s.str.* and the assembly of the Australian arid flora. The third objective of the thesis is to reconstruct a dated phylogeny of the aervoid clade, thereby allowing estimation of ancestral areas correlated with time, to elucidate when and where the aervoids originated and to test hypotheses of dispersal of the Australian genera *Omegandra* and *Ptilotus* from Asia to Australia and the diversification of *Ptilotus* within Australia. In Part 3, I analyse climatic data for all aervoid species represented on the phylogeny, reconstruct the climatic evolution of the clade, and identify climatic niche shifts along the phylogeny. These analyses test the hypotheses that *Ptilotus* was pre-adapted for arid conditions and diversified within an aridifying Australia, shedding light on what has driven *Ptilotus* to become the most diverse aervoid genus, one of the most species-rich genera in the family and an important and diverse floristic component of arid Australia.

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PART 1

Establishing a New Generic Taxonomy for the Aervoids

Wadithamnus, a new monotypic genus in Amaranthaceae

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Abstract A new monotypic genus from near-coastal areas of Oman and Yemen is here described and named *Wadithamnus*. The generitype is *Wadithamnus artemisioides comb. nov.* (basionym *Aerva artemisioides*). On the basis of morphology, *W. artemisioides* can be separated from *Aerva* by its 3(–7)-flowered cymes and flowers with two outer and four inner tepals, and four stamens alternating with the inner tepals. Molecular data (nuclear ITS and chloroplast *trnK–matK* sequences) confirm *Wadithamnus* as a distinct genus outside *Aerva*, the latter marker placing it at a basal position to the achyranthoid clade. The name *Aerva artemisioides* is lectotypified on a specimen preserved at WU.

INTRODUCTION

Amaranthaceae Juss. *s.str.* (i.e., excluding Chenopodiaceae Vent.; Caryophyllales Berchtold & J.Presl) is a family of about 65 genera and 900 species (Robertson & Clemants 2003), which are distributed in tropical and subtropical regions with most of the species diversity in the Neotropics, eastern and southern Africa, and Australia (Hernández-Ledesma *et al.* 2015: 303). The classification of the family remains only partially resolved. While subfam. *Gomphrenoideae* Schinz has been revealed to be monophyletic (see Sánchez-Del Pino *et al.* 2009), subfam. *Amaranthoideae* is largely paraphyletic and includes clades that are not well-resolved in phylogenetic analyses (see Hernández-Ledesma *et al.* 2015 and literature therein). Four major clades have been resolved within subfam. *Amaranthoideae*, informally named as follows: "achyranthoid" [genus *Achyranthes* Linnaeus [(1753: 204); 8–15 species (see e.g., Iamonico 2014) and relatives], "aervoid" [*Aerva* Forsskål (1775: 170); 12 species (see e.g., Thiv *et al.* (2006) and relatives], "amaranthoid" [*Amaranthus* (1753: 989; about 70 species (see e.g., Iamonico 2015) and relatives], and "celosioid" [*Celosia* Linnaeus (1753: 205; about 45 species (see e.g., Iamonico & Jarvis 2012) and relatives; this clade represents the only natural one, formally tribe Celosioideae Lindl.]. In addition, subfam. *Polycnemoideae* Ulbr., which was previously included in Chenopodiaceae (4 genera and 13–17 species, see e.g., Hernández-Ledesma *et al.* 2015: 303, 305–307) was recently added to Amaranthaceae.

The "aervoid" clade includes the following taxa (Müller & Borsch 2005, Sage *et al.* 2007): *Aerva*, with 12 species, *Ptilotus* R.Brown (1810: 415), with about 110 species, and *Nothosaerva brachiata* (Linnaeus 1767: 50) Wight (1853: 1). A comprehensive phylogenetic analysis of the aervoids is not yet published, awaiting sequencing of two Australian monotypic genera—*Omegandra* Leach & Towns (1993: 787), and *Kelita* Bean (2010: 105)—which most likely belong within the aervoid clade and may prove invaluable in understanding the evolution of the group (see Leach *et al.* 1993, Bean 2010).

Thiv *et al.* (2006), in a biogeographic study of species endemic to the Socotran archipelago, produced a molecular phylogeny of *Aerva* recognizing 12 species, and including some endemics from Madagascar [*A. coriacea* Schinz (1931: 141), *A. humbertii* Cavaco (1952: 253), and *A. triangularifolia* Cavaco (1952: 253)], and from Socotra [*A. revoluta* Balfour filius (1884: 92), and *A. microphylla* Moquin-Tandon (1849: 301)], and a number of neotropical species [e.g., *A. javanica* (Burman filius 1768: 212) Jussieu (1808: 131), and *A. lanata* (Linnaeus 1753: 204) Jussieu ex Schultes (1819: 564)]. Two *Ptilotus* species were

used as outgroups. However, because of the limited outgroup sampling, this study was unable to test the monophyly of *Aerva*. More recent phylogenetic studies of *Ptilotus* (Hammer *et al.* 2015) and the Amaranthaceae (see Sage *et al.* 2007) provided strong evidence that *Aerva* is paraphyletic with respect to *Ptilotus*.

Aerva species are perennial herbs or subshrubs with unisexual or bisexual flowers which are borne solitary, are bracteate, and are arranged in terminal and axillary spike-like structures. Flowers are actinomorphic with five stamens opposite to five tepals and alternating with staminal cup appendages ("pseudostaminodes"). The stigma is bilobed on a short style, differing from that of *Ptilotus* species where it is capitate on a short to long style (Townsend 1993).
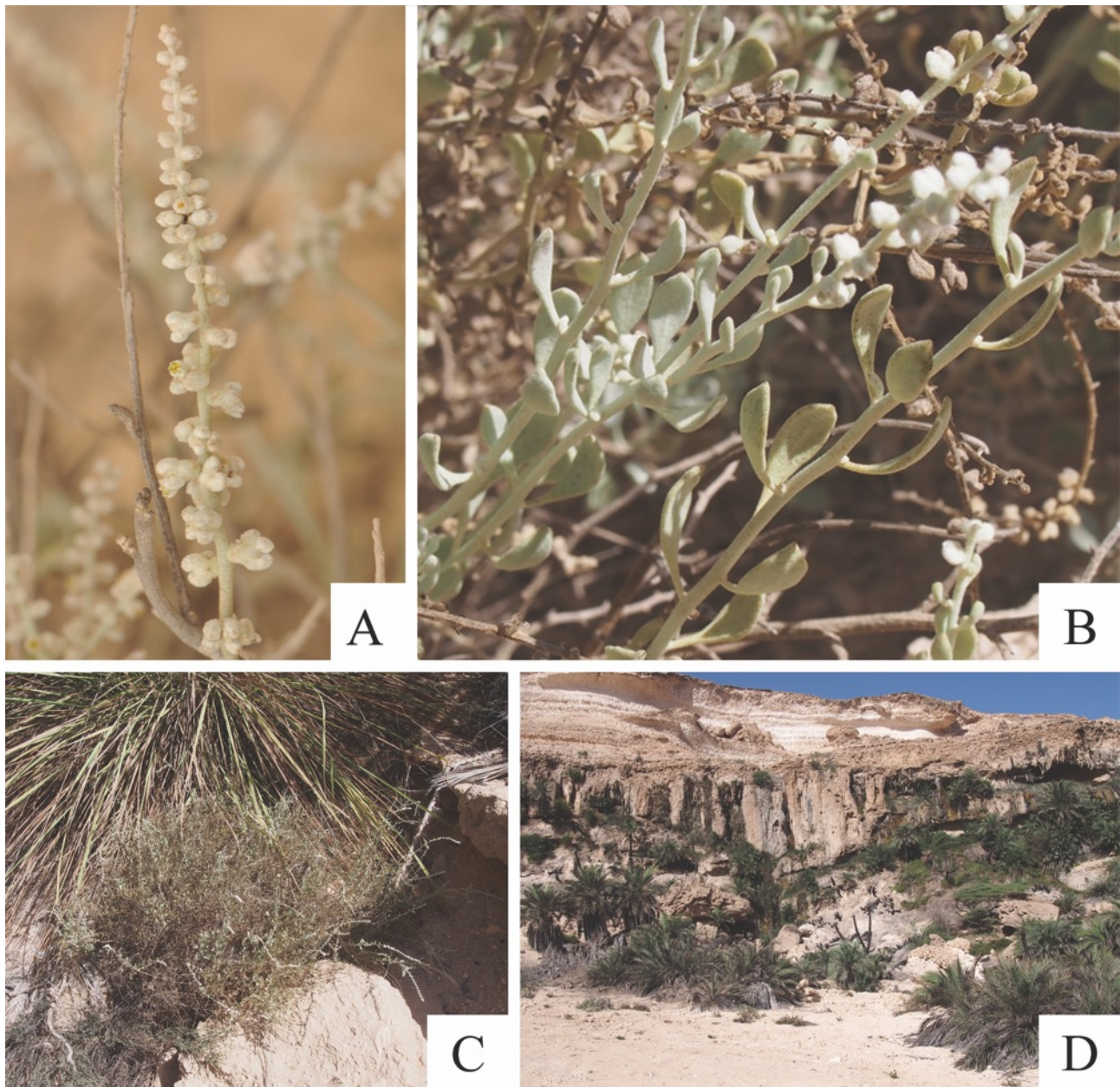
Fig. 1. *Aerva artemisioides* subsp. *batharitica*. **A.** Flowering inflorescence, **B.** Young stems and leaves, **C.** Shrubby habit, **D.** Habitat (photos from Wadi Al-Shuwaymiyah, Oman by Alena Vydrova and Vit Grulich).

Aerva artemisioides Vierhapper & Schwartz in Schwartz (1939: 43) is a species endemic to Yemen and Oman. It displays very unusual features in *Aerva*. Schwartz (1939), in the protologue, stated that *A. artemisioides* was close to *A. javanica*, but differs in having flowers arranged in 3(–7)-flowered

glomerules. He described the flowers as having the usual complement of five tepals and five stamens. Miller (1996), however, described the flowers with 6 tepals (2 outer and 4 inner) and four stamens. No other known genus in Amaranthaceae has 6 tepals (see e.g., Townsend 1993). The stigma of *A. artemisioides* is sessile and capitate, a combination that is unique within the aervoids. Two subspecies of *A. artemisioides* are recognized: the subsp. *batharitica* Miller & Nyberg (1994: 35) (coastal Oman) has elliptic to ovate leaves (Fig. 1), while the subsp. *artemisioides* (Yemen) has linear leaves (Fig. 2, Miller & Nyberg 1994).

In this paper we show that *Aerva artemisioides* has a non-aervoid morphology and is phylogenetically closer to the achyranthoids than the aervoids. As a consequence, we here propose *A. artemisioides* the new monotypic genus *Wadithamnus gen. nov.* (see the "Taxonomic treatment").

MATERIALS AND METHODS

Sampling

Sequences from 70 accessions of 43 species in 26 genera of Amaranthaceae *s.str.* were obtained from GenBank (Table 1) for inclusion in the molecular study, sampled to reflect diverse genera within the aervoids, gomphrenoids and achyranthoids. Relatively few species were available with both ITS and *trnK–matK* sequences; in some cases, different species were used to represent a genus for each of the two sequences.
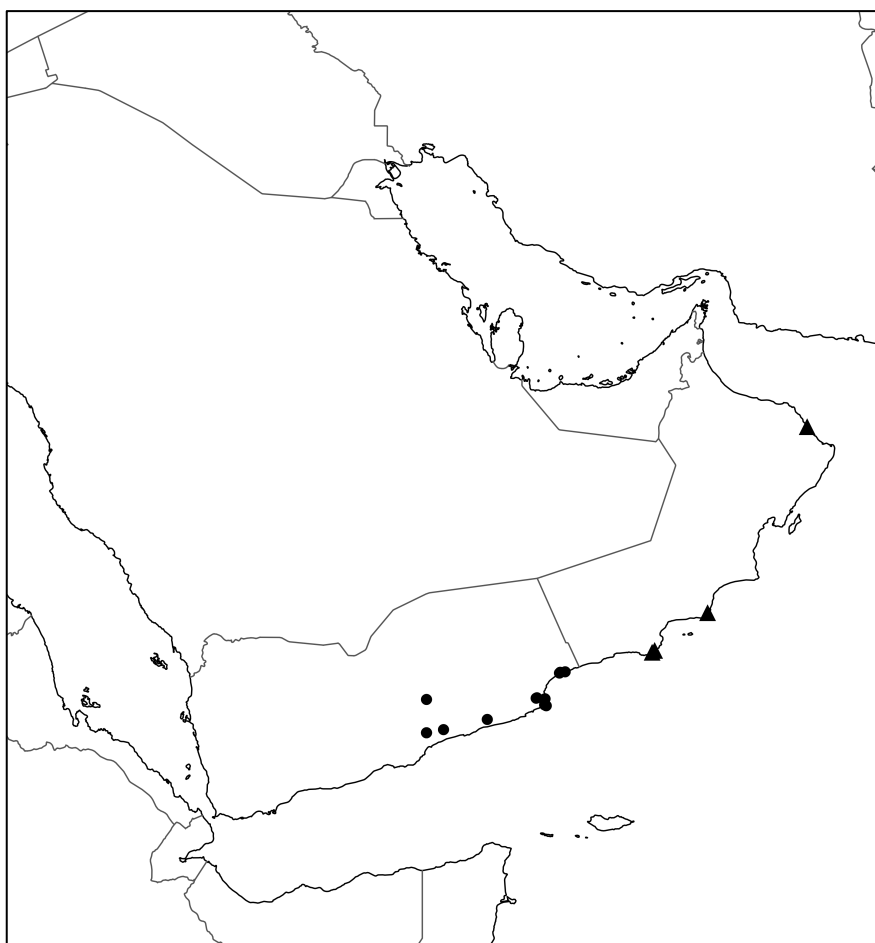
Figure 2. Distribution map of *Aerva artemisioides* specimens examined in this study for which there was GPS locality data. Circles show subsp. *artemsioides*, triangles show subsp. *batharitica*.

Phylogenetic analyses

Sequences were aligned using default settings of webPRANK multiple sequence aligner (Löytynoja & Goldman 2010). The alignment was inspected and manually adjusted as necessary in Geneious v.6.0 (Kearse *et al.* 2012). JModelTest v.2.1.4 was used to assess nucleotide substitution models for ITS and *trnK–matK* (Darriba *et al.* 2012).

Bayesian inference (BI) analyses were performed using MrBayes v.3.2.1 (Ronquist *et al.* 2012). Analyses were run for 15 million Markov chain Monte Carlo (MCMC) generations with trees sampled every 1000 generations. Run completion was determined by the average standard deviation of split frequencies falling below 0.01. Tracer v.1.6 was used to analyze the trace files generated by the Bayesian MCMC runs 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 v.2.01 (Zwickl 2006) with eight 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 *et al.* 2010). Summation of the bootstrap values was calculated using SumTrees v.3.3.1 in the DendroPy v.3.12.0 python package (Sukumaran & Holder 2010). All trees were rooted at *Amaranthus caudatus* Linnaeus (1753: 990).

Table 1 (1/2). Genbank Accessions used in the present study for the phylogenetic analysis.

Taxon Name	ITS (or ITS1, ITS2)	*trnK/matK*	Collector	Herbarium
Achyranthes aspera	--	AY514815	*Mueller 900*	BONN
Achyranthes aspera	KP875780	--	*Barrett 8308*	PERTH
Achyranthes bidentata	FJ572039	--	BFDA A-4-A01	--
Achyropsis leptostachya	--	AY998117	*Mueller 876*	BONN
Aerva artemisioides	AJ829504, AJ829477	AJ833649	*Kilian 6882*	B
Aerva congesta	AJ829504, AJ829477	AJ833650	Hansen s.n.	MAU
Aerva coriacea	AJ829506, AJ829506	AJ833651	*Phillipson & Rabes. 3141*	WAG
Aerva javanica	AJ829514, AJ829487	AJ833658	*Thiv 3190*	STU, Z
Aerva lanata	AJ829519, AJ829492	AJ833662	*Thiv 3118*	STU, Z
Aerva leucura	AJ829522, AJ829495	AJ833665	*Schneller 16*	Z
Aerva microphylla	AJ829524, AJ829497	AJ833666	*Thiv 3233*	STU, Z
Aerva revoluta	AJ829525, AJ829498	AJ833667	*Thiv 3179*	STU, Z
Aerva sanguinolenta	AJ829526, AJ829499	AJ833668	*Grierson & Long 4536*	E
Aerva triangularifolia	AJ829528, AJ829501	AJ833670	*Randrianaivo 362*	MO
Alternanthera sessilis	--	AY514796	Bot. Gard. Mainz s.n.	--
Alternanthera sessilis	GU724285	--	PS0652MT01	--
Amaranthus caudatus	--	AY514809	Bot. Gard. Mainz s.n.	--
Amaranthus caudatus	KC747415	--	*Ames 13860*	BARC
Arthraerua leubnitziae	--	AY998115	*Leuenberger & al. 3330*	PRE
Blutaparon vermiculare	--	AY514798	*Borsch & al. 3444*	ISC, BONN
Blutaparon vermiculare	AY174406	--	*McCauley 154*	BHO
Calicorema capitata	--	AY514807	*Neinhuis* s.n.	BONN
Calicorema squarrosa	--	AY998114	*Volk 12630*	M
Centemopsis micrantha	--	AY998105	*Gillet & Hemming 24874*	PRE
Cyathula achyranthoides	--	AY514862	*Mueller 865*	BONN
Cyathula officinalis	DQ497187	--	BFDA B-1-D04	--

Table 1 (2/2). Genbank Accessions used in the present study for the phylogenetic analysis.

Taxon Name	ITS (or ITS1, ITS2)	*trnK/matK*	Collector	Herbarium
Froelichia arizonica	AY174395	--	*McCauley 37*	BHO
Froelichia floridana	--	AY514799	*Thiel* s.n.	BONN
Gomphrena kanisii	KP875880	--	*Byrne 2773*	PERTH
Gomphrena pulchella	--	AY514802	*Zardini 45564*	BONN, MO
Guilleminea densa	--	AY514803	*Borsch & al. 3437*	ISC, BONN
Guilleminea densa	AY174411	--	*McCauley 167*	BHO
Hebanthe occidentalis	--	AY514821	*Zardini 45377*	BONN, MO
Kyphocarpa angustifolia	--	AY998111	*Mueller 860*	BONN, PRE
Mechowia grandiflora	--	AY998113	*Mendes dos Santos 1780*	PRE
Nothosaerva brachiata	--	AY514806	Anonymous s.n.	FR
Nototrichium divaricatum	AY174422	--	*McCauley* s.n.	BHO
Nototrichium sandwicense	--	AY514817	*Prigge 15250*	LA
Pandiaka angustifolia	--	AY514818	*Muller 324*	FR
Psilotrichum africanum	--	AY514822	*Muller 878*	BONN, PRE
Ptilotus manglesii	KP875940	--	*Davis & Jobson 12005*	PERTH
Ptilotus manglesii	--	AY514824	*Borsch 3543*	BONN
Ptilotus obovatus	--	AY514823	Bot. Gard. Bonn s.n.	BONN
Ptilotus obovatus	KP875947	--	*Davis & al.12268*	PERTH
Pupalia lappacea	--	AY514858	*Borsch 3544*	BONN
Sericorema sericea	--	AY998110	*Mueller 863*	BONN, PRE
Sericostachyus scandens	--	AY514819	*Fischer* s.n.	BONN
Tidestromia lanuginosa	--	AY514797	*Muller 3439*	ISC, BONN, MEXU
Tidestromia rhizomatosa	EU567672	--	*Olvera HF02-14*	MEXU

Morphological analysis

22 specimens of *A. artemisioides*, which are deposited at B and E, were examined and compared to specimens (deposited at BRI, E, PE and PERTH) representing all aervoid genera (herbaria acronyms according to Thiers 2016+). Flowers for dissection were rehydrated in hot water with a little detergent. The keys to the Amaranthaceae genera in Townsend (1993) were used to assess other genera that may be morphologically similar.

A scanning electron microscope (SEM) was used to study the indumentum of *A. artemisioides* and four other aervoid species, *A. javanica*, *A. lanata*, *Ptilotus mollis* Benl (1970: 4) and *P.* sp. Goldfields (*R. Davis 10796*), which represent the major clades of, respectively, *Aerva* and *Ptilotus*. Stem hairs were obtained from herbarium specimens, mounted on stubs using carbon tape, and coated with gold for two minutes using a Joel Smart Coater. A JEOL JCM-5000 NeoScope bench-top SEM at the Western Australian Herbarium (PERTH) was used to image individual hairs.

RESULTS AND DISCUSSION

The aligned ITS region comprised 718 base pairs (bp), and the aligned *trnK–matK* region comprised 2961 bp. A combined ITS and *trnK–matK* analysis was not run, because different taxa were used for each marker. Bayesian and Akaike information criteria (BIC and AIC) in jModelTest agreed that the best nucleotide substitution models were GTR + I + G for ITS and GTR + G for *trnK–matK*; these models were used in the MrBayes analyses. Both methods (BI and ML) gave largely congruent results; the BI 50% majority-rule consensus trees are shown in Figs. 3 and 4. Support values reported are Bayesian posterior probabilities and ML bootstrap support respectively (BI/ML).
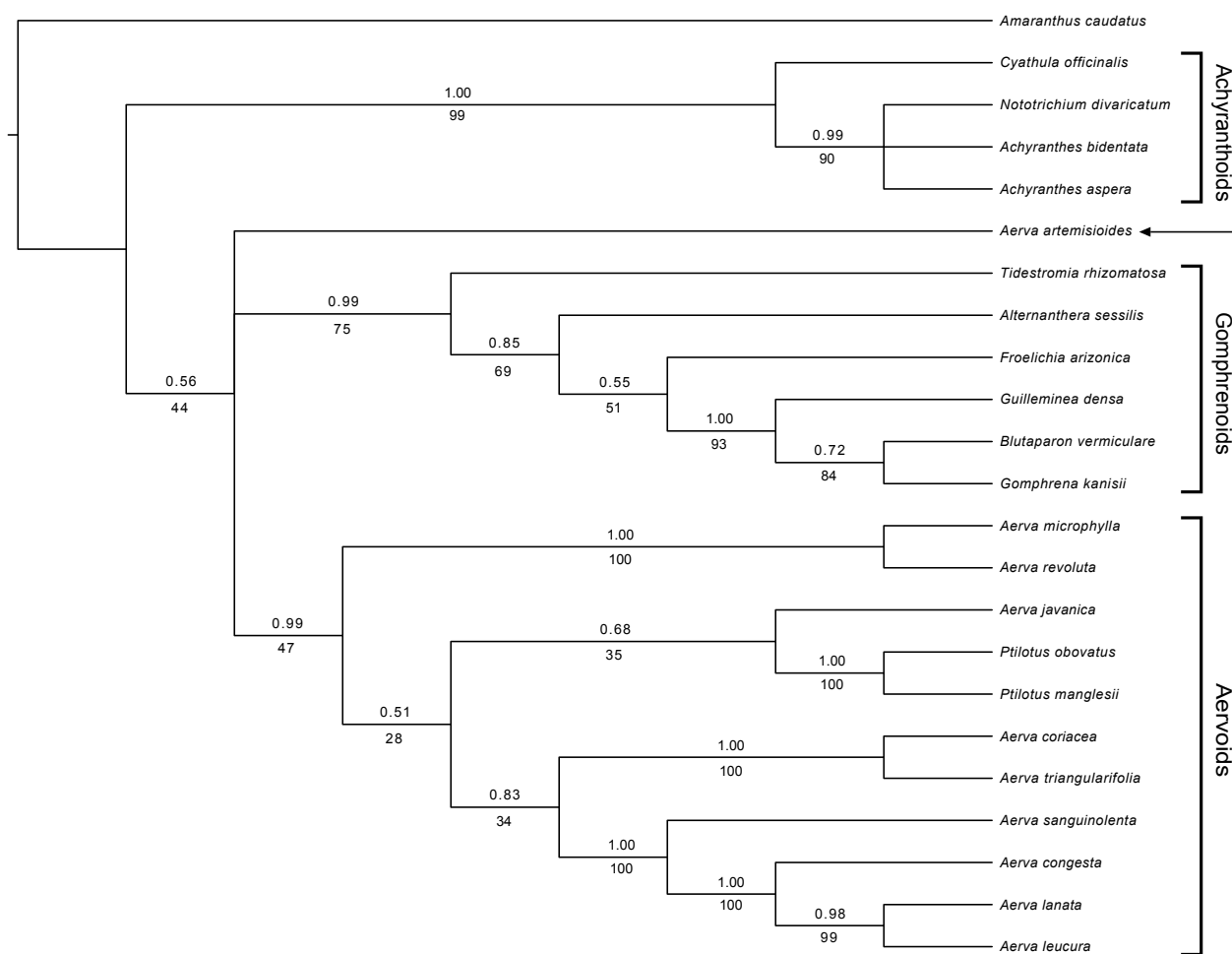
Fig. 3. ITS mrBayes 50% majority-rule consensus tree. Bayesian posterior probabilities and maximum likelihood bootstrap values are given above and below the branches, respectively. An arrow indicates the position of *Aerva artemisioides*; major clades within Amaranthaceae are labeled on the right.

Both of the reconstructed trees are broadly congruent with the family-level phylogenies of Müller & Borsch (2005) and Sage *et al.* (2007), placing the aervoids sister to the achyranthoids and gomphrenoids. These phylogenies had limited sampling of the aervoids and did not include *A. artemisioides*. The Australian genus *Ptilotus* is monophyletic (confirming Hammer *et al.* 2015), and nested within the African-Asian *Aerva. Nothosaerva brachiata* resolves in the chloroplast tree within the aervoids at a basal polytomy.

The ITS tree recovered the achyranthoids (1.00/99), gomphrenoids (0.99/75) and aervoids (0.99/47), all with high support in the Bayesian analysis. *Aerva artemisioides* was placed on an unsupported polytomy (0.56/44) at the base of the gomphrenoids+aervoids. The *trnk–matK* tree, which included more taxa, especially in the achyranthoid clade, was better resolved than the ITS tree, the two trees being otherwise congruent. A highly supported clade (1.00/91) comprises *A. artemisioides* plus the achyranthoids. The gomphrenoids likewise have good support (1.00/78) as monophyletic and are resolved as sister to the achyranthoids (1.00/76). The aervoids, without *A. artemisioides*, are resolved as monophyletic (0.99/76). Although node support within some parts of the achyranthoid clade is low, *A. artemisioides* did not group with any included genus. The high support in the *trnk–matK* tree for the aervoids without *A. artemisioides* and for the *A. artemisioides*+achyranthoid clade indicates that *A. artemisioides* does not belong in the aervoids; retaining *A. artemisioides* in *Aerva* would render the genus and the aervoids non-monophyletic.
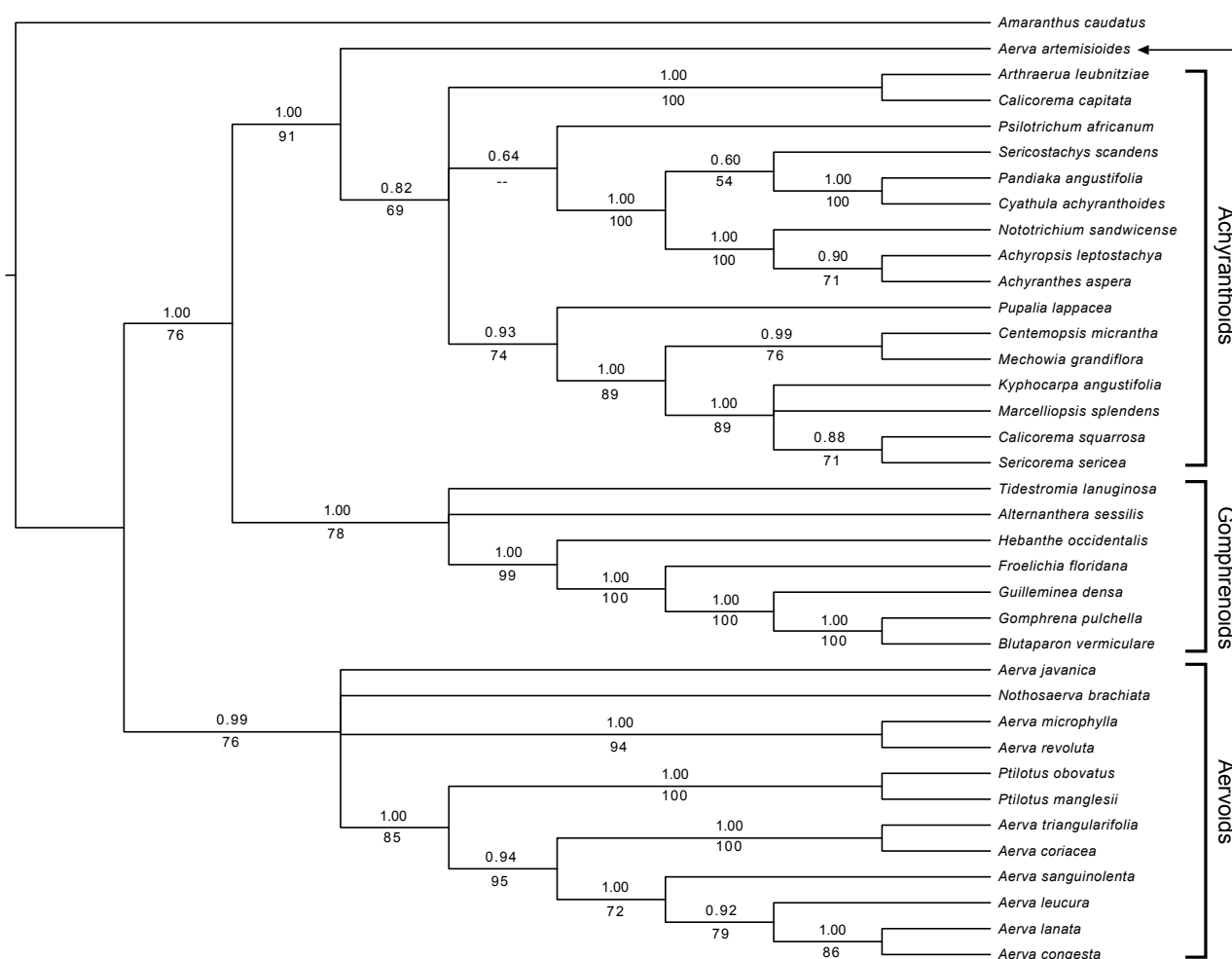
Fig. 4. *trnK-matK* mrBayes 50% majority-rule consensus tree. Bayesian posterior probabilities and maximum likelihood bootstrap are given above and below the branches, respectively. An arrow indicates the position of *Aerva artemisioides*; major clades within Amaranthaceae are labeled on the right.

Morphologically, *A. artemisioides* displays inflorescence and floral structures that are dissimilar from any other known aervoid. The inflorescence (panicle-like) includes 3- or 7-flowered cymes widely spaced along the elongate axes (Fig. 5), while in all other aervoids the flowers are borne solitary in simple or branched bracteate spike-like or raceme-like inflorescences. Flowers in *A. artemisioides* have 6 tepals (2 outer and 4 inner), and 4 stamens alternate with the inner tepals, while all the other aervoids have 5 tepals, which are opposite to 5 stamens (or infertile staminodes, as in some taxa belonging to *Ptilotus*). The indumentum of *A. artemisioides* consists of dendritic hairs, which are usually shortened to appear like a stellate hair on a simple stalk (tepal hairs can be of a more elongated form). This hair type is clearly different from that observed in the other aervoids, which usually have simple and multicellular hairs (in *Ptilotus* the hairs of some species bear whorls of short side-branches at the cell junctures but are not dendritic). In *Aerva,* the cell walls of hairs have small projections (larger at the base), which cover the hairs, but *A. artemisioides* and most *Ptilotus* species lack this feature. The stigma of *A. artemisiodes* is sessile and capitate, while all other aervoids have either simple, elongate styles terminated by capitate stigmas (e.g. *Ptilotus*), or bifid styles (e.g. other *Aerva* species).

Both morphology and the molecular phylogenies thus provide support to treat *A. artemisioides* separately from *Aerva* and the aervoids, while the chloroplast phylogeny supports its relationship to the achranthoids. Morphologically, it does not match any other genus of achyranthoid, and in floral morphology is unique within the Amaranthaceae (see also Townsend 1993). This, and its placement

sister to all the achyranthoids included in the molecular study, lead us to believe that it is best accommodated in a monotypic genus.
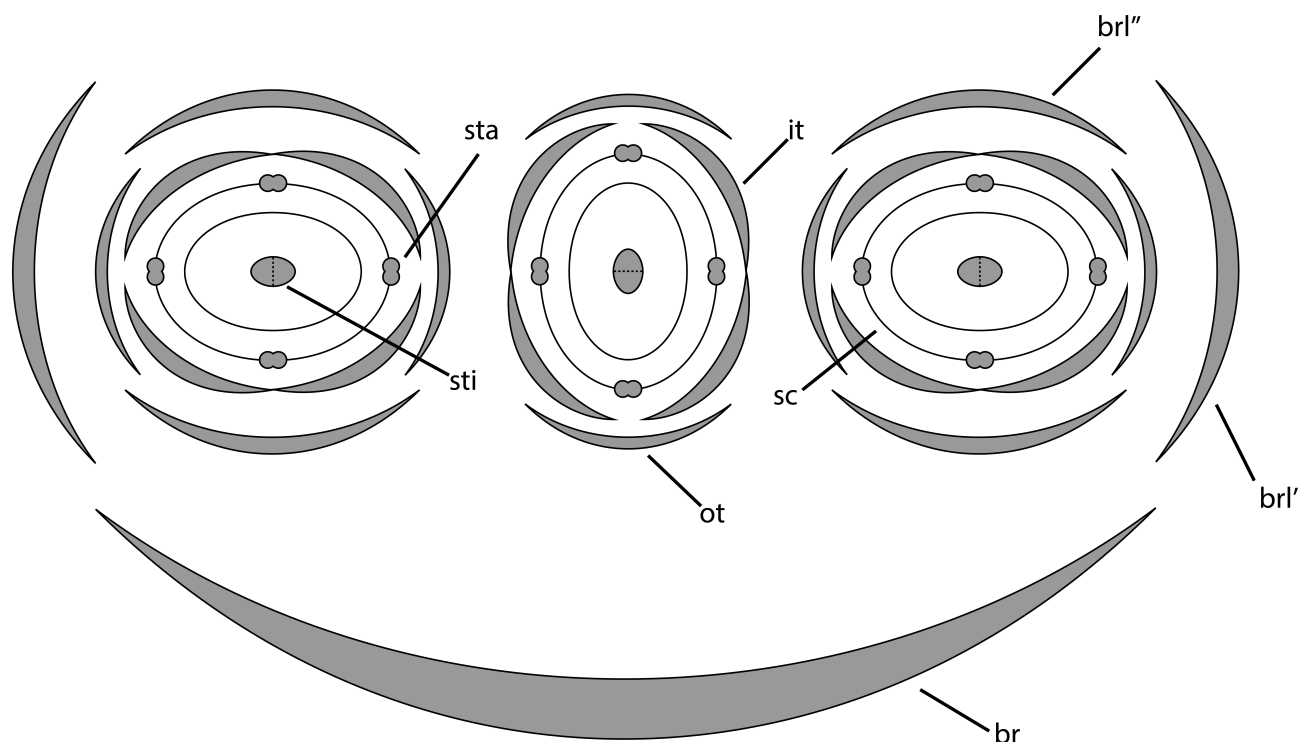
Fig. 5. Floral diagram of a 3-flowered cyme of *Aerva artemisioides.* Parts labeled are as follows: cymule bract (br), bracteole of central flower (brl'), bracteole of lateral flowers (brl''), outer tepal (ot), inner tepal (it), stamen (sta), staminal cup (sc), and stigma (sti).

TAXONOMIC TREATMENT

Wadithamnus T.Hammer & R.W.Davis *gen. nov.*

Generitype: *Wadithamnus artemisioides* (Vierh. & O.Schwartz) T.Hammer & R.W.Davis

Diagnosis:—Erect shrubs; young stems, leaves, inflorescence axes and outer surfaces of tepals densely but shortly white-tomentose with dendritic hairs, these sometimes contracted and appearing stellate. Leaves simple, alternate, entire. Flowers bisexual, bracteolate, arranged in loose and panicle-like, 3(7)-flowered cymes each borne distantly along elongate axes, each cymule subtended by a bract, the bracteoles of the central flower enclosing the lateral flowers. Tepals 6 (4 inner and 2 outer), free, glabrous adaxially. Stamens 4, alternate to the inner tepals, all fertile, united at their base into a short staminal cup and alternating with short, triangular staminal cup appendages. Ovary glabrous, compressed-ovoid, that of the central flower compressed at right angles to the cyme bract, those of the lateral flowers at right angles to the central flower; ovule 1; stigma sessile, capitate. Capsule 1-seeded, thin-walled, rupturing irregularly.
Etymology:—The generic epithet derives from the Arabic *wadi* (a seasonally wet valley or ravine) and the Greek *thamnos* (a shrub).
Distribution:—A monotypic genus from S-Arabia.

Wadithamnus artemisioides (Vierh. & O.Schwartz) T.Hammer & R.W.Davis, *comb. nov.*

Basionym: *Aerva artemisioides* Vierhapper & Schwartz in Schwartz (1939: 43).

Type (lectotype here designated):—YEMEN. Hadramaut: Wadi Dhaurûten nordwestlich von Râs Farták., 07 March 1897, *S. Paulay s.n.* (WU-0066481!, image of the lectotype available at http://herbarium.univie.ac.at/database/detail.php?ID=306580). Syntypes: YEMEN. Hadramaut: Wadi Dhaurûten nordwestlich von Râs Farták., 07 March 1899, *S. Paulay s.n.* (WU-0066482!, WU-0066483!, WU-0066484!, HBG-503185!); Fût angeblich vom Djebel Rikbêt, 15° N 50° E, 25 February 1902, *W. Hein s.n.*(WU-0066485!).
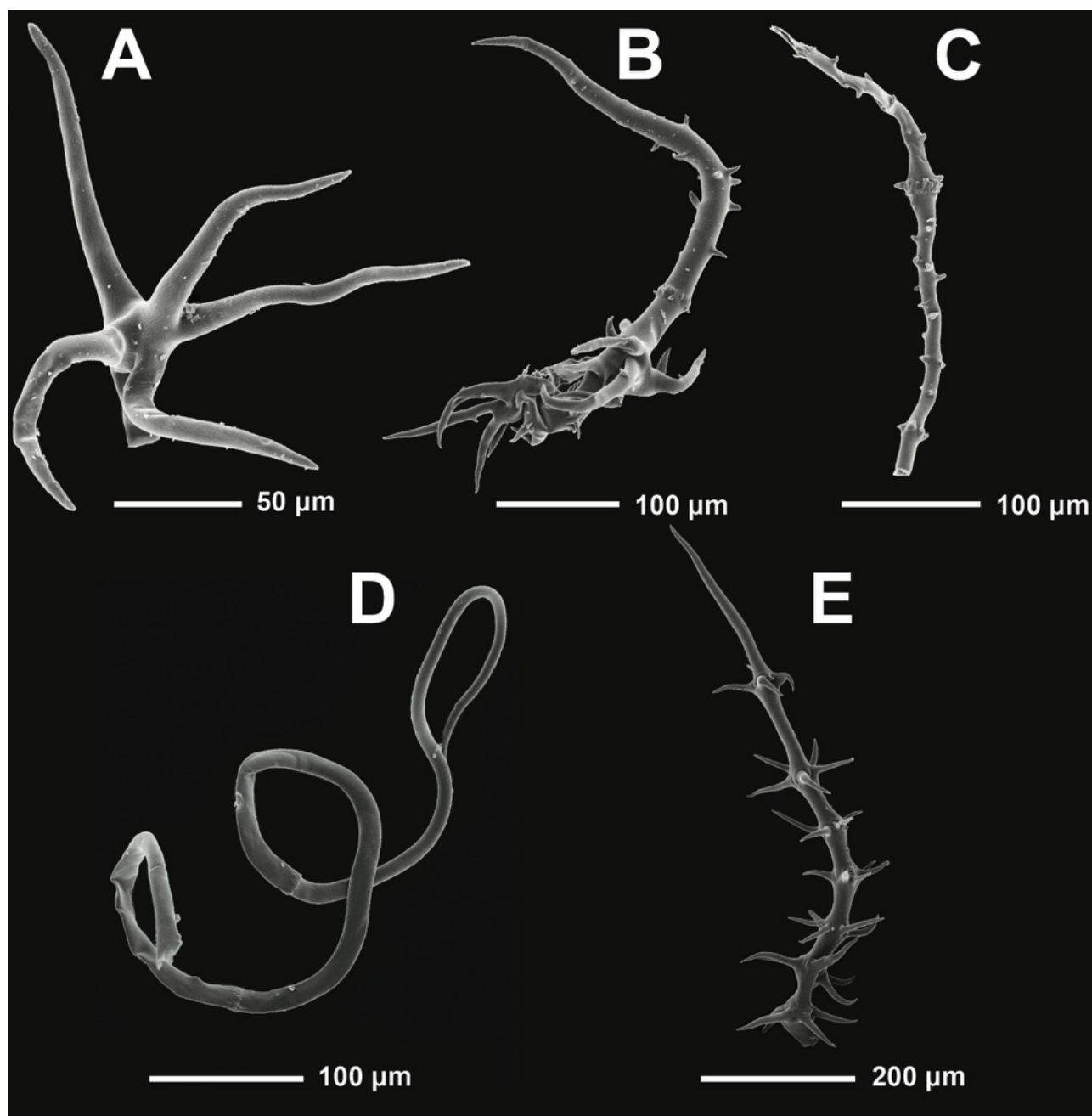
Fig. 6. SEM images of the hairs from the stem indumentum. **A.** *Aerva artemisioides* (E-00687199), **B.** *Aerva javanica* (E-00540230), **C.** *Aerva lanata* (E-00161942), **D.** *Ptilotus mollis* (PERTH-08642478), **E.** *Ptilotus* sp. Goldfields (PERTH-08387591).

Description:—Shrub, up to 1 m high. Stems terete, white-tomentose with dendritic hairs, these sometimes contracted so appearing stellate. Leaves flat, alternate, grey-green, linear-oblong, obovate, narrowly oblanceolate to elliptic (4–40 × 2–12 mm), with dense, shortly dendritic hairs; base cuneate to long-attenuate, sessile to petiolate (petiole to 4 mm long); apex rounded. Synflorescences terminal (loosely panicle-like), dense with 3(7)-flowered cymes, each cyme 1.5–8.0 mm long, 4–7 mm wide; inflorescence branches, bracts, bracteoles and flowers densely covered with short, white or pale grey, dendritic hairs; bracts ovate (1.0–1.4 × 0.7–1.1 mm); bracteoles ovate (1.1–1.4 × 0.6–1 mm). Flowers

shortly pedicellate; tepals 6; outer tepals 2, oblong to broadly ovate (1.2–2.0 × 0.6–1 mm), adaxially glabrous, broadly membranous at margins; inner tepals 4, linear to narrowly lanceolate (1.0–1.5 × 0.4–0.6 mm), adaxially glabrous, narrowly membranous-margined. Staminal cup symmetrical, glabrous, 0.3–0.5 mm long; appendages present, triangular. Stamens 4; filaments glabrous, dilating slightly towards base, 0.2–0.4 mm long; anthers 0.20–0.35 mm long. Ovary globose-compressed (0.4–0.9 × 0.4–0.7 mm), glabrous; stigma sessile, capitate. Seeds globose (0.8 × 0.7 mm), pale brown, smooth and glossy.

Typification:—The protologue of *Aerva artemisioides* consisted of a detailed diagnosis, the provenance ("In Arabien"), and the citation of specimens collected by W. Hein and S. Paulay. The collections by S. Paulay are mounted on four sheets at WU (one from 1897 and three from an expedition in 1899) and one sheet at HBG from the 1899 expedition; the collection from W. Hein is also deposited at WU and from 1902. These specimens are syntypes according to the Art. 9.5 of ICN (McNeill *et al.* 2012). The earliest specimen collected by S. Paulay is preserved at WU (code 0066481) and it bears two pieces (detached flowering shoots) of the same plant, and a label reporting "Wadi Dhaurûten nordwestlich von Rås Farták / 7 iii 1897 / leg. St. Paulay"; an additional label is marked "Typus!" and "!O. Schwartz 1934" (presumably in Schwartz' handwriting). The label data matches those reported in the protologue, and the exsiccatum morphologically matches the diagnosis. As a consequence, we here designate the WU-0066481 as the lectotype of the name *Aerva artemisioides*.

Distribution:—*Wadithamnus artemisioides* is restricted to near-coastal mountain ranges along the Arabian Sea coastline of the Arabian Peninsula.

Habitat:—Dry rocky slopes and wadi banks at 20–650 m a.s.l.

Morphological notes:—Two subspecies are here recognized according to Miller & Nyberg (1994, sub *Aerva*):

> 1. Leaves linear-oblong to linear-oblanceolate, 10–40 × 2–5 mm, long-attenuate at base into petiole or sessile .... subsp. *artemisioides*
> - Leaves elliptic to ovate, 4–25 × 5–12 mm, base cuneate, petiole short.. subsp. *batharitica*

Wadithamnus artemisioides subsp. *artemisioides*

Distribution:—Yemen between 14°51′N 49°03′E and 16°31′N 52°43′E

Habitat:— Dry rocky slopes and wadi banks at 50–650 m a.s.l.

Specimens examined:—YEMEN: 260 km from Say'un on the way to Al-Mukalla, 20 January 1985, *Sanadiki s.n.* (E-00687213); Hadramaut, Sunah 1 oil well in the Masila oil field, 15°47' N 49°03' E, 850 m, 12 October 1992, *Thulin, Erikson, Gifri & Långström 8306* (E-00003876); Al Mahra, between Ghaydah and Hawf mountain ridge 20 km E of Al Faydami, 16°30' N 52°35' E, 26 September 1998, *Hein, Kilian & Ghazanfar 4970* (B-10-0132033); Al Mahra, track from Ghaydah to Hawf, mountain 20 km E of Al Faydami, 16°29' N 52°34' E, 50–230 m, 26 September 1998, *Kilian, P. Hein, Ghazanfar 5164* (B-10-0132028); Hadhramout, Mukalla-Sayun road, Jol escarpment 30 km N of Riyan, 14°54' N 49°03' E, 550–600 m, 08 November 1999 *Kilian & Hein 5859* (B-10-0132026); Hadhramout, Al Mukalla, 14°54' N 49°03' E, 08 November 1999, *Hein & Kilian 6187* (B-10-0132032); Al Mahra, Jabal Fartak between Haswayn and Al Ghaydah, near the highest point of the track, 15°49' N 51°57' E, 600–800 m, 19 November 1999, *Hein, Bahah, Kilian & Mohammed 6547* (B-10-0132031); Al Mahra, Jabal Fartak, NE flank SW of Nishtun, 15°47' N 52°09' E, 200–500 m, 20 November 1999, *Kilian & Hein 6175* (E-00540232); Al Mahra, costal mountains, 2 km E of the village Al Fatk, 16°31'N, 52°43' E, < 10 m, 15 November 2000, *Kilian, Bahah, Hein, Hubaishan & Naumann 6850* (B-10-0132029); Al Mahra, Fartak Mts., Wadi Nishtun, a few km SW of Nishtun, 15°48' N 52°11' E, 16 November 2000, *Kilian, Hein & Naumann 6882* (E-00540233); Al mahra, southern-most plateau on the Ras Fartaq, E of Qadifut, 15°37' N 52°13' E, 16 November 2000, *Mies 1675* (B-10-0132024); Al Mahra, Jabal Faydami, S flank, ascent

from the coastal plain northeastwards, 16°30' 12'' N 52°35' 06.2'' E, 05 October 2001, *Kilian, Hein & Kürschner YP865* (B-10-0132022); Al Mahra, Nortnern Fartak Mts, dissected table-land W below J. Karmoun with the disused radio tower, 15°49' 39.2'' N 51°58' 14.4' E, 600–650 m, 06 October 2001, *Kilian & Meister YP908* (B-10 0132023); Hadhramout, Wadi Skoui (Shchawi), 15°15' 12.5'' N 50 39' 26'' E, 400–450 m, 16 August 2002, *Kilian, Hein & Hubaishan YP2746* (B-10-0380206); Al Mahra, Southern Fartak Mts., wadi immediately above the village Kadifut, 15°38' 21.4'' N, 52°10' 24.2'' E, 50–80 m, 05 September 2002, *Rabe YP3377* (B-10-0380835); Hadhramout, Wadi Arf, at wadi bed of a small tributary, 14°59' N 49°30' E, 250–550 m, 23 March 2003, *Kilian, Hein & Hubaishan YP4211* (B-10-0451691).

Wadithamnus artemisioides subsp. ***batharitica*** (A.G.Mill. & J.A.Nyberg) T.Hammer & R.W.Davis, *comb. nov.*

Basionym: *Aerva artemisioides* subsp. *batharitica* Miller & Nyberg (1994: 35).
Type:—OMAN. Dhofar: above Wadi Schuwaimayah, 17°56'N 55°32'E, 50 m, 28 April 1984, *Miller 6421* (holotype E!, image of the holotype available at http://data.rbge.org.uk/herb/E00687200); isotypes K, ON, UPS (*fide* Miller & Nyberg 1994).

Distribution:—Oman between 17°02′N 55°01′E and 18°05′N 56°29′E, with an outlying record near 23°01′N 59°07′E.
Habitat:—Dry limestone cliffs and wadi banks at 20–300 m a.s.l.
Specimens examined:—OMAN: Sharbitat, path and wadi below (E of) airstrip, 18°05' N 56°29' E, 10 May 1983, *Gallagher 6750/3* (E-00687199); Jabal Quinqari near Sudh, 17°02' N 55°01' E, 04 November 1983, *Lawton 2520* (E-00687258); Dhofar, Wadi Qumtat above Schuwaimayah, 17°56' N 55°32' E, 50–60 m, 28 April 1984, *A. Miller 6421* (E-00687200); Dhofar, Schwamiya, dry/wet wadi, 26 June 1985, *I. McLeish 547* (E-00687201); Dhofar, Edge of escarpment above Schuwaymiyah, 17 September 1989, 300 m, *Miller & Nyberg 9408* (E-00161905); 2km south of Bimmah, 23°01' N 59°07' E, 02 October 1993, *McLeish 2284* (E-00113662); Dhofar, sadh (Sudah), coastal rocks, 17°05' N 55°05' E, 0–250 m, 31 October 1993, *McLeish 2960* (E-00100967).

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Resolving intergeneric relationships in the aervoid clade and the backbone of *Ptilotus* (Amaranthaceae): Evidence from whole plastid genomes and morphology

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Abstract The informally named "aervoid clade" in Amaranthaceae includes ~134 species in five genera: *Ptilotus* (~120 spp.), *Aerva* (11 spp.) and the monotypic *Nothosaerva*, *Omegandra*, and *Kelita*. The relationships of the small aervoid genera to the large genus *Ptilotus*, and relationships between major clades within *Ptilotus*, are poorly resolved. The aims of this study were to: (1) elucidate relationships between genera and within *Ptilotus* using a phylogenomic approach; (2) identify morphological characters within each genus to help delimit generic boundaries; and (3) provide an updated taxonomic framework for the aervoids. A well-supported coding DNA sequence (CDS) phylogeny was constructed for 36 aervoid and 5 outgroup species based on 69 gene sequences derived from assembled whole-plastid genomes. The CDS tree was used to constrain relationships on a larger phylogeny based on Sanger-sequenced ITS and *matK* for 135 taxa, comprising near-comprehensive sampling within the aervoids. Both datasets were analysed using maximum likelihood and Bayesian inference. Morphological characters were assessed from herbarium specimens. Our study demonstrates that *Aerva* is polyphyletic; this is resolved by reinstating *Ouret* and erecting a new genus, *Paraerva*. *Kelita* is found to be deeply nested within *Ptilotus* and is formally synonymised. The well-resolved phylogeny of *Ptilotus* presented here will inform future studies in biogeography and character evolution. A taxonomic treatment is provided for all aervoid genera, and new combinations are made.

INTRODUCTION

Amaranthaceae Juss. s.str. (i.e., excluding Chenopodiaceae Vent.) is a family of approximately 79 genera and 800 species within the Caryophyllales Berchtold & J.Presl (see Hernández-Ledesma & al., 2015 for a recent treatment). The aervoids (Fig. 1) are an informal species-rich clade within Amaranthaceae that includes five genera: *Ptilotus* R.Br. (~120 spp.), *Aerva* Forssk. (11 spp.), and the monotypic *Kelita* A.R.Bean, *Nothosaerva* Wight and *Omegandra* G.J.Leach & C.C.Towns. (Leach & al., 1993; Bean, 2010; Hammer & al., 2017). Taxonomic and morphological diversity within the aervoids is concentrated in Australia (*Kelita*, *Omegandra* and *Ptilotus*); *Aerva* and *Nothosaerva* are native to Africa and South Asia. A comprehensive taxonomic treatment of the aervoids has been made more urgent by a recent initiative to develop a global synthesis for the Caryophyllales (Hernández-Ledesma & al., 2015; see also http://www.caryophyllales.org).

Endlicher (1837) provided an early family-level classification for the Amaranthaceae, dividing the family into three tribes based on ovary and anther characters. Genera assigned to tribe Gomphreneae (i.e., *Gomphrena* L. and allies) possessed uniovulate ovaries and unilocular anthers; tribe Achyrantheae (i.e., *Achyranthes* L. and allies; Amarantheae in Moquin-Tandon, 1849) included genera with uniovulate ovaries and bilocular anthers; while tribe Celosieae (i.e., *Celosia* L. and allies) included genera with multi-ovulate ovaries and bilocular anthers. Endlicher further divided tribe Achyrantheae into four

subtribes: Aervinae (as "Aerveae"), Amaranthinae ("Amarantheae"), Desmochaetinae ("Desmochaeteae") and Polycneminae ("Polycnemeae"). The genera known to Endlicher and currently included in the aervoids (*Aerva*, *Ptilotus* and the now subsumed genus *Trichinium* R.Br.) were included in subtribe Aervinae along with *Achyranthes*, *Centrostachys* Wall., *Nyssanthes* R.Br. and *Psilotrichum* Blume, based on their uniovulate ovaries, bilocular anthers, 3-bracteate flowers (1 bract and 2 lateral bracteoles), and indehiscent fruits.
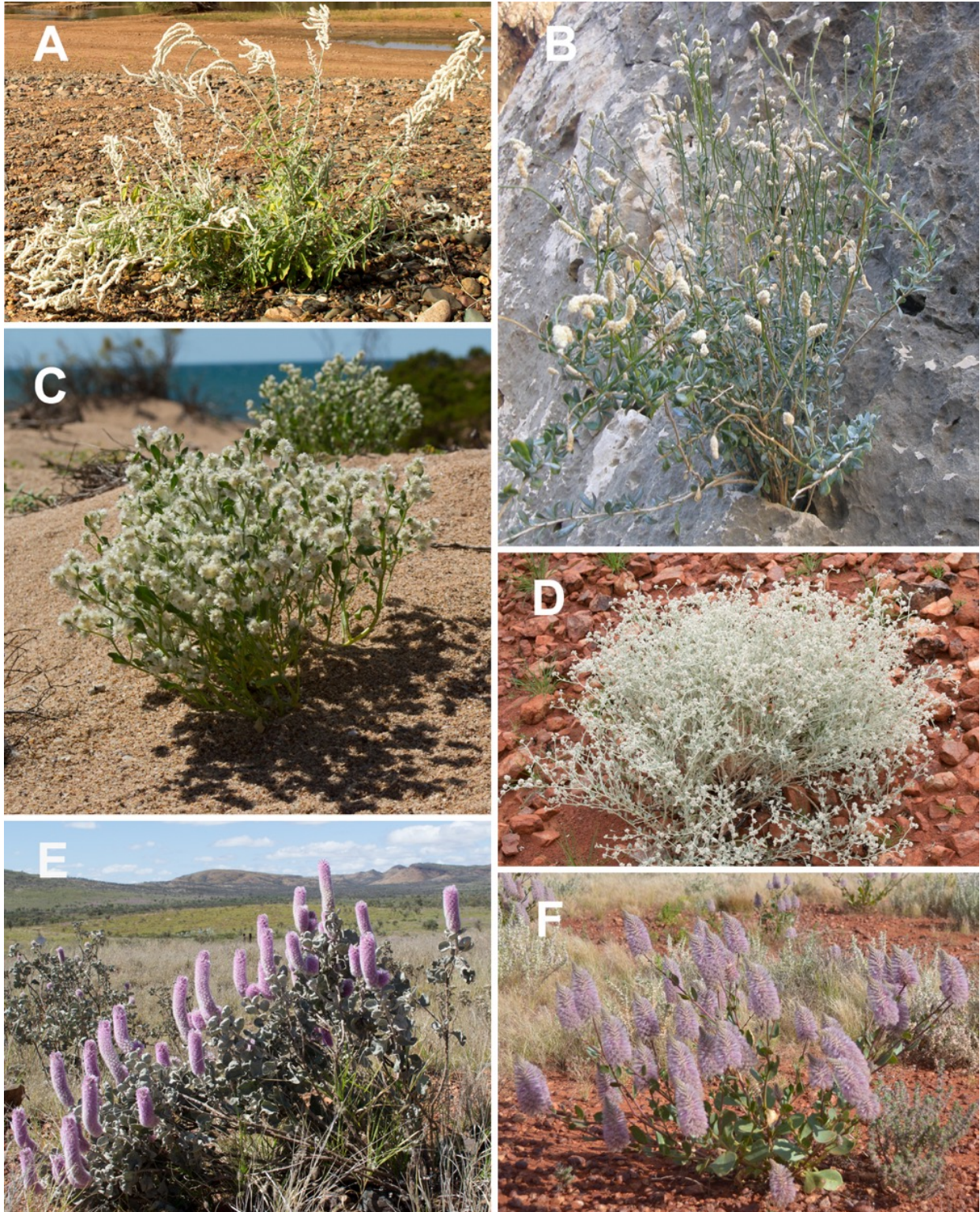
Fig. 1. Species representing major clades within the aervoids. **A,** *Aerva javanica*; **B,** *A. microphylla*; **C,** *Ptilotus villosiflorus*; **D,** *P. astrolasius*; **E,** *P. rotundifolius*; **F,** *P. exaltatus*. — Photos taken by T. Hammer in Western Australia (A, C–E) and South Australia (F) and by Alena Vydrova and Vit Grulich on Socotra (B).

Bentham & Hooker (1880) followed Endlicher (1837) and Moquin-Tandon (1849) in dividing the family into the tribes Amarantheae (= Achyrantheae), Celosieae, and Gomphreneae, but differed in the subtribal classification of Amarantheae, establishing subtribe Amaranthinae ("Euamarantheae") for taxa with an erect ovule and downwardly directed radicle, and subtribe Achyranthinae ("Achyrantheae") for taxa with a pendulous ovule and upwardly directed radicle, the aervoid genera being placed in the latter.

Schinz (1893) established the most widely accepted and long-standing classification of Amaranthaceae, largely based on the preceding classifications but adding a subfamily rank (establishing subfam. Amaranthoideae for taxa with bilocular anthers and subfam. Gomphrenoideae for taxa with unilocular anthers). Subfamily Amaranthoideae was divided into the tribes Amarantheae (one-seeded) and Celosieae (multi-seeded), the former including two subtribes following Bentham & Hooker (1880), but with "Euamarantheae" renamed as Amaranthinae. Schinz placed the aervoid genera known at that time (*Aerva*, *Nothosaerva* and *Ptilotus*) in subfamily Amaranthoideae, tribe Amarantheae and subtribe Achyranthinae, along with 12 other genera (e.g., *Achyranthes* and *Arthraerua* (Kuntze) Schinz). Townsend (1993), in the most recent treatment of the family, largely followed Schinz's classification, and accepted 38 genera in subtribe Aervinae.

The advent of molecular phylogenies allowed these classifications to be tested. Several studies (e.g., Kadereit & al., 2003; Müller & Borsch, 2005; Sage & al., 2007) have indicated that most infra-familial taxa of previous authors (including Amaranthoideae, Amarantheae and Aervinae) are paraphyletic. In anticipation of a substantially revised, formal infra-familial classification, Müller & Borsch (2005) established informal clades within the family, including the achyranthoids, aervoids, amaranthoids and gomphrenoids (for clades centred on *Achyranthes*, *Aerva*, *Amaranthus* L. and *Gomphrena* respectively). They recovered two clades within the Aervinae of previous authors, one comprising *Aerva*, *Nothosaerva* and *Ptilotus* and the other comprising *Achyranthes* and related genera (their achyranthoids). The achyranthoids, aervoids and gomphrenoids are together sister to the amaranthoids and Celosieae, the only monophyletic tribe recovered in Amaranthoideae (Kadereit & al., 2003; Müller & Borsch, 2005). We continue to use these informal clade names while awaiting an updated classification for the family.

Early, family-wide phylogenetic studies included relatively few species within genera, and often relatively few genera. Kadereit & al. (2003) included *Aerva javanica* (Burm.f.) Juss. ex Schult. and *Ptilotus manglesii* (Lindl.) F.Muell. as the only representatives of the aervoids and did not resolve *Aerva* and *Ptilotus* as a clade, instead placing them in an unresolved polytomy with the achyranthoids and gomphrenoids. This lack of resolution was perhaps due to the lack of informative characters for these taxa on the *rbcL* marker. Müller & Borsch (2005) included *A. javanica*, *N. brachiata*, *P. manglesii* and *P. obovatus* (Gaudich.) F.Muell. The two *Ptilotus* species were resolved as sisters with 100% bootstrap support, but relationships between the three genera were unresolved.

Thiv & al. (2006), in an attempt to assess phylogenetic relationships within *Aerva*, included multiple accessions for 10 of the 12 species that they recognised within the genus (i.e., *A. artemisioides* Vierh. & O.Schwartz, *A. congesta* Balf.f, *A coriacea* Schinz, *A. javanica* (Burm.f.) Juss. ex Schult., *A. lanata* (L.) Juss. ex Schult., *A. leucura* Moq., *A. microphylla* Moq., *A. revoluta* Balf.f., *A. sanguinolenta* (L.) Blume and *A. triangularifolia* Cavaco, excluding only *A. glabrata* Hook.f. and *A. humbertii* Cavaco due to a lack of suitable material for sequencing*)*. They used two *Ptilotus* species as outgroups. In their ITS, *matK* and *atpB-rbcL* phylogenies, two clades of *Aerva* were resolved and informally named clades "A" and "B" (referred to here as "clade I" and "clade II" respectively). Clade I of *Aerva* included three subclades on a polytomy: *A. artemisioides*, *A. javanica* and the two Socotran endemics *A. microphylla* and *A. revoluta*.

Clade II included all other *Aerva* species sampled. Because their tree was rooted on *Ptilotus* and did not sample more widely within the family, the monophyly of *Aerva* was not tested.

Sage & al. (2007) sampled widely for a family-level phylogeny based on *trnK/matK* sequences and provided the first indication that *Aerva* was paraphyletic with respect to *Ptilotus* (with *A. leucura* and *A. sanguinolenta* forming a highly supported clade with *P. manglesii* and *P. obovatus*, while *A. javanica* and *N. brachiata* resolved separately as a clade with weak support). Sage & al. (2007) also confirmed the monophyly of the aervoids.

Hammer & al. (2015) reconstructed the phylogeny of 87 species of *Ptilotus* using ITS and *matK*. Sampling covered the morphological and geographic diversity of the genus, and included three species of *Aerva*, two species of *Gomphrena*, *Achyranthes aspera* L. and *Amaranthus caudatus* L. (on which the tree was rooted). *Aerva* was confirmed as paraphyletic in this study, with *A. lanata* resolving as more closely related to *Ptilotus* than either *A. javanica* or *A. artemisioides*, albeit with weak support. *Ptilotus* was strongly supported as monophyletic. Four major clades (informally named A–D) were resolved within *Ptilotus*. The largest of these (D; 73% of species) comprised four subclades (D1–D4), related by a polytomy. Hammer & al. (2015) speculated that *Ptilotus* had radiated rapidly, probably after its ancestor arrived on the Australian continent following a divergence in Southeast Asia from a common ancestor with one of the clades of *Aerva*.

A follow-up study by Hammer & al. (2017) reconstructed an ITS and *trnK/matK* phylogeny for all species of *Aerva* that had been accepted by Thiv & al. (2006), as well as extra gomphrenoid and achyranthoid taxa. This study placed one species, *A. artemisioides*, in a position sister to the rest of the achyranthoids, distant from the aervoids. A morphological examination revealed that *A. artemisioides* has an unusual combination of characters, unlike any aervoid or any other known genus in Amaranthaceae: accordingly, the genus *Wadithamnus* T.Hammer & R.W.Davis was erected to accommodate it. With *W. artemisioides* removed, the remaining species of *Aerva* were still paraphyletic with respect to *Ptilotus*.

The phylogenies of Hammer & al. (2015) and Hammer & al. (2017), based on Sanger sequencing of a small number of markers, provided a good understanding of major clades within the aervoids. However, relationships between clades were in many cases poorly supported, precluding a robust taxonomic solution to the paraphyly of *Aerva*, and the monotypic genera *Kelita* and *Omegandra* were not included.

To address this, we present here a nearly taxon-complete phylogeny of the aervoids and use next-generation sequencing of the chloroplast genome to resolve, with high support, backbone relationships among clades. Such an approach has been utilized successfully to resolve backbone relationships within various plant families where phylogenies based on Sanger-sequenced traditional markers were inadequate (e.g., Ma & al., 2014; Gardner & al., 2016; Williams & al., 2016; Tonti-Filippini & al., 2017), particularly when rapid radiations obscure relationships (Wei & al., 2017; Léveillé-Bourret & al., 2018). This is the most comprehensive and strongly supported phylogeny of the aervoids to date. It provides a basis for a generic taxonomic treatment of the aervoids and will inform future biogeographic analyses and studies into the evolution of floral characters and pollination syndromes, especially within *Ptilotus*.

MATERIALS AND METHODS

Taxon sampling and DNA extraction. — Phylogenetic analyses were conducted on three datasets: (1) coding DNA sequences (CDS) of the plastid genome for 41 selected taxa, and Sanger sequences of (2) ITS for 128 taxa, and (3) *matK* for 135 taxa (sequencing of ITS failed for some taxa). The Sanger sequencing section of this study incorporated all ITS and *matK* sequences from Hammer & al. (2015),

some of the outgroup species used in Hammer & al. (2017), and an additional 26 species not previously sequenced. ITS and *matK* were used because they have been widely sequenced in previous studies, have been shown by Hammer & al. (2015, 2017) to be highly congruent, and because of their complementary nature in resolving deep and shallow nodes (Hammer & al., 2015). A near-comprehensive sampling of the aervoids included 112 (of ~120) *Ptilotus* species, 10 of 11 recognised *Aerva* species (i.e., excluding only the rare Madagascan *A. humbertii*), and the monotypic genera *Nothosaerva*, *Omegandra* and *Kelita*. In addition to the aervoids, 10 outgroup species were chosen to provide broad representation of the achyranthoids, gomphrenoids and amaranthoids. A full list of accessions used in the ITS and *matK* analyses is provided in Appendix 3.1.

Sampling for the plastid CDS phylogeny included 30 *Ptilotus* species, *Aerva javanica*, *A. glabrata* and *A. microphylla* (representing the major clades of *Aerva* from Hammer & al., 2017), *Kelita uncinella* A.R.Bean and *Omegandra kanisii* G.J.Leach & C.C.Towns. Outgroup species were *Gomphrena affinis* subsp. *pilbarensis* J.Palmer and *Gomphrena cunninghamii* (Moq.) Druce representing the gomphrenoids, *Achyranthes aspera* L. and *Alternanthera nodiflora* R.Br. representing the achyranthoids, and *Amaranthus cuspidifolius* Domin representing the amaranthoids. Most of the included *Ptilotus* species and the *Gomphrena* outgroups were sequenced as part of a project to sequence full chloroplast genomes for plants from the Pilbara region of Western Australia (see https://s3-ap-southeast-2.amazonaws.com/pilbara-cpt-website/index.html). As this region is home to approximately one-third of *Ptilotus* species, representing every major clade in the genus (Hammer & al., 2015), adequate coverage for resolving the backbone could be achieved from this narrow geographic sampling. A full list of accessions used in the CDS dataset is provided in Appendix 3.2.

DNA was extracted from silica-dried leaf material collected in the field, and from herbarium specimens at AD, BRI, E, PE and PERTH. Approximately 20 mg of dried plant material was pulverized for 1 minute in a mini-bead beater. Total genomic DNA was extracted using DNeasy Mini Plant Kits (Qiagen, Valencia, California, U.S.A.) following the manufacturer's protocol. DNA quantity and quality were assessed using a Qubit dsDNA HS Assay Kit (ThermoFisher Scientific, Waltham, Massachusetts, U.S.A.) and agarose gel electrophoresis.

NGS sequencing, genome assembly, annotation and alignment. — A Covaris S220 sonicator was used to shear 200 ng of DNA into ~500 base pair (bp) fragments. Libraries were prepared with an Illumina TruSeq Nano DNA LT Library Preparation Kit according to the manufacturer's directions, their sizes checked on a 4200 TapeStation System (Agilent Technologies, Santa Clara, California, U.S.A.), quantified with a KAPA SYBR FAST Universal qPCR Kit (Sigma-Aldrich, St. Louis, Missouri, U.S.A.), pooled, and run on a MiSeq sequencer (Illumina, San Diego, California) using a MiSeq Reagent Kit v3 (Illumina).

Sequence pre-processing included the following: removing adapter sequences using Cutadapt v.1.9.1 (Martin, 2011), correcting read errors using SPAdes v.3.6.1 (Bankevich & al., 2012) and merging overlapping paired-end reads using BBMerge v.8.82 (Bushnell & al., 2017). The clean reads were independently assembled into circular or fragmented contigs with the *de novo* assemblers NOVOPlasty v.2.5.9 (Dierckxsens & al., 2016), ORGanelle ASeMbler v.2.2 (Org.asm; https://pythonhosted.org/ORG.asm/), and Velvet v.1.2.08 (Zerbino & Birney, 2008). Velvet was run with k-mer values between 51 and 111 and a coverage cut-off of 10. Org.asm was run with the parameters minread = 5, smallbranches = 15, and seed = protChloroArabidopsis (i.e., chloroplast protein sequences of *Arabidopsis thaliana* (L.) Heynh.).

In addition, a reference-guided approach was utilized to order, orient and merge contigs to produce a single sequence draft genome, if needed, based on the alignment between the contigs and a high-quality reference chloroplast genome from the closest related species. Pair-wise alignments were generated using MUMmer v.3.23 (Kurtz & al., 2004). The assemblies were refined further and verified by automatically analysing consistency between original reads and these draft genomes using Pilon v.1.16 (Walker & al., 2014), and by visually inspecting for discrepancies between them and among multiple assembled sequences of the same organism using Geneious v.9.1.8 (Kearse & al., 2012). Finally, the best assembly for each species was selected by considering accuracy, integrity and continuity at base level. Mapping of reads to assembled sequences was carried out with BWA v.0.7.15 (Li, 2013), and multiple sequence alignments using MAFFT v.7.273 (Katoh & Standley, 2013). Genomes were annotated by the "Transfer Annotations" function in Geneious using the previously published *Amaranthus hypochondriacus* L. annotation as a reference, with manual corrections.

Coding regions were extracted from the annotated plastid genomes and aligned separately using MAFFT prior to concatenation using Geneious. The best partitioning scheme for the CDS data was found using PartitionFinder v.2.1.1 (Lanfear & al., 2016), with all genes and codon positions as separate data blocks and using the "greedy" search algorithm.

Sanger sequencing and alignment. — Amplification of ITS (ITS1, 5.8S, ITS2) was accomplished using ITS5A (forward) and 26S-25R (reverse) primers (Motley & al., 2005). Primers used to amplify *matK* were matK-AF (Thiv & al., 2006) and matK1R (Sang & al., 1997). Each 50 µl PCR reaction was performed using 5 µl 10x buffer, 0.5 µl dNTPs (2.5 mM), 41.25 µl nuclease-free water, 0.25 µl Taq, 0.5 µl of each 10 µM primer, and 2 µl DNA template. Thermocycler programs for amplification were as outlined in Hammer & al. (2015). Sequencing was performed by Macrogen (Seoul, Korea). Sequence chromatograms were manually edited using Geneious. Sequences were aligned using the default settings of MAFFT, and aligned sequences inspected and manually adjusted as needed. The best nucleotide substitution models were determined using jModelTest2 v.2.1.6 (Darriba & al., 2012).

Phylogenetic analyses. — Bayesian inference (BI) was performed using MrBayes v.3.2.2 (Ronquist & al., 2012). Analyses were run for 15 million Markov chain Monte Carlo (MCMC) generations with trees sampled every 1000 generations. Analyses were conducted on partitioned datasets with appropriate substitution models determined by jModelTest2 for each partition. Completion was determined by the average standard deviation of split frequencies falling below 0.01. Trace files generated by the MCMC runs were analysed using Tracer v.1.6 to determine adequate convergence and mixing (Rambaut & Drummond, 2007). The initial 25% of topologies before reaching likelihood stationarity were discarded; 50% majority-rule consensus trees were constructed to summarize the remaining topologies. Maximum likelihood (ML) analyses were implemented on RAxML v.8.2.10 (Stamatakis, 2014), with options for GTRGAMMA and proportion of invariable sites estimation options enabled, and run for 1000 bootstrap iterations.

Hammer & al. (2015) showed that ITS and *matK* were congruent and could be concatenated using similar taxon sampling. For this reason, and because a few species could not be sequenced for both markers, a congruence analysis was not performed and the ITS and *matK* sequences were concatenated. The CDS tree was used as a topological constraint for analyses of the concatenated Sanger dataset. Topological constraints for the MrBayes block were generated using the paleotree R package (Bapst, 2012). MAFFT alignments, jModelTest2 and BI and ML analyses were conducted on the Cyberinfrastructure for Phylogenetic Research (CIPRES) portal (Miller & al., 2010). Bootstrap values were summed using SumTrees v.3.3.1 in the DendroPy v.3.12.0 python package (Sukumaran & Holder, 2010). CDS trees were rooted at *Amaranthus cuspidifolius* and Sanger trees at *Amaranthus caudatus*.

Morphological examinations. — Specimens from AD, BRI, E, PE and PERTH (listed in Appendix 3.3), representing all major clades of aervoids, were morphologically examined. Flowers for dissection were rehydrated in hot water with a little detergent. Due to the small size of flowers in many species, a JEOL JCM5000 NeoScope bench-top scanning electron microscope (SEM) at the Western Australian Herbarium (PERTH) was used to image whole flowers and reproductive parts. Multiple gynoecia were obtained from herbarium specimens from bisexual or female individuals of each species, mounted on stubs using carbon tape, and coated with gold for two minutes using a JEOL Smart Coater.

Some terminology used in the Discussion and Taxonomic treatment sections in this paper is different from that conventionally used for this group, particularly within Australia. The term "staminal cup appendages" is used here in favour of "pseudostaminodes", as this more aptly describes these projections from the staminal cup, which are ontogenetically different from staminodes. We have adopted "sepals" instead of the conventional "tepals" to describe the perianth in this group, following recent studies (e.g., Ronse De Craene, 2013), which indicate that the uniseriate perianths in the Caryophyllales are calycine in origin (see also Borsch & al., 2018).

RESULTS

CDS and Sanger sequencing datasets. — The aligned CDS dataset comprised 69 genes with a combined length of 51,996 bp (suppl. Appendix S1). The aligned ITS region comprised 773 bp, the aligned *matK* region comprised 798 bp, and the combined ITS+*matK* dataset was 1571 bp long (suppl. Appendix S2). Amplification of ITS failed for seven species, which were therefore represented by *matK* sequences alone. Bayesian and Akaike information criteria (BIC and AIC) in jModelTest2 agreed that the best nucleotide substitution models for use in MrBayes were GTR+I+Γ for ITS and GTR+Γ for *matK*.

Phylogenetic analyses. — All phylogenetic analyses gave largely congruent results. Major clades resolved on the CDS phylogeny (Fig. 2) agreed with those resolved from previous studies (Hammer & al., 2015, 2017) and with the unconstrained ML and BI ITS+*matK* phylogenies (suppl. Figs. S1, S2). The constrained ITS+*matK* phylogeny (henceforth "constrained tree"; Fig. 3) maintained the relationships resolved in the CDS phylogeny. Species in *Amaranthus*, *Alternanthera* and *Gomphrena* in the CDS dataset were represented in the ITS+*matK* dataset by different species in the same genus; this did not affect intergeneric relationships on the tree. Because topological constraints are handled differently in MrBayes and RAxML, BI posterior probabilities (PP) and ML bootstrap support (BS) on the constrained branches (bolded in Fig. 3) vary dramatically in some cases. In MrBayes, the constraints are applied universally to the MCMC runs, with only topologies that agree with the constraints being considered, ensuring higher PP on the branches for those constrained relationships. In RAxML, the ML search tree is constrained to the topology of an input tree (i.e., the CDS tree), but no constraint is applied when bootstrapping the trees. Therefore, the BS on constrained nodes reflects support in the unconstrained concatenated Sanger dataset. In both analyses, unconstrained taxa are free to change position on the tree during the analysis.

Intergeneric relationships. — The aervoids are well supported as monophyletic (1.00/100) on the CDS phylogeny (Fig. 2) and comprise three major clades: the "basal aervoids" (represented by *O. kanisii* sister to the *Aerva* clade I taxa *A. javanica* and *A. microphylla*); *Aerva* clade II (represented by *A. glabrata*); and *Ptilotus* including *Kelita*. In the constrained tree (Fig. 3), the basally branching aervoid clade is also well supported, but the placement of *N. brachiata* within this clade with poor support causes it to collapse to a polytomy comprising *O. kanisii, N. brachiata, A. javanica*, and a strongly supported sister pair of the Socotran species *A. microphylla* and *A. revoluta* (1.00/100). The poor

placement of *N. brachiata* may be because repeated attempts to extract and sequence ITS from herbarium specimens of this species failed.

Aerva clade II is strongly supported as monophyletic (1.00/99), and sister to *Ptilotus* (1.00/99). Relationships within the clade are well resolved. One accepted species, the Madagascan endemic *A. humbertii*, was unavailable for sequencing for this study but can be confidently placed in this clade, along with the other species endemic to Madagascar (i.e., *A. coriacea* and *A. triangularifolia*), based on its morphology.
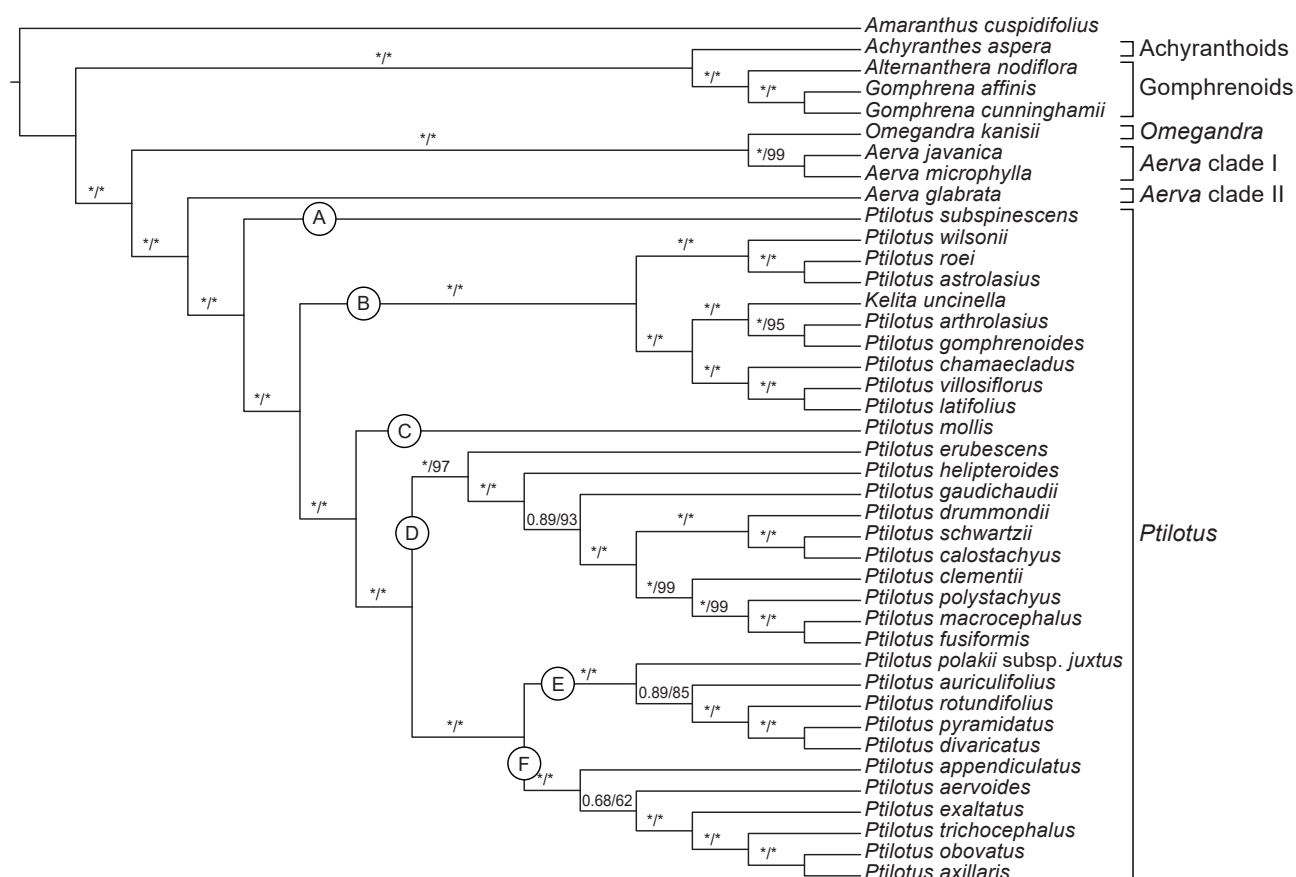
Fig. 2. The MrBayes 50% majority-rule consensus tree of the CDS dataset with support values above the branches (BI posterior probabilities/ML bootstrap percentages). Asterisks (*) indicate maximum support values (1.00 or 100% respectively). Major clades outside of *Ptilotus* indicated on the right and major clades within *Ptilotus* (A–F) are indicated on the branches.

Relationships within *Ptilotus*. — *Ptilotus* clades A, B and C of Hammer & al. (2015) are recovered on the CDS phylogeny with good support (Fig. 2). Clades A and C were each represented in the CDS tree by one of the three species in each clade (*P. subspinescens* R.W.Davis and *P. mollis* Benl respectively), and no new species sampled were placed within these clades. Relationships within clade B recovered by Hammer & al. (2015) are also recovered here (with the inclusion of newly sampled species; see Appendix 3.1). The nine species from clade B on the CDS phylogeny resolves the clade's backbone, and strongly supports the inclusion of *Kelita uncinella* within *Ptilotus* and in this clade. Only the *P. conicus* R.Br. species group, comprising seven species from northern Australia, was not represented on the CDS phylogeny. The relationships of these northern species were nevertheless well supported (1.00/82) in the constrained phylogeny, where they resolved with strong PP support (1.00/67) as a clade sister to an arid clade comprising *P. astrolasius* F.Muell. and three other species, mostly from the Pilbara region of Western Australia (Fig. 3). A group of tropical species from northern Western Australia and the Northern Territory (*P. lanatus* A.Cunn. ex Moq., *P. rotundatus* Benl, *P. lophotrichus* Benl and *P. comatus* Benl), which were not sequenced in Hammer & al. (2015), were resolved as monophyletic (1.00/82).

This tropical clade is nested within a chiefly Eremean clade (e.g., *P. arthrolasius*, *P. gomphrenoides* F.Muell. ex Benth.), rather than being more closely related to the *P. conicus* clade.

This study provides significantly improved backbone resolution within *Ptilotus* for clades D, E and F, which in Hammer & al. (2015) formed an unresolved polytomy with *P. declinatus* Nees. *Ptilotus erubescens*, a species from South Australia that shares a strong morphological affinity with the Western Australian species *P. declinatus*, was sequenced for the CDS phylogeny and resolved as sister to the rest of clade D (1.00/97). On the constrained phylogeny, *P. erubescens* is sister to *P. declinatus*, with *P. barkeri* Benl sister to both (1.00/100), these three species comprising a clade sister to the rest of clade D. A small clade of 10 species, including *P. drummondii* F.Muell., *P. leucocoma* (Moq.) F.Muell. and close relatives, are newly resolved on the constrained phylogeny (0.98/56).

Clade E includes two subclades (comprising *P. auriculifolius* (Moq.) F.Muell. and allies, and *P. polakii* F.Muell. and allies, respectively) that are newly resolved as sisters (1.00/95 and 1.00/100 respectively). The subclade with *P. polakii* includes a few species (e.g., *P. parvifolius* (F.Muell.) F.Muell.) newly sequenced for this study, but as only one taxon (i.e., *P. polakii* subsp. *juxtus* Lally) could be included in the CDS dataset, the interspecific relationships between these close and morphologically similar species could not be resolved. This subclade has been previously referred to as the "*Ptilotus parvifolius* complex" (e.g., Lally, 2008). Most species in this subclade are long-lived rigid shrubs, which occur in arid regions of Australia. The related *P. auriculifolius* species group includes the arid-zone herb *P. auriculifolius* and shrubs *P. rotundifolius* (F.Muell.) F.Muell. and *P. marduguru* Benl, which are sister to a group of species native to the biodiversity hotspot of the Southwest Australian Floristic Region (except for *P. symonii* Benl from southeastern Western Australia and southwest South Australia and the putative new species *P.* sp. Arckaringa (D.J.Duval 1958) from the Lake Eyre region of South Australia). It includes the threatened species *P. pyramidatus* (Moq.) F.Muell., known from only one population in the Perth metropolitan area, Western Australia (Davis, 2012).

Clade F was represented by six species on the CDS phylogeny (Fig. 2). *Ptilotus appendiculatus* Benl was resolved at the base of the clade in the CDS phylogeny with good support. The relationship of *P. aervoides* (F.Muell.) F.Muell. with the remaining species is ambiguous due to a poorly supported node (0.68/62). The constrained tree has better backbone support than in Hammer & al. (2015), however many of the relationships still need to be fully resolved, particularly within the derived group that includes *P. axillaris* (Benth.) F.Muell.
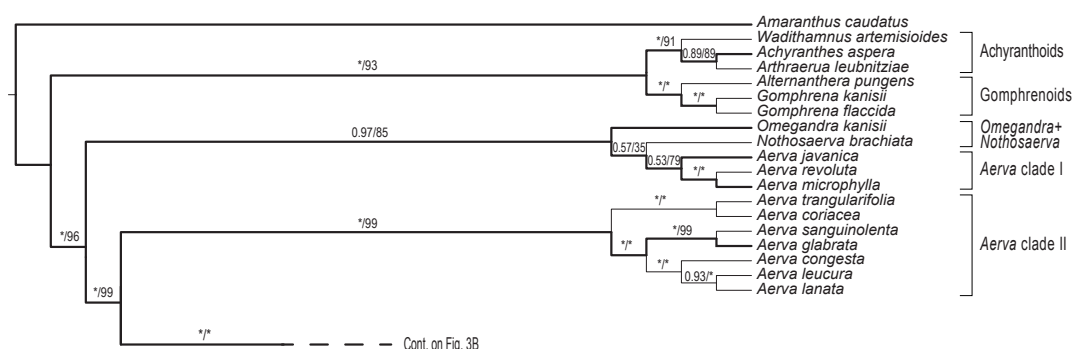
Fig. 3. The MrBayes 50% majority-rule consensus tree of the Sanger ITS+*matK* dataset with support values above branches (BI posterior probabilities/ML bootstrap percentages). **A**, The whole tree with *Ptilotus* collapsed and major clades indicated on the right; **B** (next page), The expanded tree of *Ptilotus* with major clades of *Ptilotus* (A–F) indicated on the branches. Asterisks (*) indicate maximum support values (1.00 or 100% respectively) and a hyphen (-) indicates ML support below 50%. Branches in bold indicate relationships resolved in the CDS tree and the topological constraints implemented in the analyses.

Fig. 3. Continued from previous page.

Cont. from
Fig. 3A
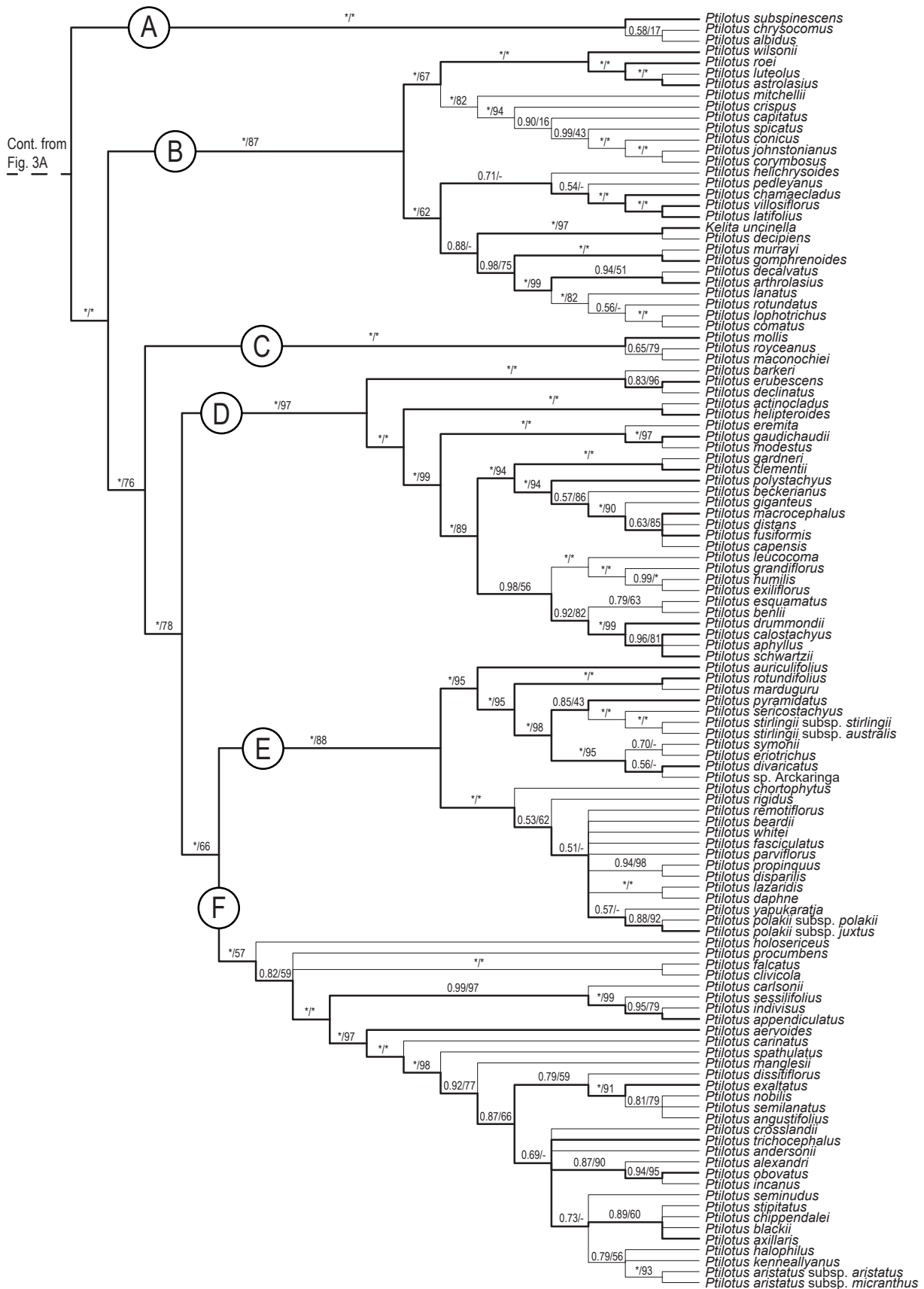
Morphology. — A number of morphological characters examined were congruent with major clades of the phylogeny and provide synapomorphies or combinations of characters that support a revised taxonomy, particularly the segregation of the traditionally circumscribed *Aerva* into three genera. Stigma morphology is particularly useful in this respect, as discussed below.

DISCUSSION

Intergeneric relationships. — The present study establishes phylogenetic relationships for the major aervoid clades, allows delimitation of clades based on morphology, and demonstrates the need for revised taxonomic delimitations of some genera.

Aerva clade I forms a monophyletic group sister to *Omegandra* in the CDS tree (Fig. 2), with *Nothosaerva* clearly placed in this group in the constrained tree (Fig. 3), but without support for a clear position, probably due to the inability to sequence ITS in this species. *Nothosaerva* and *Omegandra* are morphologically distinct from all other aervoids and from each other, and we retain them as monotypic genera. *Omegandra kanisii* and *Nothosaerva brachiata* are unique within the aervoids in having four sepals (five in all other genera) and two stamens. *Nothosaerva brachiata* differs from *O. kanisii* in having an unlobed, capitate stigma (Fig. 4B), whereas *O. kanisii* has a minutely bilobed stigma (Fig. 4A). *Omegandra kanisii* and *N. brachiata* can also be distinguished from each other based on staminal cup morphology and orientation of the stamens on the cup. *Nothosaerva brachiata* has a staminal cup that forms a circle with two stamens on opposite sides of the ovary, and no appendages. *Omegandra kanisii* has a cup that is open on one side of the ovary, attached to which are two stamens alternating with three staminal cup appendages (see Leach & al., 1993: 788, fig. 1J).

Aerva clade I was resolved as monophyletic in the present phylogeny and includes three species, the widespread *Aerva javanica* and two Socotran endemics, *A. microphylla* and *A. revoluta*. *Aerva javanica* and the Socotran species are morphologically divergent. *Aerva javanica* is unisexual, with female plants having a deeply bilobed, feathery stigma (Fig. 4D; males lack a style and functional stigma), unlike any other aervoid species. *Aerva microphylla* and *A. revoluta* are bisexual plants with succulent leaves (especially noticeable in the larger-leaved *A. revoluta*). Miller (1996) described *A. microphylla* and *A. revoluta* as having bilobed ("bifid") stigmas; however close examinations of multiple specimens of both species under SEM revealed these species to consistently possess unlobed, capitate stigmas (Fig. 4C), markedly different from the other species of *Aerva* and *Omegandra*, but similar to *Ptilotus* (see Fig. 4F). A single genus for these three species, while monophyletic, would be morphologically heterogeneous in this important character. For this reason, we prefer to segregate the two Socotran species from *A. javanica* at genus level and describe the new genus *Paraerva* to accommodate them. *Aerva* s.str. thus becomes monotypic, comprising only the morphologically divergent *A. javanica* (= *A. tomentosa* Forssk., the type of the genus).

Molecular evidence has indicated for some time that *Aerva* clade II is sister to *Ptilotus* (Sage & al., 2007; Hammer & al., 2015, 2017). All species examined in this clade have shortly bilobed stigmas (Fig. 4E), distinctly different from other aervoids. *Ptilotus* species have unlobed stigmas (Fig. 4F). *Aerva* clade II includes the type of *Ouret* Adans. (*O. lanata* (L.) Kuntze = *A. lanata*). *Ouret* is the earliest genus name in *Aerva* but was rejected when *Aerva* was conserved (Appendix III of the *ICN*, https://naturalhistory2.si.edu/botany/codes-proposals/). With our segregation of *Aerva* into distinct genera, *Ouret* again becomes available as a generic name, and is the earliest available for this clade. We therefore reinstate *Ouret* to accommodate the eight species recognised in *Aerva* clade II.

Taxonomic status of *Kelita*. — *Kelita uncinella* is a prostrate annual herb known from only two populations 6 km apart in central-eastern Queensland, Australia. *Kelita* was described by Bean (2010) as being "closely allied to both *Ptilotus* and *Omegandra*" (p. 105). Bean clearly regarded that *K. uncinella* was most closely related to *Ptilotus*, but he excluded the new species from *Ptilotus* primarily on the basis of it having inconspicuously denticulate leaf margins, zygomorphic flowers, a conspicuous sepal midrib and uncinate sepal apices, none of which he believed occurred elsewhere in *Ptilotus*. He also contrasted

the hard, indehiscent fruit, two fertile stamens, lack of staminal cup appendages, and axillary inflorescences of *Kelita*, all of which he considered to be rare in *Ptilotus*. These comparisons ignore much of the morphological variation within *Ptilotus*. Many species in *Ptilotus* have two fertile stamens (~25 spp.) and many species lack staminal cup appendages (~60 spp.). Leaf margins with small denticulations are also found in *P. polystachyus* (Gaudich.) F.Muell. Zygomorphic flowers occur in various *Ptilotus* spp. (e.g., *P. declinatus*, *P. polystachyus* and *P. sericostachyus* (Nees) F.Muell.), and fruit dehiscence is variable (see below). The only character that is truly unique to *Kelita* is the uncinate sepal apices; this is an autapomorphy within the aervoids, and hence uninformative of relationships.
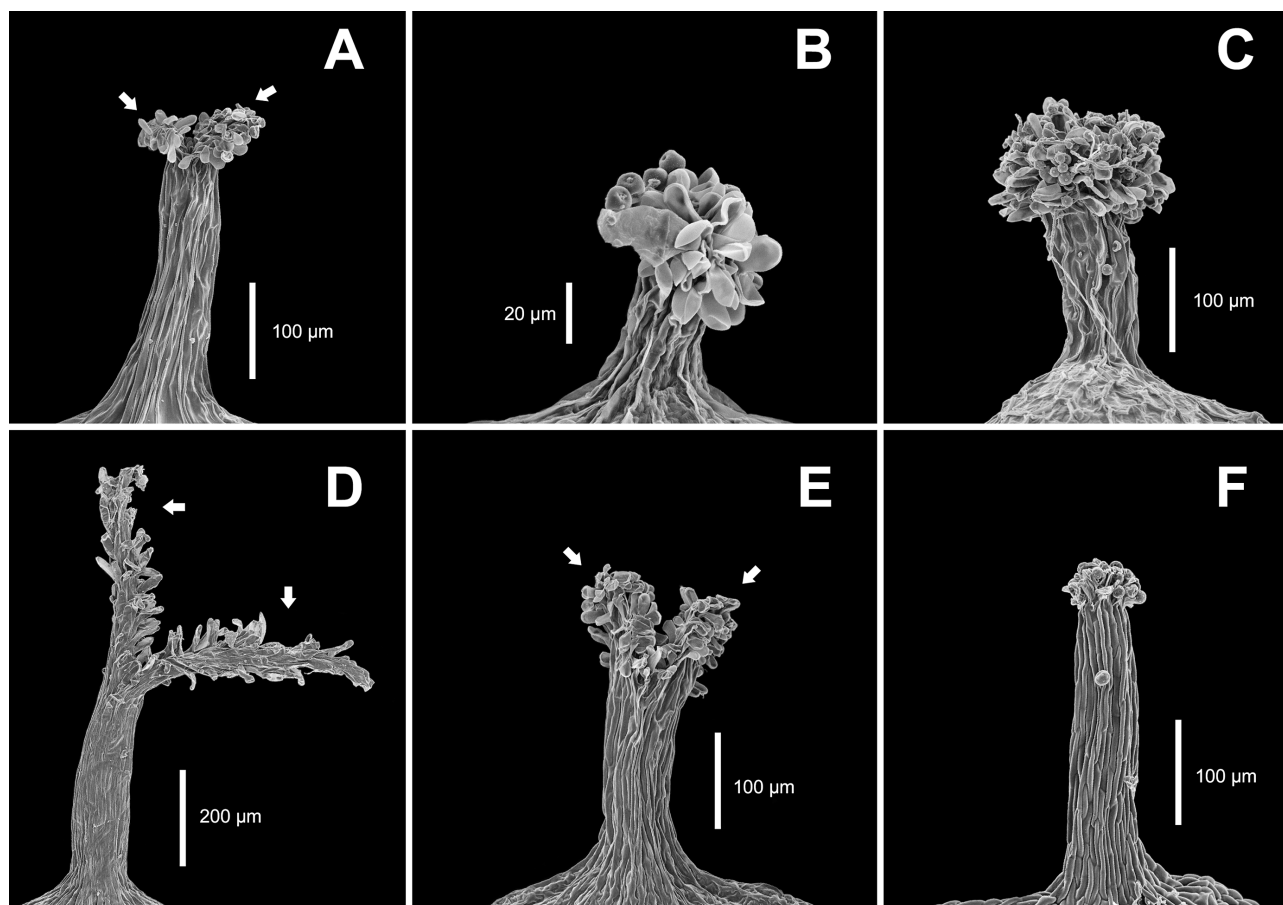
Fig. 4. SEM images of style and stigma morphology in the various clades resolved in the aervoids. **A,** *Omegandra kanisii* (BRI AQ0766448); **B,** *Nothosaerva brachiata* (E 00732828); **C,** *Aerva revoluta* (E 00161902); **D,** *A. javanica* (♀; PERTH 08743924); **E,** *A. lanata* (♀; E 00732832); **F,** *Ptilotus decipiens* (PERTH 08854165). — Arrows indicate lobes on each stigma.

In both the CDS and constrained phylogenies (Figs. 2, 3), *K. uncinella* is nested within clade B of *Ptilotus*. In the latter phylogeny, *K. uncinella* is sister to *P. decipiens* (Benth.) C.A.Gardner, a low annual herb that occurs widely in arid Australia, including Queensland. These species share axillary inflorescences, but instead of uncinate sepal apices, *P. decipiens* has straight-aristate bracteoles and sepals. Sepals of both species have conspicuous midribs. The uncinate sepal apices in *K. uncinella* and aristate apices in *P. decipiens* may aid in dispersal of the fruit, which remains within the persistent sepals, by animals (i.e., epizoochory; Bean, 2010). Most other species of *Ptilotus* have sepals that are densely hairy outside, the hairs aiding wind dispersal of the propagules (Hammer & al., 2015, 2018b). Uncinate sepal apices, probably also to aid in epizoochory, are seen in some species in the achyranthoids (e.g., *Cyathula uncinulata* (Schrad.) Schinz; see Di Vincenzo & al., 2018). *Ptilotus decipiens* and other related species in clade B (e.g., *P. villosiflorus* F.Muell. and *P. chamaecladus* Diels) also have a hard, indehiscent pericarp, as described for *K. uncinella*. Species in some other clades of *Ptilotus* (e.g., *P. helipteroides* (F.Muell.) F.Muell., *P. gaudichaudii* (Steud.) J.M.Black and allies in clade D) also have hardened propagules, but in

these the persistent sepals harden around the enclosed fruit and pinch inward above the ovary to form a tight, indurated covering around the fruit, which has a papery pericarp (see Hammer & Davis, 2018; Hammer & al., 2018a). These are not homologous with the fruits of *K. uncinella* and its close relatives.

Kelita uncinella has a reduced number of fertile stamens (two) compared with other species in clade B (five); however, a reduction in fertile stamen number (to as low as one) is common in the more derived clades D–F (see also Hammer & al., 2015). The position of *Kelita* within clade B of *Ptilotus* is highly supported, both with respect to morphology and the molecular data, and we thus synonymise *Kelita* within *Ptilotus* and make the new combination *Ptilotus uncinellus* (A.R.Bean) T.Hammer below.

TAXONOMIC TREATMENT

The polyphyly of *Aerva* could be resolved by sinking all aervoid genera into a single genus, the correct name of which would be *Aerva*. However, such a genus would be strikingly heterogeneous, and would necessitate the loss of the largest, most recognisable and morphologically distinctive genus, *Ptilotus*. Given that all clades are morphologically diagnosable, we prefer to split *Aerva* s.l. into the three genera discussed above, viz. *Aerva* s.str., *Ouret* and *Paraerva* gen. nov. We believe that this generic treatment provides the best taxonomic and nomenclatural resolution of the aervoids, consistent with their phylogeny, morphology and biogeography.

The following is a list of accepted genera within the aervoids, diagnoses for each genus, lists of accepted species (excluding *Ptilotus*), and new combinations resulting from this study. Critical characters to differentiate the genera are provided in Table 1. The scope of this current work is to resolve the generic relationships and provide a taxonomic framework for future research. A full revisionary study within each genus is forthcoming, including a treatment of *Ptilotus* for the *Flora of Australia* (see https://profiles.ala.org.au/opus/foa).

Table 1. A comparison of characters between each of the genera recognised in the aervoids.

Character	*Omegandra*	*Nothosaerva*	*Paraerva*	*Aerva* s.str.	*Ouret*	*Ptilotus*
Sexuality	Hermaphrodite	Hermaphrodite	Hermaphrodite	Dioecious	Dioecious, gynodioecious or hermaphrodite	Dioecious, gynodioecious or hermaphrodite
Habit	Perennial herbs	Annual herbs	Subshrubs	Perennial herbs or subshrubs	Perennial herbs	Shrubs, annual or perennial herbs
Leaf orientation	Alternate	Alternate or opposite	Alternate	Alternate	Alternate or opposite	Alternate
Inflorescence axis	Sessile	Sessile	Pedunculate	Sessile	Sessile	Pedunculate or sessile
Inflorescence orientation	Axillary	Axillary or terminal	Terminal	Terminal	Axillary or terminal	Axillary or terminal
Sepal No.	4	4	5	5	5	5
Stamen No.	2	2	5	5	5	1–5
Staminodes	Absent	Absent	Absent	Absent	Absent	Absent to 4
Staminal cup appendages	Present	Absent	Present	Present	Present	Absent or present
Stigma lobes	Minutely bilobed	Unlobed	Unlobed	Long-bilobed	Shortly bilobed	Unlobed

Aerva Forssk., Fl. Aegypt.-Arab.: 170. 1775, nom. cons. – Type: *A. tomentosa* Forssk. (= *A. javanica* (Burm.f.) Juss. ex Schult.).

Diagnosis. – Erect dioecious perennial herbs or subshrubs. Leaves alternate, simple, not succulent, obovate to oblanceolate; margins entire. Inflorescences spiciform, cylindrical, sessile, terminal, solitary or in clusters, often terminally paniculate. Cymes 1-flowered, subtended by a bract. Flowers unisexual, subtended by 2 bracteoles. Sepals 5 (3 inner and 2 outer), free. Male flowers lacking developed style and stigma; female flowers lacking developed anthers and filaments. Stamens 5, all fertile, united at their base into a short staminal cup and alternating with short, triangular staminal cup appendages. Ovary slightly laterally compressed; ovule 1; style long, straight, emergent; stigma feathery, deeply bilobed. Capsule 1-seeded, thin-walled, rupturing irregularly.

Distribution. – Occurs in W Africa, E Africa, S Asia, maritime S Asia and N Australia.

Diversity. – 1 species.

Aerva javanica (Burm.f.) Juss. ex Schult. in Roemer & Schultes, Syst. Veg. 5: 565. 1819 ≡ *Iresine javanica* Burm.f., Fl. Indica: 212, t. 65, fig. 2. 1768 ≡ *Illecebrum javanicum* (Burm.f.) L., Syst. Veg., ed. 13: 206. 1774 ≡ *Achyranthes javanica* (Burm.f.) Pers., Syn. Pl. 1: 259. 1805 – Lectotype (designated by Iamonico & Friis in Taxon 66(5): 1210. 2017): [illustration] "*Iresine javanica*" in Burman, Fl. Indica: t. 65, fig. 2. 1768 (image!).

= *Celosia lanata* L., Sp. Pl.: 205. 1753, non *Aerva lanata* (L.) Juss. ex Schult. 1819 – Lectotype (designated by Townsend in Polhill, Fl. Trop. East Afr., Amaranth.: 84. 1985): Sri Lanka. Herb. Hermann Vol. 4: 52 (BM barcode BM000628252 [image!]).

= *Aerva tomentosa* Forssk., Fl. Aegypt.-Arab.: 170. 1775 – Lectotype (designated by Townsend in Polhill, Fl. Trop. East Afr., Amaranth.: 84. 1985): Egypt. Cairo, *Forsskål 918* (C barcode C10001593).

= *Iresine persica* Burm.f., Fl. Indica: 212, t. 65, fig. 2. 1768 ≡ *Ouret persica* (Burm.f.) Kuntze, Revis. Gen. Pl. 2: 544. 1891 ('*Uretia*') ≡ *Aerva persica* (Burm.f.) Merr. in Philipp. J. Sci. 19(3): 348. 1921 – Holotype (vide Townsend in Polhill, Fl. Trop. East Afr., Amaranth.: 84. 1985): Iran. Herb. Burman (G).

Notes. – Schultes ascribed this species to Jussieu (1803), but A.L. de Jussieu only stated that he believed *Illecebrum javanicum* (Burm.f.) L. to be in *Aerva* but did not specifically assign the genus name with the specific epithet, as required in Art. 35.2 in the *ICN* (Turland & al., 2018). Iamonico & Friis (2017) includes a discussion on the nomenclature and lectotypification of *Aerva tomentosa* and *Iresine javanica* Burm.f.

Nothosaerva Wight, Icon. Pl. Ind. Orient. 6: 1. 1853 ≡ *Pseudanthus* Wight, Icon. Pl. Ind. Orient. 5(2): 3. 1852, nom. illeg., non Sieber ex A.Spreng. – Type: *N. brachiata* (L.) Wight.

Diagnosis. – Erect annual herbs. Leaves simple, opposite or alternate, not succulent, narrowly ovate to lanceolate; margins entire. Inflorescences spiciform, cylindrical, terminal or axillary, clustered. Cymes 1-flowered, subtended by a bract. Flowers bisexual, subtended by 2 bracteoles. Sepals 4 (2 inner and 2 outer), free. Stamens 2, both fertile, united at their base into a short staminal cup, opposite the two outer sepals; staminal cup appendages absent. Ovary laterally compressed; ovule 1; style very short, straight; stigma unlobed, capitate. Capsule 1-seeded, thin-walled, rupturing irregularly.

Distribution. – Occurs throughout Sub-Saharan Africa, the Maldives, Mauritius, and southern parts of Asia (from Arabia to Borneo).

Diversity. – 1 species.

Nothosaerva brachiata (L.) Wight, Icon. Pl. Ind. Orient. 6: 1. 1853 ≡ *Achyranthes brachiata* L., Mant. Pl.: 50. 1767 ≡ *Illecebrum brachiatum* (L.) L., Mant. Pl.: 213. 1771 ≡ *Aerva brachiata* (L.) Mart., Beitr. Amarantac.: 83. 1825 ≡ *Pseudanthus brachiatus* (L.) Wight, Icon. Pl. Ind. Orient. 5(2): 3. 1852 –

Lectotype (designated by Townsend in Nasir & Ali, Fl. W. Pakistan 71: 32. 1974): Herb. Linn. No. 290.1 (LINN [image!]).

= *Illecebrum bengalense* L., Mant. Pl.: 213. 1771 ≡ *Achyranthes bengalensis* (L.) Lam., Encycl. 1: 549. 1785 – Lectotype (designated by Iamonico & Friis in Taxon 66(5): 1209. 2017): Herb. Linn. No. 290.18 (LINN [image!]).

Omegandra G.J.Leach & C.C.Towns. in Kew Bull. 48(4): 787. 1993 – Type: *O. kanisii* G.J.Leach & C.C.Towns.

Diagnosis. – Prostrate to decumbent bisexual perennial herbs. Leaves simple, alternate, elliptic to lanceolate; margins entire. Inflorescences spiciform, cylindrical, axillary, solitary or in clusters. Cymes 1-flowered, subtended by a bract. Flowers bisexual, subtended by 2 bracteoles. Sepals 4 (2 inner and 2 outer), free. Stamens 2, both fertile, united at their base into a short staminal cup that is open on the abaxial side with 3 staminal cup appendages alternating with the stamens. Ovary slightly laterally compressed; ovule 1; style straight; stigma minutely bilobed. Capsule 1-seeded, thin-walled, rupturing irregularly.

Distribution. – Occurs in the northern parts of Queensland and the Northern Territory, Australia.

Diversity. – 1 species.

Omegandra kanisii G.J. Leach & C.C.Towns. in Kew Bull. 48(4): 787. 1993 – Holotype: Australia. Northern Territory: South Alligator Floodplain, Kakadu National Park, 7 Aug 1990, *I. Cowie 1334* (DNA barcode DNA-D0053029 [image!]; isotypes: BRI barcode BRI-AQ0511960 [image!], CANB barcode CANB00402446 [image!], K barcode K000587476 [image!]).

Ouret Adans., Fam. Pl. 2: 268, 586. 1763 ('*Uretia*' Kuntze, Revis. Gen. Pl. 2: 544. 1891, orth. var.) – Type (designated by Rickett & Stafleu in Taxon 8: 268. 1959): *O. lanata* (L.) Kuntze.

Diagnosis. – Decumbent to erect bisexual or dioecious annual or perennial herbs. Leaves simple, alternate or opposite, not succulent, ovate, obovate or elliptic; margins entire, sometimes revolute. Inflorescences spiciform, cylindrical, sessile, terminal or axillary, solitary or in clusters. Cymes 1-flowered, subtended by a bract. Flowers unisexual or bisexual, subtended by 2 bracteoles. Sepals 5 (3 inner and 2 outer), free. Male flowers (when unisexual) lacking developed stigma; female flowers lacking developed anthers and filaments. Stamens 5, all fertile, united at their base into a short staminal cup and alternating with short to long, broadly to narrowly triangular staminal cup appendages. Ovary slightly laterally compressed; ovule 1; style straight; stigma shortly bilobed. Capsule 1-seeded, thin-walled, rupturing irregularly.

Distribution. – Occurs in tropical W Africa, S Africa, E Africa, Madagascar, Mauritius and nearby islands, and southern Asia (i.e., including Arabia, India, Indochina and maritime S Asia).

Diversity. – 8 species.

Notes. – Adanson (1763: 586) described *Ouret* based on *Achyranthes lanata* L. and *Celosia lanata* L. Adanson's name was given a latinised form, "*Uretia*", by Rafinesque (1837: 40), who cited *Achyranthes alternifolia* L. (≡ *Uretia alternifolia* (L.) Raf.) as type. *Achyranthes alternifolia* is now an accepted synonym of *Digera muricata* (L.) Mart. subsp. *muricata* by way of *D. alternifolia* (L.) Aschers. (see Miller, 1996: 293). Kuntze (1891: 544) combined several *Aerva* species under "*Uretia*" (again, a latinised orthographic variant of *Ouret*) and cited *Achyranthes lanata* and *Celosia lanata*, presumably unaware of Rafinesque's previous treatment. Hiern (1900: 883) included three of Kuntze's species under *Ouret*, giving Kuntze's "*Uretia*" names as synonyms*.*

The conservation of the name *Aerva* over *Ouret* was proposed by Ascherson (1892: 333; *Ouret*, as "*Oureti*") and Harms (1904: 17) and later ratified in 1905 by the II International Botanical Congress, Vienna (Briquet, 1906: 241). Rickett & Stafleu (1959: 268) later specified *Achyranthes lanata* as the type for *Ouret*. As the name *Ouret* is validly published, it remains the earliest available for our segregate

genus. Species within our concept of *Ouret* that Kuntze (1891) formally included in "*Uretia*" were *O. lanata* (L.) Kuntze, *O. sanguinolenta* (L.) Kuntze, *O. glabrata* (Hook.f.) Kuntze, *O. congesta* (Balf.f. ex Baker) Kuntze and *O. leucura* (Moq.) Kuntze.

Thiv & al. (2006) included multiple accessions of *O. lanata* in their phylogenetic analysis of *Aerva* s.l. and found that some of these grouped with specimens of *O. sanguinolenta* while others grouped with *O. leucura*. A thorough investigation of taxonomic boundaries among these species is warranted, which is outside the scope of the current study. For this reason, we have chosen not to designate lectotypes for the species of *Ouret* at this time.

Ouret congesta (Balf.f. ex Baker) Kuntze, Revis. Gen. Pl. 2: 544. 1891 ('*Uretia*') ≡ *Aerva congesta* Balf.f. ex Baker, Fl. Mauritius: 267. 1877 – Syntypes: Mauritius. "*Rodrigues, only on coral, near the shore*", 1874, *Balfour s.n.* (K barcode K000243711 [image!], M barcode M-0088599 [image!]).

Ouret coriacea (Schinz) T.Hammer, **comb. nov.** ≡ *Aerva coriacea* Schinz in Vierteljahrsschr. Naturf. Ges. Zürich 76: 141. 1931 – Syntypes: Madagascar. "Prov. Tuléar, collines calcaires du Fiherena", *F. Geay 5308* (P barcode P04942221 [image!]); *F. Geay 5308 bis* (P barcode P00347809 [image!]).

Ouret glabrata (Hook.f.) Kuntze, Revis. Gen. Pl. 2: 544. 1891 ('*Uretia*') ≡ *Aerva glabrata* Hook.f., Fl. Brit. India 4(12): 728. 1885 – Syntype: Myanmar. *Griffith 4185* (K barcode K000848082 [image!]).

Ouret humbertii (Cavaco) T.Hammer, **comb. nov.** ≡ *Aerva humbertii* Cavaco in Bull. Soc. Bot. France 99: 253. 1952 – Syntypes: Madagascar. "Vallée de la Manambolo", *H. Humbert 12848* (P barcodes P00346821 [image!] & P00346822 [image!]).

Ouret lanata (L.) Kuntze, Revis. Gen. Pl. 2: 544. 1891 ('*Uretia*') ≡ *Achyranthes lanata* L., Sp. Pl.: 204. 1753 ≡ *Illecebrum lanatum* (L.) L., Mant. Pl.: 344. 1771 ≡ *Aerva lanata* (L.) Juss. ex Schult. in Roemer & Schultes, Syst. Veg. 5: 564. 1819 – Lectotype (designated by Iamonico in Taxon 63(2): 407. 2014): [illustration] "*AMARANTUS spicatus Zeylanicus, foliis obtusis, &c*" in Burman, Thes. Zeylan.: t. 26, fig. 1. 1736 ("1737") (image!).
= *Achyranthes villosa* Forssk., Fl. Aegypt.-Arab.: 48. 1775 – Syntypes: Yemen. Al Hadiyah, Mar 1763, *P. Forsskål 203* (C barcode C10001571 [image!], LD barcode LD1756837 [image!]).
= *Aerva elegans* Moq. in Candolle, Prodr. 13(2): 303. 1849 – Syntypes: Yemen. "Arabie [Arabia] Taifa Mont Moamara", 1838, *P.E. Botta s.n.* (P barcodes P00609991 [image!] & P00609992 [image!]).

Ouret leucura (Moq.) Kuntze, Revis. Gen. Pl. 2: 544. 1891 ('*Uretia*') ≡ *Aerva leucura* Moq. in Candolle, Prodr. 13(2): 302. 1849 ≡ *Illecebrum leucurum* Hort. Berol. ex Moq. in Candolle, Prodr. 13(2): 302. 1849 ≡ *Aerva burchellii* Moq., Prodr. 13(2): 302. 1849 – Syntype: South Africa. Griqualand West: "at Griquatown [Griekwastad] in Leeuwenkuil Valley", 14 Dec 1811, *W.J. Burchell 1892* (K barcode K000243712 [image!]).
= *Aerva ambigua* Moq. in Candolle, Prodr. 13(2): 302. 1849 – Syntype: South Africa. Transvaal: "In Africa australi ad Vaal River", *Burke s.n.* (K barcode K000243714 [image!]).

Ouret sanguinolenta (L.) Kuntze, Revis. Gen. Pl. 2: 544. 1891 ('*Uretia*') ≡ *Achyranthes sanguinolenta* L., Sp. Pl., ed. 2: 294. 1762 ≡ *Illecebrum sanguinolentum* (L.) L., Mant. Pl.: 344. 1771 ≡ *Aerva sanguinolenta* (L.) Blume, Bijdr. Fl. Ned. Ind.: 547. 1826 – Lectotype (designated by Townsend in Nasir & Ali, Fl. W. Pakistan 71: 30. 1974): Herb. Linn. No. 290.3 (LINN [image!]).
= *Achyranthes scandens* Roxb., Fl. Ind. 2: 503. 1824 ≡ *Aerva scandens* (Roxb.) Wight, Icon. Pl. Ind. Orient. 2: t. 724. 1843 ≡ *Aerva scandens* (Roxb.) Moq., Prodr. 13(2): 302. 1849, nom. superfl. ≡ *Aerva scandens* (Roxb.) Wall. ex Hook.f., Fl. Brit. India 4(12): 727–728. 1885, nom. superfl. ≡ *Ouret scandens*

(Roxb.) Hiern, Cat. Afr. Pl. 1: 893. 1900 – Syntype: s. loc., *N. Wallich 6911* (BR barcode BR00000006950354 [image!]).

= *Aerva timorensis* Moq. in Candolle, Prodr. 13(2): 301. 1849 – Syntype: Indonesia. Timor: "Ile de Timor", *J. Decaisne s.n.* (P barcode P00610525 [image!]).

= *Aerva velutina* Moq. in Candolle, Prodr. 13(2): 301. 1849 – Syntypes: Philippines. "Is of Panay", Mar 1837, *H. Cuming 1635* (K barcode K000848079 image!); "Indes orientales", *N. Wallich 833* (P barcodes P00609997 [image!] & P00609998 [image!]).

Ouret triangularifolia (Cavaco) T.Hammer, **comb. nov.** ≡ *Aerva triangularifolia* Cavaco in Bull. Soc. Bot. France 99: 253. 1953 – Syntypes: Madagascar. "Environs de Tuléar", Aug 1919, *H. Perrier de la Bâthie 12790* (P barcodes P00347804 [image!] & P00347805 [image!]).

Paraerva T.Hammer, **gen. nov.** – Type: *P. revoluta* (Balf.f.) T.Hammer (≡ *Aerva revoluta* Balf.f.).

Diagnosis. – Erect bisexual subshrubs. Leaves alternate, succulent or semi-succulent, obovate, simple, discolorous; margins entire, sometimes revolute. Inflorescences spiciform, cylindrical to ovoid, sessile or pedunculate, terminal, solitary or in clusters. Cymes 1-flowered, subtended by a bract. Flowers bisexual, subtended by 2 bracteoles. Sepals 5 (3 inner and 2 outer), free. Stamens 5, all fertile, united at their base into a short staminal cup and alternating with short, triangular staminal cup appendages. Ovary slightly laterally compressed; ovule 1; style short, straight; stigma unlobed, capitate (Fig. 4C). Capsule 1-seeded, thin-walled, rupturing irregularly.

Distribution. – Endemic to the island of Socotra.

Diversity. – 2 species.

Etymology. – From the Greek *para*- ("beside" or "next to") and the genus name *Aerva*, referring to the phylogenetic placement of the new genus with respect to *Aerva*.

Paraerva microphylla (Moq.) T.Hammer, **comb. nov.** ≡ *Aerva microphylla* Moq. in Candolle, Prodr. 13(2): 301. 1849 ≡ *Ouret microphylla* (Moq.) Kuntze, Revis. Gen. Pl. 2: 544. 1891 ('*Uretia*') – **Lectotype (designated here)**: Yemen. Socotra: *Nimmo s.n.* (K barcode K000243701 [image!]).

Paraerva revoluta (Balf.f.) T.Hammer, **comb. nov.** ≡ *Aerva revoluta* Balf.f., Proc. Roy. Soc. Edinburgh 12: 92. 1884 ≡ *Ouret revoluta* (Balf.f.) Kuntze, Revis. Gen. Pl. 2: 544. 1891 ('*Uretia*') – **Lectotype (designated here):** Yemen. Socotra: "in montibus Haghier", Dec 1883, *B.C.S. 478* (K barcode K000243697 [image!]).

Notes. – Of the two specimens cited in the protologue by Balfour (1884), we nominate *B.C.S. 478* (K000243697) as the lectotype. It consists of four flowering woody stems with leaves and matches the protologue. The specimen *Schweinfurth 558* (M-0107324) consists of a single plant with several flowering stems attached to a woody base.

Ptilotus R.Br., Prodr.: 415. 1810 – Type (designated by Iamonico in Phytotaxa 350(2): 151. 2018): *P. corymbosus* R.Br.

= *Trichinium* R.Br., Prodr.: 414. 1810 – Type: none designated.

= *Goniotriche* Turcz. in Bull. Soc. Imp. Naturalistes Moscou 22(3): 37 1849 – Type: *G. tomentosa* Turcz. (= *Ptilotus obovatus* (Gaudich.) F.Muell.).

= *Hemisteirus* F.Muell. in Linnaea 25: 434. 1853 – Type: *H. psilotrichodes* F.Muell. (≡ *Ptilotus modestus* T.Hammer).

= *Dipteranthemum* F.Muell. ex Oliv. in Hooker's Icon. Pl. 16: t. 1541. 1886 – Type: *D. crosslandii* F.Muell ex Oliv. (≡ *Ptilotus crosslandii* (F.Muell. ex Oliv.) Benl).

= *Kelita* A.R.Bean in Muelleria 28(2): 105. 2010, **syn. nov.** – Type: *K. uncinella* A.R.Bean (≡ *Ptilotus uncinellus* (A.R.Bean) T.Hammer).

– "*Arthrotrichum*" F.Muell. in Trans. Bot. Soc. Edinburgh 7: 500. 1863, not validly published (Art. 36.1).

Diagnosis. – Erect, decumbent or prostrate bisexual, rarely dioecious (1 sp.) or gynodioecious shrubs or annual or perennial herbs. Leaves simple, alternate or rarely subopposite, not or rarely succulent, linear to orbicular, concolorous or rarely discolorous; margins entire or rarely denticulate, sometimes revolute or undulate. Inflorescences spiciform, ovoid to cylindrical, sessile or pedunculate, terminal or axillary, solitary or in clusters. Cymes 1-flowered, subtended by a bract. Flowers bisexual or unisexual, subtended by 2 bracteoles, often showy and coloured. Sepals 5 (3 inner and 2 outer), free. Stamens 5, all fertile or 1–4 fertile and the others reduced to inconspicuous or showy staminodes, united at their base into a staminal cup; staminal cup appendages absent or present and alternating with stamens. Ovary slightly laterally compressed; ovule 1; style centrally or eccentrically fixed on the ovary, short or long, straight, curved or sigmoid; stigma unlobed, capitate. Capsule 1-seeded, thin-walled and rupturing irregularly, rarely indurate and indehiscent.

Distribution. – All species are native to continental Australia; some species also occur on adjacent islands (e.g., *P. capensis* (Benl) A.R.Bean extending to the Torres Strait Islands) and Tasmania (i.e., *P. spathulatus* (R.Br.) Poir.), with only *P. conicus* extending outside of Australia to the Lesser Sunda Islands (e.g., Timor) and Aru Islands (i.e., Trangan).

Diversity. – Approximately 120 species.

Notes. – A full list and an electronic identification key to the species of *Ptilotus* is maintained and updated regularly on the online platform KeyBase (available at http://keybase.rbg.vic.gov.au/keys/show/6609). See Hammer & al. (2015) for a discussion on the taxonomic history of *Ptilotus*, *Trichinium* and *Dipteranthemum* F.Muell. ex Oliv. and Hammer & al. (2018a) for discussion of *Hemisteirus* F.Muell. *Ptilotus corymbosus* R.Br., one of the two original species of the genus, was recently designated as the type of the genus name by Iamonico (2018).

Dipteranthemum is sometimes attributed to F.Muell. (in S. Sci. Rec. 3: 281. 1883 or 1884). However, it appears that the relevant portion of Volume 3 of the *Southern Science Record* was never published. Although Mueller distributed "extra-prints" of his own paper to a small number of correspondents this is not regarded as meeting the requirements of effective publication under *ICN* Art. 29. The names were later validly published by Oliver as a descriptio generico-specifica under *ICN* Art. 38.5 (Turland & al., 2018).

Ptilotus uncinellus (A.R.Bean) T.Hammer, **comb. nov.** ≡ *Kelita uncinella* A.R.Bean in Muelleria 28(2): 106–109, fig. 1. 2010 – Holotype: Australia. Queensland: WNW of Glenden [precise locality withheld for conservation reasons], 8 Jun 2009, *A.R. Bean 29009* (BRI barcode BRI-AQ0821100 [image!]; isotypes: CANB barcode CANB00725761 [image!], K barcode K000913312 [image!], L barcode L.3709750 [image!], MEL barcode MEL 2358710 [image!], NY barcode 01842825 [image!]).

AUTHOR CONTRIBUTION STATEMENT

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SUPPLEMENTARY MATERIALS

The following supplementary materials are available as electronic downloads at https://www.taxonomytim.com/thesis (as Chapter 3): **Suppl. Appendix S1.** The alignment of the CDS dataset of 69 plastid genes extracted from the chloroplast genomes of 41 taxa sequenced for this study; **Suppl. Appendix S2.** The concatenated alignment of ITS nrDNA and matK cpDNA for the 135 taxa in this study; **Suppl. Figure S1.** Unconstrained maximum likelihood (RAxML) tree of the ITS+matK dataset. Bootstrap supports are indicated on the branches, and major clades are indicated on the right; **Suppl. Figure S1.** Unconstrained MrBayes 50% majority census tree of the ITS+matK dataset. Posterior probabilities are indicated on the branches, and major clades are indicated on the right.

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Appendix 3.1. Voucher information and GenBank accession numbers for taxa used in the Sanger dataset.

Taxon names follow changes outlined in the taxonomic treatment. Taxon name, collection country (state), collector(s) and collection number (herbarium code), GenBank accession number for ITS (or ITS1 & ITS2) and *matK*. An asterisk (*) indicates newly generated sequences for this study.

Achyranthes aspera L., Australia (Western Australia), *R. Barrett 8308* (PERTH), KP875780, KP875780; ***Aerva javanica*** (Burm.f.) Juss. ex Schult., Australia (Western Australia), *L. Hunt LH 20* (PERTH), KP875878, KP875781; ***Alternanthera pungens*** Kunth, U.S.A., *T. Borsch, D. Pratt & K. Müller 3449* (BONN, ISC), –, AY514795; ***Amaranthus caudatus*** L., Bolivia, *Ames 13860* (BARC), KC747415, KC747132; ***Arthraerua leubnitizae*** Schinz, Namibia (Swakopmund), *D. Long & D. Rae 760* (E), MK013148*, MK013122*; ***Gomphrena flaccida*** R.Br., Australia (Western Australia), *P. Courtney 203* (PERTH) KP875879, KP875782; ***Gomphrena kanisii*** J.Palmer, Australia (Western Australia), *G. Byrne 2773* (PERTH), KP875880, KP875783; ***Nothosaerva brachiata*** (L.) Wight, Burkina Faso, *Anonymous s.n.* (FR), –, AY514806; ***Omegandra kanisii*** G.J.Leach & C.C.Towns., Australia (Northern Territory), *P. Jobson & C. Martine 12480* (NT), MK013149*, MK013123*; ***Ouret congesta*** (Balf.f. ex Baker) Kuntze, Mauritius, *Hansen s.n.* (MAU), AJ829504 & AJ829477, AJ833650; ***Ouret coriacea*** (Schinz) T.Hammer, Madagascar, *Phillipson & Rabes 3141* (WAG), AJ829506 & AJ829506, AJ833651; ***Ouret glabrata*** (Hook.f.) Kuntze, China (Guizhou), *S. Tan 0472* (PE), MK013150*, MK013124*; ***Ouret lanata*** (L.) Kuntze, Yemen (Socotra), *M. Thiv 3118* (STU, Z), AJ829519 & AJ829492, AJ833662; ***Ouret leucura*** (Moq.) Kuntze, Zimbabwe, *Schneller 16* (Z), AJ829522 & AJ829495, AJ833665; ***Ouret sanguinolenta*** (L.) Kuntze, Bhutan, *Grierson & Long 4536* (E), AJ829526 & AJ829499, AJ833668; ***Ouret triangularifolia*** (Cavaco) T.Hammer, Madagascar, *Randrianaivo 362* (MO), AJ829528 & AJ829501, AJ833670; ***Paraerva microphylla*** (Moq.) T.Hammer, Yemen (Socotra), *M. Thiv 3233* (STU, Z), AJ829524 & AJ829497, AJ833666; ***Paraerva revoluta*** (Balf.f.) T.Hammer, Yemen (Socotra), *M. Thiv 3179* (STU, Z), AJ829525 & AJ829498, AJ833667; ***Ptilotus actinocladus*** T.Hammer & R.W.Davis, Australia (Western Australia), *G. Byrne 307* (PERTH) KP875954, KP875857; ***Ptilotus aervoides*** (F.Muell.) F.Muell., Australia (Western Australia), *R. Davis, T. Hammer & B. Anderson RD 12267* (PERTH), KP875881, KP875784; ***Ptilotus albidus*** (C.A.Gardner) Benl, Australia (Western Australia), *K. Thiele 3289* (PERTH), KP875882, KP875785; ***Ptilotus alexandri*** Benl, Australia (Western Australia), *C. Page DHI 78* (PERTH), KP875883, KP875786; ***Ptilotus andersonii*** R.W.Davis, Australia (Western Australia), *S. Patrick 2787* (PERTH), –, MK013125*; ***Ptilotus angustifolius*** T.Hammer, Australia (South Australia), *K. Brewer 895* (PERTH) MK013151*, MK013126*; ***Ptilotus aphyllus*** Benl, Australia (Western Australia), *R. Davis 2007* (PERTH), KP875884, KP875787; ***Ptilotus appendiculatus*** Benl, Australia (Western Australia), *R. Davis 11812* (PERTH), KP875885, KP875788; ***Ptilotus aristatus*** Benl subsp. ***aristatus***, Australia (South Australia), *R. Bates 58592* (AD), KP875886, KP875789; ***Ptilotus aristatus*** subsp. ***micranthus*** Albr. & Lally, Australia (Northern Territory), *G. Leach & M. Leach 1851* (CANB), KP875887, KP875790; ***Ptilotus arthrolasius*** F.Muell., Australia (Western Australia), *R. Davis 11821* (PERTH), KP875888, KP875791; ***Ptilotus astrolasius*** F.Muell., Australia (Western Australia), *R. Davis 11795* (PERTH), KP875889, KP875792; ***Ptilotus auriculifolius*** (A.Cunn. ex Moq.) F.Muell., Australia (Western Australia), *R. Davis 11815* (PERTH), KP875890, KP875793; ***Ptilotus axillaris*** (F.Muell. ex Benth.) F.Muell., Australia (Western Australia), *R. Davis 11793* (PERTH), KP875891, KP875794; ***Ptilotus barkeri*** Benl, Australia (South Australia), *E. James 153* (NT), MK013152*, MK013127*; ***Ptilotus beardii*** Benl, Australia (Western Australia), *R. Davis 11343* (PERTH), KP875892, KP875795; ***Ptilotus beckerianus*** (F.Muell.) F.Muell. ex J.M.Black, Australia (South Australia), *F. Davies 1432* (CBG), KP875893, KP875796; ***Ptilotus benlii*** R.W.Davis & T.Hammer, Australia (Western Australia), *R. Davis 10952* (PERTH), MK013153*, MK013128*; ***Ptilotus blackii*** Benl, Australia (Western Australia), *R. Davis 11966* (PERTH), KP875894, KP875797; ***Ptilotus calostachyus*** F.Muell., Australia (Western Australia), *R. Davis 11816* (PERTH) KP875895, KP875798; ***Ptilotus capensis*** (Benl) A.R.Bean, Australia (Queensland), *D. Crayn & S. Worboys 1346* (CNS), –, MK013129*; ***Ptilotus capitatus*** (F.Muell.) C.A.Gardner, Australia (Western Australia), *G. Byrne 1642* (PERTH), KP875896, KP875799; ***Ptilotus carinatus*** Benl, Australia (Western Australia), *D. Edinger 5125* (PERTH), KP875897, KP875800; ***Ptilotus carlsonii*** F.Muell., Australia (Western Australia), *R. Meissner & R. Coppen 4224* (PERTH), MK013154*, MK013130*; ***Ptilotus chamaecladus*** Diels, Australia (Western Australia), *R. Davis, T. Hammer & B. Anderson RD 12296* (PERTH), KP875898, KP875801; ***Ptilotus chippendalei*** Benl, Australia (Western Australia), *R. Chinnock 572* (AD), KP875899, KP875802; ***Ptilotus chortophytus*** (Diels) Schinz Australia (Western Australia), *R. Davis 10923* (PERTH), KP875900, KP875803; ***Ptilotus chrysocomus*** R.W.Davis, Australia (Western Australia), *J. Williams s.n.* (PERTH) KP875901, KP875804; ***Ptilotus clementii*** (Farmar) Benl, Australia

(Western Australia), *R. Davis 11817* (PERTH), KP875902, KP875805; ***Ptilotus clivicola*** R.W.Davis & T.Hammer, Australia (Western Australia), *C. Payne s.n.* (PERTH), KP875903, KP875806; ***Ptilotus comatus*** Benl, Australia (Northern Territory), *K. Brennan 10363* (DNA), MK013155*, MK013131*; ***Ptilotus conicus*** R.Br., Australia (Western Australia), *R. Barrett & M. Barrett 3134* (PERTH), KP875904, KP875807; ***Ptilotus corymbosus*** R.Br., Australia (Western Australia), *G. Byrne 3400* (PERTH), KP875905, KP875808; ***Ptilotus crispus*** Benl, Australia (Western Australia), *R. Barrett & M. Barrett 3156* (PERTH), KP875906, KP875809; ***Ptilotus crosslandii*** (F.Muell. ex Oliv.) Benl, Australia (Western Australia), *R. Davis, T. Hammer & B. Anderson RD 12286* (PERTH), KP875907, KP875810; ***Ptilotus daphne*** Lally, Australia (Western Australia), *N. Gibson 6505* (PERTH), KP875908, KP875811; ***Ptilotus decalvatus*** Benl, Australia (Western Australia), *P. Chukowry BES PC 039* (PERTH), KP875909, KP875812; ***Ptilotus decipiens*** (Benth.) C.A.Gardner, Australia (Western Australia), *D.J. Edinger 1870* (PERTH), KP875910, KP875813; ***Ptilotus declinatus*** Nees Australia (Western Australia), *R. Davis & P. Jobson RD 12004* (PERTH), KP875911, KP875814; ***Ptilotus disparilis*** Lally, Australia (South Australia), *K. Brewer 722* (AD), MK013156*, MK013132*; ***Ptilotus dissitiflorus*** (F.Muell.) F.Muell., Australia (Northern Territory), *P. Jobson 11869* (NT), –, MK013133*; ***Ptilotus distans*** (R.Br.) Poir., Australia (Western Australia), *R. Barrett 737* (PERTH), KP875912, KP875815; ***Ptilotus divaricatus*** (Gaudich.) F.Muell., Australia (Western Australia), *T. Hammer & R. Davis TH 71* (PERTH), MK013157*, MK013134*; ***Ptilotus drummondii*** (Moq.) F.Muell., Australia (Western Australia), *G. Byrne 2040* (PERTH), KP875913, KP875816; ***Ptilotus eremita*** (S.Moore) T.Hammer & R.W.Davis, Australia (Western Australia), *R. Davis 12114* (PERTH), KP875921, KP875824; ***Ptilotus eriotrichus*** (W.Fitzg. ex Ewart & J.White) P.S.Short, Australia (Western Australia), *I. Fordyce MU 55-37* (PERTH) KP875914, KP875817; ***Ptilotus erubescens*** Schltdl., Australia (South Australia), *K. Graham BS117-1227* (AD), MK013158*, MK013135*; ***Ptilotus esquamatus*** (Benth.) F.Muell., Australia (Western Australia), *R. Davis 11292* (PERTH), KP875915, KP875818; ***Ptilotus exaltatus*** Nees, Australia (Western Australia), *R. Davis, T. Hammer & B. Anderson RD 12295* (PERTH), KP875945, KP875848; ***Ptilotus exiliflorus*** R.W.Davis, Australia (Western Australia), *J. Firth 692* (PERTH), KP875916, KP875819; ***Ptilotus falcatus*** R.W.Davis & T.Hammer, Australia (Western Australia), *R. Warner & S. Werner WWF 12-14* (PERTH), KP875917, KP875820; ***Ptilotus fasciculatus*** W.Fitzg. Australia (Western Australia), *J. Richardson s.n.* (PERTH 07292767), KP875918, KP875821; ***Ptilotus fusiformis*** (R.Br.) Poir., Australia (Western Australia), *R. Davis & R. Butcher RD 11819* (PERTH), KP875919, KP875822; ***Ptilotus gardneri*** Benl, Australia (Western Australia), *R.W. Purdie 4895* (PERTH), KP875920, KP875823; ***Ptilotus gaudichaudii*** (Steud.) J.M.Black, Australia (Western Australia), *R. Davis, T. Hammer & B. Anderson RD 12283* (PERTH), KP875922, KP875825; ***Ptilotus giganteus*** (A.Cunn. ex Moq.) R.W.Davis & R.Butcher, Australia (Western Australia), *K.F. Kenneally K 11831* (PERTH), KP875924, KP875827; ***Ptilotus gomphrenoides*** F.Muell. ex Benth., Australia (Western Australia), *R. Davis, T. Hammer & B. Anderson RD 12280* (PERTH), KP875925, KP875828; ***Ptilotus grandiflorus*** F.Muell., Australia (Western Australia), *M. Trudgen & M. Guest 22546* (PERTH) KP875926, KP875829; ***Ptilotus halophilus*** R.W.Davis, Australia (Western Australia), *I. Fordyce KL 25-1* (PERTH) KP875927, KP875830; ***Ptilotus helichrysoides*** (F.Muell.) F.Muell., Australia (Western Australia), *J. Jackson, A. Brown & B. Moyle DLP 128* (PERTH), KP875928, KP875831; ***Ptilotus helipteroides*** (F.Muell.) F.Muell., Australia (Western Australia), *R. Davis, T. Hammer & B. Anderson RD 12274* (PERTH), KP875929, KP875832; ***Ptilotus holosericeus*** (Moq.) F.Muell., Australia (Western Australia), *R. Davis 11829* (PERTH), KP875930, KP875833; ***Ptilotus humilis*** (Nees) F.Muell., Australia (Western Australia), *R. Davis 12361* (PERTH), KP875931, KP875834; ***Ptilotus incanus*** (R.Br.) Poir., Australia (Western Australia), *P. Hurter WP15* (PERTH), KP875932, KP875835; ***Ptilotus indivisus*** Benl, Australia (New South Wales), *T. Lally 1675* (CANB), MK013159*, MK013136*; ***Ptilotus johnstonianus*** W.Fitzg., Australia (Western Australia), *H. Dauncey H337* (PERTH), KP875933, KP875836; ***Ptilotus kenneallyanus*** Benl, Australia (Western Australia), *G. Byrne 1484* (PERTH), KP875934, KP875837; ***Ptilotus lanatus*** A.Cunn. ex Moq., Australia (Western Australia), *G. Byrne 1349* (PERTH), MK013160*, MK013137*; ***Ptilotus latifolius*** R.Br., Australia (Western Australia), *R. Davis 11791* (PERTH), KP875935, KP875838; ***Ptilotus lazaridis*** Benl, Australia (Western Australia), *P. Hurter s.n.* (PERTH 08406812), KP875936, KP875839; ***Ptilotus leucocoma*** (Moq.) F.Muell., Australia (New South Wales), *R. Purdie 7832* (CANB), KP875937, KP875840; ***Ptilotus lophotrichus*** Benl, Australia (Northern Territory), *K. Brennan 9293* (DNA), MK013161*, MK013138*; ***Ptilotus luteolus*** (Benl & H.Eichler) R.W.Davis, Australia (Western Australia), *S. Kern & D. True 12043* (PERTH), MK013162*, MK013139*; ***Ptilotus maconochiei*** Benl, Australia (Queensland), *D. Kelman 141* (BRI), KP875938, KP875841; ***Ptilotus macrocephalus*** (R.Br.) Poir., Australia (Western Australia), *R. Davis 11787* (PERTH), KP875939, KP875842; ***Ptilotus manglesii*** (Lindl.) F.Muell., Australia (Western Australia), *R. Davis & P. Jobson RD 12005* (PERTH), KP875940, KP875843; ***Ptilotus marduguru*** Benl, Australia (Western Australia), *Sweedman LSJ 6574* (PERTH), KP875941, KP875844; ***Ptilotus mitchellii*** Benl, Australia (Western Australia), *E. Thoma ET 1335* (PERTH), KP875942, KP875845;

Ptilotus modestus T.Hammer, Australia (New South Wales), *R. Purdie 7847* (CANB), KP875923, KP875826; *Ptilotus mollis* Benl, Australia (Western Australia), *J. Bull ONS JSF 338.02* (PERTH), KP875943, KP875846; *Ptilotus murrayi* F.Muell., Australia (Western Australia), *K. Thiele 4583* (PERTH), KP875944, KP875847; ***Ptilotus nobilis*** (Lindl.) F.Muell., Australia (South Australia), *T. Hammer & K. Thiele TH 84* (PERTH), MK013163*, MK013140*; ***Ptilotus obovatus*** (Gaudich.) F.Muell., Australia (Western Australia), *R. Davis, T. Hammer & B. Anderson RD 12268* (PERTH), KP875947, KP875850; ***Ptilotus parvifolius*** (F.Muell.) F.Muell., Australia (South Australia), *D. Duval 17* (AD), –, MK013141*; ***Ptilotus pedleyanus*** Benl & H.Eichler, Australia (Queensland), *J. Silcock JLS680* (BRI), KP875948, KP875851; ***Ptilotus polakii*** subsp. ***juxtus*** Lally, Australia (Western Australia), *R. Davis 11797* (PERTH), KP875949, KP875852; ***Ptilotus polakii*** F.Muell. subsp. ***polakii***, Australia (Western Australia), *R. Davis 11788* (PERTH), KP875950, KP875853; ***Ptilotus polystachyus*** (Gaudich.) F.Muell., Australia (Western Australia), *R. Davis 12276* (PERTH), KP875951, KP875854; ***Ptilotus procumbens*** Benl Australia (Western Australia), *P. Armstrong 06/860* (PERTH), KP875952, KP875855; ***Ptilotus propinquus*** Lally, Australia (South Australia), *H. Vonow 2184* (AD), KP875953, KP875856; ***Ptilotus pyramidatus*** (Moq.) F.Muell., Australia (Western Australia), *C. Tauss 4102* (PERTH), KP875955, KP875858; ***Ptilotus remotiflorus*** Benl, Australia (Queensland), *A. Emmott B689A* (BRI) KP875956, KP875859; ***Ptilotus rigidus*** Lally, Australia (Western Australia), *J. Jackson 290* (PERTH) KP875957, KP875860; ***Ptilotus roei*** (F.Muell. ex Benth.) F.Muell., Australia (Western Australia), *R. Davis, T. Hammer & B. Anderson RD 12277* (PERTH), KP875958, KP875861; ***Ptilotus rotundatus*** Benl, Australia (Northern Territory), *K. Brennan 9109* (DNA), MK013164*, MK013142*; ***Ptilotus rotundifolius*** (F.Muell.) F.Muell., Australia (Western Australia), *R. Davis, T. Hammer & B. Anderson RD 12288* (PERTH), KP875959, KP875862; ***Ptilotus royceanus*** (Benl), Australia (Northern Territory), *P. Jobson 10779* (NT), KP875960, KP875863; ***Ptilotus schwartzii*** (F.Muell.) Tate, Australia (Western Australia), *R. Davis, T. Hammer & B. Anderson RD 12273* (PERTH), KP875961, KP875864; ***Ptilotus semilanatus*** (Lindl.) J.M.Black, Australia (New South Wales), *R. Purdie 7814* (CANB), KP875946, KP875849; ***Ptilotus seminudus*** (J.M.Black) J.M.Black, Australia (Western Australia), *G. Cockerton & N. McQuoid LCH 15892 (*PERTH), KP875962, KP875865; ***Ptilotus sericostachyus*** (Nees) F.Muell., Australia (Western Australia), *R. Davis 11298* (PERTH), KP875963, KP875866; ***Ptilotus sessilifolius*** (Lindl.) Benl, Australia (Western Australia), *R. Cranfield 21749* (PERTH), KP875964, KP875867; ***Ptilotus* sp. Arckaringa** (D.J.Duval 1958), Australia (South Australia), *D. Duval 1958* (AD), –, MK013143*; ***Ptilotus spathulatus*** (R.Br.) Poir., Australia (Western Australia), *W. Thompson & J. Allen 1445* (PERTH), KP875965, KP875868; ***Ptilotus spicatus*** F.Muell. ex Benth., Australia (Western Australia), *G. Byrne 3052* (PERTH), KP875966, KP875869; ***Ptilotus stipitatus*** Benl, Australia (Western Australia), *R. Davis 11184* (PERTH), KP875967, KP875870; ***Ptilotus stirlingii*** subsp. ***australis*** R.W.Davis & R.Butcher, Australia (Western Australia), *R. Davis 10956* (PERTH), KP875968, KP875871; ***Ptilotus stirlingii*** (Lindl.) F.Muell. subsp. ***stirlingii***, Australia (Western Australia), *R. Davis 10956* (PERTH), KP875969, KP875872; ***Ptilotus subspinescens*** R.W.Davis, Australia (Western Australia), *E. Thoma & A. Joder 145* (PERTH), KP875970, KP875873; ***Ptilotus symonii*** Benl, Australia (Western Australia), *K. Thiele 3722* (PERTH) KP875971, KP875874; ***Ptilotus trichocephalus*** Benl, Australia (Western Australia), *R. Davis 10988* (PERTH), KP875972, KP875875; ***Ptilotus uncinellus*** (A.R.Bean) T.Hammer, Australia (Queensland), *B. Thomson s.n.* (BRI AQ0830746), MK013165*, MK013144*; ***Ptilotus villosiflorus*** F.Muell., Australia (Western Australia), *T. Hammer, S. Dillon & K. Thiele TH 24* (PERTH), MK013166*, MK013145*; ***Ptilotus whitei*** (J.M.Black) Lally, Australia (Western Australia), *P. Jobson 10452* (NT), KP875973, KP875876; ***Ptilotus wilsonii*** Benl, Australia (Western Australia), *H. Adjuk HA 75* (PERTH), MK013167*, MK013146*; ***Ptilotus yapukaratja*** R.W.Davis & T.Hammer, Australia (Western Australia), *M. Griffiths & S. Cherriman s.n.* (PERTH 08513848), MK013168*, MK013147*; ***Wadithamnus artemisioides*** (Vierh. & O.Schwartz) T.Hammer & R.W.Davis, Yemen (Al Mahra), *Kilian 6882* (B), AJ829477 & AJ829504, AJ833649.

Appendix 3.2. Voucher information and GenBank accession numbers for taxa used in the CDS dataset. Taxon names follow changes outlined in the taxonomic treatment. Taxon name, collection country (state), collector(s) and collection number (herbarium code), and GenBank accession number. All accessions were newly sequenced for this study.

Achyranthes aspera L., Australia (Western Australia), *S. Dillon & A. Markey CR 9167* (PERTH), MK410010; ***Aerva javanica*** (Burm.f.) Juss. ex Schult., Australia (Western Australia), *T. Hammer, S. Dillon & K. Thiele TH 26* (PERTH), MK410028; ***Alternanthera nodiflora*** R.Br., Australia (Western Australia), *S. van Leeuwen & al. PBS 0410* (PERTH), MK410015; ***Amaranthus cuspidifolius*** Domin, Australia (Western Australia), *B. Mathews & R. Orifici BES BDM 029* (PERTH), MK410001; ***Gomphrena affinis*** subsp. ***pilbarensis*** Kanis ex J.Palmer, Australia (Western Australia), *C. Flaherty & E. Ridley CEF 064* (PERTH), MK410012; ***Gomphrena cunninghamii*** (Moq.) Druce, Australia (Western Australia), *T. Edwards 38* (PERTH), MK410014; ***Omegandra kanisii*** G.J.Leach & C.C.Towns., Australia (Northern Territory), *P. Jobson 12480* (NT), MK410034; ***Ouret glabrata*** (Hook.f.) Kuntze, China (Guizhou), *S. Tan 0472* (PE), MK410031; ***Paraerva microphylla*** (Moq.) T.Hammer, Yemen (Socotra), *A. Miller 10287* (E), MK410032; ***Ptilotus aervoides*** (F.Muell.) F.Muell., Australia (Western Australia), *T. Hammer & S. Dillon TH 28* (PERTH), MK410022; ***Ptilotus appendiculatus*** Benl, Australia (Western Australia), *T. Hammer & S. Dillon TH 50* (PERTH), MK410029; ***Ptilotus arthrolasius*** F.Muell., Australia (Western Australia), *S. Dillon & A. Markey CR 9154* (PERTH), MK410016; ***Ptilotus astrolasius*** F.Muell., Australia (Western Australia), *T. Hammer, S. Dillon & K. Thiele TH 23* (PERTH), MK410023; ***Ptilotus auriculifolius*** (A.Cunn. ex Moq.) F.Muell., Australia (Western Australia), *A.R. Bean 24989* (PERTH), MK410002; ***Ptilotus axillaris*** (F.Muell. ex Benth.) F.Muell., Australia (Western Australia), *S. Dillon & A. Markey CR 9151* (PERTH), MK410008; ***Ptilotus calostachyus*** F.Muell., Australia (Western Australia), *T. Hammer, S. Dillon & K. Thiele TH 18* (PERTH), MK410030; ***Ptilotus chamaecladus*** Diels, Australia (Western Australia), *T. Hammer & S. Dillon TH 53* (PERTH), MK410025; ***Ptilotus clementii*** (Farmar) Benl, Australia (Western Australia), *B. Morgan Bmor 1067* (PERTH), MK409999; ***Ptilotus divaricatus*** (Gaudich.) F.Muell., Australia (Western Australia), *T. Hammer & R. Davis TH 71* (PERTH), MK410020; ***Ptilotus drummondii*** (Moq.) F.Muell., Australia (Western Australia), *T. Hammer & R. Davis TH 75* (PERTH), MK410021; ***Ptilotus erubescens*** Schltdl., Australia (South Australia), *K. Graham BS117-1227* (AD), MK410035; ***Ptilotus exaltatus*** Nees, Australia (Western Australia), *A.R. Bean 25246* (PERTH), MK410003; ***Ptilotus fusiformis*** (R.Br.) Poir., Australia (Western Australia), *S. Dillon & A. Markey CR9156* (PERTH), MK409998; ***Ptilotus gaudichaudii*** (Steud.) J.M.Black, Australia (Western Australia), *S. van Leeuwen 3866* (PERTH), MK410004; ***Ptilotus gomphrenoides*** F.Muell. ex Benth., Australia (Western Australia), *K. McMaster LCH 25882* (PERTH), MK410011; ***Ptilotus helipteroides*** (F.Muell.) F.Muell., Australia (Western Australia), *K. Thiele 4145* (PERTH), MK410007; ***Ptilotus latifolius*** R.Br., Australia (Western Australia), *T. Hammer & S. Dillon TH 49* (PERTH), MK410027; ***Ptilotus macrocephalus*** (R.Br.) Poir., Australia (Western Australia), *B. Morgan Bmor 1076* (PERTH), MK410037; ***Ptilotus mollis*** Benl, Australia (Western Australia), *S. Coultas SC 12* (PERTH), MK410005; ***Ptilotus obovatus*** (Gaudich.) F.Muell., Australia (Western Australia), *A. Douglas & R. Graham LCH 14916* (PERTH), MK410006; ***Ptilotus polakii*** subsp. ***juxtus*** Lally, Australia (Western Australia), *T. Hammer & S. Dillon TH 51* (PERTH), MK410024; ***Ptilotus polystachyus*** (Gaudich.) F.Muell., Australia (Western Australia), *A.R. Bean 25397* (PERTH), MK419082; ***Ptilotus pyramidatus*** (Moq.) F.Muell., Australia (Western Australia), *C. Tauss 4102* (PERTH), MK410036; ***Ptilotus roei*** (F.Muell. ex Benth.) F.Muell., Australia (Western Australia), *R. Davis, T. Hammer & B. Anderson RD 12277* (PERTH), MK410017; ***Ptilotus rotundifolius*** (F.Muell.) F.Muell., Australia (Western Australia), *G. Cassis PILB 202* (PERTH), MK410000; ***Ptilotus schwartzii*** (F.Muell.) Tate, Australia (Western Australia), *B. Vincent BV 108* (PERTH), MK410013; ***Ptilotus subspinescens*** R.W.Davis, Australia (Western Australia), *M. Trudgen & P. Chukowry BES PC 054* (PERTH), MK410009; ***Ptilotus trichocephalus*** Benl, Australia (Western Australia), *R. Davis & F. Obbens RD 12329* (PERTH), MK410018; ***Ptilotus uncinellus*** A.R.Bean, Australia (Queensland), *B. Thomson s.n.* (BRI: AQ0830743), MK410033; ***Ptilotus villosiflorus*** F.Muell., Australia (Western Australia), *T. Hammer, S. Dillon & K. Thiele TH 24* (PERTH), MK410026; ***Ptilotus wilsonii*** Benl, Australia (Western Australia), *H. Adjuk HA 75* (PERTH), MK410019.

Appendix 3.3. Selected specimens examined from the major clades within the aervoids for the morphological examinations. Taxon names follow changes outlined in taxonomic treatment.

Aerva javanica (Burm.f.) Juss. ex Schult. – AFRICA. Egypt, Aswan, Abraq area, 6 March 1986, *Sheded 3254* (E: E00161900). ASIA. Iran, Kerman, Esfandaqeh to Jiroft, 25 km W to Jiroft (Sabzvaran), 11 June 1977, *Assadi & al. 1893* (E: E00161898); United Arab Emirates, Abu Dhabi, Wadi Mubarraz SE of Al Ayn at Zoological Gardens, 19 February 1980, *Edmondson 3055* (E: E00687230). AUSTRALIA. Western Australia, Pilbara, Roadside 100 m E of Turner River, on Great Northern Highway 18 km SW of South Hedland, 18 August 2015, *Hammer & al. TH26* (PERTH: PERTH 08743924). *Nothosaerva brachiata* (L.) Wight – ASIA. India, Punjab, "Karnal Jungle", 29 November 1886, *Drummond 26464* (E: E00732824); Sri Lanka, North Central Province, Anuradhapura District, roadside 100 miles from Kandy on road to Jaffna, S of Punawa, 24 February 1973, *Townsend 73/115* (E: E00732826); E of the road from Dambulla to Anuradhapura [at] milestone 53, 8 March 1973, *Townsend 73/181* (E: E00732828). *Omegandra kanisii* G.J.Leech & C.C.Towns. – AUSTRALIA. Northern Territory, Batten Point, near junction of Batten Creek and McArthur River, 45 km NNE of Borroloola, 13 May 2016, *Jobson & Martine 12480* (NT: D0273611); Queensland, 2 km east of Burketown, 10 May 2003, *Thompson & Newton BUR103* (BRI: AQ 769593); Frog site waterhole, N of Normanton on Burke development road, 30 April 2004, *McDonald KRM2274* (BRI: BRI-AQ766448). *Ouret glabrata* (Hook.f.) Kuntze – ASIA. China, Guizhou Province, Ceheng, Zhe Lou Zhen, 7 August 2004, *Wang 0333* (PE: PE00874853); Zhenfeng, Lianhuan Xiang, 5 June 2005, *Tan 0472* (PE: PE01522637); Yunnan, Mengzi, Jinpo temple, 21 November 2003, *Shui & al. 40525* (PE: PE01869486). *Ouret lanata* (L.) Kuntze – ASIA. Sri Lanka, Anuradhapura District, Aukana, 17 February 1973, *Townsend 73/65* (E: E00732833); Yemen, Socotra Wadi Deneghan, ~7 km SE of Hadiboh, 19 February 1989, *Miller & al. 8246* (E: E00161942). AFRICA. South Africa, Natal [KwaZulu-Natal Prov.], Dunford Native Reserve, 5 September 1970, *Strey 9917* (E: E00732832). *Ouret leucura* (Moq.) Kuntze – AFRICA. Botswana, Ngamiland District, 8.5 km W of Tsau [Tsao], 13 March 1987, *Long & Rae 248* (E: E00161939); Moremi road between south gate and main road, 24 March 1987, *Long & Rae 439* (E: E00161938). *Ouret sanguinolenta* (L.) Kuntze – ASIA. Bhutan, Punakha District, near Wache, Tang Chu, 20 April 1982, *Grierson & Long 4536* (E: E00161940); China, Guangxi, Yangshou Park, 19 October 1988, *Skyes Ch548* (E: E00271791); Taiwan, Pingtung Hsien, Chunjih Hsiang, Tahan forest road, 4 December 1994, *Hsiao & al. 1007* (E: E00025998). *Paraerva microphylla* (Moq.) T.Hammer – ASIA. Yemen, Socotra, Wadi Irih draining S into Nogad plain due S of Hadiboh, 28 February 1989, *Miller & al. 8524* (E: E00161904); Nogad plain, mouth of Wadi Irih, 5 February 1990, *Miller & al. 10287* (E: E00161903). *Paraerva revoluta* (Balf.f.) T.Hammer – ASIA. Yemen, Socotra, Mugadrihon Pass, ~5 km SSW of Hadiboh, 21 February 1989, *Miller & al. 8297* (E: E00161902); Wadi Daneghan, ~10 km SE of Hadiboh, 10 February 1990, *Miller & al. 10377* (E: E00161901); Mugadrihon Pass, 28 January 1994, *Thulin & Gifri 8809* (E: E00687203). *Ptilotus aervoides* (F.Muell.) F.Muell. – AUSTRALIA. Western Australia, Pilbara, Powerline track S off Rio Tinto Rail Access Road ~250 m N of Fortescue River, 19 August 2015, *Hammer & Dillon TH 28* (PERTH: PERTH 08735123). *Ptilotus astrolasius* F.Muell. – AUSTRALIA. Western Australia, Pilbara, 300 m S of intersection with the North West Coastal Highway, 26 km SW of South Hedland, 16 August 2015, *Hammer & al. TH 23* (PERTH: PERTH 08735301). *Ptilotus appendiculatus* Benl – AUSTRALIA. Western Australia, Pilbara, on the roadside of North West Coastal Highway, 14 km SW of Nanutarra Roadhouse, 22 August 2015, *Hammer & Dillon TH 50* (PERTH: PERTH 08743940). *Ptilotus decipiens* (Benth.) C.A.Gardner – AUSTRALIA. Western Australia, Central Ranges, camp 1 at Townsend Ridges, 42 km SE of Warburton, 6 May 2000, *Edinger 1870* (PERTH: PERTH 05619289); 3 km W of Mount Webb, 17 July 2016, *Trickett DD966* (PERTH: PERTH 08854165). *Ptilotus divaricatus* (Gaudich.) F.Muell. – AUSTRALIA. Western Australia, Jibberding Natural Area Reserve parking bay 25 km N of Wubin, 30 August 2015, *Hammer & Davis TH 71* (PERTH: PERTH 08619654). *Ptilotus drummondii* (Moq.) F.Muell. – AUSTRALIA. Western Australia, Chittering, 19.5 km E along Bindoon-Dewars Pool Road from Great Northern Highway, 25 October 2015, *Hammer & Davis TH 75* (PERTH: PERTH 08619476). *Ptilotus erubescens* Schltdl. – AUSTRALIA. South Australia, Barossa, [4.2 km direct WNW of Williamstown], 25 October 2000, *Graham & Kajar BS117-1227* (AD: AD 154486). *Ptilotus exaltatus* Nees – AUSTRALIA. Western Australia, Pilbara, 54 km from Newman on road to Marble Bar, 1 May 2006, *Bean 25246* (PERTH: PERTH 07484038). *Ptilotus helipteroides* (F.Muell.) F.Muell. – AUSTRALIA. Western Australia, De La Poer Range Nature Reserve, Dumbung Soak, 24 April 2011, *Thiele 4145* (PERTH: PERTH 08430225). *Ptilotus mollis* Benl – AUSTRALIA. Western Australia, Pilbara, Ironstone hill slope 270 m ESE of Marble Bar Road, ~55 km SE of Marble Bar and 208 km SE of Port Hedland, 29 April 2013, *Coultas SC 12* (PERTH: PERTH 08568863). *Ptilotus polakii* subsp. *juxtus* Lally – AUSTRALIA. Western Australia, Carnarvon, On track 2 km E of North West Coastal

Highway and 11.4 km SSE of Minilya roadhouse, 22 August 2015, *Hammer & Dillon TH 51* (PERTH: PERTH 08743762). **Ptilotus polystachyus** (Gaudich.) F.Muell. – AUSTRALIA. Western Australia, Mid West region, 80 km N of Mullewa, 29 August 2015, *Hammer & Davis TH 64* (PERTH: PERTH 08619611). **Ptilotus rotundifolius** F.Muell. – AUSTRALIA. Western Australia, Junction of Great Northern Highway and Karijini Drive, 31 August 2005, *Cassis PILB 202* (PERTH: PERTH 07273231). **Ptilotus subspinescens** R.W.Davis – AUSTRALIA. Western Australia, Pilbara, 35.91 km NW Mt Turner, 38.27 km SW Mt Sheila, 62.17 km NW of Tom Price, *Trudgen & Chukowry BES-PC054* (PERTH: PERTH 08377200). **Ptilotus uncinellus** (A.R.Bean) T.Hammer – AUSTRALIA. Queensland [precise localities withheld for conservation reasons], Collinsville, 25 June 2011, *Thomson s.n.* (BRI: BRI-AQ830743); Collinsville, 26 June 2011, *Thomson s.n.* (BRI: BRI-AQ0830746). **Ptilotus villosiflorus** F.Muell. – AUSTRALIA. Western Australia, Pilbara, On a sand dune on Pretty Pool Beach near the "clothing optional" sign, 17 August 2015, *T. Hammer, S. Dillon & K. Thiele TH 24* (PERTH: PERTH 08743886).

PART 2

Resolving the Taxonomy of Two Widespread Species Complexes within *Ptilotus*

The noble and the exalted: a multidisciplinary approach to resolving a taxonomic controversy within *Ptilotus* (Amaranthaceae)

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Abstract. A molecular study on *Ptilotus nobilis* (Lindl.) F.Muell. var. *nobilis* and *P. exaltatus* Nees var. *exaltatus* led to the conclusion that these taxa are conspecific, resulting in the synonymisation of the latter under the former as *P. nobilis* subsp. *nobilis*. In the present study, we test previous taxonomic concepts in the *P. nobilis-P. exaltatus* species group by examining (1) the morphology of specimens in the herbarium and field, and (2) ecological and geographic partitioning of two widespread and broadly sympatric taxa in the group using Maxent and CART models. We provide strong evidence supporting the reinstatement of *P. exaltatus* as distinct from *P. nobilis*, on the basis of multiple morphological characters and strong ecological and geographic partitioning, the latter showing how large-scale ecological data can be used to help resolve taxonomic issues. In addition, we raise *P. nobilis* subsp. *angustifolius* (Benl) Lally & W.R.Barker to the rank of species as *P. angustifolius* (Benl) T.Hammer and reinstate *P. semilanatus* (Lindl.) F.Muell. ex J.M.Black.

INTRODUCTION

Ptilotus R.Br. (Amaranthaceae) comprises ~115 species of annual and perennial herbs and shrubs endemic to Australia, mostly in arid regions, with one species (*P. conicus* R.Br.) that extends outside of Australia to the Lesser Sunda Islands and the Aru Islands (Hammer *et al.* 2015; Hammer *et al.* 2018). Arguably the most iconic member of the genus is *P. nobilis* (Lindl.) F.Muell., commonly known by a variety of names including pussytails, foxtails and tall mulla mulla, which produces spectacular massed floral displays, especially in the Pilbara region of Western Australia, and is cultivated for its cut flowers and as a garden plant (Hentig *et al.* 1995; Lee *et al.* 2008).

As currently circumscribed, *P. nobilis* comprises three subspecies (subsp. *nobilis*, subsp. *semilanatus* (Lindl.) A.R.Bean and subsp. *angustifolius* (Benl) Lally & W.R.Barker), with the previously recognised *P. exaltatus* Nees var. *exaltatus* synonymised under *P. nobilis* subsp. *nobilis* (see Lally and Barker 2010).

Ptilotus nobilis was named by Lindley (in Mitchell 1838: 22; as *Trichinium nobile* Lindl.) and described in the protologue as having cylindrical spikes of yellow (now commonly described as "cream" or "green") flowers (Fig. 1B). *Ptilotus exaltatus* was described by Nees von Esenbeck (1845) as having a cylindrical inflorescence of purple flowers with densely woolly hairs at the base of the interior surface of the tepals (Fig. 1A). Lindley subsequently described *T. semilanatum* Lindl., noting in the protologue its linear leaves and hemispherical inflorescences with relatively few, pink flowers (Mitchell 1848: 45).

Poiret (1816) was the first to recognise that Brown's genera *Ptilotus* and *Trichinium* could not be supported as distinct, treating them instead at sectional level under *Ptilotus* and making the required combinations for a number of *Trichinium* species in *Ptilotus*. Mueller (1868, 1882) followed Poiret's lead and, among other species, synonymised Lindley's *T. nobile* and *T. semilanatum* (with a third species, *T. macrocephalum* Moq.), in *Ptilotus* as *P. nobilis*.

Fig. 1. A. Flowers of *P. exaltatus* var. *exaltatus* (PERTH 08877998) at anthesis. B. Flowers of *P. nobilis* var. *nobilis* (PERTH 08877920) at anthesis. C. *Ptilotus nobilis* var. *nobilis* flowering *en masse* on a stony plain in South Australia (27°46'10.7"S, 134°0'18.8"E). Photographs by T. Hammer.

Bentham (1870), however, retained the genera as distinct, mainly because there were differing views as to which genus name was preferable (both were published simultaneously). Noting the close similarity between *T. semilanatum* and Nees von Esenbeck's *P. exaltatus*, he transferred the latter into *Trichinium* as *T. exaltatum* (Nees) Benth., indicating in a note under *T. exaltatum* that the two species may be better treated as varieties. He placed these two species and *T. nobilis* in different series, *T. nobilis* in ser. *Straminea* and *T. exaltatum* and *T. semilanatum* in ser. *Rhodostachya*. The two series were differentiated on inflorescence colour and tepal indumentum: inflorescences yellow or greenish, tepals without dense woolly hairs inside for ser. *Straminea*; inflorescences pink or red, tepals with dense woolly hairs inside for ser. *Rhodostachya*). While modern phylogenies (e.g. Hammer *et al.* 2015) do not support Bentham's series, the morphological differences he noted between these three species are significant (see below).

Maiden and Betche (1916) synonymised *Trichinium* with *Ptilotus* and reduced *T. semilanatum* to *P. exaltatus* var. *semilanatus* (Lindl.) Maiden & Betche. Black (1924) recognised *Trichinium* as distinct from *Ptilotus* and included *T. semilanatus* as a separate species based on its shorter inflorescences. He later subsumed *Trichinium* into *Ptilotus* and provided the combination *P. semilanatus* (Lindl.) F.Muell. ex J.M.Black (Black 1948). No subsequent authors have reinstated *Trichinium*, and modern phylogenies support treating them as a single genus (see Hammer *et al.* 2015).

Benl (1959) followed Maiden and Betche (1916), considering *P. semilanatus* as merely a variety of *P. exaltatus* (i.e. as *P. exaltatus* var. *semilanatus*) with shorter flowers and inflorescences, without commenting on the vegetative differences between the two taxa noted in Lindley's protologues.
Benl (1959) also named a second variety, *P. nobilis* var. *angustifolius* Benl, citing a smaller stature, bushier habit, much narrower leaves and smaller inflorescences than the typical variety and noting that the differences between *P. nobilis* var. *nobilis* and var. *angustifolius* on the one hand, and *P. exaltatus* var. *exaltatus* and var. *semilanatus* on the other, were in many respects parallel. He regarded that there were transitional specimens between *P. nobilis* var. *nobilis* and var. *angustifolius*.

Benl used flower colour and the presence or absence of dense woolly hairs on the interior surface of the inner tepals to separate *P. nobilis* and *P. exaltatus* (Benl 1959, 1971). A fifth taxon in the group, *P. exaltatus* var. *villosus* Benl, was erected by Benl (1970) for plants from Western Australia with dense hairs on vegetative parts that persist to maturity. He subsequently described a further two varieties within *P. exaltatus*: *P. exaltatus* var. *pallidus* Benl, based on larger, pallid bracts and bracteoles, and shorter inflorescences (Benl 1979), and; *P. exaltatus* var. *glaber* Benl, based on the complete lack of hairs on the bracts, perianth and gynoecium (Benl 1984).

Ptilotus exaltatus var. *exaltatus* and *P. nobilis* var. *nobilis sensu* Benl are widespread in mainland Australia: the former occurring widely in Western Australia, Northern Territory and South Australia, in western Queensland and New South Wales, and in north-western Victoria; the latter occurring mainly in the Northern Territory and South Australia, extending to south-western Queensland and north-western New South Wales. *P. exaltatus* var. *semilanatus sensu* Benl is widespread on the western slopes of the Great Dividing Range in Queensland and New South Wales, extending into the Wimmera region of Victoria and with minor extensions into far-eastern South Australia. It occurs east of the range of, and mostly allopatric to, *P. exaltatus* var. *exaltatus*, although the two taxa appear to co-occur in parts of central New South Wales, central Queensland and northern Victoria. By comparison with these widespread taxa, *P. nobilis* var. *angustifolius sensu* Benl is narrowly distributed in South Australia, along the coastal plain from the southern end of Fleurieu Peninsula to west of the southern Flinders Ranges. It is allopatric with all other taxa, occurring south of the range of *P. nobilis* var. *nobilis*, in an area that receives higher rainfall.

A significant change to the taxonomy of the species group was made by Bean (2008), citing a molecular study by Lee *et al.* (2007) that concluded, based on a phenetic neighbor-joining (NJ) analysis of internal transcribed spacer (ITS) sequences, that *P. nobilis* and *P. exaltatus* were not reciprocally monophyletic. Bean (2008) regarded that the morphological differences used to separate these taxa by previous authors were inconsistent and concluded that the taxa were merely colour morphs of the same species; accordingly, he synonymised *P. exaltatus* var. *exaltatus* under *P. nobilis* subsp. *nobilis*. At the same time, he raised *P. exaltatus* var. *semilanatus* to subspecies rank as *P. nobilis* subsp. *semilanatus*. *Ptilotus nobilis* var. *angustifolius* and *P. exaltatus* var. *villosus* were not dealt with in Bean's treatment (although he commented that the former may be the same taxon as subsp. *semilanatus*); as a result, the latter lacked a published combination under *P. nobilis* and was given the phrase name *P.* sp. Goldfields (R.Davis 10796) at the Western Australian Herbarium, awaiting an investigation of its taxonomic status.

Lally and Barker (2010) subsequently raised *P. nobilis* var. *angustifolius* to subspecies rank, distinguishing it from subsp. *semilanatus* based on differences in perianth and style length. They reduced *P. exaltatus* var. *pallidus* to a taxonomic synonym of *P. nobilis* subsp. *nobilis*, regarding it as falling within the natural range of variability in that taxon. *Ptilotus exaltatus* var. *glaber* was synonymised under *P. nobilis* for the Australian Plant Census (Council of Heads of Australasian Herbaria 2009); anomalously glabrous individuals have been found in normal populations of a number of species in *Ptilotus* (e.g. *P. helipteroides* (F.Muell.) F.Muell.; T. Hammer pers. obs.).

Field and herbarium observations of the members within the *P. nobilis-P. exaltatus* species group led us to critically re-examine the circumscriptions and status of all taxa in the group (hereafter referred to as the *P. nobilis* complex), and particularly to revisit the decision by Bean (2008) to synonymise *P. exaltatus* under *P. nobilis*. Field observations also led us to believe that Benl's taxa *P. nobilis* var. *nobilis* and *P. exaltatus* var. *exaltatus* (subsumed into Bean's *P. nobilis* subsp. *nobilis*) may be ecologically and geographically partitioned, potentially lending support for their recognition as distinct taxa.

During the course of this study we used the general lineage species concept (de Queiroz 1998, 2007; for recent examples, see Anderson *et al.* 2016, 2017) to inform our designation of species boundaries. In this concept, partitions between metapopulation lineages, recognised on the basis of differences in morphological characters, ecology, or nucleotide sequences, can be considered sufficient, but not necessary, evidence for lineage divergence and hence recognition of species. Our re-evaluation of *P. nobilis sensu* Bean has provided consistent evidence supporting the separation of *P. nobilis* var. *nobilis* and *P. exaltatus* var. *exaltatus* based on multiple morphological characters, which in turn has allowed an analysis of habitat preferences and the elucidation, using a geospatial species modelling approach, of distinct ecological partitioning of these two taxa across their combined range. In addition, this study supports the recognition of *P. nobilis* subsp. *angustifolius* and *P. nobilis* subsp. *semilanatus* at species rank, based on morphological and geographical evidence. *Ptilotus* sp. Goldfields (R. Davis 10796) is found to be not distinct from *P. exaltatus*.

MATERIALS AND METHODS

Morphological examinations

To assess morphological variation within the *P. nobilis* complex, specimens from the State Herbarium of South Australia (AD), Australian National Herbarium (CANB), Northern Territory Herbarium at Alice Springs (NT), Queensland Herbarium (BRI), and Western Australian Herbarium (PERTH) were examined, including material from throughout thegeographic ranges of all taxa. Specimens from the New South Wales National Herbarium (NSW) and National Herbarium of Victoria (MEL) were not examined, but material collected from these states was adequately represented in the other herbaria. A total of 1,382 fertile specimens were examined, including 1,224 specimens of *P. nobilis* subsp. *nobilis* (*sensu* Bean 2008 and Lally and Barker 2010, i.e. including Benl's *P. exaltatus* var. *exaltatus*), 42 specimens of *P. nobilis* subsp. *angustifolius*, 53 of *P. nobilis* subsp. *semilanatus* and 63 of *Ptilotus* sp. Goldfields (R.Davis 10796). Voucher specimens used in Lee *et al.* (2007) were borrowed from BRI, critically re-examined and, where necessary, re-determined.

Fieldwork was conducted in South Australia and the Northern Territory in the spring of 2016 to assess patterns of variation in areas where members of the group occur in close proximity, including homogeneity of characters within populations, floral visitor preferences and habitat specificity.

Geospatial analyses

Records of all specimens of *P. nobilis* subsp. *nobilis sensu* Bean (2008) examined in the morphological assessment were obtained from the Australasian Virtual Herbarium (see http://avh.ala.org.au/, accessed 19 May 2017). Specimens with a georeferenced precision ≤1000 m (n=395; Supplementary Material S1) were used in Maximum Entropy Modeling (Maxent; Phillips *et al.* 2004) and Classification and Regression Tree (CART; Breiman *et al.* 1984; see also De'ath and Fabricius 2000) analyses to assess the ecological and geographic partitioning between specimens determined by the herbarium specimen observations to match Benl's concepts of *P. nobilis* var. *nobilis* and *P. exaltatus* var. *exaltatus* across their range. The other currently-recognised subspecies of *P. nobilis* (subsp. *angustifolius* and subsp. *semilanatus*) were not included in the analyses, as these are allopatric to other members of the group and are clearly geographically and environmentally distinct (see below). A set of 35 climate variables (from the CliMond dataset; Kriticos *et al.* 2012) and 10 soil layers (from Grundy *et al.* 2015) were used for predictions. While both Maxent and CART can be used to predict potential distributions of species, there is a critical difference between the methods: Maxent estimates a probability of occurrence of a species at each point in space, while CART attempts to identify environmental conditions that differentiate the target species by recursively partitioning the data into increasingly homogeneous subsets. Maxent can thus identify where a species is unlikely to occur, while CART makes no assumptions on likelihood of occurrence, instead classifying each point in space as being occupied by one or the other species.

Maxent analyses were conducted using Maxent v.3.3.3k (Phillips and Dudík 2008). Prior to analysis, the soil predictive layers were resampled to the same spatial resolution as the CliMond data using ESRI ArcMap v.10.3 (Environmental Systems Research Institute, Redlands, CA, USA). No feature selection was conducted on the environmental data. Multiple occurrence records for each taxon from any given location cell were ignored, leaving 229 and 92 unique records for *P. exaltatus* var. *exaltatus* and *P. nobilis* var. *nobilis,* respectively. 25% of records for each taxon (57 for *P. exaltatus* var. *exaltatus*, 23 for *P. nobilis* var. *nobilis*) were randomly withheld for model testing. All other settings were left as default.

Accuracy of models was assessed using the Area Under Curve (AUC) statistic. The AUC measures how well the created model fits the data by plotting the number of real positive occurrences against the number of false positives (see Fielding and Bell 1997). Two AUC values were generated, one for the training dataset and one for the withheld (test) dataset. Because the model will naturally be better at predicting the data that was used to train it, an AUC test of a random, unseen dataset achieves a more realistic measure of the model's real-world performance.

CART analyses were conducted on the geospatial dataset (Supplementary Material S1) using Salford's Predictive Modeller v.8.0 (Salford Systems, San Diego, CA, USA). Environmental values at each occurrence location were estimated using the Point Sampling Tool in QGIS v.2.16. Prior to construction of the CART model, uninformative predictors were removed using the Random Forests algorithm in Salford's Predictive Modeller, and 20% of the points representing each species were randomly withheld from model construction to use as a validation dataset (62 and 21 records of *P. exaltatus* var. *exaltatus* and *P. nobilis* var. *nobilis* respectively). The final CART tree was converted to an if-then rule set, and eCognition v.9.0 (Trimble, Munich, Germany) was used to predict which species should occur, based on the rule set, for every location within continental Australia. A combined map was constructed from both the Australia-wide CART and Maxent analyses, to compare the modelled distribution in Maxent with the classifications produced by the CART tree and subsequent CART map. As Maxent creates a model of probable occurrence, and CART predicts occurrence based on the environmental variables at each point, we wanted to compare these two analyses. The combined map was constructed by including only those map cells for which Maxent predicted ≥50% probability that each species occurs at the given location.

These grid cells were then coloured based on the species that were assigned by CART. Record occurrences used in the analyses were overlaid on the map for each taxon.

In addition to Australia-wide analyses, a second CART analysis was based on specimens from South Australia only. Most specimens determined as *P. nobilis* var. *nobilis* in this study occur in South Australia, our determined specimens included a thorough sampling from South Australia, and mapping of determined specimens indicated that *P. nobilis* var. *nobilis* and *P. exaltatus* var. *exaltatus* are narrowly parapatric, with their distributions finely interdigitated, in that state. The wider geographic range of *P. exaltatus* var. *exaltatus* may obscure environmental conditions determining the species distributions in this area of overall sympatry. Of the specimens in our morphological study, 69 *P. exaltatus* var. *exaltatus* records and 66 *P. nobilis* var. *nobilis* records were available for this analysis, selected based on georeference precision as for the full analysis. This analysis did not use the climatic variables of the prior CART analysis, as we predicted that edaphic differences were primarily driving the fine-scale interdigitation. All other parameters of the South Australian analysis were identical to the Australia-wide analysis.

RESULTS

Morphology

All fertile specimens within the current concept of *P. nobilis* subsp. *nobilis sensu* Bean could be reliably segregated into the prior concepts of *P. nobilis* var. *nobilis* (n=282) and *P. exaltatus* var. *exaltatus* (n=942), with no intermediates found. The most clear-cut and consistent difference between these taxa is the adaxial indumentum towards the base of the tepals, as previously noted by Nees von Esenbeck (1845), Bentham (1870) and Benl (1959, 1971). In *P. exaltatus* (i.e. both Benl's var. *exaltatus* and var. *semilanatus*), a dense "plug" of crisped, woolly hairs occludes the base of the tepal tube (and hence obscures the ovary; Fig. 1A), while in *P. nobilis* (i.e. both var. *nobilis* and var. *angustifolius*) the indumentum is sparser and comprises straighter, more erect hairs that usually do not occlude the tepal tube or obscure the ovary (Fig. 2C–E). Inflorescences of *P. nobilis* are usually green (sometimes tinged pink in var. *nobilis*), while those of *P. exaltatus* are consistently pink to purple. In the field, two further morphological characters consistently separate the taxa: tepals in *P. exaltatus* consistently gape more widely at anthesis than in *P. nobilis* and are usually more or less straight, while those of *P. nobilis* are distinctively slightly falcately down-curved (Figs 1B, 2D, E)*.* Both features can be discerned on most herbarium specimens, with practice.

Field observations also indicate that the two taxa have different floral scents, with *P. nobilis* var. *nobilis* more strongly sweetly-scented than *P. exaltatus* var. *exaltatus*, particularly at night. Benl (1971) included the floral fragrance of *P. nobilis* as one of the characters separating the species. Other green-flowered *Ptilotus* species that are night-scented, such as *P. macrocephalus* (R.Br.) Poir. and *P. polystachyus* (Gaudich.) F.Muell., are moth-pollinated, and night-flying moths were observed pollinating *P. nobilis* in the field in South Australia (T. Hammer pers. obs.). By contrast, *P. exaltatus* var. *exaltatus* is pollinated by day-flying bees (including European honeybees and the native *Amegilla cingulata*; T. Hammer pers. obs.).
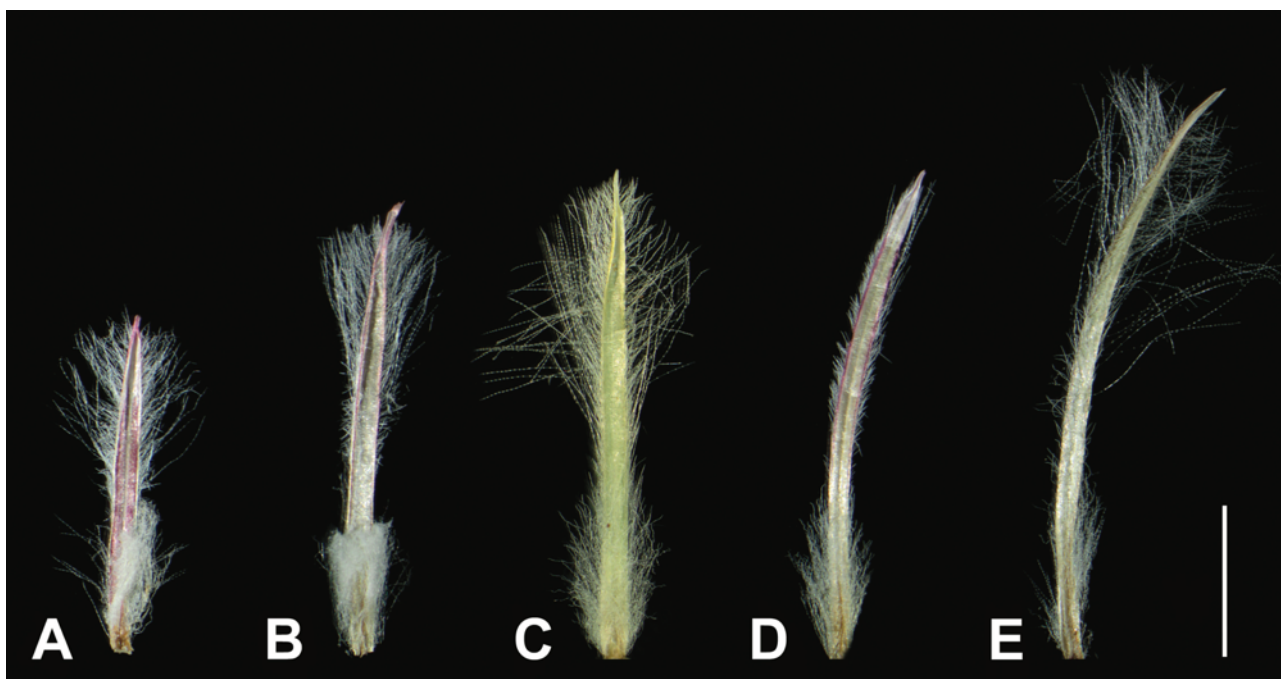
Fig. 2. A, B, D, E. Interior surface of dissected inner tepals of voucher specimens from Lee *et al.* (2007). C. A specimen collected for this study. A. *Ptilotus exaltatus* var. *semilanatus* (BRI AQ751386), B. *P. exaltatus* var. *exaltatus* (BRI AQ699313), C. *P. nobilis* var. *angustifolius* (AD 277212), D. Pink-flowered *P. nobilis* var. *nobilis* (BRI AQ609643) and E. Green-flowered *P. nobilis* var. *nobilis* (BRI AQ751211). The voucher specimens for D & E were re-determined in this study (see Box 1). Scale bar = 5 mm.

Of the eight voucher specimens from Lee *et al.* (2007) that were available for study, two were originally determined as *P. nobilis* var. *nobilis*, five as *P. exaltatus* var. *exaltatus* (four from Queensland and one from Western Australia) and one as *P. exaltatus* var. *semilanatus*. All four of the *P. exaltatus* var. *exaltatus* vouchers from Queensland were determined in our study, based on the morphological characters and concepts of previous authors (discussed above), to be *P. nobilis* var. *nobilis* (Box 1). Other than a varying degree of pinkish hue of the tepals, these vouchers were identical in all respects to those identified by Lee *et al.* (2007) as *P. nobilis* var. *nobilis* (see Fig. 2 for images of tepal indumentum, colour and shape). The two specimens determined as *P. nobilis* var. *nobilis* and the specimen determined as *P. exaltatus* var. *semilanatus*, were correctly identified; the study thus appears to have included only one correctly identified specimen of *P. exaltatus* var. *exaltatus* (i.e. the specimen from Western Australia).

Ptilotus nobilis subsp. *angustifolius* shares the floral characters of *P. nobilis* var. *nobilis,* having cream to green flowers with narrowly-gaping tepals and relatively sparse, straight, erect hairs at the inner base of the tepals (Fig. 2C). As noted by Benl (1959; as var. *angustifolius*) and Lally and Barker (2010), this taxon differs from *P. nobilis* var. *nobilis* in having narrower leaves, shorter inflorescences, and a lower and bushier habit. Field observations of plants growing in South Australia immediately above the strand-line at Sellicks Beach in the south of its range, and inland near Tarcowie in the north of its range (K. Brewer pers. comm.) reveal that, in addition to these differences noted above, it differs substantially from *P. nobilis* var. *nobilis* in being a long-lived, rounded subshrub or tufted perennial herb, with annual flowering stems arising from perennial, woody, branching stems either above-ground or at ground level, and with a branching, woody taproot. *Ptilotus nobilis* var. *nobilis*, by contrast, is an annual with herbaceous flowering stems arising from a short-lived taproot (plants may occasionally persist for a second year under good conditions). In our view the magnitude of these differences is sufficient for recognition of *P. nobilis* subsp. *angustifolius* at species rank, and thus provide the required new combination *P. angustifolius* (Benl) T.Hammer below.

Ptilotus nobilis subsp. *semilanatus* is morphologically close to *P. exaltatus* var. *exaltatus* sharing the purple to pink, relatively straight, widely-gaping tepals with woolly basal indumentum (Fig. 2A). Examination of herbarium specimens clearly showed that, like *P. angustifolius*, it is a long-lived perennial, with annual flowering stems arising from a perennial, branching, woody rootstock (see also Lally and Barker 2010), quite distinct from the annual (rarely short-lived perennial), tap-rooted habit of *P. exaltatus* var. *exaltatus*. It also has distinctively narrow basal leaves 1–5 mm wide with attenuate leaf bases more than half as long as the leaf lamina (leaves 20–60 mm wide with attenuate bases less than a third as long as the leaf lamina in *P. exaltatus* var. *exaltatus*), and usually hemispherical to ovoid inflorescences (cylindrical in *P. exaltatus* var. *exaltatus*) with truncate or obtuse apices (acute in *P. exaltatus* var. *exaltatus*). It has a distribution that is distinct from that of the more arid habitats of *P. nobilis* var. *nobilis* and *P. exaltatus* var. *exaltatus*, as well as *P. angustifolius*. The combination of floral and vegetative characters and its geographic disjunction lead us to reinstate it at the rank of species, as *Ptilotus semilanatus* (Lindl.) F.Muell. ex J.M.Black.

Specimens of *P.* sp. Goldfields (R. Davis 10796) have a leaf and stem indumentum that is denser and more persistent than is typical for *P. exaltatus* var. *exaltatus*. However, both herbarium and field observations showed that indumentum density and persistence are not consistent characters, with many individuals within single populations having a variable degree of indumentum persistence, and with pubescence grading continuously between dense and sparse. For this reason, we consider that *P.* sp. Goldfields comprises part of the normal range of variation of *P. exaltatus s.str.*

Maxent distribution models

Maxent models for *P. exaltatus* var. *exaltatus* and *P. nobilis* var. *nobilis* performed very well with Australia-wide training data, returning an AUC of 0.938 for *P. exaltatus* var. *exaltatus* and 0.951 for *P. nobilis var.* nobilis. Testing the models using the withheld records reduced the AUC values to 0.780 and 0.915 respectively, indicating that the predicted distributions had good support with both known and withheld occurrences.

The predictive model for *P. exaltatus* var. *exaltatus* was primarily driven by the climatic variables annual mean moisture index (Bioclim 28; contribution = 21.5%), radiation of warmest quarter (Bioclim 26; contribution = 13.3%) and radiation seasonality (Bio23; contribution = 8.5%). The model for *P. nobilis* var. *nobilis* was driven by radiation of wettest quarter (Bioclim 24; contribution = 36.8%), the soil layer "level of sand" (Sand; contribution = 11.8%) and soil pH (pH; contribution = 7.9%).

Fig. 3. Australia-wide geospatial analyses of potential species within current *Ptilotus nobilis* subsp. *nobilis*; A. Maxent model for *P. nobilis* var. *nobilis*; B. Maxent model for *P. exaltatus* var. *exaltatus*.

The predicted occurrence map from the Maxent model of *P. nobilis* var. *nobilis* (Fig. 3A) indicates a high probability of occurrence in parts of inland South Australia, western New South Wales and south-western Queensland. Other areas of high probability of occurrence are concentrated around Alice Springs, Northern Territory and along the Northern Territory-South Australia border, extending slightly across the border into Western Australia (e.g. near the Cavenagh Range). The South Australian coastal plain between Adelaide and Port Augusta, where *P. angustifolius* is endemic, was not predicted as probable habitat for *P. nobilis* var. *nobilis*.

The predicted distribution of *P. exaltatus* var. *exaltatus* (Fig. 3B) is noticeably different to that of *P. nobilis* var. *nobilis*, with high probabilities of occurrence across much of Western Australia, in South Australia mostly excluding the northeast and mesic south and southeast, in the Northern Territory south of the Top End, and in southwestern New South Wales. Some areas of high probability for *P. nobilis* var. *nobilis*, such as northeastern and parts of central South Australia, are not predicted for *P. exaltatus* var. *exaltatus*. *Ptilotus exaltatus* var. *exaltatus* is noticeably absent from higher rainfall areas of southern and eastern Australia. The western slopes of the Great Dividing Range, where *P. semilanatus* occurs, were not included in the predicted distribution of *P. exaltatus* var. *exaltatus.* Some areas where *P. exaltatus* var. *exaltatus* is known to occur (e.g. near-coastal areas of the Kimberley) were not predicted in this model due to a lack of representative records in our dataset (i.e. no records met the coordinate uncertainty criterion). The predicted distribution can thus be considered a slightly conservative model of the true distribution of *P. exaltatus* var. *exaltatus*.

Australia-wide CART analysis

Feature selection for the CART analysis using the Random Forest process removed 37 environmental variables as uninformative, leaving eight informative variables that were used to construct the final tree (Table 1). The optimum CART tree (as selected by lowest relative error) had 18 terminal nodes with an accuracy of 86%. As CART is a data-hungry algorithm that over-splits trees, the optimal tree was manually pruned to 13 nodes with an accuracy of 84% (Supplementary Material S2).

Records of *P. exaltatus* var. *exaltatus* are more environmentally heterogeneous than those of *P. nobilis* var. *nobilis* (as expected given the wider geographic range of the former). *Ptilotus nobilis* var. *nobilis* records are in general associated with a moisture index seasonality ≤0.19 (Bio31; n=80, 85%) and soils of pH >5.68 (n=77, 82%). There are two major environmental groups within the training records of *P. exaltatus* var. *exaltatus*. The first group (n=182, 83%) is associated with moisture index seasonality >0.19; this group is almost completely separate from any records of *P. nobilis* var. *nobilis* (i.e. except for 14 records). The second group (n=36, 17%) share lower moisture index seasonality with *P. nobilis var. nobilis*.

To generate distribution maps based on the CART tree (Fig. 4A), areas were scored dichotomously as being probable habitat for either *P. nobilis* var. *nobilis* or *P. exaltatus* var. *exaltatus* (but not both). The map concurs with Maxent in predicting a widespread *P. exaltatus* var. *exaltatus*, with *P. nobilis* var. *nobilis* more restricted in east-central Australia. The combination map of the Maxent and CART models (Fig. 4B) shows areas of probability of occurrence ≥50% for each species from Maxent, with the associated species distribution from the CART analyses. Both Maxent and CART show ecological partitioning of these taxa and have high fidelity with the original records.

Table 1. Climatic and edaphic variables (from Kriticos *et al.* 2012 and Grundy *et al.* 2015 respectively) used in the geospatial analyses and a description for each. Abbreviations indicated in parentheses next to the variable name.

Variable name	Description
Bio01	Annual mean temperature (°C)
Bio02	Mean diurnal temperature range (mean[period max-min]) (°C)
Bio03	Isothermality (Bio02 ÷ Bio07)
Bio04[A]	Temperature seasonality (C of V)
Bio05	Max temperature of warmest week (°C)
Bio06	Min temperature of coldest week (°C)
Bio07	Temperature annual range (Bio05-Bio06) (°C)
Bio08	Mean temperature of wettest quarter (°C)
Bio09[A]	Mean temperature of driest quarter (°C)
Bio10	Mean temperature of warmest quarter (°C)
Bio11	Mean temperature of coldest quarter (°C)
Bio12	Annual precipitation (mm)
Bio13	Precipitation of wettest week (mm)
Bio14	Precipitation of driest week (mm)
Bio15	Precipitation seasonality (C of V)
Bio16	Precipitation of wettest quarter (mm)
Bio17	Precipitation of driest quarter (mm)
Bio18	Precipitation of warmest quarter (mm)
Bio19	Precipitation of coldest quarter (mm)
Bio20	Annual mean radiation (W m^{-2})
Bio21	Highest weekly radiation (W m^{-2})
Bio22	Lowest weekly radiation (W m^{-2})
Bio23	Radiation seasonality (C of V)
Bio24[A]	Radiation of wettest quarter (W m^{-2})
Bio25[A]	Radiation of driest quarter (W m^{-2})
Bio26	Radiation of warmest quarter (W m^{-2})
Bio27	Radiation of coldest quarter (W m^{-2})
Bio28	Annual mean moisture index
Bio29[A]	Highest weekly moisture index
Bio30	Lowest weekly moisture index
Bio31[A]	Moisture index seasonality (C of V)
Bio32	Mean moisture index of wettest quarter
Bio33	Mean moisture index of driest quarter
Bio34	Mean moisture index of warmest quarter
Bio35	Mean moisture index of coldest quarter
Bulk Density (BD)[B]	Bulk Density of the whole soil (including coarse fragments) in mass per unit volume by a method equivalent to the core method
Soil Organic Carbon (SOC)[B]	Mass fraction of carbon by weight in the < 2 mm soil material as determined by dry combustion at 90° C
Clay[A]	< 2 μm mass fraction of the < 2 mm soil material determined using the pipette method
Silt[B]	2–20 μm mass fraction of the < 2 mm soil material determined using the pipette method
Sand	20 μm–2 mm mass fraction of the < 2 mm soil material determined using the pipette method
pH[A]	pH of 1:5 soil/0.01M calcium chloride ($CaCl_2$) extract
Available Water Capacity (AWC)[B]	Available water capacity computed for each of the specified depth increments
Total Nitrogen (TN)	Mass fraction of total nitrogen in the soil by weight
Total Phosphorus (TP)[B]	Mass fraction of total phosphorus in the soil by weight
Effective Cation Exchange Capacity (ECEC)[B]	Cations extracted using barium chloride ($BaCl_2$) plus exchangeable H + Al

[A] Variable informative in the Australia-wide CART analysis.

[B] Edaphic variable that was informative in the South Australian CART analysis.
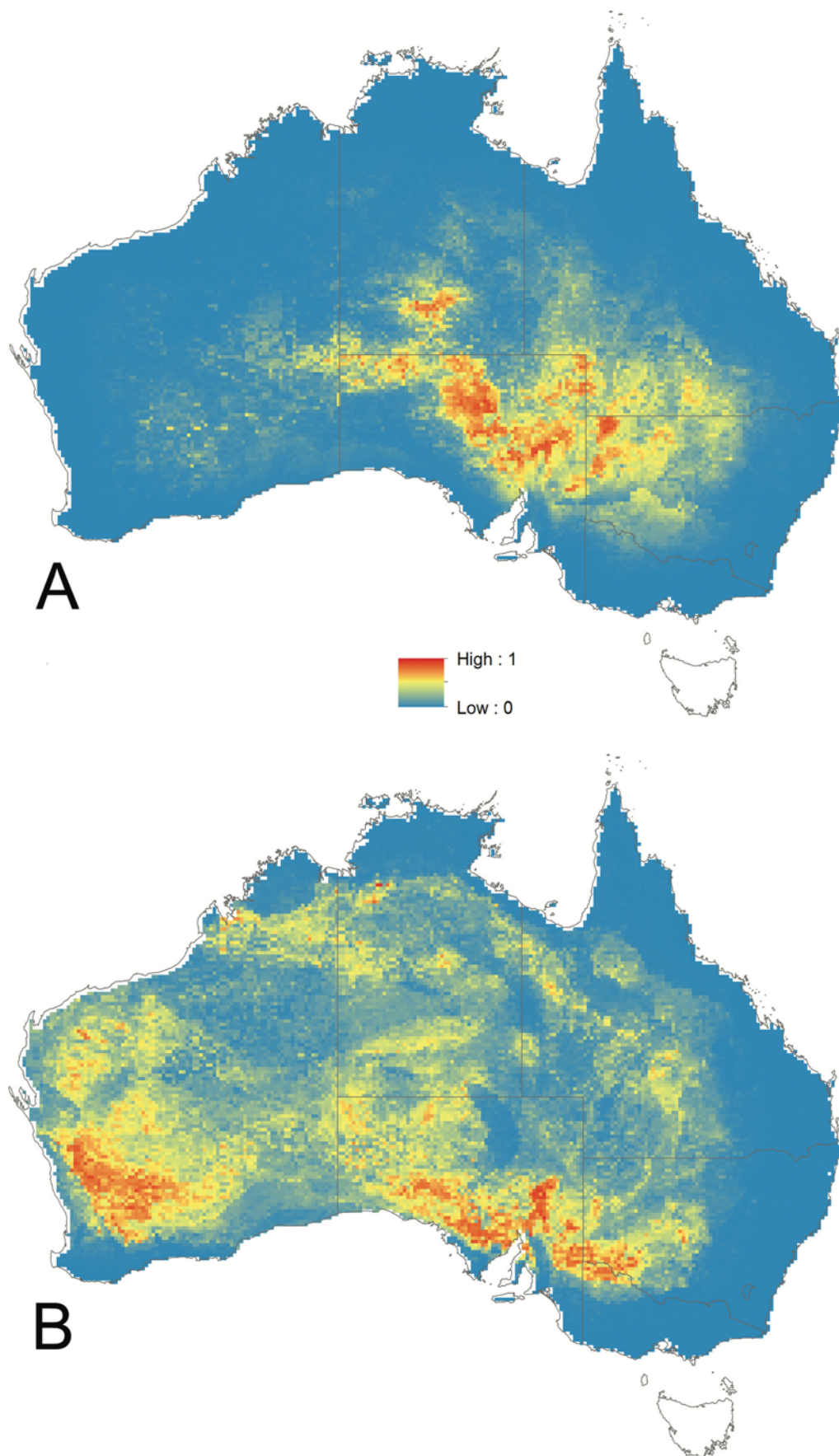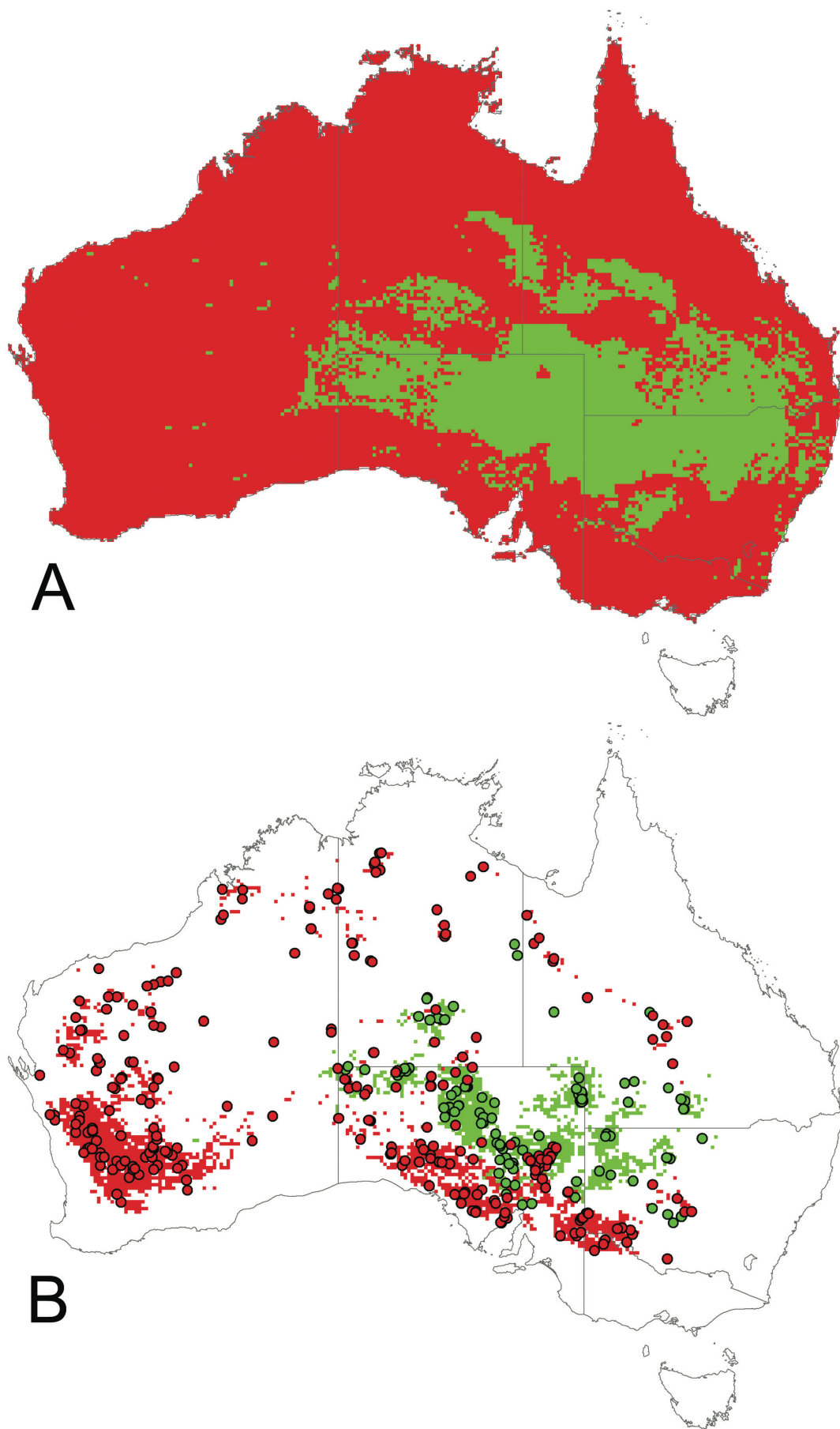
Fig. 4. Australia-wide geospatial analyses of potential species within current *Ptilotus nobilis* subsp. *nobilis*: A. CART analysis of *P. nobilis* var. *nobilis* (green) and *P. exaltatus* var. *exaltatus* (red); B. Combined Maxent and CART analysis of *P. nobilis* var. *nobilis* (green) and *P. exaltatus* var. *exaltatus* (red).

All analyses indicate that the two taxa are geographically partitioned at both coarse and fine scales. The Maxent models predict that *P. nobilis* var. *nobilis* is unlikely to occur in Western Australia, other than in an area close to the Northern Territory/South Australia border. One collection of *P. nobilis* var. *nobilis* was identified occurring within the predicted distribution in Western Australia, at the foot of the Cavenagh Range (PERTH 05585813), the only currently known record of this taxon from Western Australia. The analyses also show that in some areas, particularly in South Australia, the taxa are likely to be broadly sympatric but narrowly parapatric and locally partitioned by fine-scale soil patterns. This was confirmed in the field by observations along the Stuart highway in northern South Australia (e.g. the specimens *T. Hammer & K.Thiele TH87–TH90*) where the two taxa (readily discriminated morphologically, with no intermediates) occupied different parts of the landscape, with *P. exaltatus* var. *exaltatus* on lighter, coarser-textured soils in lowlands and along ephemeral watercourses and *P. nobilis* var. *nobilis* on heavier, finer-textured soils on local stony uplands (Fig. 1C). The same pattern is likely to occur in the Flinders Ranges where the two species co-occur (this could not be confirmed in the field due to poor seasonal conditions there during the period of this study but is consistent with herbarium label data from this area). Similar fine-scale patterning is possible in areas of Queensland and New South Wales, where the species are narrowly parapatric and are predicted by the Maxent modelling to co-occur.

South Australia CART analysis

Soil variables were used exclusively in the CART model constructed for the South Australian records (Fig. 5). Feature selection removed four uninformative variables leaving six variables (Table 1) that were considered informative for the model. The optimum CART tree (as selected by lowest relative error) had eight terminal nodes with an accuracy of 84%. Inspection of the tree showed that it was not significantly over-fitted, hence it was not pruned (Supplementary Material S3).

Fig. 5. The South Australian CART map using soil variables showing the cells classified as *Ptilotus exaltatus* var. *exaltatus* (black) and *P. nobilis* var. *nobilis* (grey).

As with the Australia-wide analyses, *P. exaltatus* var. *exaltatus* occurs across a wider range of soil conditions than *P. nobilis* var. *nobilis*. Associated with this heterogeneity, two major environmental groups were identified within *P. exaltatus* var. *exaltatus,* with 55% (n=38) of the records from areas with soils with organic carbon higher than 0.76 and the remaining records from areas with soils with lower organic carbon levels. The records from areas associated with lower organic carbon show further heterogeneity with two major groupings present: 61% (n=19) of the records are from areas having an available water capacity (AWC) of ≤13.48 while the remaining records are from areas with higher AWC. *Ptilotus nobilis* var. *nobilis* is much more homogenous, with 70% (n=46) of the records being from regions with an organic carbon level ≤0.76 and an AWC >13.48. Of the remaining records, only 9% (n=6) are from areas with high organic carbon and the remaining 21% (n=14) are from areas with a lower AWC.

DISCUSSION

The taxonomic status of P. exaltatus *and* P. nobilis

Bean (2008), in uniting *P. exaltatus* and *P. nobilis*, noted their traditional morphological separation based on tepal indumentum but regarded that this feature was "not consistent" (p. 241). He ascribed a supposed difference between the two taxa in the "odour of the leaves" to Benl (1971), with no further comment. Regarding flower colour, he noted that populations of mixed colour had been observed, citing "M. Johnston pers. comm.". With all morphological features apparently inconsistent and variable, Bean (2008) stated that he relied largely on the "strong genetic evidence" (p. 241) provided by Lee *et al.* (2007) to merge the two taxa.

Bean's comment that tepal indumentum is "inconsistent" leaves open the question as to what it is inconsistent with; presumably, he regarded that tepal indumentum and flower colour were not correlated. However, our assessment of herbarium specimens from throughout the range of the species shows that tepal indumentum and flower colour are highly consistent, with a woolly tepal base being closely associated with pink flowers and a non-woolly tepal base with greenish flowers, as noted by numerous authors since Bentham (1870). Moreover, tepal indumentum alone allowed all fertile specimens to be assigned to one of the two taxa, with no intermediates or specimens of uncertain placement. Field observations support this, including in parts of South Australia where the two taxa are closely parapatric (see above).

Herbarium and field observations show that flower colour, while generally a reliable indicator of species, is somewhat variable in both *P. exaltatus* and *P. nobilis*, varying in the former from pale to deep pink or purple, and in the latter generally cream-green but with some individuals pale pink, especially noticeable at the tepal apices. Similar colour variations in otherwise mostly greenish-coloured flowers have also been noted in *P. macrocephalus* and *P. polystachyus*. It is unclear whether the mixed populations noted by M. Johnston (cited in Bean 2008 and discussed in Lee *et al.* 2007) comprise slight natural variability within *P. nobilis,* misidentifications (see below), or cases where the two taxa co-occur either in mixed or adjacent stands.

The comment in Bean (2008) regarding leaf odour appears to be derived from a similar comment in Lee *et al.* (2007: 72): "Benl (1971) distinguishes the two species by the pungent odour of the leaves". However, there is no reference to a "pungent [leaf] odour" as a character for these species in Benl (1971). Benl (1988), in an unpublished manuscript for the *Flora of Australia*, did use "pungent leaves" (along with tepal indumentum) to segregate *P. exaltatus* and *P. nobilis* in his key to species (p. 14). We believe, however, that Lee *et al.* (2007) and Bean (2008) misinterpreted Benl's use of "pungent"; Benl is clearly referring to pungent (sharp) leaf *apices*, not odour, as elucidated in his species descriptions (e.g. Benl

1988: 45–47). Leaves of both *P. nobilis* and *P. exaltatus* have a distinct, hard, pungent, fragile, shortly mucronate apex, which is usually visible on young leaves but often lost from older ones. In both species, the leaves have a faintly herbaceous smell both fresh and dried.

In citing "strong genetic evidence" as support for the conspecificity of *P. nobilis* and *P. exaltatus*, Bean (2008) failed to take into account a number of manifest inadequacies in the study of Lee *et al.* (2007). Firstly, the study was poorly designed for answering a question of species delimitation, with substantially inadequate population sampling. Only three specimens identified as *P. nobilis*, from a single population, were compared to 15 specimens of *P. exaltatus* (ten identified as var. *exaltatus*, three as var. *semilanatus*, and two as var. *villosus*). It is unlikely that such a small sample would be able to adequately characterise the taxa. Secondly, a misunderstanding of flower colour variation within *P. nobilis* led to four specimens being incorrectly assigned to *P. exaltatus*. Of the 18 *P. nobilis* and *P. exaltatus* specimens used in the analysis, 11 were unvouchered and therefore their identities cannot be assessed, highlighting the importance of vouchering specimens for use in molecular studies.

Finally, ITS is a poor marker for answering questions of species delimitation in *Ptilotus*. In the ITS phylogeny reconstructed by Hammer *et al.* (2015), relationships between clearly distinct species within the closely-related *P. polakii* clade (e.g. *P. polakii* F.Muell., *P. propinquus* Lally, *P. remotiflorus* Benl, *P. rigidus* Lally, and *P. whitei* (J.M.Black) Lally) could not be resolved due to a lack of sequence divergence. Similarly the sister species pairs *P. rotundifolius* (F.Muell.) F.Muell. and *P. marduguru* Benl, *P. clementii* (Farmar) Benl and *P. gardneri* Benl, and *P. falcatus* R.W.Davis & T.Hammer and *P. clivicola* R.W.Davis & T.Hammer have no or few base pair differences despite being morphologically distinct. ITS appears to be relatively slowly-evolving in *Ptilotus* in the timeframe relevant to lineage divergence of closely related and probably recent species groups, such as the *P. nobilis* complex. Future attempts to resolve species boundaries within this complex using molecular data should take this into account by judiciously choosing molecular markers, employing broader sampling within populations and across geographic ranges, and utilising multiple species delimitation analyses (for relevant discussions see de Queiroz 2007; Carstens *et al.* 2013).

Contribution of geospatial analyses

The geospatial analyses confirm that *P. exaltatus* var. *exaltatus* and *P. nobilis* var. *nobilis*, as delimited morphologically on the basis of tepal indumentum, are ecologically partitioned. In South Australia, where these species are narrowly parapatric, the CART tree using only edaphic variables segregated most records of *P. nobilis* var. *nobilis* from those of *P. exaltatus* var. *exaltatus* based on the former occurring on soils with less soil organic carbon and higher available water capacity. These variables are consistent with *P. nobilis* var. *nobilis* occurring on soils that are more sparsely vegetated and heavier. When the distributions are compared to soil landscapes from the Digital Atlas of Australian Soils (see http://data.bioregionalassessments.gov.au/dataset/9e7d2f5b-ff51-4f0f-898a-a55be8837828; accessed 20 November 2017), *P. nobilis* var. *nobilis* occurs predominantly on stony plains or tablelands with crusty loamy soils (e.g. Dr1.13 and Dr1.33). These landscapes are typically sparsely vegetated, and this was confirmed by field observations in South Australia, where we collected *P. nobilis* var. *nobilis* in this habitat (see Fig. 1C), and observations from the Northern Territory (D. Albrecht pers. comm.). Such soils are likely to have lower organic carbon (being more sparsely vegetated) and higher available water capacity (being heavier-textured), consistent with the CART analysis. *Ptilotus exaltatus* var. *exaltatus* occurs in a wide variety of landscapes and soil types, including those in which *P. nobilis* var. *nobilis* does not occur in our dataset, such as on sandy or calcareous soils, and laterites.

Our analyses show that *P. nobilis* var. *nobilis* has a narrower ecological and environmental range than the more variable and widely distributed *P. exaltatus* var. *exaltatus*. Potential explanations include *P.*

nobilis var. *nobilis* being relatively recently derived from a widespread, ancestral *P. exaltatus* var. *exaltatus*, or *P. exaltatus* var. *exaltatus* having an adaptive advantage that has allowed it to occupy a wider range of habitats. Within the clade of *Ptilotus* that includes *P. exaltatus* var. *exaltatus* and *P. nobilis* var. *nobilis*, (D4; Hammer *et al.* 2015) pink flowers with a woolly indumentum are probably plesiomorphic, lending support to the hypothesis that *P. nobilis* var. *nobilis* is derived. Genetic isolation resulting from the ecological partitioning of *P. nobilis* var. *nobilis* may have allowed compounding selection pressures (e.g., from different pollinators), to influence speciation, as evidenced by the differences in floral morphology and scent. The evolution of pollination syndromes and niche preferences within *Ptilotus* will be topics of subsequent papers.

This study is an example of the use of large-scale ecological datasets to support taxonomic decisions underpinned by classical morphological evidence, and of the reciprocity of ecology and morphology. Morphological segregation of *P. exaltatus* var. *exaltatus* from *P. nobilis* var. *nobilis* was necessary to allow the Maxent and CART analysis to adequately partition the taxa; conversely, the success of this partitioning lends support to the morphological separation of the taxa. The analysis also leads the way to further ecological and evolutionary studies, by revealing the environmental variables supporting the ecological separation between *P. exaltatus* var. *exaltatus* and *P. nobilis* var. *nobilis*, thereby forming a solid base for further ecological studies and testing the putative processes of adaptive radiation that might have led to such separation.

The role of ecological evidence in taxonomy has been misunderstood (e.g. Richards 1939) as well as embraced (e.g. Jones and Luchsinger 1979; Stace 1989). Ecological traits provide many characters that are not traditionally evaluated by taxonomists and may provide additional evidence for lineage separation of closely related taxa and the role of natural selection on their populations (Andersson 1990; de Queiroz 2007). Incorporation of ecological data into taxonomic studies has yielded promising results in species delimitation of cryptic or otherwise complex taxa (e.g. Rissler and Apodaca 2007). With the availability of large, aggregated, online, georeferenced specimen datasets coupled with high-resolution environmental layers (e.g. in the Atlas of Living Australia, available at http://www.ala.org.au), and increasingly available ecological trait libraries for a wide range of species, we expect the formal integration of ecology and morphology in taxonomic studies to become more common.

Taxonomic implications

Our study demonstrates that the decision by Bean (2008) to combine *P. exaltatus* and *P. nobilis* was based on an over-reliance on a flawed and inadequate molecular analysis, and an inadequate understanding of the discrete and correlated morphological differences between the taxa. The clear geographic and ecological partitioning of the taxa provides further support for their recognition. We here recognise both as distinct species, reinstating *P. exaltatus* and reverting to the traditionally more narrowly defined concept of *P. nobilis*. Taxonomic treatments and a key to each recognised species of the *P. nobilis* complex are given below.

TAXONOMY

Ptilotus exaltatus Nees in J.G.C.Lehmann (ed.), *Pl. Preiss.* 1: 630 (1845)

Trichinium exaltatum (Nees) Benth., *Fl. austral.* 5: 227 (1870). *Type:* In sublimosis ad flumen Avon inter praedia rustica cl. cl. Heals et Whitfield [Western Australia, Avon River, between the farms of Messrs Heals and Whitfield], 15 March 1840, *L.Preiss 1367* (syn: LD 1029820 *n.v.*, image!, retrieved from https://plants.jstor.org/stable/10.5555/al.ap.specimen.ld1029820).

Trichinium burtonii F.M.Bailey, *Bot. Bull. Dept. Agric., Queensland* 2: 14 (1891). *Type*: Queensland, between Camooweal and Urandangie [*sic*; Urandangi] towards Georgina River, Mar.–Aug. 1890, *R.C.Burton s.n.* (syn: BRI AQ0332690 *n.v.*, image!, retrieved from https://plants.jstor.org/stable/10.5555/al.ap.specimen.bri-aq0332690; iso: MEL 1058704 *n.v.*, image!, retrieved from https://plants.jstor.org/stable/10.5555/al.ap.specimen.mel1058704, MEL 2281650 [fragment] *n.v.*, image!, retrieved from http://plants.jstor.org/stable/10.5555/al.ap.specimen.mel2281650).

Trichinium nervosum F.M.Bailey, *Queensland Agric. J.* 25: 287 (1910). *Types*: Queensland, Georgina River, Sep. 1910, *E.W.Bick 50* (syn: BRI AQ0332692 *n.v.*, image!, retrieved from http://plants.jstor.org/stable/10.5555/al.ap.specimen.bri-aq0332692); Queensland, Georgina River, Sep. 1910, *E.W.Bick 51* (syn: BRI AQ0332691 *n.v.*, image!, retrieved from http://plants.jstor.org/stable/10.5555/al.ap.specimen.bri-aq0332691); Queensland, Georgina River, Oct. 1912 [*sic*; possibly 1910, see discussion under Typification below] , *E.W.Bick s.n.* (syn: K 000348671 *n.v.*, image!, retrieved from http://plants.jstor.org/stable/10.5555/al.ap.specimen.k000348671).

Ptilotus exaltatus var. *pallidus* Benl, *Mitt. Bot. Staatssamml. München* 15: 164 (1979). *Type*: Northern Territory, Mt Olga, *s. dat., B.Barlow 1877* (holo: AD 97733105 *n.v., fide* Lally and Barker 2010).

Ptilotus exaltatus var. *glaber* Benl, *Muelleria* 5(4): 260 (1984). *Type*: Northern Territory, 4.8 miles (7.7 km) E. of Ormiston Gorge turnoff on Glen Helen road, 23° 42' S 132° 42' E, 19 Jun. 1972, *N.M.Henry 438* (holo: NT 35691 *n.v.*, digital image at CANB!; iso: NY, MO, both *n.v.*).

Ptilotus exaltatus var. *villosus* Benl, *Mitt. Bot. Staatssamml. München* 7: 314 (1970). *Type*: Western Australia, Mollerin, Sep. 1939, *C.A.Gardner s.n.* (holo: PERTH 01555073!; iso: PERTH 01555081!).

Ptilotus sp. Goldfields (R.Davis 10796) Western Australian Herbarium, in *FloraBase*, https://florabase.dpaw.wa.gov.au/ [accessed 28 May 2018].

Erect annual or short-lived perennial *herbs* 10–120 cm high, 10–100 cm wide; taproot fleshy and little-branched. *Stems* ribbed, villous or glabrescent; hairs simple or verticillate. *Basal leaves* linear-lanceolate or spathulate, 50–150 mm long, 20–60 mm wide, glabrous or sometimes with dense verticillate hairs; bases shortly attenuate; margins entire and sometimes undulate; apex mucronate, the mucro 1–3 mm long. *Cauline leaves* narrowly elliptic to obovate, 5–115 mm long, 3–40 mm wide, glabrous or sometimes with dense verticillate hairs; bases sessile or subsessile; margins entire and sometimes undulate; apex mucronate to pungent with a fragile mucro 1–3 mm long. *Inflorescences* spiciform, terminal, cylindrical, pink to purple, 20–130 mm long, 30–50 mm wide, sweetly scented; apex acute. *Bracts* ovate-lanceolate, 5–10.5 mm long, 2.5–4.3 mm wide, brown grading to transparent at margins; indumentum of scattered verticillate hairs especially towards base; apex mucronate, the mucro 0.5–1 mm long. *Bracteoles* ovate, 7.5–10.5 mm long, 3.5–5 mm wide, brown along midvein grading to transparent at margins, with dense to scattered verticillate hairs abaxially; apex mucronate, the mucro ~0.5 mm long. *Tepals* widely gaping at anthesis, 17–22 mm long, 1–1.5 mm wide, ± straight, purple to pink throughout, abaxially villous with verticillate hairs, the longest hairs to 4 mm long underlain by shorter, denser, and more densely-branched hairs, the indumentum shorter and sparser along the midline; apex truncate-serrate, glabrous; inner tepals adaxially lanate with hairs to ~4 mm long. *Fertile stamens* 3; *filaments* purple to pink, 9–17 mm long, unequal in length, filiform; *anthers* pink, 1.2–1.7 mm long, 0.5–1 mm wide. *Staminodes* 2, 1–4 mm long, obscured by the woolly tepal hairs, unequal in length, filiform. *Staminal cup* symmetrical, not lobed, 2–2.5 mm long, strongly adnate to tepal tube, ciliate above. *Ovary* obovoid, 1–2 mm long, 0.7–1.5 mm wide, pilose or glabrous apically*;* stipe ~1.5 mm long. *Style* slightly sigmoidal, 13–17 mm long, white to pale pink, excentrically fixed on the ovary. *Stigma* capitate. *Seed* smooth, glossy brown, ~1.6 mm long, ~1 mm wide.

Distribution and habitat

Ptilotus exaltatus is widespread throughout arid and semi-arid Australia, in New South Wales, the Northern Territory, Victoria, Queensland, South Australia and Western Australia (see Fig. 3B) It is found in a variety of habitats, including acacia shrublands, eucalypt woodlands, and grasslands, and can be found on red sands, brown or red sandy clays and calcareous loams, and on stony or gravelly sites including laterites. It is generally found on lighter-textured soils than the related *P. nobilis.*

Notes

Ptilotus exaltatus can be readily distinguished from *P. nobilis* by its widely gaping, purple to pink flowers with ±straight tepals and a dense, woolly indumentum plug obscuring the ovary (Figs 1A, 2B). Flowers have been observed being pollinated by day-flying bees including honey bees and the native *Amegilla cingulata*, in contrast to *P. nobilis,* which is pollinated mainly by night-flying moths.

Male-sterile (gynodioecious) individuals (e.g. PERTH 08735220) have been observed in some areas. These have noticeably smaller flowers and inflorescences, with small stamens and shrivelled, under-developed anthers. Gynodioecy has also been observed in many other species of *Ptilotus* (e.g. *P. obovatus*; Stewart and Barlow 1976).

Bean (2008) cited "*Trichinium macrocephalum* Moq. ... *nom. illeg.*, *non* R.Br. (1810)" as a synonym of *P. nobilis* subsp. *nobilis.* This citation, however, is in error. Moquin-Tandon (1849), in a treatment of *Trichinium* for de Candolle's *Prodromus*, listed *T. macrocephalum* with a direct reference to Brown (1810) and cited three specimens, one of which ("[i]n Novae-Hollandiae orâ meridionali, R. Brown") is the type of *T. macrocephalum* R.Br. (=*P. macrocephalus* (R.Br.) Poir.). In so doing, Moquin-Tandon did not publish a new name but partially misapplied Brown's concept of *T. macrocephalum,* given the cited specimens.

Typification

A specimen at K of *Trichinium nervosum* (K 000348671) is recognised here as a probable syntype. The label on the specimen reads "Georgina River, E.W. Bick, October 1912, Comm. F.M. Bailey", with no collection number. The type specimens of *T. nervosum* were collected at this location by Bick in 1910 (BRI AQ0332692 and BRI AQ0332691). Bick made several collections along the Georgina River in 1910, including the syntypes of *T. nervosum* (Australasian Virtual Herbarium; http://avh.ala.org.au/, accessed 1 December 2017). In 1912, however, his collections were all from Cape York Peninsula. In 2005, J.A.Wege annotated the sheet with "Holotype", overlooking the anomalous date (*T. nervosum* having been published in 1910). It is likely, however, that this specimen was collected in 1910 along with the other type material from Georgina River, was available to Bailey when the species was described, and was subsequently sent to Kew in 1912 (the year given for the collection possibly being in error for the year received).

Selected specimens examined

NORTHERN TERRITORY: 12.8 miles SW of Angas Downs Homestead, Central Australia, *G.Chippendale 7390* (PERTH 221805); Roper Valley Station, 63 Mile Well, *C.S.Robinson 770* (PERTH 221813). QUEENSLAND: 17.6 km S of Mount Isa on road to Boulia, 20° 52′ 12.0″ S 139° 27′ 00.0″ E, *D.Halford Q450* (AD 99217052). SOUTH AUSTRALIA: Immarna, *E.S. Simpson s.n.* (PERTH 00214027); Stuart Highway, 97 km S. of Marla, 28° 01' 49.4" S 134° 06' 35.2" E, *T.Hammer & K.Thiele TH87* (PERTH 8877998). WESTERN AUSTRALIA: 1 km SE of Dairy Creek Homestead, 25° 19′ 4.4″ S 115° 54′ 46.0″ E, *R.J.Cranfield 9717* (PERTH 04349059); On rocky hill, near upper Rudall River, 22° 20′ 0.0″ S 122° 40′ 0.0″ E, *A.S.George 10833* (PERTH 00222216); Flats E of Paraburdoo, 23° 11′ 35.0″ S 117° 40′ 18.0″ E, *K.J.Atkins 491* (PERTH 04181115); On track SW of Shay Gap Road and 1.3 km E of Coonieena Creek, 20° 31′ 46.2″ S 120° 8′ 54.4″ E, *T.Hammer, S.Dillon & K.Thiele TH20* (PERTH 08735220); Road verge, 0.5 km from Meekatharra on Meekatharra–Carnarvon Road, 26° 35′ 37.3″ S 118° 29′ 16.0″ E, *G.Byrne 161* (PERTH

06574653); ~22 km N of Mt Gould on Carnarvon-Meekatharra road, 25° 36′ 36.2″ S 117° 15′ 38.4″ E, *R.Davis, T.Hammer & B.Anderson RD 12287* (PERTH 08552045); Stony crab hole country, Mardabia paddock, Towera Station, 23° 17′ 00.0″ S 115° 32′ 00.0″ E, *R.J.Cranfield 1799* (PERTH 220337).

Ptilotus semilanatus (Lindl.) F.Muell. ex J.M.Black, *Fl. S. Australia* ed. 2, 2: 327–328 (1948)

Trichinium semilanatum Lindl. in T.L.Mitchell, *J. exped. trop. Australia* 45 (1848); *Ptilotus exaltatus* var. *semilanatus* (Lindl.) Maiden & Betche, *Census N.S.W. pl.* 72 (1916); *Ptilotus nobilis* subsp. *semilanatus* (Lindl.) A.R.Bean, *Telopea* 12(2): 242 (2008). *Type:* New South Wales, Subtropical New Holland, Cannonba Ponds, 20 miles W of Macquarie River, 27 Jan. 1846, *W.Stephenson 74* (Syn: CGE *n.v.*, digital image at BRI!, MEL 2281653 *n.v.*, image!, retrieved from http://plants.jstor.org/stable/10.5555/al.ap.specimen.mel2281653, MEL 2235246 *n.v.*, image!, retrieved from http://plants.jstor.org/stable/10.5555/al.ap.specimen.mel2235246).

Trichinium setigerum A.Cunn. ex Moq., *Prodr.* 13(2): 290 (1849). *Type*: New South Wales, Hunters River, 19 Apr. 1825, *A.Cunningham s.n.* (syn: K 000356871, image!, retrieved from http://plants.jstor.org/stable/10.5555/al.ap.specimen.k000356871)

Trichinium pulchellum A.Cunn. ex Moq., *Prodr.* 13(2): 290 (1849). Type: New South Wales, swampy plains near Lachlan River, May 1817, *A.Cunningham s.n.* (syn: P 00609971 *n.v.*, image!, http://plants.jstor.org/stable/10.5555/al.ap.specimen.p00609971, BM 000900549 *n.v.*, image!, retrieved from http://plants.jstor.org/stable/10.5555/al.ap.specimen.bm000900549, K 000348673 *n.v.*, image!, http://plants.jstor.org/stable/10.5555/al.ap.specimen.k000348673).

Clumping, perennial, upright to decumbent *herbs,* 18–35 cm high, 30–80 cm wide; taproot branching and woody. *Stems* ribbed, glabrescent; hairs simple or verticillate. *Basal leaves* narrowly oblanceolate, 20–60 mm long, 1–5 mm wide, glabrous; bases attenuate, to ~10 mm long; margins entire and sometimes undulate; apex mucronate, the mucro ~0.5 mm long. *Cauline leaves* narrowly elliptic to oblanceolate, 10–30 mm long, 1–8 mm wide, glabrous or sometimes with sparse verticillate hairs; bases sessile or subsessile towards stem apex, long-attenuate towards stem base; margins entire and commonly undulate; apex mucronate, the mucro ~0.5 mm long. *Inflorescences* spiciform, terminal, hemispherical or ovoid (less commonly cylindrical), pink to purple, 5–25(–80) mm long, 30–35 mm wide, with flowers rarely interrupted at the base; apex truncate or obtuse. *Bracts* narrowly ovate to ovate, 4–6 mm long, 1.9–3 mm wide, brown and opaque, with abaxial scattered verticillate hairs especially at the base; apex mucronate, the mucro 0.5–1 mm long. *Bracteoles* broadly ovate, 4.4–6 mm long, 2.9–3.2 wide, transparent, with dense to scattered verticillate hairs abaxially; apex mucronate, the mucro ~0.5 mm long. *Tepals* widely gaping at anthesis, 14–17 mm long, 1.1–2 mm wide, ± straight, purple or pink throughout, abaxially villous with verticillate hairs, the longest hairs to 4 mm long underlain by shorter, denser, and more densely-branched hairs, the indumentum shorter and sparser along the midline; apex truncate-serrate, glabrous; inner tepals adaxially lanate with hairs to ~5 mm long. *Fertile stamens* 3; *filaments* cream to pink, 7–9.5 mm long, unequal in length, filiform; *anthers* pink, 1.2–1.5 mm long, 0.7–1 mm wide. *Staminodes* 2, 2.5–2.8 mm long, obscured by woolly hairs, unequal in length, filiform. *Staminal cup* symmetrical, not lobed, 1–1.5 mm long. *Ovary* obovoid 1.4–2 mm long, 1.1–1.2 mm wide, pilose apically; stipe 0.6–1 mm long. *Style* slightly sigmoidal, 8–10.5 mm long, white to pale pink, excentrically fixed on the ovary. *Stigma* capitate. *Seed* smooth, dark brown, ~2 mm long, ~1.25 mm wide.

Distribution and habitat
Ptilotus semilanatus occurs west of the Great Dividing Range in central and eastern Queensland and New South Wales, and in Victoria and adjacent parts of South Australia east of Bordertown. It typically occurs in eucalypt woodlands or grasslands and on plains of orange or brown loamy or clayey soils. It is

typically found in higher rainfall areas than *P. exaltatus*, though their distributions overlap slightly in some areas.

Notes

Ptilotus semilanatus can be readily distinguished from other species in the group by the combination of a perennial, clumping habit, branching woody taproot (Fig. 6), typically shorter, truncate or rounded (or less commonly cylindrical) inflorescences, shorter pink tepals and style, and densely woolly indumentum on the interior surface of the inner tepals (see Fig. 2A). In its perennial habit it is similar to *P. angustifolius*; in *P. semilanatus*, however, the above-ground stems are mostly annual and arise from a perennial, underground, branching rootstock, while in *P. angustifolius* the annual flowering stems arise from an above-ground, branched, woody base or from a woody crown at ground level. *Ptilotus angustifolius* thus develops a shrubbier habit than *P. semilanatus*. Floral morphology, indumentum and tepal colour indicate that *P. semilanatus* is most closely related to *P. exaltatus*.

Bean (2008) synonymised *P. dissitiflorus* var. *longifolius* Benl under *P. nobilis* subsp. *semilanatus*, stating that it "indisputably belongs here" (p. 242), but did not give his reasoning. He described *P. nobilis* subsp. *semilanatus* as having truncate or obtuse inflorescence apices (in contrast to the conical inflorescence apices of *P. nobilis* subsp. *nobilis*); however, examination of the type (MEL 2281812) of *P. dissitiflorus* var. *longifolius*, and specimens borrowed from BRI, shows that it consistently has conical inflorescences (often quite interrupted), as does the typical variety of *P. dissitiflorus*. Bean did not address this inconsistency nor provide a detailed description for his concept of *P. nobilis* subsp. *semilanatus*. Inflorescence apex shape and density of flowers at anthesis tend to be relatively consistent within species of *Ptilotus*, while inflorescence length can vary due to differences in the duration of the growing season (Hammer and Davis 2017). Further study of *P. dissitiflorus* var. *longifolius* is warranted before it can be adequately placed, but we consider this work to be outside the scope of the present study.

Selected specimens examined

NEW SOUTH WALES: Yarrabandai-Forbes road at Bollingers road turn-off, 33° 18' S 147° 44' E, *G.M.Cunningham & P.L.Milthorpe s.n.* (CANB 864908); 36 km NW of Griffith on Goolgowi road next to wheatfields, 34° 04' S 145° 48' E, *A.M.Rowell 51* (CANB 801368). SOUTH AUSTRALIA: 11 miles ESE of Bordertown, 36° 22' 35.0" S 140° 57' 22.0" E, *R.L.Specht 1615* (AD 97410284). QUEENSLAND: Roadside under trees, clay soil, 1 km from Bowenville, 27° 18' 24.8" S 151° 29' 25.0" E, *M.Johnston 505* (BRI AQ751386); State Forest 127 Kerimbilla, 30 km N of Kurumbul, 28° 22' S 150° 36' E, *P.I.Forster 32227* (BRI AQ742691); 'Elgin' near Condamine, 27° 0' 40" S 150° 5' 28" E, *K.M. Stephens, K.M.Thrupp & A.Daniel s.n.* (BRI AQ855300); Kerimbilla State Forest NE of Goondiwindi, 28° 22' 53" S 150° 36' 22" E, *A.R.Bean 29573* (BRI AQ821511); 24 km NE of Roma, 26° 20' 58" S 148° 51' 14" E, *D.J.Baumgartner 33717* (BRI AQ776363); Rang Rd. toward Valpree 100 km WSW of Townsville, 19° 34' 07" S 145° 55' 50" E, *R.J.Cumming 18665* (BRI AQ860207); 7 km NW of Tomahawk, N of Lake Galilee, 22° 13' S 145° 56' E, *R.J.Cumming 13274* (BRI AQ637672); Tregole NP Collected at Tregole Bore ~2.5 km S of Tregole House, 26° 28' 21" S 147° 2' 08" E, *C.Dollery 211* (BRI AQ550864).

Fig. 6. Scan of a specimen of *Ptilotus semilanatus* (CANB 801368), showing unique characters of the species such as the tufted habit, branching perennial taproot, truncate inflorescences and narrow leaves.

Ptilotus nobilis (Lindl.) F.Muell, *Fragm.* 6: 227–228 (1868)

Trichinium nobile Lindl. in T.L..Mitchell, *Three exped. Australia* 2: 23 (1838). *Type*: New South Wales, Interior of New Holland, 2 Apr. 1836, *T.L.Mitchell 50* (syn: CGE *n.v.*, digital image at BRI!).

Trichinium densum A.Cunn. ex Moq., *Prodr.* 13(2): 289 (1849). *Type*: New South Wales, swampy-plains near Lachlan River, June 1817, *A.Cunningham s.n.* (syn: P 00609984 *n.v.*, image!, retrieved from http://plants.jstor.org/stable/10.5555/al.ap.specimen.p00609984, BR 0000013464882 *n.v.*, image!, retrieved from http://plants.jstor.org/stable/10.5555/al.ap.specimen.br0000013464882).

Erect annual or short-lived perennial *herbs* 10–120 cm high, 20–70 cm wide; taproot fleshy. *Stems* ribbed, glabrescent; hairs simple or verticillate. *Basal leaves* lanceolate or spathulate, 50–150 mm long and 20–60 mm wide, glabrous; bases shortly attenuate; margins entire; apex mucronate, the mucro 0.5–1 mm long. *Cauline leaves* elliptic, oblanceolate or obovate, 15–100 mm long, 3–40 mm wide, glabrous or sometimes with sparse verticillate hairs; bases sessile or subsessile; margins entire; apex mucronate, the mucro 0.5–1 mm long. *Inflorescences* spiciform, terminal, cylindrical, cream-green or pale pink, 30–200 mm long, 40–50 mm wide, sweetly scented; apex acute or less commonly truncate or obtuse. *Bracts* ovate-lanceolate, 8–10 mm long, 3–5 mm wide, brown grading to transparent at margins, with sparse to dense verticillate hairs abaxially; apex mucronate, the mucro 0.5–1 mm long. *Bracteoles* broadly ovate, 9–11 mm long, 4–5.5 mm wide, brown along midvein and grading to transparent at margins, with abaxial verticillate hairs especially at the base; apex mucronate, the mucro 0.5–1 mm long. *Tepals* narrowly gaping at anthesis, 20–25 mm long, 1.2–1.5 mm wide, usually slightly to distinctly falcate, cream-green throughout or pale pink, abaxially villous with verticillate hairs, the longest hairs to 6 mm long underlain by shorter, denser, and more densely-branched hairs, the indumentum shorter and sparser along the midline; apex truncate-serrate, glabrous; inner tepals adaxially with scattered indumentum with ±erect hairs of varying lengths. *Fertile stamens* 3; *filaments* white or pale pink, 13–19 mm long, unequal in length, filiform; *anthers* cream or pale pink, 1–2 mm long, 0.5–1 mm wide. *Staminodes* 2, 2–5 mm long, unequal in length, filiform. *Staminal cup* symmetrical, not lobed, 2–2.5 mm long, strongly adnate to tepal tube, ciliate above. *Ovary* obovoid, 1.2–2.2 mm long, 0.9–1.5 mm wide, pilose or glabrous apically; stipe 1–1.5 mm long. *Style* slightly sigmoidal, 16.5–20 mm long, white or pale pink, excentrically fixed on the ovary. *Stigma* capitate. *Seed* smooth, glossy brown, ~1.6 mm long, ~1 mm wide.

Distribution and habitat
Ptilotus nobilis is widespread in southern and eastern Northern Territory and northern, central and eastern South Australia extending to central New South Wales and south-central Queensland (see Fig. 3A). One specimen (PERTH 05585813) is recorded from the Cavenagh Range, Western Australia. It is found in open shrublands, grasslands, open stony plains and breakaways, typically on fine-textured, brown or orange loamy or clayey soils (Fig. 1C).

Notes
Ptilotus nobilis can be readily distinguished from *P. exaltatus* on the basis of the indumentum on the interior base of the inner tepals. In *P. nobilis* the hairs are sparse, scattered, and erect, while in *P. exaltatus* they form a densely woolly plug. It can also be separated by usually having green flowers (sometimes to pale pink) and slightly to distinctly falcate-curved tepals that do not gape widely at anthesis (Figs 1B, 2D–E); the tepals of *P. exaltatus* are always pink to purple, are ± straight, and gape widely at anthesis (Figs 1A, 2B).

Pollination is likely to be entirely or predominantly by night-flying moths. The flowers are strongly sweetly scented at night, much less so during the day. The narrowly-gaping tepals render the flowers ±tubular at anthesis, a common feature of moth-pollinated flowers. This feature is also seen in other green-flowered, moth-pollinated species in this genus, such as *P. macrocephalus*.

Conservation status

Ptilotus nobilis is common where it occurs in South Australia, Northern Territory, Queensland and New South Wales. Its distribution has been shown to extend over the border into Western Australia, where it is only known from one collection. Its conservation status in Western Australia should be assessed.

Selected specimens examined

NORTHERN TERRITORY: On skeletal soils below schistose hills, Paynter Springs, Hamilton Downs, *N.Ford 119* (PERTH 00226181); In rocky red sandy soil, Atherrita Bore, 52 miles SE of Todd River Homestead, *D.J.Nelson 1299* (PERTH 00337102); ~25 km SW of Arltunga 64 km ENE of Alice Springs, 23° 32′ 32.8″ S 134° 29′ 48.8″ E, *R.Davis, R.Butcher & P.Jobson 12703* (PERTH 08619387); NE end of Waterhouse Range, 1 km West of Whitepoint Bore, 23° 57′ 30″ S 133° 28′ 21″ E, *P.Latz 18899* (NT A0105236); Waterhouse Range; 45 km SSW of Alice Springs, 24° 0′ 20″ S 133° 38′ 22″ E, *P.Latz & D.Randall 23928* (NT D0190495). QUEENSLAND: 36 miles W of Camooweal, *G.M.Chippendale 1494* (PERTH 00226165); 10 km from Longreach on road to Winton, 23° 21' 29'' S 144° 11' 10'', *M.Johnston 504-1* (BRI AQ609643); 50 km N of Cunnamulla on highway to Charleville-Mitchell Highway, 27° 40' 0.7" S 145° 54' 00.1" E, *M.Johnston s.n.* (BRI AQ751208); 15 km E of Cunnamulla on Ballon Highway to St. George, 28° 03' 0.4" S 145° 51' 0.0" E, *M.Johnston s.n.* (BRI AQ751209). SOUTH AUSTRALIA: 13 km S. of Coober Pedy on Stuart Highway, 29° 5' 8.1'' S 134° 52' 7.1'' E, *T.Hammer & K.Thiele TH84* (PERTH 8877971); Stuart Highway, 100 km S. of Marla, 28° 4' 9.8'' S 134° 6' 38.0'' E, *T.Hammer & K.Thiele TH86* (PERTH 8877963); Stuart Highway, 27.4 km N. of Cadney Park & 54 km S. of Marla, 27° 41' 5.8" S 133° 56' 48.1" E, *T.Hammer & K.Thiele TH92* (PERTH 8878005). WESTERN AUSTRALIA: Foot of Cavenagh Range, 26° 9' S 127° 59' E, *Morcombe s.n.* (PERTH 05585813).

Ptilotus angustifolius (Benl) T.Hammer, *comb. et stat. nov.*

Ptilotus nobilis var. *angustifolius* Benl, *Mitt. Bot. Staatssamml. München* 3: 43 (1959); *Ptilotus nobilis* subsp. *angustifolius* (Benl) Lally & W.R.Barker, *J. Adelaide Bot. Gard.* 24: 51 (2010). *Type*: South Australia, Flinders Range, Oct. 1901, *M.Koch s.n.* (holo: M 0152673 *n.v.*, *fide* Lally and Barker 2010; iso: NSW 29534 *n.v.*, image!, retrieved from http://plants.jstor.org/stable/10.5555/al.ap.specimen.nsw29534; NSW 790629 *n.v.*, image!, retrieved from http://plants.jstor.org/stable/10.5555/al.ap.specimen.nsw790629).

Long-lived rounded perennial *herbs or subshrubs* 12–40 cm high, 20–100 cm wide, with annual, herbaceous flowering stems from basally woody branches or rootstock. *Stems* ribbed, glabrescent; hairs simple or verticillate. *Basal leaves* narrowly oblanceolate, 50–90 mm long, 6–15 mm wide, glabrous; bases long-attenuate, to 60 mm; margins entire and sometimes undulating; apex mucronate, the mucro ~0.5 mm long. *Cauline leaves* oblanceolate, 20–70 mm long, 4–10 mm wide, glabrous or sometimes with sparse verticillate hairs; bases subsessile or short-attenuate towards stem apex, long-attenuate towards stem base; margins entire and commonly undulate; apex mucronate, the mucro ~0.5 mm long. *Inflorescences* spiciform, terminal, hemispherical to ovoid (less commonly cylindrical), cream-green, 22–75 mm long, 40–51 mm wide; apex acute. *Bracts* ovate-lanceolate, 9–13 mm long, 3–4 mm wide, brown grading to transparent at margins, with sparse to dense verticillate hairs abaxially; apex mucronate, the mucro to 1 mm long. *Bracteoles* broadly ovate, 10–13 mm long 4–5.5 mm wide, brown along midvein grading to transparent at margins, with abaxial verticillate hairs especially towards the base; apex mucronate, the mucro ~0.5 mm long. *Tepals* narrowly gaping at anthesis, 20–25 mm long, 1–1.7 mm wide, falcate or straight, cream-green throughout, abaxially villous with verticillate hairs, the longest hairs to 6 mm long underlain by shorter, denser, and more densely-branched hairs, the indumentum shorter and sparser along the midline; apex truncate-serrate, glabrous; inner tepals with adaxially scattered, indumentum, with ±erect hairs of varying lengths. *Fertile stamens* 3; *filaments*

cream, 12–17 mm long, unequal in length, filiform; *anthers* white or cream, 1.2–1.9 mm long, 0.5–1 mm wide. *Staminodes* 2, 7–11 mm long, unequal in length, filiform. *Staminal cup* symmetrical, not lobed, 2–2.5 mm long, strongly adnate to tepal tube, ciliate above. *Ovary* obovoid 2–2.6 mm long and 1.2–1.7 mm wide, glabrous or with a few hairs apically; stipe 1.1–1.8 mm long. *Style* slightly sigmoidal, 15–20 mm long, white, excentrically fixed on the ovary. *Stigma* capitate. *Seed* not observed.

Distribution and habitat
Ptilotus angustifolius occurs at scattered localities along the coastal and near-coastal plain from Port Augusta to just south of Adelaide on the Fleurieu Peninsula in South Australia. It has been found in eucalypt woodlands and grasslands on brown loam and clay soils.

Notes
In its floral morphology, *P. angustifolius* is most similar to *P. nobilis*, but it can be readily distinguished from that species by its rounded, tufted habit with short, herbaceous flowering stems arising from long-lived woody stems and rootstock, and narrower leaves. It is similar in habit to *P. semilanatus*, from which it can be readily separated on floral morphology (e.g. size and colour of the tepals, presence of a woolly plug within the tepals and inflorescence size and shape; Fig. 2C). Unlike *P. semilanatus*, individuals of *P. angustifolius* observed in the field (e.g. above the strandline at Sellick's Beach) are shrubby with above-ground woody stems; perennial woody stems can also be seen on the scanned isotypes (see Lally and Barker 2010). Due to this shrubby habit, true basal leaves are only seen in specimens of young plants.

Conservation status
Ptilotus angustifolius is not currently listed as a taxon of conservation concern in South Australia (State Herbarium of South Australia 2017). Attempts during this study to re-collect it from a population in remnant bush at a cemetery near Gladstone, South Australia failed despite a thorough search; five previous collections from this site are dated between 1908 and 1978, indicating that it may previously have been common there. The species is known from 74 collections since 1881 (only nine since 1990) and typically occurs on or near areas affected by human disturbance. We recommend that the conservation status of this species be urgently assessed.

Selected specimens examined
SOUTH AUSTRALIA: Sellicks Beach cliffs, 35° 20' 25'' S 138° 26' 40'' E, *M.O'Leary 3680* (AD 277212); Tarcowie, approximately 3.5 km NW of township on Price-Maurice Rd. (E. side of road), 32° 55'' 29'' S 138° 30' 30'' E, *K.H.Brewer 895* (AD 277211, PERTH 08775338); Gladstone, 33°15'36"S 138° 23'13"E, *P.Slattery 50* (AD 255518); Stone Hut, 33°06'12"S 138°17'08"E, *P.Slattery 48* (AD 255519); Southern Mt. Lofty Ranges, ~3 km south of Sellick's Hill, *H.M.Cooper s.n.* (AD 96515162); Mambray Creek, 32° 50'S 138° 4'E, *D.J.E.Whibley 4329* (AD 97436021); Marino Rocks, 35°03'S 138°31'E, *D.N.Kraehenbuehl 4587* (AD 98603116); Balaklava, 1 mile west of the town, *G.H.Clarke 3165* (AD 98592544); Gladstone cemetery, 33° 16'S 138° 21'E, *D.A.Stewart 1949* (AD 97733068); Mount Remarkable National Park, 32° 50'S 138° 4'E, *N.N.Donner 4923* (AD 97437005); Barunga Gap, ~40 km north-north-west of Port Wakefield, *B.Copley 688* (AD 96647032).

Key to species of the *P. nobilis complex*

1. Inflorescences pinkish to purple; flowers with dense plug of woolly hairs at the interior base of the inner tepals; tepals widely gaping at anthesis...2
1: Inflorescences cream-greenish or sometimes pale pink; flowers with sparse, ±erect hairs at the interior base of the inner tepals; tepals narrowly gaping at anthesis...3
2. Annual (or short-lived perennial) herbs with stems arising from a fleshy, little-branched taproot; inflorescence cylindrical; style > 12 mm long ....***P. exaltatus***
2: Long-lived, perennating herbs, with annual stems arising from a much-branched, woody rootstock; inflorescence hemispherical or ovoid (less commonly cylindrical); style < 11 mm long ... ***P. semilanatus***
3. Erect annual (or short-lived perennial) herbs, with stems arising from a fleshy taproot; mature cauline leaf bases sessile or subsessile; inflorescences cylindrical..***P. nobilis***
3: Rounded, long-lived perennial herbs or subshrubs, with annual flowering stems arising from a woody rootstock or (in older plants) above-ground, branching, woody stems; mature cauline leaf bases attenuate to long-attenuate; inflorescences hemispherical to ovoid (less commonly cylindrical) ....
....***P. angustifolius***

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SUPPLEMENTARY MATERIALS

The following supplementary materials are available as electronic downloads at https://www.taxonomytim.com/thesis (under Chapter 4): **Table S1.** The dataset used in the geospatial analyses, including the specimen barcode, species determination, state of occurrence, locality coordinates and associated environmental variables used in the analyses; **Fig. S1.** Australia-wide CART analysis using climatic and edaphic variables; **Fig. S2.** South Australia CART analysis using only edaphic variables.

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Of a different feather: two new species of featherheads from the *Ptilotus macrocephalus* (Amaranthaceae) complex

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Abstract. *Ptilotus macrocephalus* (R.Br.) Poir. (Amaranthaceae), commonly known as a featherhead, is a widespread and common species in many parts of Australia. In the present study, we assess morphological variation in *P. macrocephalus* throughout its geographic range and provide evidence for the recognition of two new species, namely, *P. psilorhachis* T.Hammer & R.W.Davis and *P. xerophilus* T.Hammer & R.W.Davis. Geospatial analyses indicated that these new species are partitioned ecologically and geographically.

INTRODUCTION

Ptilotus R.Br. comprises ~120 species, all of which are native to mainland Australia, with one species (*P. conicus* R.Br.) also occurring on the Lesser Sunda Islands and the Aru Islands, and one (*P. spathulatus* (R.Br.) Poir.) occurring in Tasmania. Species diversity is highest in arid and semi-arid regions, particularly in Western Australia (Hammer *et al.* 2015, 2018a).

Ptilotus macrocephalus (R.Br.) Poir., known by a number of common names including featherheads and green mulla mulla, is a widespread species found throughout mainland Australia. It was one of six species first described by Brown (1810) in *Trichinium* R.Br. (as *T. macrocephalum* R.Br.), on the basis of a specimen from Victoria. Poiret (1816) synonymised *Trichinium* under *Ptilotus*, making the new combination *P. macrocephalus* (R.Br.) Poir. Moquin-Tandon (1849) rejected Poiret's synonymy and retained *Trichinium* and *Ptilotus* as two distinct genera, but misapplied the name *T. macrocephalum* to specimens of *P. exaltatus* Nees (e.g. *A.Cunningham 202* at MEL; see also Hammer *et al.* 2018b). Confusion between *T. macrocephalum* and *P. exaltatus* led Moquin-Tandon to describe *T. pachocephalum* Moq., also on the basis of a specimen from Victoria, and *T. angustifolium* Moq. from the Hunter River, New South Wales. Further confusion was created when Lindley (in Mitchell 1848) misapplied the name *T. fusiforme* R.Br. to specimens of *T. macrocephalum*.

Mueller (1868) followed Poiret (1816) by including *Trichinium* within *Ptilotus*. He recognised *T. macrocephalum sensu* Moquin-Tandon (1849) as a synonym of his own concept of *P. nobilis* (Lindl.) F.Muell., and considered *T. angustifolium, T. pachocephalum* and *T. fusiforme sensu* Lindley (in Mitchell 1848) to be conspecific (including them under *P. pachocephalus* (Moq.) F.Muell.). Bentham (1870) recognised the similarity of *Ptilotus* and *Trichinium*, but nevertheless maintained them as separate genera and listed Mueller's *P. pachocephalus* as a synonym of *T. macrocephalum*. Black (1948) synonymised *Trichinium* under *Ptilotus*, reinstating the name *P. macrocephalus*, and this view has been followed by subsequent taxonomists. The phylogeny presented in Hammer *et al.* (2015) showed that all of Brown's original *Trichinium* species are nested within *Ptilotus*. *Ptilotus macrocephalus* is placed in an informal 'Clade D' and is closely related to *P. polystachyus* (Gaudich.) F.Muell., *P. giganteus* (A.Cunn. ex Moq.) R.W.Davis & R.Butcher and related species.

Bean (2008) noted that *P. macrocephalus* occurs across a wide range of environments, from arid regions of Western Australia and western Queensland to high-rainfall areas near the coast in eastern Australia and is morphologically variable. He recorded that sepals (termed 'tepals' by Bean) are longest in plants from north-eastern Queensland, nearly as long in near-coastal areas of Queensland and New South Wales, and shortest in Western Australia; he considered this variation to be clinal. In the present study, we prefer to continue the use of the term 'sepals' to the previously used 'tepals' (Hammer 2018; Hammer *et al.* 2018a), because recent studies have clarified the calycine origin of the uniseriate perianth of the Caryophyalles (e.g. Ronse de Craene 2013), the corolla having been lost, and following the change in terminology for other genera in Amaranthaceae (e.g. Vrijdaghs *et al.* 2014; Borsch *et al.* 2018).

The present study critically examines morphological variation in *P. macrocephalus* across its range and shows that there is strong morphological evidence for the recognition of three distinct geographically and ecologically partitioned taxa.

MATERIALS AND METHODS

Morphological examinations
In total, 676 fertile specimens from the National Herbarium of Victoria (MEL), the National Herbarium of New South Wales (NSW), the Queensland Herbarium (BRI), the State Herbarium of South Australia (AD) and the Western Australian Herbarium (PERTH) were examined, including material from throughout the geographic range of *P. macrocephalus*. Specimens from the Northern Territory Herbarium (DNA) were not accessed, but an adequate amount of material collected from the Northern Territory was available from other herbaria. Important morphological characters were identified, and specimens categorised into three distinct morphotypes without *a priori* consideration of geography or habitat.

Geospatial analyses
Collection localities of all specimens examined were obtained from the Australasian Virtual Herbarium (http://avh.chah.org.au/, accessed 2 August 2017). Records with a georeferenced precision >10 km were discarded, leaving n=427 samples (Supplementary Material S1). A geospatial dataset of 40 Bioclim climate variables from the CliMond dataset (Kriticos *et al.* 2012; Kriticos *et al.* 2014; available at https://www.climond.org, accessed 15 April 2019) for each included locality was constructed using the Point Sampling Tool in QGIS (ver. 3.3, QGIS Development Team 2018). Given the broadly allopatric and very wide ranges of the three morphotypes, climate variables were considered to be suitable for modelling their distributions. Maximum-entropy modelling (Maxent ver. 3.4.1; Phillips *et al.* 2004; Phillips and Dudík 2008) analyses were used to assess ecological and geographic partitioning between the three morphotypes (see also Hammer *et al.* 2018b). No feature selection was conducted on the data. Multiple occurrence records of each morphotype from any given 10 × 10-km cell were ignored. Bootstrapping was performed for each model with 10 replications and 25% of records were randomly withheld for model testing. All other settings were left as default. Model accuracy was assessed using the area under curve (AUC) statistic (Fielding and Bell 1997), and jackknife tests were used to assess variable importance. Taxon distribution maps were created using QGIS.

A principal component analysis (PCA) was conducted on the geospatial dataset using the R statistical platform (ver. 3.5.1, R Core Team 2018) in RStudio (ver. 1.1.456, RStudio Team 2016) to determine whether if the morphotypes occupied discrete environmental envelopes. Standardisation of the variables was implemented. The PCA was visualised using the R packages ggplot2 (Wickham 2016) and ggfortify (Tang *et al.* 2016).

RESULTS

Morphological examinations

All specimens of *Ptilotus macrocephalus sens. lat.* (i.e. as currently circumscribed) examined could be reliably segregated into one of three morphotypes (Fig. 1), one of which included the type specimens of *T. macrocephalum*, *T. angustifolium* and *T. pachocephalum*. The morphotypes are distributed in (1) south-eastern Australia, from far south-eastern South Australia through Victoria and into eastern New South Wales, (2) arid parts of central and western Australia, closely associated with the Eremaean botanical province, and (3) eastern Queensland from west of Brisbane to near Cairns. The morphotypes are, hereafter, referred to as (1) *P. macrocephalus*, (2) *P. xerophilus* and (3) *P. psilorhachis* (see Taxonomy section below). Distinguishing morphological characters are summarised in Table 1.

Table 1. Summary of distinguishing morphological characters for the taxa in this study

Character	*P. macrocephalus*	*P. psilorhachis*	*P. xerophilus*
Taproot woodiness	Woody	Fleshy	Fleshy
Rachis indumentum	Densely hairy	Glabrous or very sparsely hairy	Densely hairy
Flower arrangement	Tight	Loose	Tight
Adaxial sepal hairs	Present	Absent	Absent
Staminal cup hairs	Present	Present	Absent
Outer sepal length (mm)	20–24	28–45	14–25
Outer sepal width (mm)	1.5–2.0	1.3–1.8	0.7–1.1

Plants of *P. macrocephalus* are perennial herbs with a woody, sometimes branching, taproot; many specimens appear to be long-lived (Fig. 1B) on the basis of scars and dead branches from previous seasons being retained on the crown. By contrast, the other two species have fleshy taproots and seem to be mainly annuals but may persist for several seasons given favourable conditions (Fig. 1D, F). Field observations of *P. xerophilus* in Western Australia, the Northern Territory and northern South Australia (T. A. Hammer, *pers. obs.*) and of *P. psilorhachis* near Cairns, Queensland (P.J.H. Hurter, *pers. comm.*) confirm their primarily annual habit.

Inflorescences of *P. psilorhachis* are markedly different from those of *P. macrocephalus* and *P. xerophilus*. A key feature of *P. psilorhachis* is a glabrous or very sparsely hairy rachis (i.e. a few scattered hairs may be present). This is in distinct contrast to the densely villous or tomentose indumentum that obscures the surface of the rachis in the other two taxa. Flowers on pressed specimens of *P. psilorhachis* are also loosely arranged with the rachis visible between the flowers (Fig. 1C), whereas flowers in *P. macrocephalus* and *P. xerophilus* are more tightly arranged, with the overlapping bracts and bracteoles obscuring the rachis (Fig. 1A, E).

Differences in sepal indumentum between species of *Ptilotus* are often informative (e.g. Hammer 2018; Hammer et al. 2018b). The indumentum on the abaxial surface of the sepals differs among the three taxa (Fig. 2). The hairs in *P. macrocephalus* and *P. psilorhachis* are long, spreading and verticillate for almost the entire length of the sepal (Fig. 2B, F), except for glabrous apices. In *P. xerophilus*, the hairs are short, appressed and simple in the lower half of the sepal (some specimens are almost glabrous), with only the upper half having long, spreading, verticillate hairs below the glabrous apices (Fig. 2C). These variations in abaxial sepal indumentum give the inflorescences a different appearance overall (Fig. 1). The indumentum within the flowers also varies among the three taxa (Fig. 3). In *P. macrocephalus* the inner sepals are adaxially villous and the apex of the staminal cup has simple hairs (Fig. 3A). In *P. xerophilus*, the adaxial surface of the inner sepals and staminal cup are glabrous (Fig. 3B). In *P. psilorhachis*, the inner sepals are adaxially glabrous and the apex of the staminal cup has simple hairs (Fig. 3C).
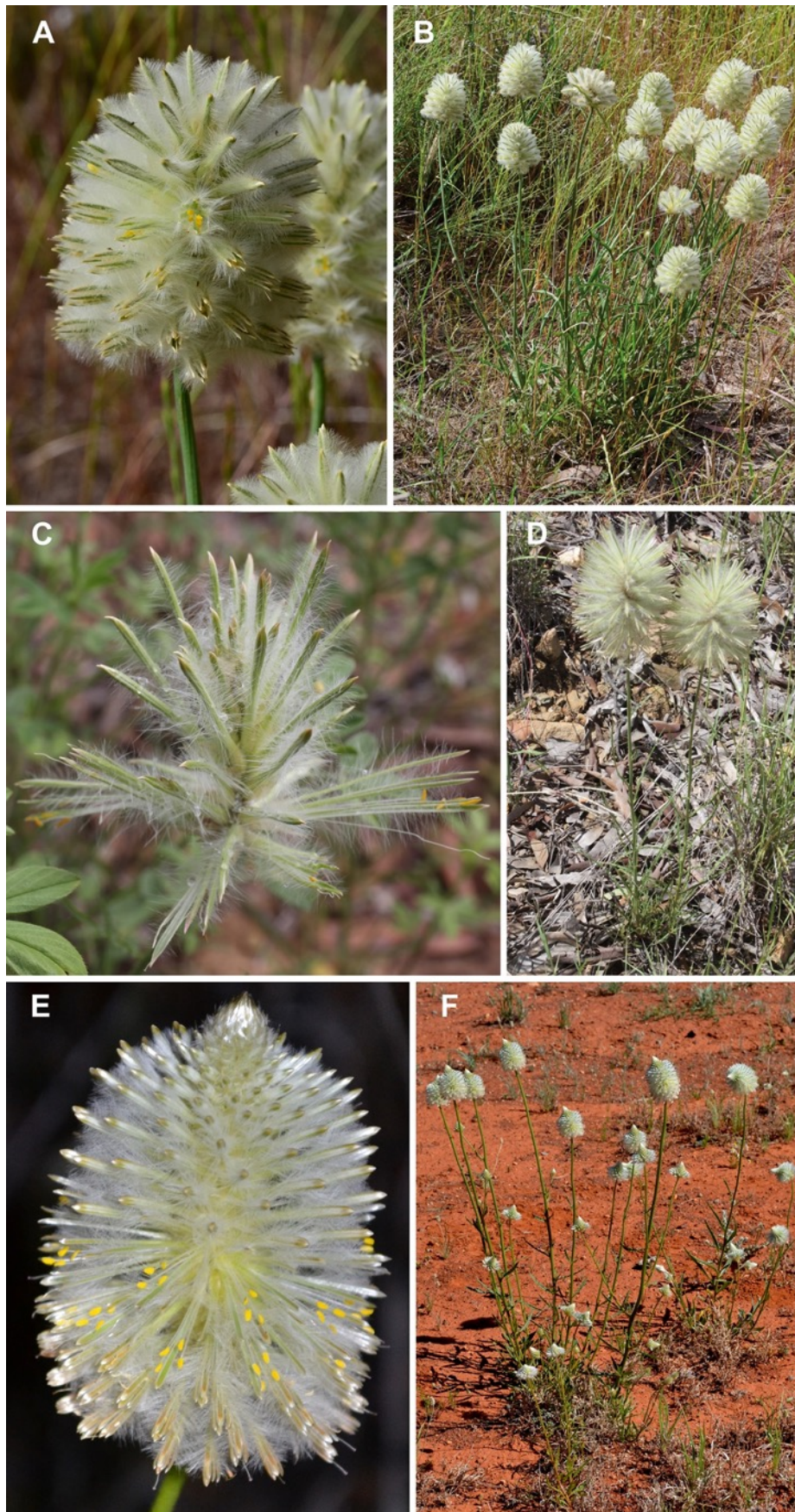
Fig. 1. Examples of the three taxa *in situ*. A. *Ptilotus macrocephalus* inflorescence in Victoria. B. *P. macrocephalus* perennial habit in Victoria. C. *P. psilorhachis* inflorescence in Queensland. D. *P. psilorhachis* annual habit in Queensland. E. *P. xerophilus* inflorescence in Western Australia. F. *P. xerophilus* annual habit in Western Australia. Photographs by G. Goods and M. Goods (A, B), J. Newland and R. Fryer (C, D) and T. Hammer (E, F).

Fig. 2.   Longitudinal sections showing the adaxial surface of inner sepals (i.e. staminal cup attached) and abaxial surface of outer sepals for each of the three taxa. A. Inner sepal of *Ptilotus macrocephalus* (*A.C.Beauglehole 86470*, MEL 1620389). B. Outer sepal of *Ptilotus macrocephalus* (*A.C.Beauglehole 86470*, MEL 1620389). C. Inner sepal of *Ptilotus xerophilus* (*D.J.Nelson 1742*, MEL 2220885). D. Outer sepal of *Ptilotus xerophilus* (*D.J.Nelson 1742*, MEL 2220885). E. Inner sepal of *Ptilotus psilorhachis* (*I.R.Telford & R.J.Rudd 11249*, BRI AQ581412). F. Outer sepal of *Ptilotus psilorhachis* (*I.R.Telford & R.J.Rudd 11249*, BRI AQ581412). Scale bar: 5 mm.

Fig. 3. Close up of the adaxial surface of the inner sepals, showing the differences in staminal cup and sepal indumentum (from Fig. 2). A. *Ptilotus macrocephalus* (*A.C.Beauglehole 86470*, MEL 1620389). B. *P. xerophilus* (*D.J.Nelson 1742*, MEL 2220885). C. *P. psilorhachis* (*I.R.Telford & R.J.Rudd 11249*, BRI AQ581412). Scale bar: 1 mm.

As Bean (2008) noted, there are clear differences in sepal length within *P. macrocephalus sens. lat* (Fig. 2). The outer sepals of *P. psilorhachis* are noticeably longer (28–45 mm) than those of *P. macrocephalus sens. str.* (20–24 mm) and *P. xerophilus* (14–25 mm). Bean (2008) suggested that sepal length was clinal in *P. macrocephalus sens. lat.*, but we found no evidence of clinal change in sepal length in our concepts of *P. macrocephalus sens. str.* or *P. xerophilus*. There is a general clinal trend in sepal length in *P. psilorhachis* (with long sepals predominantly at the northern end of the range and shorter sepals in the south); however, we found that there were exceptions at both ends of the geographic range. In addition to the differences in sepal length, the outer sepals of *P. xerophilus* are narrower (0.7–1.1 mm wide) than those of *P. macrocephalus* (1.5–2.0 mm) and *P. psilorhachis* (1.3–1.8 mm).

Geospatial analyses

Ptilotus macrocephalus was represented in the geospatial dataset for the Maxent modelling by 130 specimens, *P. psilorhachis* by 35 specimens and *P. xerophilus* by 262 specimens.

The distribution model for *P. macrocephalus* largely fits its known distribution (Fig. 4A), with areas in the southeast of mainland Australia and in Tasmania fitting well with the climatic conditions modelled for the species. The model returned an average AUC of 0.899 with a standard deviation of 0.008. Variables contributing most to the model were Bio27 (radiation of coldest quarter; 34.8%) and Bio28 (annual mean moisture index; 29.5%). The highest probability areas were in eastern Victoria and eastern to central New South Wales; an apparent disjunction, on the basis of known specimens between southern Victoria and the Northern and Central Tablelands of New South Wales appears to not be a sampling artefact, with reduced probabilities of occurrence in the gap. A specimen from Toowoomba in southern Queensland (BRI AQ0178569) was determined as *P. macrocephalus* in the present study, but it was not included in the geospatial analysis because of the georeferenced precision being > 10 km.

The model for *P. psilorhachis* predicted its occurrence through much of eastern Queensland, with lower probabilities on Cape York Peninsula extending west across the northern end of the Northern Territory to the Kimberley region of Western Australia (Fig. 4B). The model returned an average AUC of 0.976 with a standard deviation of 0.004. Variables contributing most to the model were Bio18 (precipitation of warmest quarter; 48.0%) and Bio28 (21.8%). There is an apparent gap between the occurrences of *P. psilorhachis* in northern Queensland inland of Cairns and southern Queensland, which may be due to

under-collecting or perhaps indicates that the available habitats there are not suitable for the species. The distribution models of *P. macrocephalus* and *P. psilorhachis* overlap around the border of northern New South Wales and southern Queensland, but no specimens of *P. psilorhachis* are known from New South Wales.
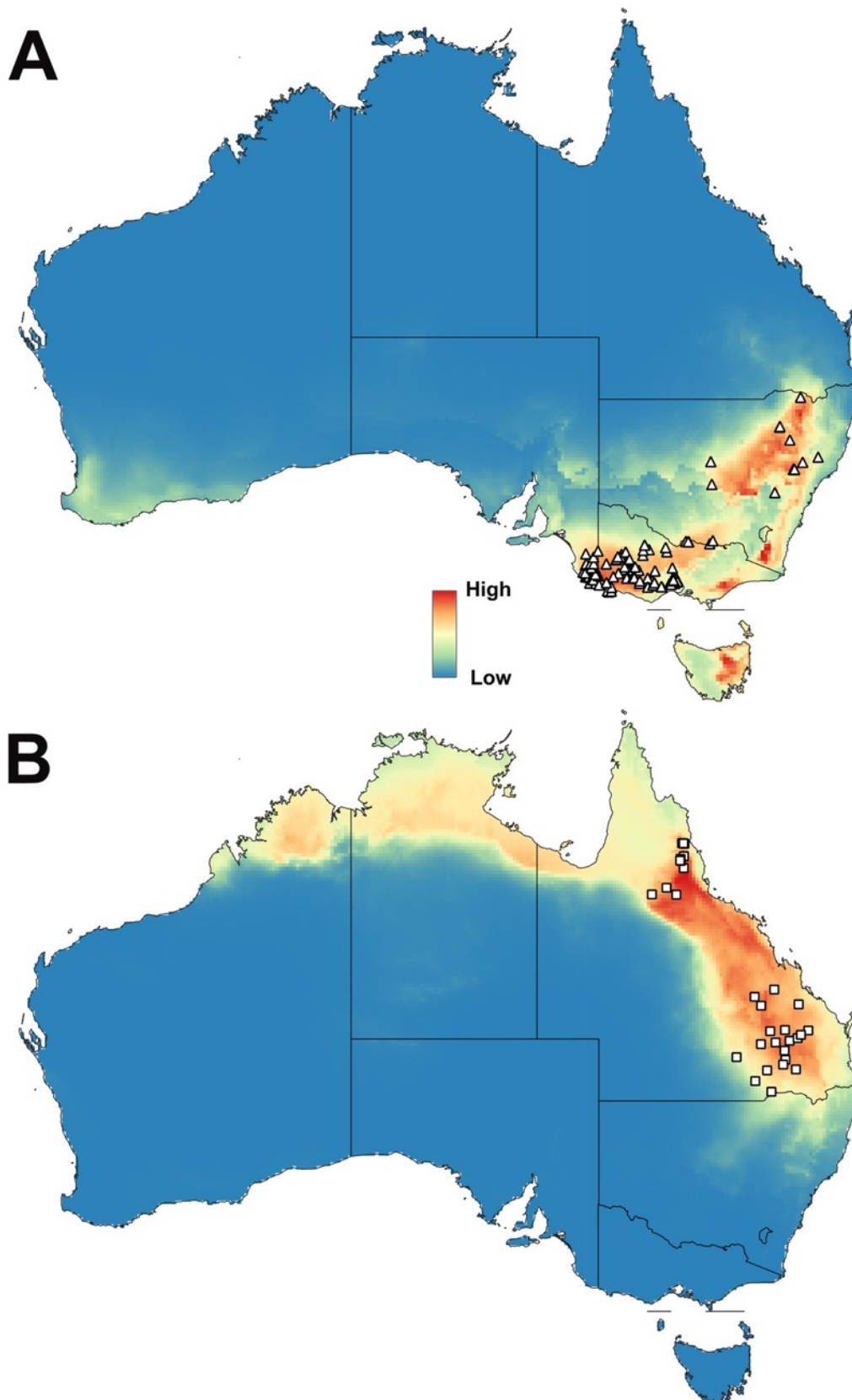
Fig. 4. Maps of the Maxent distribution models of the probability of occurrence. A. *Ptilotus macrocephalus*. B. *P. psilorhachis*. Points are the occurrence records used in the analysis for each taxon.
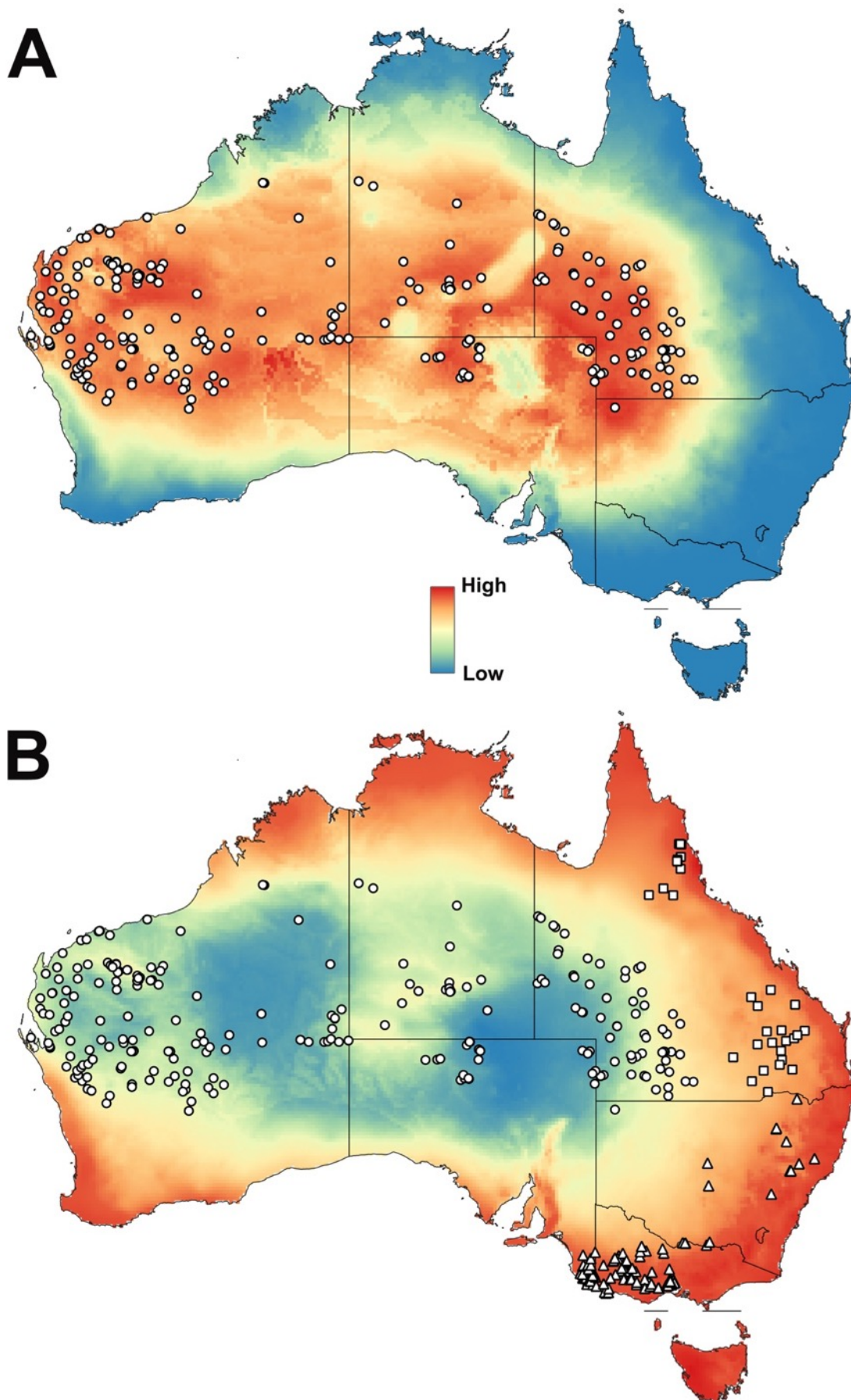
Fig. 5. A. Map of the Maxent distribution model of the probability of occurrence of *Ptilotus xerophilus*. B. The records of all three taxa plotted over a map of the variable Bio28, annual mean moisture index. Points are the occurrence records used in the analysis for each taxon: *P. macrocephalus* (triangles), *P. psilorhachis* (squares) and *P. xerophilus* (circles).

The model for *P. xerophilus* predicted its occurrence accurately throughout central and western Australia, excluding the far north, south and east (Fig. 5A). The model returned an average AUC of 0.791 with a standard deviation of 0.007. Variables that contributed most to the model were Bio21 (highest weekly radiation; 37.1%), Bio28 (22.9%) and Bio27 (12.4%). Narrow distributional gaps in northern South Australia and the south-eastern Northern Territory may be real on the basis of lower probabilities of modelled occurrence.

The species distribution models for each taxon were geographically allopatric or parapatric (Fig. 4, 5), with very little overlap at the margins of each. Across all three models, the variable Bio28 (annual mean moisture index) was an important predictor in driving the distribution of the taxa (Fig. 5B).

The PCA of the entire dataset shows that the three taxa, originally separated by differences in morphology, occupy discrete climatic envelopes (Fig. 6). *Ptilotus psilorhachis* occupies the largest climatic space of the three taxa, with specimens from southern Queensland and northern New South Wales being resolved disjunctly from those in northern Queensland and adjacent to a few specimens of *P. macrocephalus* from northern New South Wales. These latter specimens are also climatically disjunct from the main area of distribution of *P. macrocephalus* in Victoria.
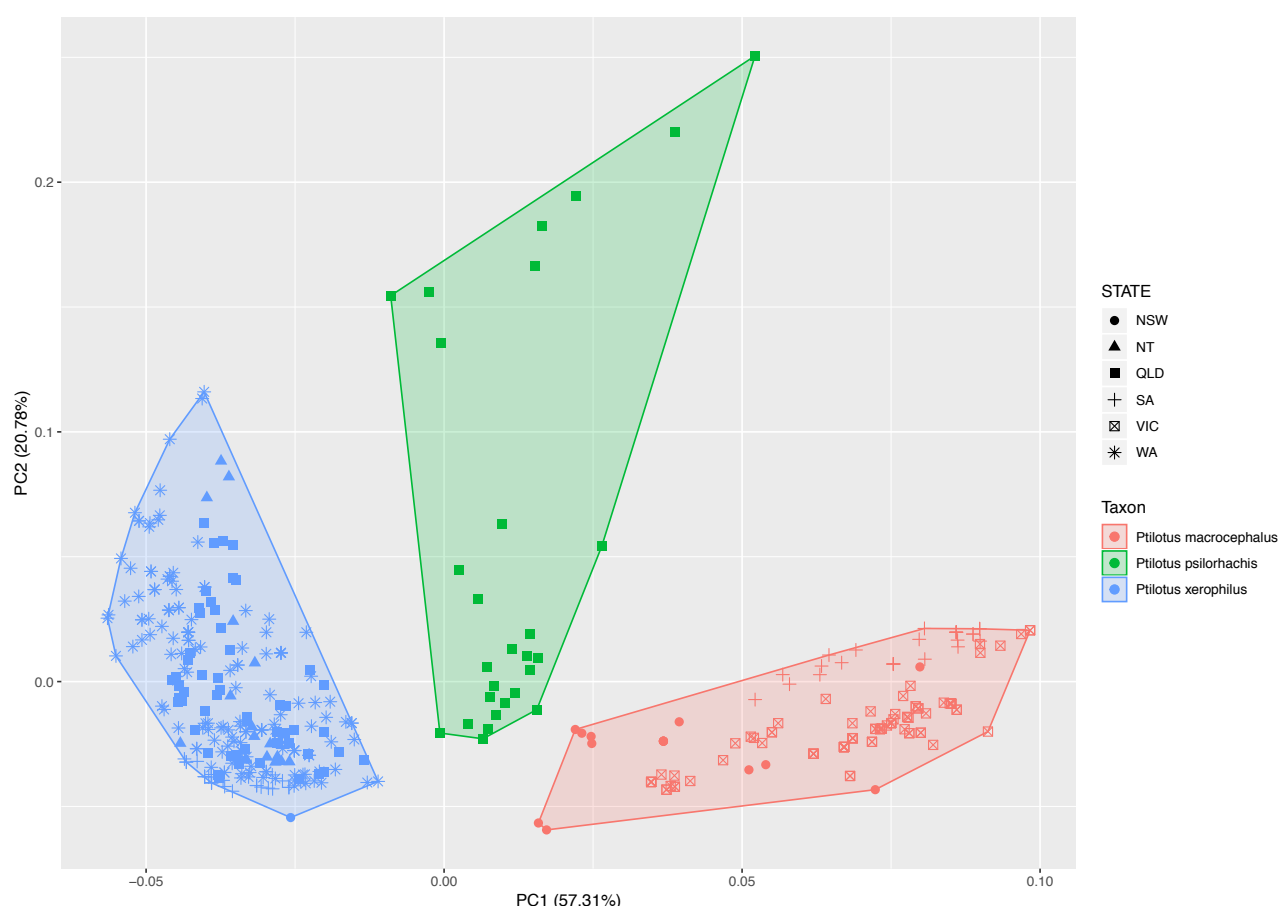
Fig. 6. Principal component analysis (PCA) ordination of the geospatial dataset, with convex hulls representing the climatic space for each taxon.

DISCUSSION

Geospatial analyses

Maxent modelling and the PCA ordination on the basis of environmental parameters showed that the three morphologically-defined taxa are also ecologically partitioned, with the partitioning matching their distributions.

Because few specimens of *P. macrocephalus* from New South Wales were available for the geospatial analysis, the climatic space occupied by that species may be more continuous than is apparent in the current PCA (see Fig. 6), although the Maxent model showed that these occurrences in northern New South Wales and those from Victoria may be separated by unsuitable environments on the Southern Tablelands and Southwest Slopes. An apparent environmental disjunction between northern and southern populations of *P. psilorhachis* may be caused by under-collecting or by a lack of suitable habitat in these areas (Fig. 4B).

The distribution of *P. xerophilus* is disjunct from the other two taxa, and it is more clearly different, in ecology, from the other two species, which occur in noticeably higher-rainfall regions of Australia. Despite its wider geographic distribution, it occupied the smallest environmental envelope on the PCA (Fig. 6), further suggesting that the geographically narrow disjunctions in its modelled distribution in northern South Australia and the southern Northern Territory are likely to be real.

Phylogenetic relationships

A single specimen of *P. xerophilus* was included (as *P. macrocephalus sens. lat.*) in the phylogeny of Hammer *et al.* (2015), where it formed a clade with *P. beckerianus* (F.Muell.) F.Muell. ex J.M.Black, *P. distans* (R.Br.) Poir., *P. capensis* (Benl) A.R.Bean, *P. fusiformis* (R.Br.) Poir., *P. giganteus* and *P. polystachyus*.

Relationships among some of these species remain unresolved or poorly supported owing to a lack of sequence variation within the markers used for the phylogeny, perhaps indicating recent speciation within the group (Hammer *et al.* 2015). Taxonomic patterns within this group are complex, as evidenced by several new species being recognised recently, on the basis of discrete but subtle segregation of morphological characters (e.g. *P. capensis* separated from *P. distans* by Bean (2008) and *P. giganteus* from *P. polystachyus* by Davis and Butcher (2010)).

Because *P. macrocephalus sens. str.* and *P. psilorhachis* were not included in these phylogenies, their relationships to the other species in the clade are uncertain. The three taxa in *P. macrocephalus sens. lat.* recognised here may not comprise a monophyletic group.

Ptilotus distans, *P. capensis* and *P. giganteus* are distributed in the monsoonal tropics of northern Australia in the northern end of the Northern Territory of the Northern Territory, Cape York Peninsula to just south of Cairns, and the Kimberley region of Western Australia respectively. These areas all fall within the predicted area of distribution of *P. psilorhachis*. It is possible that all these species comprise a single Northern Australian radiation from a common ancestor adapted to the monsoon tropics.

Ptilotus beckerianus is a rare endemic in South Australia, occurring in the southern part of the Eyre Peninsula and on Kangaroo Island, west of and disjunct from the western edge of the range of *P. macrocephalus sens. str.* (i.e. occurrences in the Naracoorte Coastal Plain IBRA bioregion). Both species are perennial herbs with woody taproots (Black 1948). *Ptilotus beckerianus* is perhaps most morphologically similar to *P. macrocephalus sens. str.* within this clade and may be closely related,

perhaps indicating a small southern radiation from a temperate-adapted ancestor. The species that are the sister group to the subclade that includes all these species (i.e. *P. clementii* (Farmar) Benl, *P. gardneri* Benl, *P. polystachyus*) all occur in northern arid or semi-arid areas (the last with a wide distribution, including temperate southern and monsoon-tropical northern Australia). It is thus possible that the clade has diversified by recent peripatric or parapatric speciation from an arid-adapted common ancestor, with radiation into new tropical and temperate niches.

Taxonomic implications

The present study has demonstrated that, as currently circumscribed, *P. macrocephalus sens. lat.* includes three distinct taxa that are partitioned morphologically, geographically and ecologically. We consider the morphological differences among these species to be significant, and the ecological differences between these taxa add validation to the concept that they are distinctly evolving metapopulation lineages, as recognised by the general-lineage species concept (de Queiroz 2007). We, therefore, regard that these taxa are most appropriately recognised at species rank. Accordingly, we formally name and describe here the new species *P. psilorhachis* T.Hammer & R.W.Davis and *P. xerophilus* T.Hammer & R.W.Davis, and recircumscribe *P. macrocephalus* in the narrow sense as discussed above.

TAXONOMY

Ptilotus macrocephalus (R.Br.) Poir., *Lam., Encycl. Suppl.* 4: 620 (1816).

Trichinium macrocephalum R.Br., *Prodr.* 415 (1810). *Type*: Port Phillip [Victoria], *s. dat., Anon. s.n.* (syn: BM 000895593, image! https://plants.jstor.org/stable/10.5555/al.ap.specimen.bm000895593; syn: GB 0047020, image! https://plants.jstor.org/stable/10.5555/al.ap.specimen.gb-0047020).

Trichinium angustifolium Moq. in A.L.P.P. de Candolle (ed.), *Prodr.* 13(2): 293 (1849). *Type*: New South Wales, the upper parts of Hunter River, 1825, *A.Cunningham s.n.* (syn: K 000356810, image! https://plants.jstor.org/stable/10.5555/al.ap.specimen.k000356810; syn: P 00609981, image! https://plants.jstor.org/stable/10.5555/al.ap.specimen.p00609981; syn: G-DC (microfiche), *fide* Bean 2008).

Trichinium pachocephalum Moq. in A.L.P.P. de Candolle (ed.), *Prodr.* 13(2): 294 (1849); *Ptilotus pachocephalus* (Moq.) F.Muell., *Fragm.* 6(49): 228 (1868). *Type*: Port Phillip [Victoria], 20 Mar. 1840, *R.C.Gunn s.n.* (syn: P 00609982, image! https://plants.jstor.org/stable/10.5555/al.ap.specimen.p00609982).

Erect perennial *herbs* with a woody, sometimes branching taproot, 30–50 cm high. *Stems* terete, ribbed, glabrous or glabrescent towards stem apex; hairs crisped-nodose or subverticillate. *Basal leaves* not seen. *Cauline leaves* linear-lanceolate to narrowly oblanceolate, sessile, 30–120 mm long, 2–14 mm wide, glabrous or glabrescent with sparse, verticillate hairs; margins entire, often undulate. *Inflorescences* spiciform, terminal, cylindrical, creamish-green (rarely with a pinkish flush), 30–90 mm long, 45–60 mm wide; rachis densely tomentose or sometimes villous; flowers tightly arranged on rachis; apex rounded or truncate. *Bracts* narrowly ovate to ovate, 7.5–11 mm long, 3.0–4.5 mm wide, transparent, glabrous or with verticillate hairs along the conspicuous, white or pale brown midrib; apex mucronate, the mucro 1.0–1.2 mm long; margins entire. *Bracteoles* broadly ovate to obovate, 6.5–9.8 mm long, 3.2–5 mm wide, transparent, glabrous; midrib conspicuous, white; apex shortly mucronate, the mucro 0.1–0.2 mm long; margins serrate. *Outer sepals* lanceolate, 20–24 mm long, 1.5–2.0 mm wide, creamish-green (rarely with a pale pink flush toward apex), adaxially villous, abaxially villous with spreading verticillate hairs almost throughout; apex acute, glabrous. *Inner sepals* lanceolate, 19–23 mm long, 0.8–1.2 mm wide, with colour and indumentum as for outer. *Fertile stamens* 4; *filaments* cream, 15–19 mm long, unequal, filiform; *anthers* yellow, 1.2–2.0 mm long, 0.4–1.0 mm wide. *Staminode* 1, 0.5–

3.5 mm long. *Staminal cup* symmetrical, unlobed, 0.4–0.5 mm long, strongly adnate to sepal tube, apically covered with simple hairs. *Ovary* obconical, 0.8–1.3 mm long, 1.0–1.8 mm wide, apically covered with simple hairs; stipe 1.0–2.0 mm long. *Style* sigmoidal, 17–22 mm long, cream, excentrically fixed on the ovary, verticillate-hairy on the lower half. *Stigma* capitate. *Seed* smooth, pale brown, ~2.7 mm long, ~1.5 mm wide. (Fig. 1A, B, 2A, B, 3A)

Distribution and habitat

Ptilotus macrocephalus occurs from south-eastern South Australia, through central and northern Victoria, into central to eastern New South Wales, and just over the border into south-eastern Queensland (Fig. 4A). On the basis of herbarium label data, the species commonly inhabits open grasslands dominated by *Austrostipa* spp. and *Themeda triandra* on brown or reddish loamy soils derived from basalt in the south of its range. In the north of the range, it is recorded on north-facing hill slopes or on flats in open eucalyptus woodlands with stony, red or brown sandy or clayey loam soils.

Conservation status

Common in South Australia and Victoria; known occurrences in New South Wales are somewhat sporadic, many being older collections, indicating that it may be restricted in occurrence and uncommon in that state. The species may be rare in Queensland, occurring only near the border with New South Wales; therefore, its conservation status should be assessed.

Notes

The epithet of *Trichinium pachocephalum* deviates from the usual construction ('*pachycephalum*'), as it derives from the Greek noun *pachos* (thickness) rather than the more usual adjective *pachys* (thick). While this construction is not strictly correct grammatically, it is valid under Art. 60.10 of the *ICN* (Turland *et al.* 2018) and is not a correctable orthographic error.

Selected specimens examined

QUEENSLAND. Toowoomba, *C.H.Hartmann s.n.* (BRI AQ0178569). SOUTH AUSTRALIA. Near Donovan's Landing, *B.Copley 1807* (AD); Desert Camp Conservation Park, *D.J.Duval & M.K.Jones 275A* (AD); Mary Seymour Conservation Park, *H.P.Vonow 2906* (AD). NEW SOUTH WALES. Carters Road, Pigna Barney, *N.Cobcroft s.n.* (NSW 441772); 8.2 miles [~13.2 km] W of Sandy Hollow on the Mudgee Road, *R.G.Coveny 2451* (NSW); alongside Carters Road east of Boonara, *J.R.Hosking 2125* (NSW); Goulburn River Valley, ~5 km SW of Baerami, *T.A.James, W.Bishop & S.V.Goodwin 660* (NSW); Narrabri, *J.H.Maiden s.n.* (NSW 29487); near Lake Urana, *W.E.Mulham W975* (NSW). VICTORIA. Hawkes Road, *C.W.Ahrens 40* (MEL); Hands Road, *C.W.Ahrens 43* (MEL); Oliver's Lake Flora and Fauna Reserve, *A.C.Beauglehole 86470* (AD); Grampian Mountains, *D.Keane s.n.* (AD 96947051); Werribee, *A.Morrison 1467* (PERTH); 1.8 km upstream from Donovan, *R.D.Pearce 395* (AD); Anglesea, *V.Stajsic 99* (MEL); Willaura–Wickliffe road, *N.G.Walsh & Z.Smith 5698* (MEL).

Ptilotus psilorhachis T.Hammer & R.W.Davis, sp. nov.

Type: Queensland, Emu Creek Station, 8.5 km NNE of Petford, 20 Jan. 2002, *P.I.Forster, R.Booth & R.Jensen PIF 28204* (holo: BRI AQ555919!; iso: AD 142716!, MEL 2291403A!).

Erect annual or short-lived perennial *herbs* with fleshy taproot, 40–60 cm high. *Stems* terete, ribbed, glabrous or glabrescent towards stem apex; hairs crisped-nodose or subverticillate. *Basal leaves* not seen. *Cauline leaves* linear-lanceolate to narrowly oblanceolate, sessile, 20–120 mm long, 1–6 mm wide, glabrescent or with sparse, verticillate hairs; margins undulating or entire. *Inflorescences* spiciform, terminal, cylindrical, creamish-green (rarely with a pinkish flush), 40–100 mm long, 60–90 mm wide; rachis glabrous or very sparsely hairy; flowers loosely arranged on rachis; apex rounded or truncate. *Bracts* narrowly ovate to ovate, 9–12 mm long, 2.5–4 mm wide, transparent, glabrous or with

verticillate hairs along midrib; midrib conspicuous, white or pale brown; apex mucronate, the mucro ~1 mm long; margins entire. *Bracteoles* obovate, 7–11.5 mm long, 2.5–5 mm wide, transparent, glabrous; midrib conspicuous, white; apex shortly mucronate, the mucro 0.1–0.2 mm long; margin serrate. *Outer sepals* lanceolate, 28–45 mm long, 1.3–1.8 mm wide, creamish-green or rarely with pale pink flush toward apex, adaxially villous, abaxially villous with spreading verticillate hairs; apex acute, glabrous. *Inner sepals* lanceolate, 27–42 mm long, 0.6–1.0 mm wide, creamish-green or rarely with pale pink flush toward apex, adaxially glabrous, abaxially villous with spreading verticillate hairs; apex acute, glabrous. *Fertile stamens* 4; *filaments* cream, 20–36 mm long, unequal, filiform; *anthers* yellow, 2.0–3.0 mm long, 0.5–1.0 mm wide. *Staminode* 1, 0.4–6 mm long. *Staminal cup* symmetrical, not lobed, 0.3–0.5 mm long, strongly adnate to sepal tube, the apex covered with simple hairs. *Ovary* obconical, 1.2–2.2 mm long, 1.4–2.0 mm wide, glabrous; stipe ~1 mm long. *Style* sigmoidal, 25–40 mm long, cream, excentrically fixed on the ovary, with verticillate hairs on lower half. *Stigma* capitate. *Seed* smooth, pale brown, 3.3–3.5 mm long, 1.9–2.0 mm wide. (Fig. 1C, D, 2E, F, 3C)

Distribution and habitat

Ptilotus psilorhachis is distributed in eastern Queensland from west of Cairns south to west of Brisbane (Fig. 4B), including the IBRA subregions of Einasleigh Uplands, Brigalow Belt North and Brigalow Belt South. On the basis of herbarium label data, the species commonly inhabits open eucalypt woodlands, commonly dominated by *Eucalyptus populnea*, *E. crebra* or *E. leptophleba*, on flats with brown or red sandy or clayey loam soil, sometimes with a gravelly surface.

Etymology

From the Greek *psilos* (naked) and *rhakhis* (a spine or rachis), referring to the conspicuous and glabrous or very sparsely hairy rachis characteristic of the species.

Conservation status

Ptilotus psilorhachis is common across its range and is not considered to be of conservation concern.

Selected specimens examined

QUEENSLAND. 3 km along Pearlinga road W of Mundubbera, *A.R.Bean 11936* (BRI); 65 km from Mitchell on road to Bollon, *A.R.Bean 24397* (BRI); 16.6 km along Roche Creek Road E of Wandoan, *A.R.Bean 29487* (BRI); Gayndah Dirnbir, *E.W.Bick s.n.* (BRI AQ0178578); 'Potters Flats' ~52 km north of Yuleba, *C.Eddie CPE2472* (BRI); ~1.5 km by road east of Dimbulah, *K.R.McDonald KRM4927* (BRI); 40 miles [~64.4 km] S of Ayr on W bank of Burdekin River, *H.C.Seton 5* (BRI); Lakeland Downs near Condamine, *K.M.Stephens, C.Thrupp & A.Daniel s.n.* (BRI AQ0855297); 4 km from Petford along road to Irvinebank, *I.R.Telford & R.J.Rudd 11249* (BRI); Nangram Station, Condamine Highway, ~10 miles [~16 km] NE of Condamine, *W.G.Trapnell s.n.* (BRI AQ0178565); 4 km south of Mount Garnet on the Gunnawarra Road, *B.S.Wannan 3998* (BRI); Old coal road between Gladstone and Biloela, *M.Worthington 1676* (BRI).

Ptilotus xerophilus T.Hammer & R.W.Davis, sp. nov.

Type: Western Australia, 700 m south along Paynes Find–Yalgoo road from junction of Geraldton–Mount Magnet road, 26 Aug. 2018, *R.Davis & T.Hammer RD12901* (holo: PERTH!; iso [to be distributed]: BRI!, CANB!)

Erect annual *herbs* with fleshy taproot, 20–120 cm high. *Stems* terete, ribbed, glabrous or glabrescent towards stem apex; hairs crisped-nodose or subverticillate. *Basal leaves* not seen. *Cauline leaves* linear-lanceolate to narrowly oblanceolate, sessile, 10–130 mm long, 2–8 mm wide, glabrescent or with sparse verticillate hairs; margins undulating or entire. *Inflorescences* spiciform, terminal, cylindrical, creamish-green or rarely with pinkish flush, 30–70 mm long, 32–50 mm wide, sweetly scented at night; rachis densely tomentose or sometimes villous; flowers tightly arranged on rachis;

apex acute, rounded or truncate. *Bracts* ovate to broadly ovate, 6–10 mm long, 2.5–4.5 mm wide, transparent, glabrous or with verticillate hairs along midrib; midrib conspicuous, white or pale brown; apex mucronate, the mucro 0.5–0.6 mm long; margins entire. *Bracteoles* obovate, 5–9 mm long, 2–4 mm wide, transparent, glabrous; midrib conspicuous, white; apex shortly mucronate, the mucro ~0.1 mm long; margins serrate. *Outer sepals* linear to lanceolate, 14–25 mm long, 0.7–1.1 mm wide, creamish-green or rarely with pale pink flush toward apex, adaxially glabrous, abaxially villous with spreading verticillate hairs in the upper half, the indumentum sparse, short and appressed in lower half to one third; apex acute, glabrous. *Inner sepals* linear to lanceolate, 13–25 mm long, 0.5–0.8 mm wide, creamish-green or rarely with pale pink flush toward apex, adaxially glabrous, abaxially villous with spreading verticillate hairs, the indumentum sparse, short and appressed in lower half to one third; apex acute, glabrous. *Fertile stamens* 4; *filaments* cream, 11–20 mm long, unequal, filiform; *anthers* yellow, 1.3–2.0 mm long, 0.5–0.6 mm wide. *Staminode* 1, 0.5–4 mm long. *Staminal cup* symmetrical, not lobed, 0.2–0.4 mm long, strongly adnate to sepal tube, glabrous. *Ovary* obconical, 0.8–1.5 mm long, 1–1.5 mm wide, glabrous or with a row of simple hairs apically; stipe ~1 mm long. *Style* sigmoidal, 12–22 mm long, cream, excentrically fixed on the ovary, with verticillate hairs on lower half. *Stigma* capitate. *Seed* smooth, pale brown, 1.8–2 mm long, 1.0–1.1 mm wide. (Fig. 1E, F, 2C, D, 3B)

Distribution and habitat

Occurs throughout arid regions of Western Australia, Northern Territory and in northern South Australia, western Queensland and north-western New South Wales (Fig. 5A), typically on plains with clayey or loamy soils and often in open mulga shrublands.

Etymology

From the Greek *xeros* (dry) and *philia* (love), referring to the distribution of this species in lower-rainfall regions of Australia than that of the other two species in the *P. macrocephalus* complex, which occur in higher-rainfall regions of eastern Australia (see Fig. 5B).

Conservation status

The species is common in Western Australia, Northern Territory, South Australia and Queensland, but may be uncommon or rare in north-western New South Wales, with only a few specimens recorded from the northwestern corner of the state, where its status should be assessed.

Notes

This species often occurs *en masse*, particularly in Western Australia. We consistently observed this species being visited by night-flying moths (e.g. in the family Erebidae) in Western Australia. Floral observations were also made in daylight, and no insect visitation was seen. The flowers of this species are conspicuously sweetly scented at night. The closely related species *P. polystachyus*, which is also green-flowered and night-scented, was also observed to be visited by moths at night. We suspect *P. xerophilus* and the related green-flowering species to be primarily pollinated by nocturnal moths (see also Hammer et al. 2018b).

Selected specimens examined

WESTERN AUSTRALIA. Eastern Hamersley Range, *N.Casson & E.M.Mattiske MCPL1002* (PERTH); 186 km S of Carnarvon on North West Coastal Highway, *R.Davis 10995* (PERTH); On Rio Tinto Rail Access Road, ~18 km N of of Nanutarra–Munjina Road, *T.Hammer & S.Dillon TH30* (PERTH); Woolgorong Station Homestead Gate, *T.Hammer & R.Davis TH61* (PERTH); 80 km N of Mullewa, *T.Hammer & R.Davis TH65* (PERTH). NORTHERN TERRITORY. Ruby Gorge, Hale River, 112 km ENE of Alice Springs, *A.C.Beauglehole 20724* (AD); 11.6 miles [~18.7 km] NE of Frewena, *G.M.Chippendale 7346* (PERTH); Mount Olga, W side at Docker River road junction, *N.N.Donner 4408* (AD); ~5 km SE of Alice Springs, *J.Z.Weber 893* (AD). SOUTH AUSTRALIA. Cordillo Downs, *J.Bates 47179* (AD); Arckaringa Station, *P.J.Lang BSOP-441* (AD); on the Indulkana Range Plateau, *P.J.Lang & P.D.Canty BS23-29163* (AD); ~16 km

W of Arckaringa Homestead along road to Evelyn Downs, *T.S.Te, D.J.Duval & D.E.Murfet 1021* (AD). QUEENSLAND. 7.2 km E of Stonehenge, *A.R.Bean 22437* (BRI); 40 km E of Quilpie towards Charleville, *A.R.Bean 29976* (BRI); Colwell Mackinlay, *D.M.Collings s.n.* (BRI AQ0178574); 67 km WNW of Mount Isa and 6 km N of Mingera, *P.L.Harris 281* (BRI); Idalia National Park, Hobbs Tank, *C.Morgan CM11* (BRI); ~11 km E of Scott's Tank, Diamantina National Park, *M.Mostert MM256* (BRI); Well Flat, 2 km S of Wathopa Homestead, *J.L.Silcock JLS1522* (BRI). NEW SOUTH WALES. 5 miles [~8 km] W of Tibooburra, *G.M.Cunningham 1164* (NSW).

Key to species of the *P. macrocephalus* group

1.   Inflorescence rachis densely villous or tomentose 2
1:     Inflorescence rachis glabrous or with a few sparse hairs present ....*P. psilorhachis*
2.   Perennial herb with a woody taproot; outer sepals >1.4 mm wide; abaxial sepal surface with spreading verticillate hairs on entire surface (except glabrous apices); hairs present on adaxial sepal surface; staminal cup hairs present... *P. macrocephalus*
2:     Annual or short-lived perennial herb with fleshy taproot; outer sepal <1.2 mm wide; abaxial sepal surface with short appressed hairs on bottom half; hairs absent on adaxial sepal surface; staminal cup hairs absent...*P. xerophilus*

ACKNOWLEDGEMENTS

SUPPLEMENTARY MATERIALS

The following supplementary materials are available as electronic downloads at https://www.taxonomytim.com/thesis (under Chapter 5): **Suppl. Table S1.** The dataset used in the geospatial analyses, including the species determination, specimen barcode, state of occurrence, locality coordinates and associated environmental variables used in the analyses.

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PART 3

Why are the aervoids so diverse in arid Australia?

Out of the arid: the origin of *Ptilotus* (Amaranthaceae) and radiations into seasonally wet Australia

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Abstract. Arid-adapted immigrant lineages were important in the assembly of the Australian biota. One such lineage is the aervoid clade of Amaranthaceae *s.str.*, which includes six genera and c. 135 species that occur from West Africa to Australia. The centre of diversity for the clade is Australia, with c. 120 *Ptilotus* species occurring in nearly every climatic region, though with most species in arid Western Australia. In the present study, we test the chronological and geographic origins of the aervoids and *Ptilotus* by reconstructing a dated phylogeny for the family with near-comprehensive sampling and estimate ancestral geographic ranges for clades. We also investigate climatic niche evolution within *Ptilotus* and identify likely climatic origins and subsequent niche shifts by reconstructing ancestral states of climatic variables on the aervoid phylogeny, visualised using a phyloecospace approach. Rates of diversification within *Ptilotus* are correlated with biogeographic events, and geospatial analyses are employed to identify probable diversification hotspots within Australia. We infer that the aervoids originated in Oligocene Africa-Asia and that *Ptilotus* arrived in northern Australia via dispersal, probably via the islands of the Sunda Shelf in the Early Miocene. Subsequent diversification of *Ptilotus* was rapid, giving rise to all major clades in the western Eremaean by the time of an aridification pulse in the Middle Miocene. Climatic niche shifts are apparent for multiple independent species groups within the four largest clades of *Ptilotus*, from the arid Eremaean into monsoonal northern and temperate southern Australia. Our analyses support the hypothesis that a pre-adaptation to aridity and early arrival in an aridifying Australia were integral to the success of *Ptilotus* when compared to its relatives, and that the Eremaean has been a source for biodiversity of the genus and for independent radiations into neighbouring climatic zones.

Keywords: arid, Australia, Eremaean, ecology, historical biogeography, niche shift, phyloecospace

INTRODUCTION

The assembly of the Australian biota during the Cenozoic is of great interest (e.g. Cracraft 1986, 1994, Byrne *et al.* 2008, Byrne *et al.* 2011, Crisp and Cook 2013, Lee *et al.* 2015, Oliver and Hugall 2017). The isolation of Australia following the breakup of Gondwana (c. 40–30 Ma) presented a unique scenario of isolated lineage evolution as the continent drifted northward (Byrne *et al.* 2011, Crisp and Cook 2013). As Australia approached Asia, a floristic exchange commenced with dispersals between the continents, particularly since the Oligocene around 25 Ma (Crayn *et al.* 2015). Approximately half of modern Australian plant lineages are derived from overseas dispersals (Crisp *et al.* 2004, Crisp and Cook 2013). Both Gondwanan and immigrant plant lineages had to adapt to a continent undergoing extensive environmental change (Byrne *et al.* 2008, 2011).

Aridification in Australia commenced around the Eocene-Oligocene boundary (c. 32 Ma), probably largely caused by a change in global ocean currents initiated by the separation of Australia from Antarctica (Martin 2006). This period was marked by a large turnover in the global biota (Sun *et al.* 2014, Oliver and Hugall 2017). Aridity in Australia intensified as it drifted northward (Lawver and Gahagan 2003, Martin 2006), with the overall trend overlain with pulses of aridification during the Miocene and Pliocene related to global events including an overall cooling, drop in sea levels, and expansion of the Antarctic ice sheets (Martin 2006). Today, approximately two-thirds of Australia is arid

or semi-arid as defined by the Köppen-Geiger climate classification (Fig. 1A; Beck *et al.* 2018), with the most arid region referred to as the Eremaean region (Fig. 1B; Ebach *et al.* 2015).

Australia is home to many species-rich plant lineages that cover the bulk of the arid continent, which have adapted to arid or semi-arid conditions, such as *Acacia* Mill. (Ariati *et al.* 2006), *Eucalyptus* L'Hér. (Mathews and Bonser 2005, Thornhill *et al.* 2019) and *Triodia* R.Br. (Toon *et al.* 2015, Anderson *et al.* 2019). These lineages could have evolved *in situ* in response to the aridifying climate. Donoghue (2008) and Edwards and Donoghue (2011), however, have argued that emerging niches are more likely to be occupied by immigrant lineages arriving with relevant adaptations rather than by *in situ* evolution from non-immigrant species. This may have been the case for arid Australia, as many successful arid plant lineages appear to be immigrants, such as *Triodia* (Toon *et al.* 2015) and members of Chenopodiaceae (Shepherd *et al.* 2004, Kadereit and Freitag 2011).

Some immigrant lineages appear to be much more species-rich within arid Australia than on other continents, such as the aervoids (Hammer *et al.* in press). This clade within Amaranthaceae *s.str.* includes *Ouret* (8 spp.; previously *Aerva s.lat.*), *Paraerva* T.Hammer (2 spp.; previously *Aerva s.lat.*), the monotypic genera *Omegandra* G.J.Leach & C.C.Towns., *Nothosaerva* Wight and *Aerva s.str.* (i.e. *A. javanica* (Burm.f.) Juss. ex Schult.; Hammer *et al.* in press) and *Ptilotus* R.Br. (mulla mulla), which includes over 120 spp. of herbs and shrubs. A recent study by Hammer *et al.* (in press) established *Ptilotus* and *Ouret* as sister to a clade of the four smaller genera (referred to here as the 'ONAP clade', using the first initial of each genus).

All species of *Ptilotus* are native to Australia, with one species (*P. conicus* R.Br.) extending to a few islands north of Australia (e.g. Timor; van Steenis 1979). Most taxonomic diversity in *Ptilotus* is in arid or semi-arid regions of Western Australia (Hammer *et al.* 2018a). Some can be locally dominant, particularly following seasonal rains, and produce spectacular *en masse* floral displays (Fig. 2A), making them an ecologically important floristic component of the deserts (Hammer *et al.* 2015, 2018b). Smaller numbers of species are found in open habitats in more mesic temperate communities (Fig. 2D, E) and tropical savannas. No species occur in alpine communities or heavily shaded forests (Hammer *et al.* 2015). *Ptilotus* is the most species-rich genus of Amaranthaceae *s.str.* in Australia. By comparison, the second largest amaranth genus in Australia is *Gomphrena* L. (c. 33 species), which is mostly restricted to the monsoonal tropics (Palmer 1998).

The other aervoid genera are much less diverse than the arid Australian genus *Ptilotus*. *Omegandra* is the only genus of the ONAP clade native to Australia, where its sole species occurs in near-coastal, monsoonal Northern Territory and Queensland (Leach *et al.* 1993). The monotypic *Nothosaerva* is morphologically similar to *Omegandra* (Hammer *et al.* in press) but occurs from West Africa (e.g. Senegal) to South Asia (e.g. India and Sri Lanka). Both occur in coastal floodplains with clay soils that are susceptible to seasonal flooding (Leach *et al.* 1993, Miller 1996). The morphologically variable, dioecious *Aerva javanica* is widespread throughout the drier parts of the old-world tropics and subtropics, in western Africa, southern Asia and northern Australia (Townsend 1974, 1985). Over much of its range it is represented solely by apomictic females, with male individuals found only in northeastern Africa and southern Arabia, the hypothesised native range of the species (Khan *et al.* 1970).
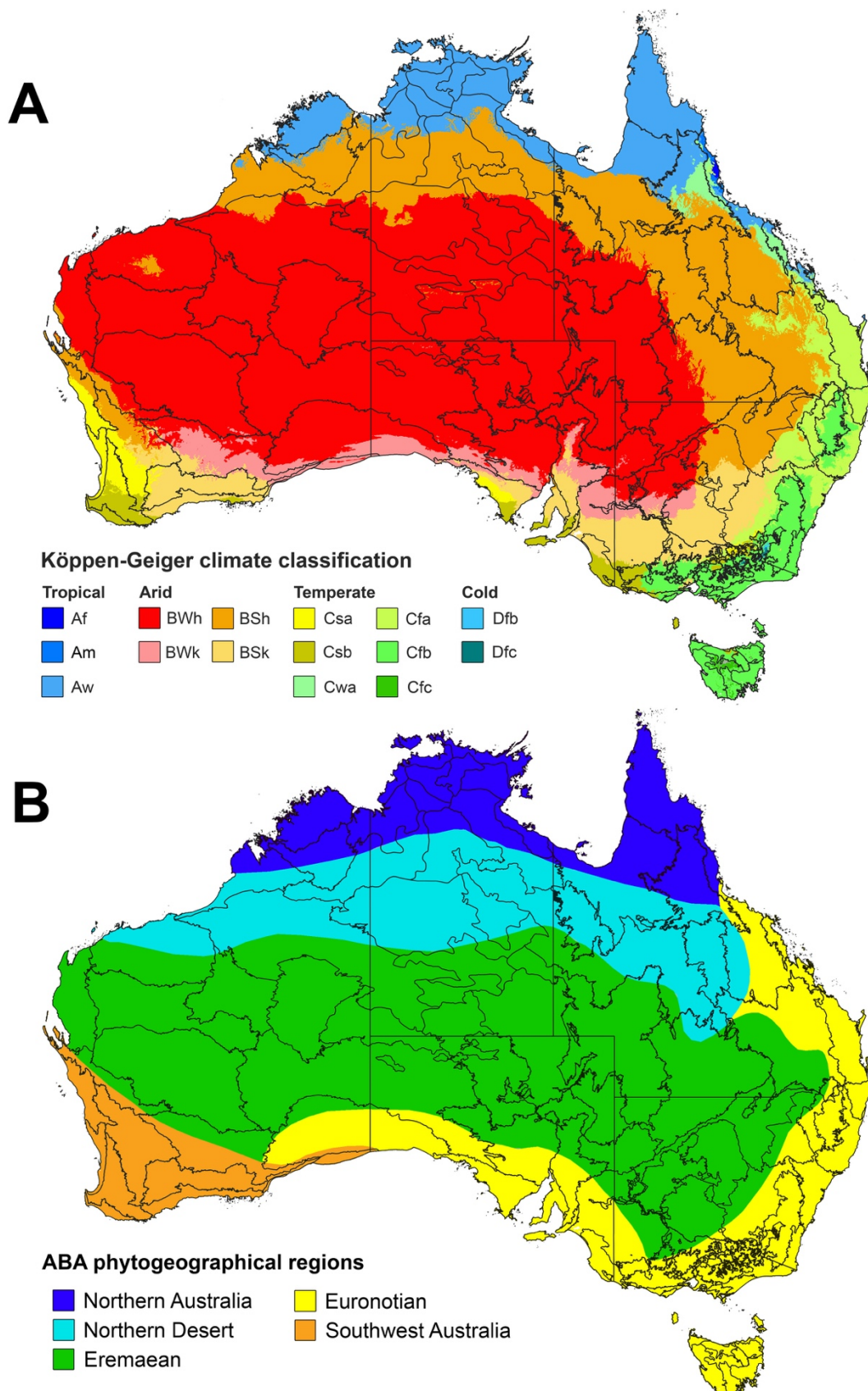
Fig. 1. Map of Australia indicating **A.** the present-day Köppen-Geiger climate classifications from Beck *et al.* (2018), and **B.** the five Australian Bioregionalisation Atlas phytogeographical regions reproduced from Ebach *et al.* (2015). Boundaries are IBRA 7.0 regions and used for reference. Köppen-Geiger climate abbreviations are as follows (primary climate in parentheses): Af – (tropical) rainforest; Am – (tropical) monsoon; Aw – (tropical) savanna; BWh – (arid) desert hot; BWk – (arid) desert cold; BSh – (arid) steppe hot; BSk – (arid) steppe cold; Csa – (temperate) dry-hot summer; Csb – (temperate) dry-warm summer; Cwa – (temperate) dry winter-hot summer; Cfa – (temperate) hot summer; Cfb – (temperate) warm summer; Cfc – (temperate) cold summer; Dfb – (cold) warm summer; Dfc – (cold) cold summer.

Fig. 2. Examples of habitat and morphology of *Ptilotus in situ*: **A.** Several annual *Ptilotus* species *en-masse* in the Pilbara, Western Australia, **B.** *Ptilotus royceanus* in rock crevices of a gorge near Kaltukatjara, Northern Territory, **C.** *Ptilotus aervoides* (F.Muell.) F.Muell. on a stony outwash plain in the Pilbara, Western Australia, **D.** *Ptilotus spathulatus* in a eucalyptus (York gum) woodland near Goomalling, Western Australia, **E.** *Ptilotus drummondii* (Moq.) F.Muell. in a eucalyptus (jarrah) forest near Gidgegannup, Western Australia, and **F.** *Ptilotus xerophilus* in a mulga shrubland near Leinster, Western Australia. Photographs by T. Hammer.

The genus *Paraerva* comprises two species of succulent subshrubs endemic to the island of Socotra, where they typically occur in cliff crevices and on rocky slopes (Miller 1996, Hammer et al. in press). *Ouret*, the sister genus of *Ptilotus*, is widely distributed from Africa (e.g. *O. leucura* (Moq.) Kuntze), Madagascar (e.g. *O. coriacea* (Schinz) T.Hammer), Indian Ocean islands (e.g. *O. congesta* (Balf.f. ex Baker) Kuntze) and southern Asia (e.g. *O. sanguinolenta* (L.) Kuntze) (Hammer et al. in press). *Ouret lanata* (L.) Kuntze has the largest distribution in the genus, extending from western Africa through southern Asia to Timor and New Guinea (Hammer et al. in press). *Ouret* species grow in open areas in woodlands, shrublands and grasslands and on rocky slopes and coastal sands (Townsend 1985).

Understanding the historical biogeography of Amaranthaceae *s.str.* has been challenging due to the lack of available fossils, including pollen (Di Vincenzo *et al.* 2018, Weselkov *et al.* 2018). Primary calibrations within Amaranthaceae have been limited to geological dating of islands with endemic taxa (Thiv *et al.* 2006). The sister family Chenopodiaceae has a better fossil and palynological records, allowing more accurate dating of phylogenies (e.g. Kadereit *et al.* 2005, Kadereit and Freitag 2011, Kadereit *et al.* 2012). Di Vincenzo *et al.* (2018) used fossil calibrations at the base of and within Chenopodiaceae to date the phylogeny of the Amaranthaceae-Chenopodiaceae alliance and investigate evolution within the achyranthoids, recovering an age for the crown group of Amaranthaceae *s.str.* of 43–58 Ma, the divergence of the aervoids from the achyranthoids and gomphrenoids at 24–37 Ma, and a date for the crown of aervoids at 18–32 Ma. The origin of the aervoids is thus in the late Oligocene or Early Miocene. However, only eight of c. 135 aervoid species were sampled for this study, limiting the inferences that can be made for the clade.

The genus *Ptilotus* and its smaller relatives provide a case study to investigate the evolution and diversification of the arid flora of Australia. A Late Oligocene date for the crown of the aervoids (Di Vincenzo *et al.* 2018) indicates that the divergence between *Omegandra* and *Ptilotus* is too late for a Gondwanan origin of *Ptilotus*, implying that the ancestral Australian aervoids arrived following long-distance dispersal and would have encountered a continent in the process of major aridification (Byrne *et al.* 2011). Extant species of *Ptilotus* are especially diverse in the arid regions of Australia, but it is not clear if the adaptation to aridity is a plesiomorphic or apomorphic trait for the genus.

In order to understand the evolution and diversification of *Ptilotus* in arid and non-arid Australia, we test the following hypotheses: 1) the ancestor of the aervoid clade occurred outside Australia and originated in the Oligocene, with *Omegandra* and *Ptilotus* being the result of independent dispersals from Asia; 2) the common ancestor of *Ptilotus* was adapted for arid conditions; and 3) arid Australia has been a source area for repeated, independent radiations of *Ptilotus* lineages into neighbouring climatic zones. We do this by testing the chronological and geographic origin of the aervoids and of *Ptilotus* and its major clades, to elucidate how *Ptilotus* became one of the most species-rich and morphologically diverse genera in Amaranthaceae *s.str.* and an important component of the arid Australian flora. The pattern of diversification uncovered for the aervoids will likely add to our knowledge of the assembly of the Australian biota and shed light on patterns seen in other diverse Australian lineages.

MATERIALS AND METHODS

We constructed a time-calibrated phylogeny of the aervoids with near-comprehensive sampling and identified time periods of high diversification by plotting lineages through time and modelling speciation rates. We reconstructed likely ancestral climates on inner nodes of the phylogeny using climatic data from extant species, and implemented a phyloecospace approach (see below) by mapping the phylogeny onto extant and inferred ancestral positions in climatic ecospace to test the hypothesis of

an arid origin for the aervoids and *Ptilotus* and to identify climatic niche shifts. Geographic centres of biodiversity within Australia for *Ptilotus* were identified using geospatial analyses of extant species.

Taxon sampling

Phylogenetic analyses were conducted on two molecular datasets. Dataset 1 was used to reconstruct the phylogeny of the aervoid genera for use in the geospatial and phyloecospace analyses. It comprised a concatenated dataset from Hammer *et al.* (in press) of *matK* cpDNA and internal transcribed space region (ITS) nrDNA for 125 species (112 *Ptilotus* spp., seven *Ouret* spp., two *Paraerva* spp., and *A. javanica*, *N. brachiata* (L.) Wight and *Omegandra kanisii* G.J.Leach & C.C.Towns.). This represents c. 92% coverage of the aervoid clade and includes all genera. The outgroup taxon *Achyranthes aspera* L. was used following Hammer *et al.* (2015). A topological constraint for the backbone of the phylogeny was derived using a set of 69 coding DNA sequences (CDS) from the plastome for 37 aervoid species (32 *Ptilotus* spp., *A. javanica*, *Omegandra kanisii*, *Ouret lanata* and *Paraerva microphylla*) and *Achryanthes aspera* (see Hammer et al. in press).

Dataset 2 was used to reconstruct a dated phylogeny of the Chenopodiaceae-Amaranthaceae alliance, with greater representation within the aervoids than previous studies, to allow estimation of divergence times for the major clades of aervoids. It comprised ITS and *trnK/matK* for 194 species, including all 125 species from Dataset 1. The remaining species were chosen to represent major clades within Amaranthaceae *s.str.* and Chenopodiaceae.

All sequences used in this study have been previously published and are available on Genbank. A full list of species and Genbank accession numbers are included in Supplementary Material S1.

Molecular clock calibration

The molecular clock calibration used primary and secondary calibration points. Primary calibration points were constrained by *Chenopodipollis multiplex* (dated 65–56 Ma), *Salicornites massalongoi* (35–23 Ma) and *Parvangula randeckensis* (23–16 Ma) (for more information, see Di Vincenzo *et al.* 2018). These were assigned as minimum constraints for the stem nodes of the clades Chenopodiaceae, Salicornioideae and Chenopodioideae respectively. Each fossil calibration was modelled as an exponential prior distribution to allow for divergence earlier than the ages assigned for the fossils (Ho and Philips 2009), prior offsets were set equal to the minimum age of the respective fossil, and parameters were assigned so that the median equalled the maximum age of the fossil. A fourth constraint, within the aervoids, was derived from *O. congesta* (Balf.f. ex Baker) Kuntze (as '*Aerva congesta*' in Thiv *et al.* 2006), which is endemic to the volcanic islands Mauritius and Rodrigues. The formation of Mauritius has been dated to 7.8 Ma (McDougall and Chamalaun 1969), and Rodrigues to 1.5 Ma (Upton *et al.* 1967). We chose the older date (Mauritius) as the calibration point, modelling it as a normal prior with a mean equivalent to half the maximum age of the island (Ho and Phillips 2009). Using the date of formation of an island with an endemic taxon assumes the most parsimonious explanation, that the taxon evolved on the island and that no extinctions took place elsewhere.

A secondary calibration point for the common ancestor of the Amaranthaceae-Chenopodiaceae alliance was based on the lognormal prior age distributions recovered by Di Vincenzo *et al.* (2018; i.e. node 16) and was modelled as a normal distribution equal to the 95% highest posterior density (HPD) interval of the ages. While we are fully aware of the shortcomings of secondary calibrations (see e.g. Schenk 2016) and agree that dating of this kind requires a reasonable level of scepticism, the historical biogeography of many large and important groups has been inferred using this type of calibration (e.g. Di Vincenzo *et al.* 2018, Hancock *et al.* 2018), and it is the best available method.

Phylogenetic analyses and estimation of divergence times

Each CDS gene region for Dataset 1 was aligned separately using MAFFT v7.402 prior to concatenation using Geneious v9.1.8 (Kearse *et al.* 2012, Katoh and Standley 2013). The best partitioning scheme and substitution model for each partition were found using PartitionFinder v2.1.1 (Lanfear *et al.* 2016), with all genes and codon positions as separate data blocks and using the 'greedy' search algorithm (Guindon *et al.* 2010; Lanfear *et al.* 2012). The Sanger-derived dataset of ITS1, 5.8S ribosomal DNA, ITS2 and *matK* were aligned separately using MAFFT and concatenated as partitions. jModelTest2 v2.1.6 was used to determine optimal nucleotide substitution models for each partition (Darriba *et al.* 2012). Bayesian inference was performed using MrBayes v3.2.2 (Ronquist *et al.* 2012) for the CDS alignment, and this used as a topological constraint for the Sanger phylogeny. The topological constraint was generated using PaleoTree v3.1.3 in R v3.5.2 (Bapst 2012, R Core Team 2019) and implemented as a mrBayes block. Both the CDS and Sanger mrBayes analyses were run for 50 million Markov Chain Monte Carlo (MCMC) generations with trees sampled every 1000 generations. Trace files generated by the MCMC runs were analysed using Tracer v1.7.1 to determine adequate convergence and mixing (Rambaut and Drummond 2007). The initial 25% of topologies before reaching likelihood stationarity were discarded. 50% majority rule consensus trees were constructed to summarise the remaining topologies. All trees were rooted at *Achyranthes aspera*.

Divergence times were estimated using a Bayesian relaxed molecular clock model in Bayesian Evolutionary Analysis by Sampling Trees (BEAST) v2.5.1 (Drummond *et al.* 2006, Bouckaert *et al.* 2014) conducted on Dataset 2, partitioned into ITS1, 5.8S rDNA, ITS2 and *trnk/matK*. The best-fit nucleotide substitution model for each partition was found using jModelTest2 (Darriba *et al.* 2012). Lineage diversification was modelled as a birth-death process, and a random starting tree employed. Two MCMC runs with 50 million generations each were performed for the estimation of divergence times, with trees sampled every 1000 generations. Major clades within the aervoids and *Ptilotus* were constrained as monophyletic to represent the CDS phylogeny produced with Dataset 1. Adequate convergence and mixing of the MCMC was established through inspection of the output using Tracer v1.7.1 (Rambaut *et al.* 2018). The initial 25% of topologies were removed as a burn-in, and the resulting two runs were combined and resampled every tenth generation using LogCombiner v2.5.1, for a total of 7,500 topologies (Bouckaert *et al.* 2014). The consensus tree was summarised as a maximum clade credibility (MCC) tree via TreeAnnotator v2.5.1 and visualised using FigTree v1.4.4 (Bouckaert *et al.* 2014, Rambaut 2018). All runs of BEAST, MAFFT, jModelTest2, mrBayes and PartitionFinder were conducted on the Cyberinfrastructure for Phylogenetic Research (CIPRES) portal (Miller *et al.* 2010; available from https://www.phylo.org/, accessed 24 April 2019).

Estimation of speciation rate and lineages through time

The BEAST consensus tree was pruned to include only *Ptilotus* species and analysed in BAMM (Bayesian Analysis of Macroevolutionary Mixtures) v2.5.0 to reconstruct the posterior density of evolutionary rates with the speciation-extinction model (Rabosky *et al.* 2014a, Shi and Rabosky 2015). BAMM was run for 50 million MCMC generations, with taxon coverage set to 90%. The R package BAMMtools v2.5.0 was used to set the priors for BAMM, assess MCMC convergence and visualise the output (Rabosky *et al.* 2014b). A Lineages Through Time (LTT) plot was constructed for *Ptilotus* using the Analyses of Phylogenetics and Evolution (ape) v5.2 package in R (Paradis and Schielp 2019).

Occurrence records and diversity mapping

Occurrence records were used to 1) map the diversity of *Ptilotus* within Australia, 2) assign biogeographic ranges of the extant aervoid species for estimation of ancestral ranges, and 3) assign climatic variables to extant aervoid species for reconstructions along the phylogeny and mapping of the aervoid phylogeny onto climatic ecospace (i.e. phyloecospace). Distributions were compiled from

available herbarium records within Australia from the Australasian Virtual Herbarium (AVH; https://avh.chah.org.au/, accessed 22 February 2019), for *Aerva*, *Omegandra* and *Ptilotus*, and outside of Australia from the Global Biodiversity Information Facility (GBIF; http://www.gbif.org/, accessed 22 February 2019) for *Aerva*, *Ouret*, *Paraerva* and *Nothosaerva*. Most of the diversity of the aervoids occurs in Australia and within *Ptilotus*, hence most data were derived from records deposited on AVH from Australian herbaria. Within Australia, only records with a coordinate uncertainty ≤ 1 km were used in the analyses. As occurrence records were limited for non-*Ptilotus* species, the same criterion of coordinate uncertainty was not applied to species outside Australia. GBIF records of most aervoid species were sparse, typically based on older specimens, and were not available for many countries for the most widespread species (e.g. *N. brachiata* and *O. lanata*). Taxa that have been the subject of recent taxonomic treatments, but whose changes have not yet been reflected in the online databases (e.g. *P. nobilis* (Lindl.) F.Muell. in Hammer *et al.* 2018b and *P. xerophilus* T.Hammer & R.W.Davis in Hammer *et al.* 2019), were manually edited to reflect the most recent taxonomy. For all species, records identified as geographic outliers or probable misidentifications (on the basis of atypical locality, habitat or label data) were withheld from the final occurrence dataset. A total of 16,400 localities were used for 124 aervoid species; data are available with citations of the original data downloads from AVH and GBIF as Supplementary Material S2.

To visualise the geospatial diversity of *Ptilotus* and identify probable hotspots of diversity for the genus and the major clades, spatial analyses were conducted using Biodiverse v2.1 (Laffan *et al.* 2010). A total of 15,186 records were used for the 112 *Ptilotus* species included in Dataset 1 (Supplementary Material S2). The cell size was set to 0.5 decimal degrees (c. 55.5 km^2 at the equator). Two neighbour sets were utilized, with neighbour set 1 set to one cell and neighbour set 2 set as a circle with a radius of one cell. Occurrences were analysed for Species Richness (SR; as 'Richness' in Biodiverse), Net Relatedness Index (NRI, as 'PHYLO_NRI1'; Webb *et al.* 2008), Phylogenetic Diversity (PD; Faith 1992) and the Difference of the Relative Phylogenetic Diversity (RPD DIFF, as 'PHYLO_RPD_DIFF1'; Mishler *et al.* 2014). The latter three indices incorporate evolutionary information into diversity mapping. NRI is a measure of mean pairwise phylogenetic distance between species in a cell (Webb *et al.* 2002). PD is a measure of the sum of the branch lengths between the root and the tips of the phylogeny for each species present in a cell (Faith 1992). RPD DIFF shows the cells with higher or lower PD than would be expected given the null hypothesis (i.e. SR; Mishler *et al.* 2014). In addition, *Ptilotus* clades A–F were analysed using the Proportional PD Clade Contribution index, which calculates the contribution of each node and its descendants to the PD calculation proportional to the entire tree, and individually using SR. Maps were visualised using QGIS v3.6 (QGIS Development Team 2019).

Estimation of ancestral ranges

Biogeographic areas were assigned to each species by mapping the occurrence records in QGIS. The geographic regions used to estimate the ancestral ranges were split among the continents occupied by the aervoids: Africa, Asia and Australasia. To investigate the biogeographic history of *Ptilotus*, Australasia (i.e. Australia and New Guinea) was included as five phytogeographical regions following Ebach *et al.* (2015) (Fig. 1B). In total, seven biogeographic regions were used for the ancestral range estimation: A–Africa, B–Asia, C–Northern Australia, D–Northern Desert, E–Eremaean, F–Euronotian and G–Southwest Australia. New Guinea was included as part of Northern Australia to represent its historical association with Australia and connection via the Sahul shelf. The biogeographic regions of Wallacea and Sundaland were included within Asia. While Madagascar, Mauritius-Rodrigues and Socotra are biogeographically interesting for the evolution of *Ouret* and *Paraerva*, these regions were included as part of Africa in this study, which was designed to focus on *Ptilotus*. A comprehensive study of species boundaries within *Ouret* is warranted before an investigation into its biogeography is conducted (Thiv *et al.* 2006, Hammer et al. in press). While *A. javanica* occurs in northern Australia, this

distribution is believed to be the result of a recent introduction. The earliest record of the species in Australia is from the De Grey River (i.e. near Port Hedland), Western Australia in 1937 (*J. Palmer s.n.*, PERTH 209430; information retrieved from AVH), and it is believed to have been introduced and spread with introduced camels (the hairy fruits were commonly used as stuffing for saddles; Keighery 2010). Given this, we limited the natural range of *A. javanica* in our analysis to Africa and Asia (see also Khan *et al.* 1970). *Ouret lanata* was found in our dataset to occur in New Guinea (included in the analysis with Northern Australia); unlike *A. javanica*, there is no available evidence to indicate this is a recent introduction of the species.

Ancestral ranges for extant species were estimated using Reconstructing Ancestral State Phylogenies (RASP) v4.1b (Yu *et al.* 2015). The best-fit model of geographic range evolution was found using the BioGeoBears model selection option in RASP and found to be Lagrange DEC (dispersal-extinction-cladogenesis) (Ree and Smith 2008, Matzke 2013, 2014), which was implemented as the "Bayes-Lagrange" statistical-DEC (S-DEC) model using 1000 random trees from the BEAST analysis of Dataset 2. Trees were trimmed in RASP to exclude all non-aervoid species. S-DEC takes into account topological uncertainty of the sampled trees when inferring ancestral states (Yu *et al.* 2015). Ancestral ranges were limited to a maximum of three combinations among the seven areas. Matrices were manually constructed for range and dispersal constraints, to represent more likely dispersal events between the continents and areas within Australia and to prevent non-adjacent ranges among ancestral taxa. Where inferred ancestral ranges included two non-adjacent areas and a third area adjacent to both, all three areas were included.

Estimation of ancestral climate
A set of 67 global climatic variables from WorldClim v1.4 (Hijmans *et al.* 2005) for current conditions (1960–1990) at 30 arc-seconds (c. 1 km^2) was used to construct a dataset using the point sampling tool in QGIS for the occurrence records. The climate dataset was subjected to a Pearson's Correlation test using the *cor* function in the 'stats' v.3.5.2 package in R to determine significant correlations between variables (R Core Team 2019). Significance was determined by assigning p-values using the *rcorr* function in the R package Hmisc v4.2-0 (https://www.rdocumentation.org/packages/Hmisc/versions/4.2-0). Variables determined as significantly correlating were represented by a single variable in the analyses to prevent correlated variable bias in the results. The values of each variable were summarised for each species as a midpoint between the minimum and maximum value, to avoid geographic sampling bias inherent in herbarium collections (sampling bias would be present using the mean or median).

Climate variables were reconstructed for internal nodes in the aervoid phylogeny (Dataset 1) using the *contMap* function in phytools v.0.6-60 (Revell 2012). The outgroup taxon, *Achyranthes aspera*, was excluded from the analyses. Principal Component Analyses (PCA) were conducted on the climate dataset for the extant aervoid species and the ancestral nodes using the *prcomp* function in R (R Core Team 2019). PCAs were visualised using the R packages ggplot2 (Wickham, 2016) and ggfortify (Tang *et al.* 2016). Phyloecospace diagrams were visualised using manually written R code to map the topology of the aervoid phylogeny onto PCAs representing the climatic ecospace occupied by the species. Variables used for all PCAs were scaled. Similar methods have been successfully used to map phylogenetic topology onto morphospace or ecospace in studies of the evolution of morphology or ecology in various groups of organisms (e.g. Sidlauskas 2008, Louys *et al.* 2013, Gómez *et al.* 2015, Baldo *et al.* 2017). The climatic dataset used for the analyses is available as Supplementary Material S3.

RESULTS

Molecular datasets
The CDS alignment of 69 genes for the 37 species in Dataset 1 had a combined length of 51,996 base pairs (bp). For the Sanger alignment of the 125 species in Dataset 1, the partitions had lengths of 128 bp, 295 bp, 291 bp and 799 bp, for 5.8S, ITS1, ITS2 and *matK* respectively. The alignments in Dataset 2 for the 194 species had lengths of 128 bp, 330 bp, 342 bp and 2831 bp for 5.8S, ITS1, ITS2 and *trnK/matK* respectively. For both datasets, Akaike information criteria (AIC) in jModelTest2 determined the best nucleotide substitution models for ITS1 and ITS2 as GTR+I+Γ, for 5.8S rDNA as SYM+I+Γ, and for *matK* (Dataset 1) and *trnK/matK* (Dataset 2) as GTR+Γ. The alignments and specifications for the mrBayes analyses and the input file for the BEAST2 analysis are available as Supplementary Materials S4 and S5.

Phylogenetic analyses
The CDS and Sanger phylogenies of Dataset 1, presented as Supplementary Material S6 and Fig. 3 respectively, reproduced the aervoid topology of Hammer *et al.* (in press), with the major clades of the aervoids recovered: the ONAP clade, *Ouret* and *Ptilotus* clades A–F. The dated phylogeny of Dataset 2 (Fig. 4) produced congruent results with Dataset 1 and presents the first attempt at molecular dating within the aervoid clade. The dated phylogeny lacks resolution within some of the major clades, which were resolved by the CDS phylogeny (e.g. between subclades of clade B). Because of this, and because the dated phylogeny is presented as a fully bifurcating tree (i.e. with no polytomies), nodes with low support (i.e. posterior probability < 0.95) should be treated with caution.

Estimates of divergence times
The crown group of Amaranthaceae *s.str.* was dated to 51.1 Ma (95% HPD 45.38–56.51 Ma), congruent with results from Di Vincenzo *et al.* (2018). The aervoid clade is estimated to have diverged from the achyranthoid and gomphrenoid clades in the Oligocene around 32 Ma (95% HPD 25.62–38.17 Ma) and started to diversify around 27 Ma (95% HPD 21.39–32.87 Ma). The crown of the ONAP clade was dated to 25.9 Ma (95% HPD 19.79–31.78 Ma), when *Omegandra* diverged from the other genera. Subsequent early divergences in the clade occurred in the Late Oligocene around 23.7 Ma (95% HPD 17.14–30.03 Ma), when *Nothosaerva* and *Aerva+Paraerva* diverged, and around 23.5 Ma (95% HPD 17.27–30.46 Ma) with the divergence of *Aerva* and *Paraerva*. *Ouret* and *Ptilotus* were dated to have diverged around 23.7 Ma (95% HPD 18.71–28.94 Ma), with the crown groups dated to 16.6 Ma (95% HPD 9.96–23.54 Ma) and 21.5 Ma (95% HPD 17.11–26.36 Ma) respectively. Within *Ptilotus*, most clades diverged in the Early Miocene with clade B at 19.3 Ma (95% HPD 15.45–23.49 Ma), clade C at 17.9 Ma (95% HPD 14.23–21.68 Ma) and clade D at 16.6 Ma (95% HPD 13.21–20.16 Ma). By the Middle Miocene, clades E and F are estimated to have diverged at 15.6 Ma (95% HPD 12.50–19.10 Ma). Inferred ages, 95% HPD intervals and posterior probabilities for all nodes are provided in Supplementary Material S7.

The Lineages Through Time (LTT) plot of *Ptilotus* (Fig. 5) indicates that cladogenesis was initially rapid, with most clades diverging early in the evolution of the genus. BAMM analysis indicated that the genus had a high initial rate of speciation at the base of the tree, with no likely subsequent shifts in rate frequencies (Fig. 5; see also Hammer *et al.* 2015). Diversification appears to have remained high through the Middle Miocene and somewhat slowed through the Late Miocene and during the Pliocene and Pleistocene.
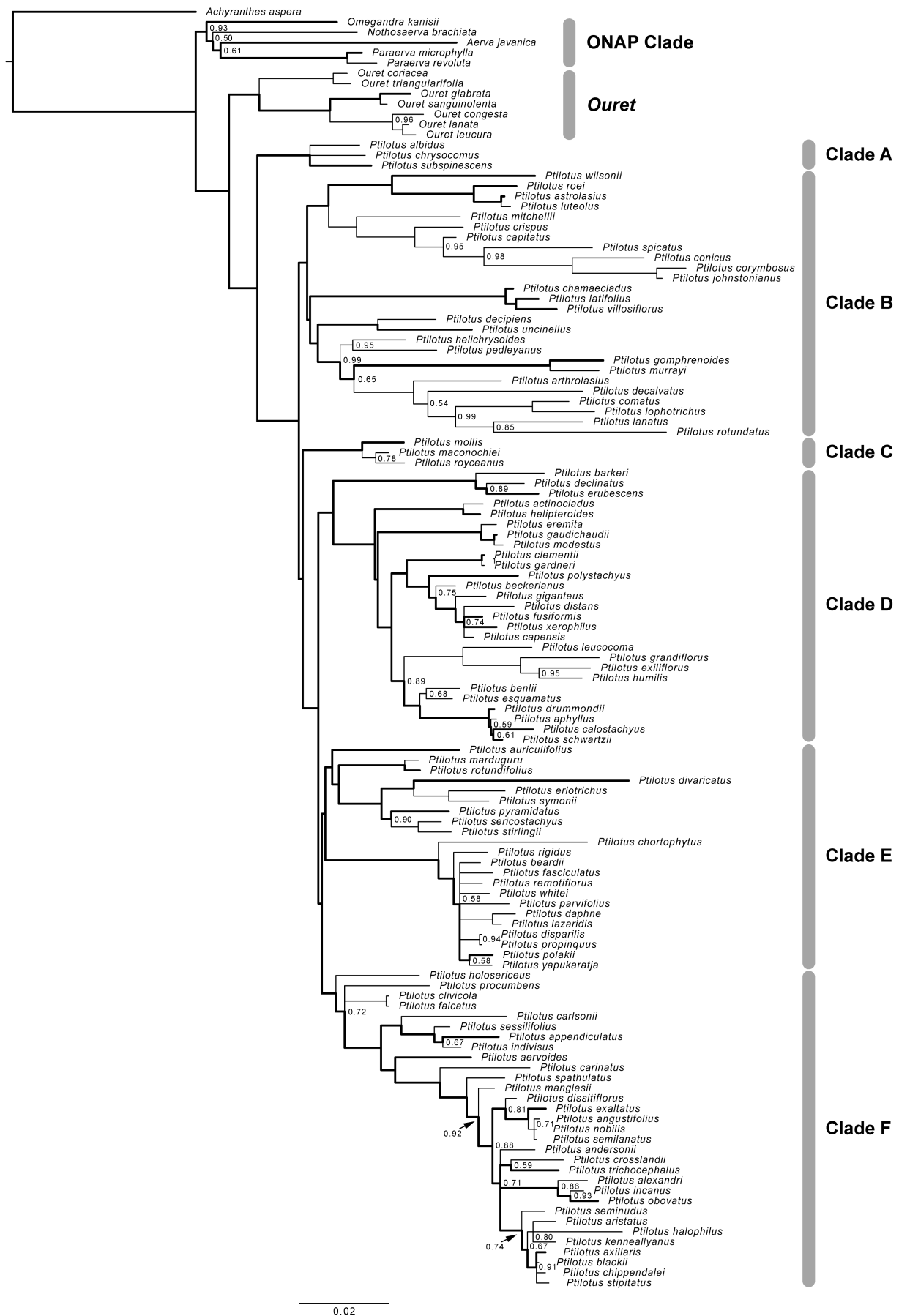
Fig. 3. The mrBayes 50% majority consensus tree of Dataset 1. All values indicated are posterior probabilities (PP). Nodes without support values indicated are maximum support (PP = 1.0). Branches in bold indicate relationships resolved in the CDS tree (Supplementary Material S6), the topological constraint implemented in the analysis. Major clades of the aervoids are indicated on the right.

Fig. 4. The dated Amaranthaceae-Chenopodiaceae phylogeny produced by BEAST2 with Dataset 2. The x-axis represents time in millions of years (Ma) with geological epochs labelled (Pliocene and Pleistocene abbreviated as 'Pl.' and 'P.' respectively). Support values of 1.0 and ≥ 0.95 PP are indicated by circles on the nodes. Calibrations are indicated on the branches with triangles. Node bars are 95% highest posterior density (HPD) interval of the ages. Ages of the crown groups of the aervoids and *Ptilotus* are indicated by arrows. Major clades of the aervoids and the families Amaranthaceae *s.str.* and Chenopodiaceae are labelled on the right.

Fig. 5. A. Plot of the log of lineages over time (LTT; black line) for *Ptilotus* on the dated phylogeny with a red dotted line indicating a linear rate. **B.** The speciation rate over time (blue line) estimated by the BAMM speciation-extinction model with grey shading as the 95% confidence interval. The divergence of the major clades of *Ptilotus* are indicated on the LTT plot.

Historical biogeographic analysis

Biogeographic reconstruction of the aervoids using the dated phylogeny (Fig. 6) indicates that the common ancestor occurred in Africa and Asia, and not Australia. The ancestor of *Omegandra*, sometime after its split with the other members of the ONAP clade c. 25.9 Ma, is inferred to have dispersed to Australia. The common ancestor of *Ptilotus* dispersed independently to Australia between 23.7 and 21.5 Ma; by 21.5 Ma the genus had spread from Northern Australia to the Eremaean. The common ancestor for each major clade of *Ptilotus* (i.e. A–F) was estimated to have an origin in the Eremaean, with many of the nodes within these clades also being estimated to have originated within the Eremaean. A few species groups were found to have diverged in the Late Miocene and Pliocene to occupy areas in Northern Australia (e.g. in clade B) or in Southwest Australia (e.g. in clade D). Radiations out of the Eremaean are also apparent in clades B, D, E and F. The occurrence of *P. conicus* in Indonesia and of *O. lanata* in New Guinea appear to be the result of recent range expansions.

Geospatial analyses of extant species

The analyses of *Ptilotus* occurrence records in Biodiverse indicates arid Western Australia (i.e. the western Eremaean and northern Southwest Australia) as species rich and phylogenetically diverse for the genus (Fig. 7A, B). *Ptilotus* is particularly diverse in the Pilbara, Carnarvon, Gascoyne, Murchison, Yalgoo, Geraldton Sandplains and Avon Wheatbelt IBRA (Interim Bioregionalisation of Australia) regions (Department of the Environment 2019). Species Richness is highest (24 spp.) in the southern Pilbara, on the Pilbara-Gascoyne border and tri-regional border of the Yalgoo, Geraldton Sandplains and Avon Wheatbelt IBRA regions. Phylogenetic Diversity is highest in the Southern Pilbara (PD = 0.69) and northern tip of the Avon Wheatbelt (PD = 0.68). Other areas of high diversity and richness occur near Alice Springs (MacDonnell Ranges and adjacent IBRA regions) in central Australia (PD = 0.47, SR to 18 spp.), and in the Dampierland IBRA region in Western Australia (PD = 0.52, SR to 10 spp.).
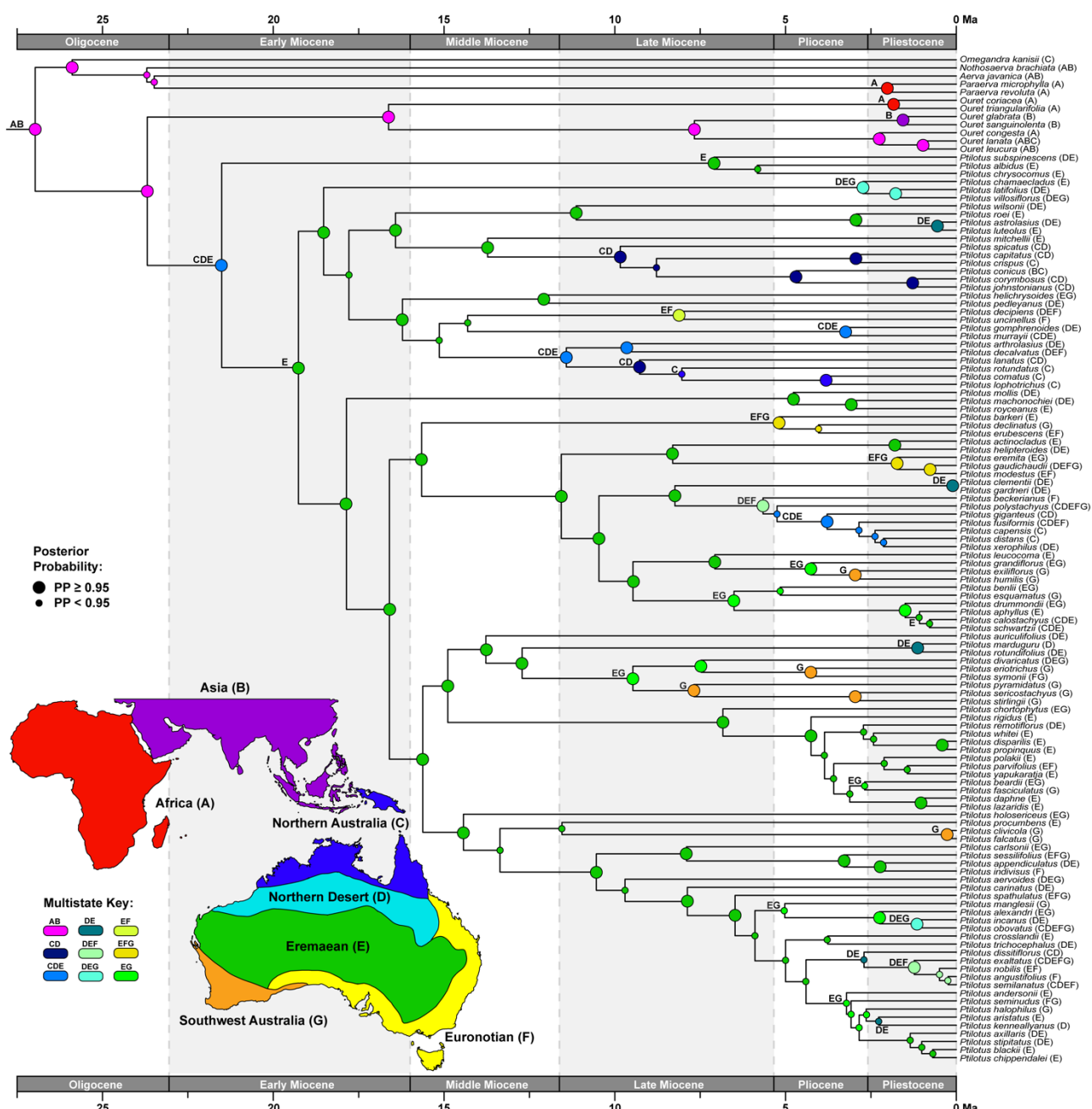
Fig. 6. Ancestral area estimation for the aervoids based on the dated phylogeny of Dataset 2 (Fig. 1B). Posterior probability for each node is indicated by circle size at each node, with the largest nodes ≥ 0.95 PP. Colour of the node indicates most likely areas recovered by the S-DEC analysis. Areas and colours are indicated in the map inlay for the five phytogeographic areas of Australia, in addition to Africa and Asia and the key to colours for multistate areas.

Phylogenetic Diversity was higher than expected in the Carnarvon, Gascoyne and Murchison IBRA regions (Fig. 7C; compare Fig. 8A, B) and in coastal Pilbara and Dampierland. Species in northern Australia (especially Dampierland) are more closely related than expected, while species in southeastern Australia (southern South Australia to central New South Wales) are less closely related than expected (Fig. 7D). There were also clade-specific diversity hotspots (Figs 8 & 9). Clade B shows the highest contribution to SR and PD in Northern Australia and parts of the Northern Desert (Figs 8B & 9B). Parts of the semi-arid transition zone on the northern boundary of Southwest Australia to the Eremaean are particular species rich and phylogenetically diverse for clades D–F (Figs 8D–F & 9D–F).
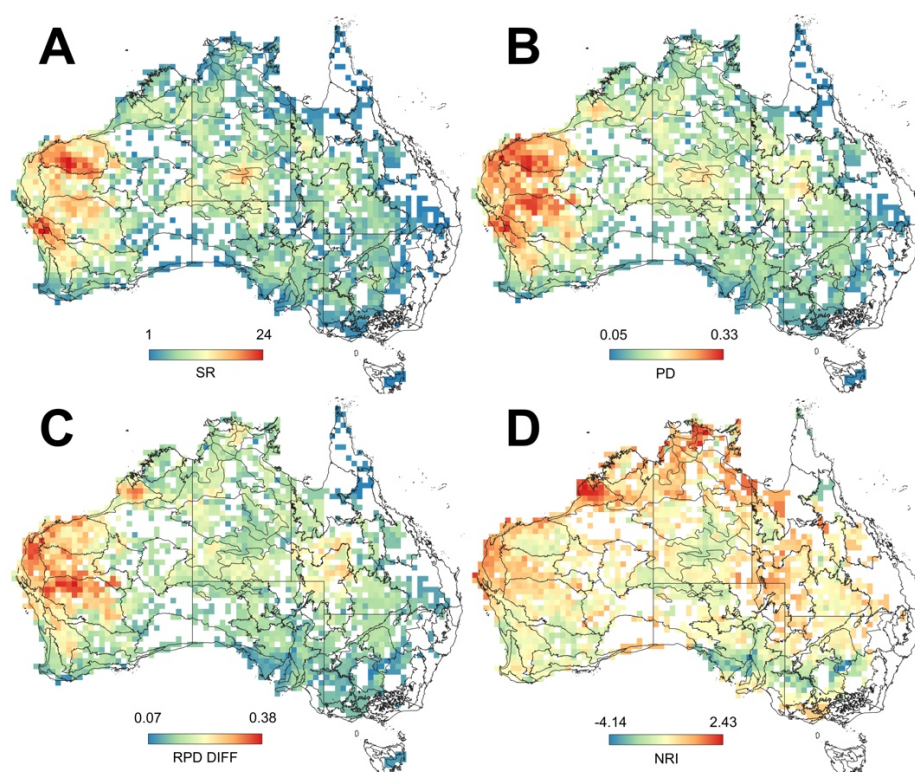
Fig. 7. Maps of Australia for the indices analysed using Biodiverse: **A.** Species Richness (SR), **B.** Phylogenetic Diversity (PD), **C.** the Difference of Relative PD (RPD DIFF), and **D.** Net Relatedness Index (NRI). Boundaries are IBRA 7.0 regions.

Analyses of climatic data

The Pearson's Correlation test showed that 32 of 67 climate variables were not significantly correlated; these were thus used in subsequent analyses (Supplementary Material S8; descriptions for the variables used in the final analyses are shown in Table 1). There is substantial overlap in ecospace between the major clades of *Ptilotus* (Fig. 10A), and to a lesser extent also within the ONAP clade and *Ouret*. For the Australian species, clustering in ecospace is noticeable among those species that occur in one of the five ABA phytogeographical regions (Fig. 10B), with widely distributed species occurring in intermediate positions. Every clade of *Ptilotus* overlaps with the Eremaean climatic envelope.

Ancestral state reconstructions of the 32 climatic variables on the phylogeny of Dataset 1 are presented in Supplementary Material S9. The common ancestor of the aervoids occupied an environment most similar to that of the ONAP clade and *Ouret* in Africa (Fig. 11A). The common ancestor of *Ptilotus* is located near, but not within, the Eremaean ecospace, with subsequent nodes, from which clades B–F diverged, well within the Eremaean (Fig. 11A, B).

Mapping the aervoid phylogeny onto the climatic ecospace of extant species and reconstructed nodes (Figs 12–14) indicates that adaptation to differing climates has driven the evolutionary history of the aervoids. The common ancestor of *Ptilotus* diverged climatically from the common ancestor of the aervoids (Fig. 12A), tending towards a more arid and less tropical climate. Within the ONAP clade, *Omegandra* and *Nothosaerva* have a similar trajectory in phyloecospace (Fig. 12B), despite *Omegandra* having diverged from the other members of the ONAP clade before *Nothosaerva* and occurring in Northern Australia. Similarly, *Paraerva* and *Aerva* diverged from their common ancestor, with the former now occupying a more arid phyloecospace and the latter a more semi-arid one (Fig. 12B). The Madagascan species of *Ouret* (*O. triangularifolia* (Cavaco) T.Hammer and *O. coriacea* (Schinz) T.Hammer) and the African *O. leucura* (Moq.) Kuntze remain close in ecospace to the common ancestor of *Ouret*, while the Asian species (*O. sanguinolenta* (L.) Kuntze and *O. glabrata* (Hook.f.) Kuntze) are the most divergent in phyloecospace (Fig. 12C).
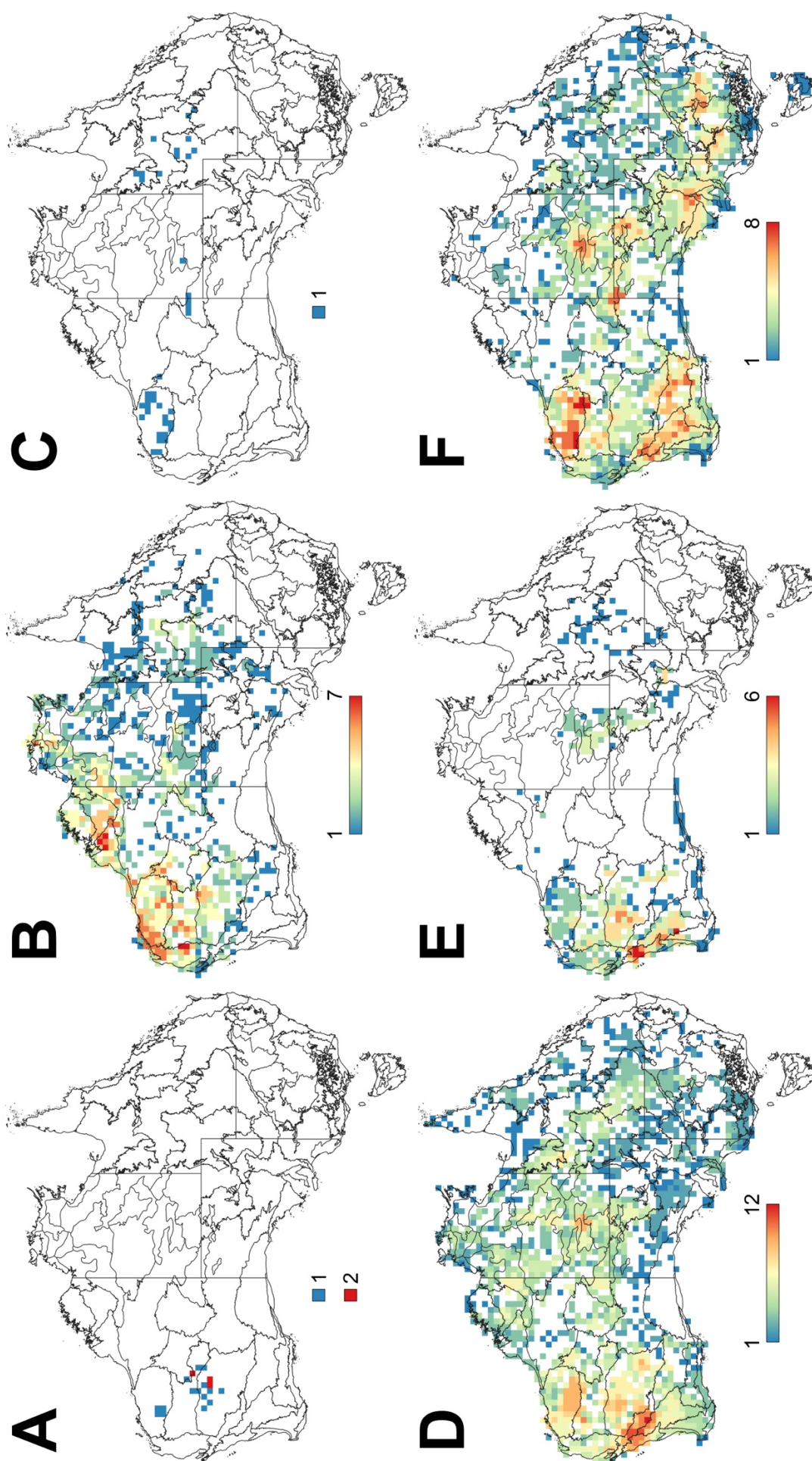
Fig. 8. Maps of Australia for phylogenetic diversity (PD) analysed using Biodiverse for the clades of *Ptilotus*. Lettering corresponds to *Ptilotus* clades A–F. Boundaries are IBRA v7 regions.
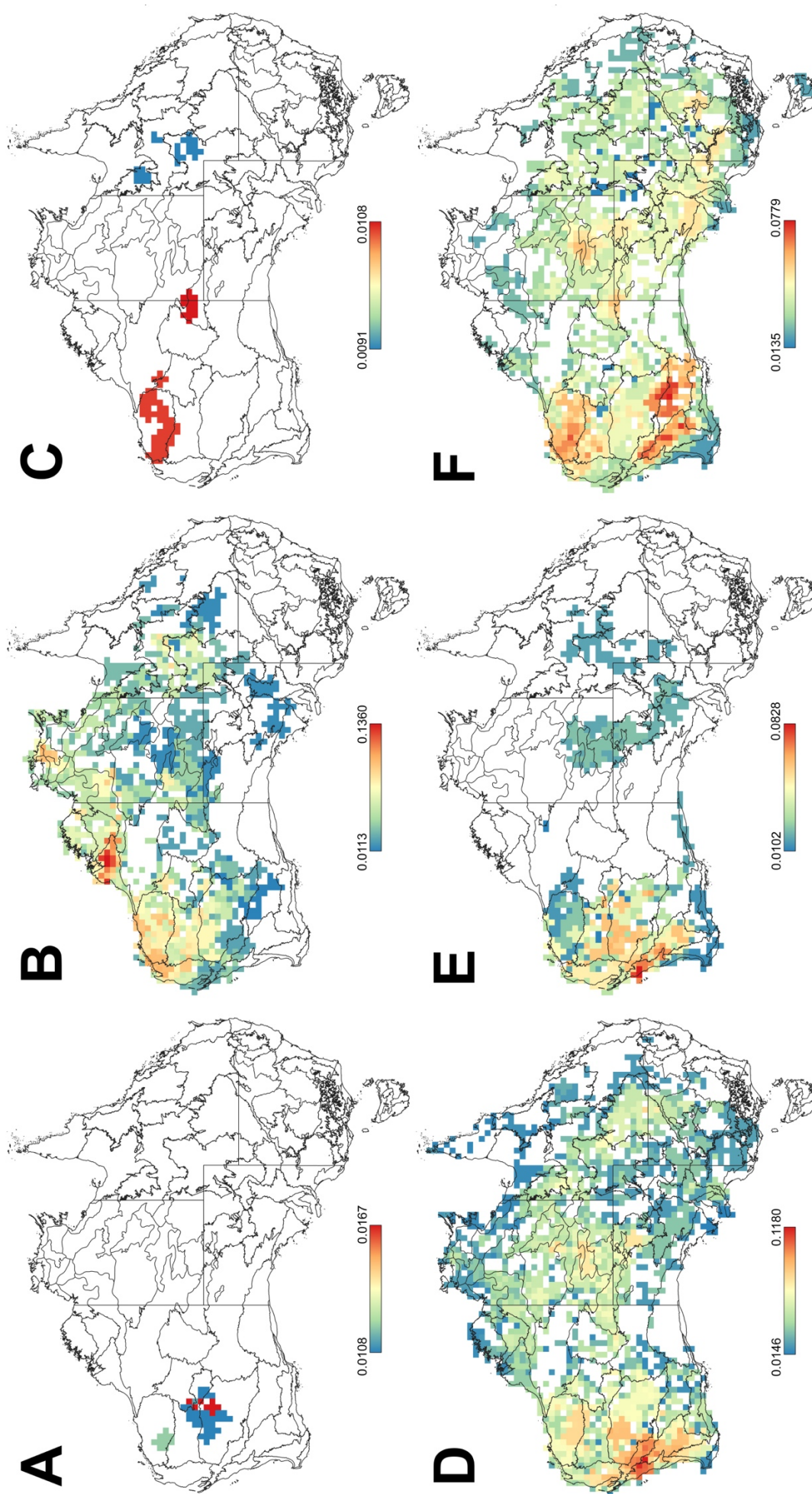
Fig. 9. Maps of Australia for species richness (SR) analysed using Biodiverse for the clades of *Ptilotus*. Lettering corresponds to *Ptilotus* clades A–F. Boundaries are IBRA v7 regions.

Table 1. WorldClim 1.4 variables and meanings (i.e. excluding altitude) used in the current study. Variables of precipitation in millimeters and temperature in °C × 10. All variables are based on averages over ~1960–1990 (for more information, see Hijmans *et al.* 2005).

Variable Name	Description
BIO 3	Isothermality (BIO 2/BIO 7) (× 100)
BIO 4	Temperature seasonality (standard deviation × 100)
BIO 5	Maximum temperature of warmest month
BIO 8	Mean temperature of wettest quarter
BIO 9	Mean temperature of driest quarter
BIO 10	Mean temperature of warmest quarter
BIO 12	Annual precipitation
BIO 13	Precipitation of wettest month
BIO 14	Precipitation of driest month
BIO 16	Precipitation of wettest quarter
BIO 17	Precipitation of driest quarter
PREC 1	Mean precipitation of January
PREC 2	Mean precipitation of February
PREC 3	Mean precipitation of March
PREC 4	Mean precipitation of April
PREC 6	Mean precipitation of June
PREC 7	Mean precipitation of July
PREC 8	Mean precipitation of August
PREC 12	Mean precipitation of December
TMIN 2	Minimum temperature of February
TMIN 6	Minimum temperature of June
TMIN 9	Minimum temperature of September
TMIN 10	Minimum temperature of October
TMEAN 2	Mean temperature of February
TMEAN 4	Mean temperature of April
TMEAN 10	Mean temperature of October
TMEAN 11	Mean temperature of November
TMAX 2	Maximum temperature of February
TMAX 3	Maximum temperature of March
TMAX 6	Maximum temperature of June
TMAX 7	Maximum temperature of July
TMAX 11	Maximum temperature of November

Clades B–F of *Ptilotus* all diverged in an Eremaean ecospace (Fig. 12A). Clades A and C show a similar pattern of an Eremaean origin (Fig. 13A, C), with some extant species occurring in a phyloecospace consistent with the environment of the Northern Desert (e.g. *P. subspinescens* in clade A and *P. mollis* in clade C). Clade B has an origin in the Eremaean, but with two distinct subclades of Northern Australian species radiating into an environment that is tropical and with higher precipitation (Fig. 13B). The *P. fusiformis* (R.Br.) Poir. species group in clade D shows a high degree of divergence in phyloecospace, with *P. capensis* (Benl) A.R.Bean, *P. giganteus* (A.Cunn. ex Moq.) R.W.Davis & R.Butcher, *P. distans* (R.Br.) Poir. and *P. fusiformis* in more northern and higher rainfall climates than the Eremaean *P. xerophilus* and southern-temperate *P. beckerianus* (F.Muell.) F.Muell. (Fig. 14A). *Ptilotus polystachyus* (Gaudich.) F.Muell. occupies an intermediate position in this species group and is distributed in every phytogeographic region analysed in the present study (Fig. 6). Several species in clade D radiated independently into a more mesic, winter-rainfall climate (Fig. 14A). Some species in clade E (e.g. *P. marduguru* Benl) radiated from the Eremaean into the Northern Desert, while the *P. pyramidatus* (Moq.) F.Muell. species group radiated into Southwest Australia (Fig. 14B). Clade F shows a similar pattern to clades D and E (Fig. 14C), with an origin in the Eremaean, radiations throughout the arid ecospace, and conspicuous excursions of species groups into Northern Australia and the Northern Desert on one hand (e.g. *P. dissitiflorus* (F.Muell.) F.Muell.) and Southwest Australia and Euronotian on the other (e.g. *P. spathulatus* (R.Br.) Poir.).
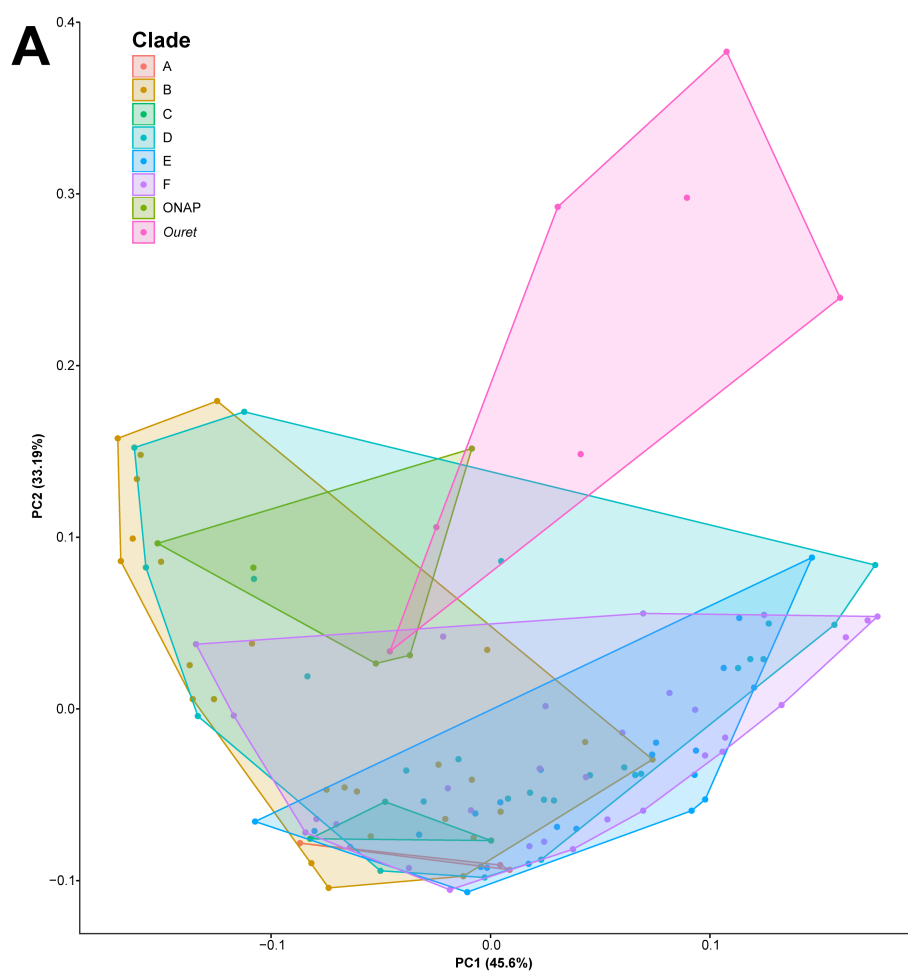
Fig. 10. Principal component analysis of the climatic data for the species (i.e. terminal nodes) on aervoid phylogeny. **A.** Major aervoid clades are coloured and indicated by bounding hulls. **B.** Geographic distributions for species with a single state (i.e. those endemic to a single area) are coloured and all species with a particular single state are indicated by hulls. Species with multistate distributions (i.e. over multiple geographic regions) are indicated as grey dots and factor loadings for climatic variables (see Table 1) are included as arrows.
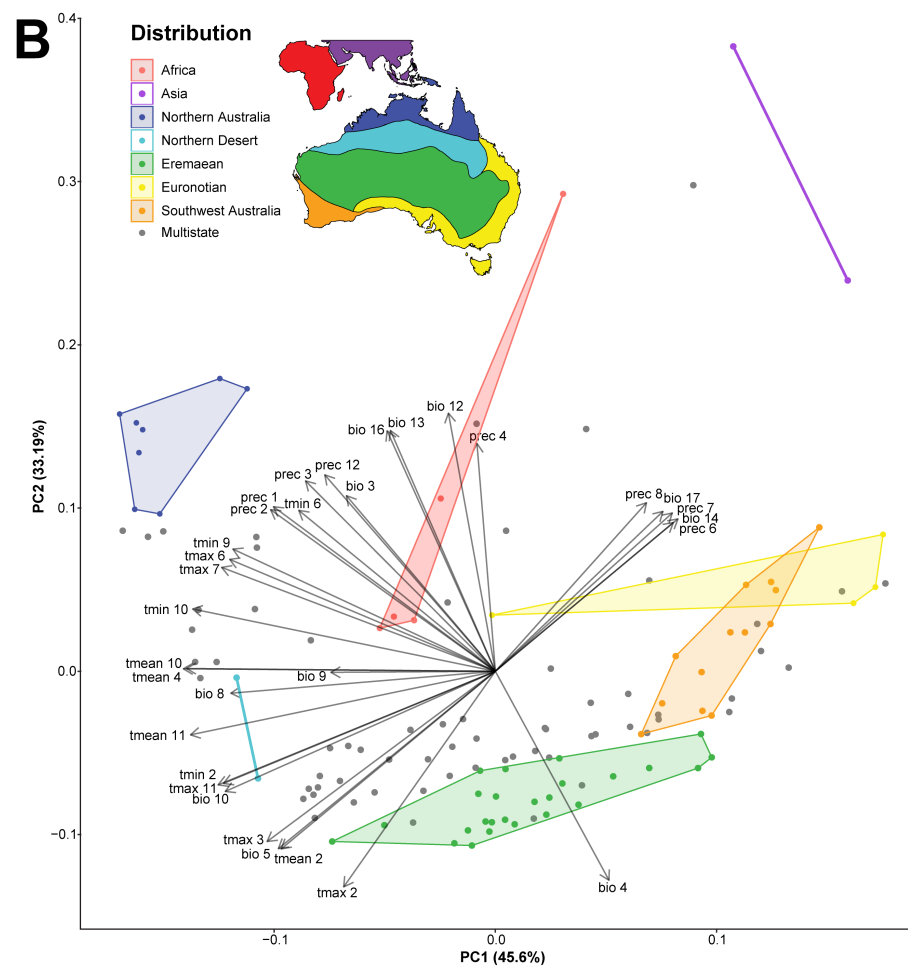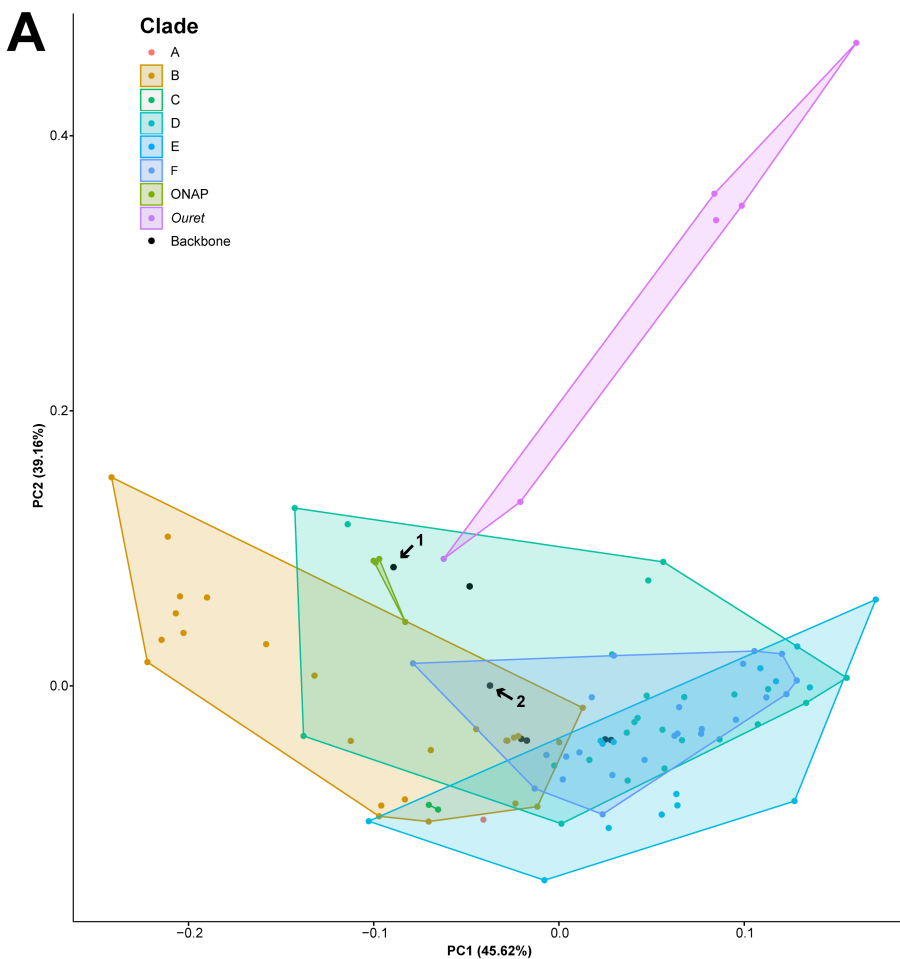
Fig. 11. Principal component analysis of the reconstructed climatic data for the internal nodes of the aervoid phylogeny. **A.** Major aervoid clades are coloured and/or indicated by hulls; black dots indicate backbone nodes with no clade membership and arrows indicate common ancestors of the aervoids (1) and *Ptilotus* (2). **B.** Geographic distributions for species with a single state (i.e. those endemic to a single area) are coloured and all species with a particular single state are indicated by hulls. Geographic distributions are based on the ancestral area estimation for each node. Species with multistate distributions (i.e. over multiple geographic regions) are indicated as grey dots and factor loadings for climatic variables are included as arrows.
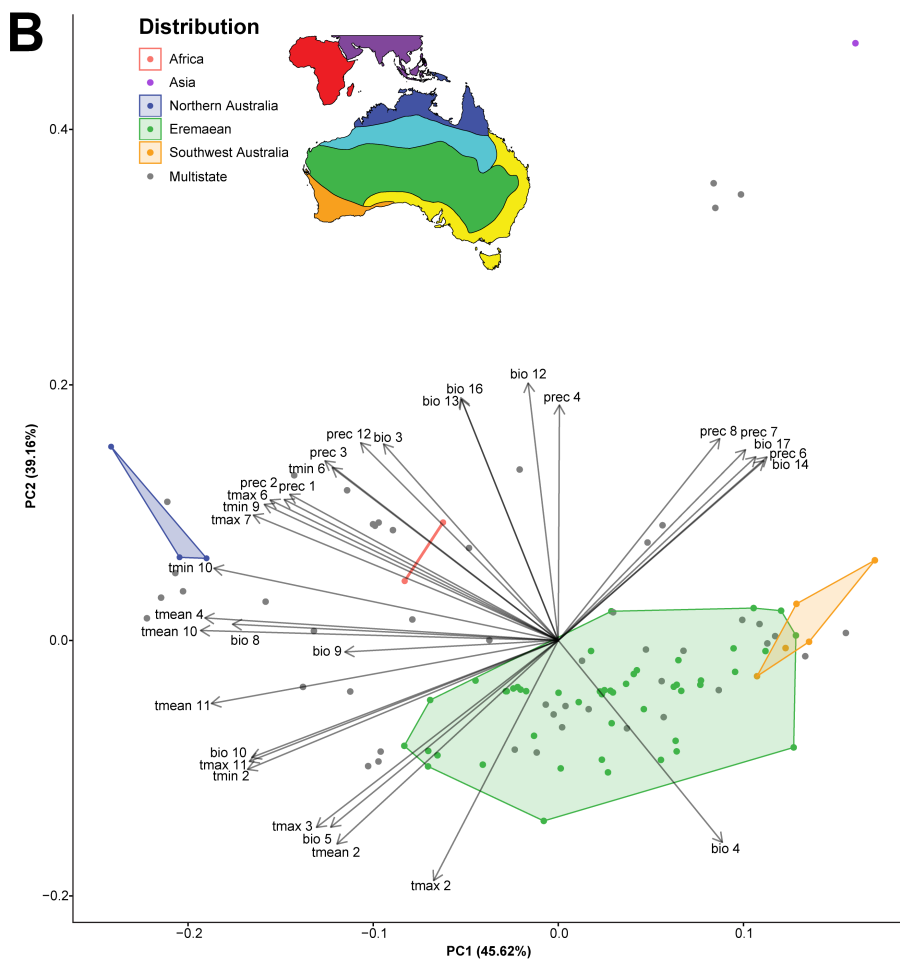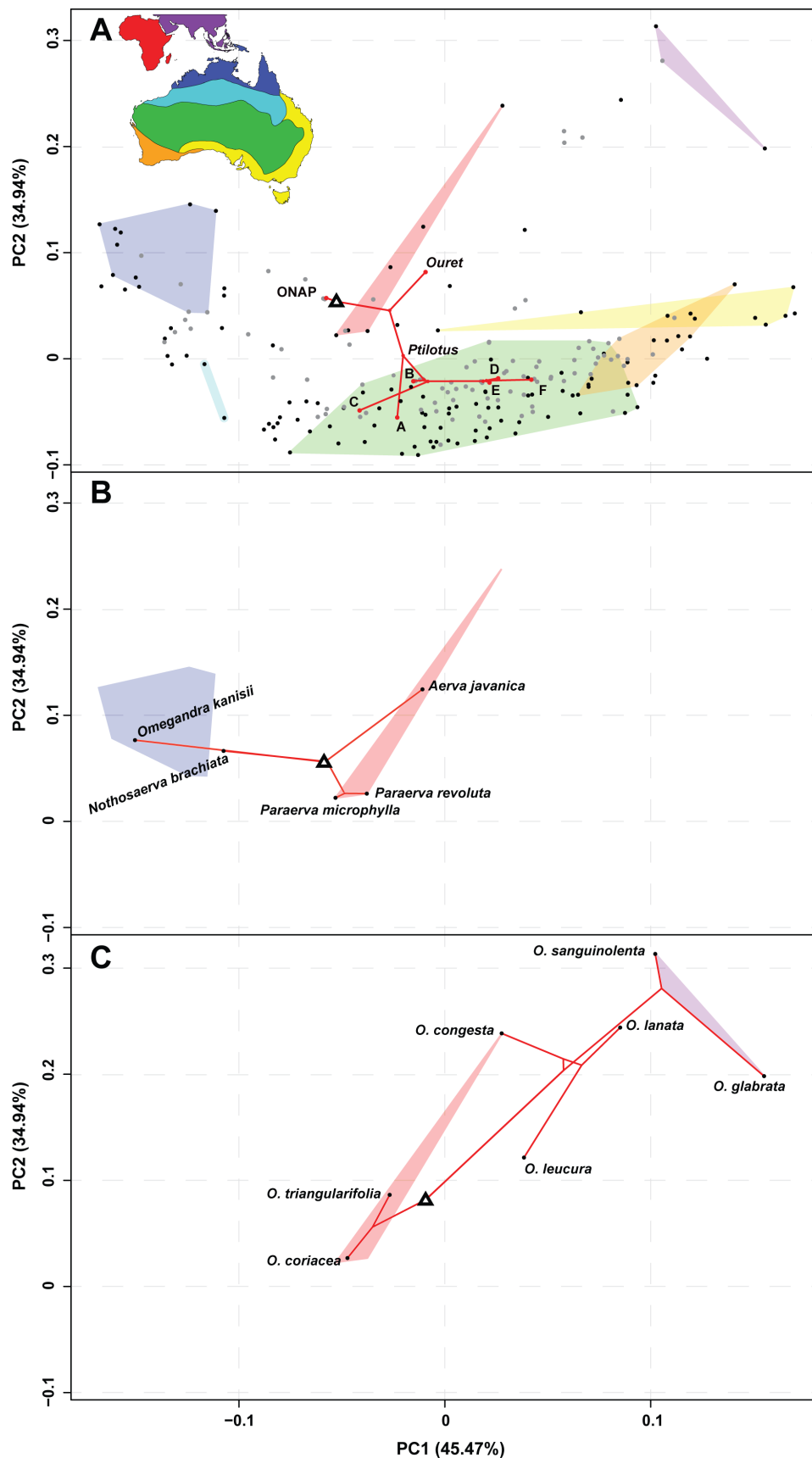
Fig. 12. The PCA of extant and reconstructed climatic datasets for all extant species (i.e. terminal nodes) and internal nodes on the aervoid phylogeny (Dataset 1). **A.** All nodes on the tree are plotted, with extant species as black dots and internal nodes as grey dots. The backbone of the phylogeny is drawn in red with the crown nodes of the major aervoid clades indicated by the relevant letter or name. **B.** The topology and extant species of the ONAP clade. **C.** The topology and extant species of *Ouret*. Triangles represent common ancestors of the illustrated clades. Coloured convex hulls indicate geographic areas of Africa and Asia and the five phytogeographic regions of Australia (Fig. 1B) represented in ecospace by the nodes that are endemic to the respective area.
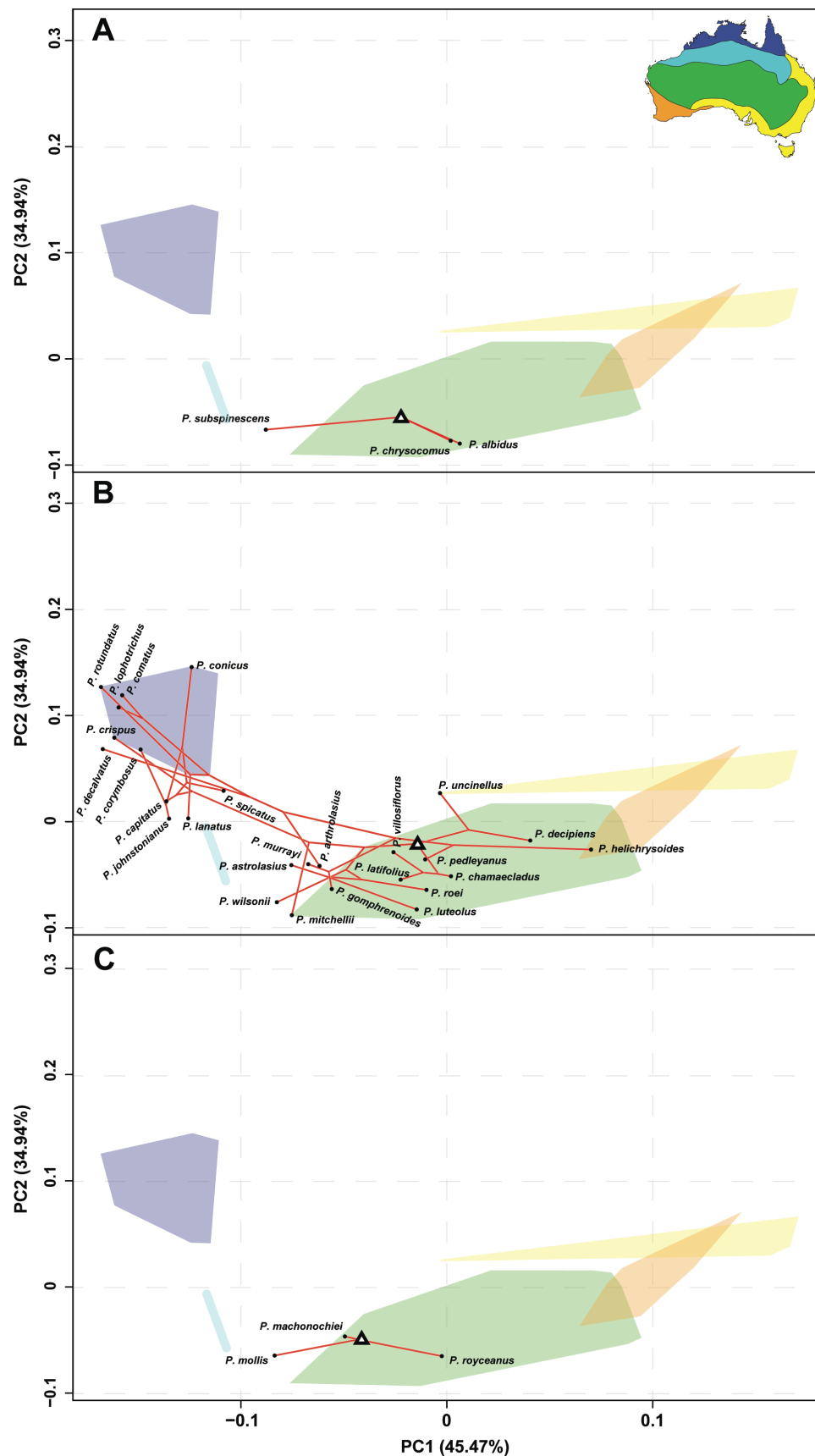
Fig. 13. The PCA of extant and reconstructed climatic datasets for all extant species (i.e. terminal nodes) and internal nodes on the aervoid phylogeny (Dataset 1). **A.** The topology and species of *Ptilotus* clade A. **B.** The topology and species of *Ptilotus* clade B. **C.** The topology and species of *Ptilotus* clade C. Triangles represent common ancestors of the respective clades. Coloured convex hulls indicate the five phytogeographic regions of Australia (Fig. 1B) represented in ecospace by the nodes that are endemic to the respective area.
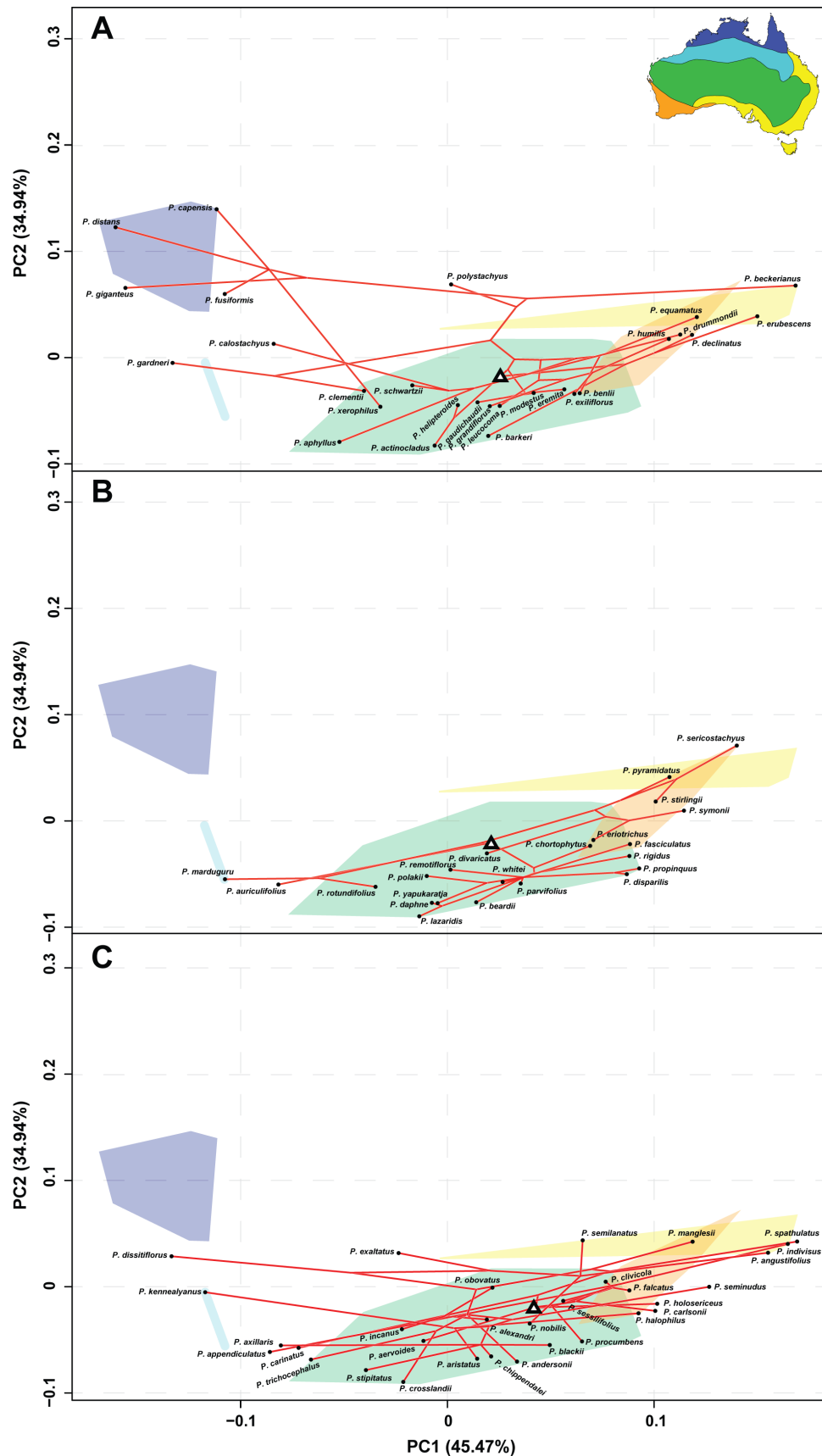
Fig. 14. The PCA of extant and reconstructed climatic datasets for all extant species (i.e. terminal nodes) and internal nodes on the aervoid phylogeny (Dataset 1). **A.** The topology and species of *Ptilotus* clade D. **B.** The topology and species of *Ptilotus* clade E. **C.** The topology and species of *Ptilotus* clade F. Triangles represent common ancestors of the respective clades. Coloured convex hulls indicate the five phytogeographic regions of Australia (Fig. 1B) represented in ecospace by the nodes that are endemic to the respective area.

DISCUSSION

The origin of the aervoids

The present study indicates that the common ancestor of the aervoids occurred outside of Australia, with an origin in the Oligocene around 27 Ma and an estimated range of Africa–Asia (Fig. 5), similar to the ranges of the extant genera *Aerva*, *Nothosaerva* and *Ouret* (Hammer *et al.* in press). The phyloecospace analysis estimated the common ancestor of the aervoids to likely inhabit a semi-arid, tropical or subtropical climate, similar to the ONAP clade and African *Ouret* species (Fig. 12B). If the ancestral species was widespread, it may have inhabited a broad range of climates, as seen in the widespread species *A. javanica* and *O. lanata*.

The backbone of the ONAP clade is unresolved (Figs 3 & 4), limiting inference of the order of divergence of *Omegandra* and *Nothosaerva* from *Aerva* and *Paraerva*. The phyloecospace analysis shows that *Omegandra* and *Nothosaerva* share similar climates and similar evolutionary trajectories from the common ancestor of the aervoids (Fig. 12B). *Nothosaerva* is more widespread than *Omegandra*, occurring throughout tropical Africa and southern Asia, while *Omegandra* is restricted to near-coastal tropical Northern Territory and Queensland. The two species of *Paraerva*, both succulents, occur on Socotra, characterised by a climate with the least amount of annual precipitation (indicated by BIO 12) of all the aervoids studied (94 mm annum^{-1} for *Paraerva microphylla* and 123 mm annum^{-1} for *Paraerva revoluta*; see Supplementary Material S3). Several species of *Ptilotus* in the Eremaean also show varying degrees of succulence (e.g. *P. yapukaratja* R.W.Davis & T.Hammer; Davis and Hammer 2018).

Ouret and *Ptilotus* diverged around 23.7 Ma with a common ancestral range of Africa–Asia (Fig. 6). The crown group of *Ouret* is dated to around 16.6 Ma, likewise with an African–Asian distribution (Fig. 6). A few species in *Ouret* have widespread distributions, although a clade sister to the rest of the genus is restricted to Madagascar (e.g. *O. coriacea*). Phyloecospace analysis of *Ouret* shows climatic divergence from its common ancestor with *Ptilotus* (Fig. 12A), with radiation into climates with much higher precipitation (Fig. 10); the Asian species of *Ouret* occupy climates with the highest precipitation of all aervoids (Fig. 11C), e.g. with the range midpoint for annual precipitation (BIO 12) for *O. sanguinolenta* at 2,595 mm annum^{-1} (see Supplementary Material S3). The Madagascan lineage within *Ouret* is most similar to the reconstructed ancestor of the genus and of the ONAP clade. The historical biogeography of *Ouret* and the ONAP clade warrants more detailed analysis, which may provide insights into the evolutionary history of the floras of Africa, southern Asia and islands of the Indian Ocean. Inferences from our current study are hampered by a lack of resolution at the base of the ONAP clade and the need for taxonomic clarification of species boundaries within *Ouret* (see Hammer *et al.* in press). Further taxonomic revisions within *Ouret* may affect the distributions of its species.

The origin of the Australian aervoids

Ptilotus and *Omegandra* are the result of independent dispersals into Australia (Fig. 6). While the arrival of *Omegandra* in Australia may have happened any time after its divergence within the ONAP clade around 25.9 Ma, it is likely that *Ptilotus* arrived in Northern Australia prior to its most recent common ancestor, dated at 21.5 Ma. These ages, for the divergence of *Omegandra* and *Ptilotus* from their aervoid relatives and subsequent dispersal to Australia from Asia, are consistent with the collision of Sahul with Sundaland at c. 25 Ma (Byrne *et al.* 2011), the floristic exchange between the Sunda and Sahul shelves perhaps commencing as early as 34 Ma (Crayn *et al.* 2015). Most aervoid species have small, light fruits with a seed that can persist within the hairy sepals, perhaps aiding in anemochory (Hammer *et al.* in press). Dispersal of the aervoids from Sunda to Sahul is thus plausible.

By the Early Miocene (c. 21.5 Ma), the common ancestor of *Ptilotus* probably occurred in Northern Australia, the Northern Desert and Eremaean phytogeographical regions (Fig. 6). It is important to note that the boundaries of the phytogeographical regions used to estimate the ancestral ranges would have been different in the past. The aridification of Australia probably commenced in near-coastal north-western Australia at the Eocene-Oligocene boundary c. 33 Ma as seasonally dry, semi-arid conditions (Martin 2006, Byrne *et al.* 2011, Crisp and Cook 2013). These may have been early habitats for the common ancestor of *Ptilotus* (Fig. 12A).

By the Middle to Late Miocene, the interior of Australia had become more arid, evidenced by the loss of major drainage systems over much of Western Australia (Martin 2006; Moa and Retallack 2019). For extant *Ptilotus* species, species richness and phylogenetic diversity are highest in Western Australia in the southern Pilbara, Gascoyne and Murchison IBRA regions and northern parts of the Southwest Australia phytogeographical region (Fig. 7), suggesting these regions as likely origins of diversification and indicating the geographic origin of the genus as the western Eremaean. Comparatively fewer species, in fewer clades, occur in the eastern Eremaean, where phylogenetic diversity and species richness are low (Figs 7–9). Since the aridification of Australia probably progressed from west to east (see Crisp and Cook 2013) and the majority of the diversity of the genus is in Western Australia, we find a western Eremaean origin for the crowns of the extant clades of *Ptilotus* to be likely.

Our biogeographic and phyloecospace analyses of *Ptilotus* provide support for niche conservatism, i.e. the tendency to retain ancestral ecologies (Wiens and Graham 2005). Niche conservatism may be high for most lineages (e.g. Prinzing *et al.* 2001, Crisp *et al.* 2009, Petitpierre *et al.* 2013), resulting in emerging niches being more likely to be occupied by immigrant lineages arriving with relevant adaptations rather than by *in situ* evolution from native species (Donoghue and Edwards 2008, Donoghue 2011). *Ptilotus* may have arrived in Australia at an opportune time for a lineage adapted to arid or semi-arid conditions, settling in north-western Australia and able to take advantage of subsequent expansions of the arid zone. Similar radiations have been hypothesised for other arid-adapted Australian lineages (Byrne *et al.* 2011, Ladiges *et al.* 2011, Toon *et al.* 2015, Brennan and Oliver 2017).

Ptilotus underwent relatively rapid cladogenesis (Fig. 5A), with all major clades diverging within six million years by the Middle Miocene. Speciation rates were initially high and have slowed over time (Fig. 5B). A likely explanation for this is explosive diversification due to increasing ecological opportunity for an arid-adapted lineage in an aridifying climate (Kozak and Wiens 2010, Yoder *et al.* 2010). *Ptilotus* shows a clear pattern of diversification within the Eremaean and subsequent radiations into peripheral climatic regions, which is also seen in other arid Australian lineages e.g. *Calandrinia* Kunth (Montiaceae; Hancock *et al.* 2018) and *Goodenia* Sm. (Goodeniaceae; Jabaily *et al.* 2014).

Shifts into monsoonal Australia
We find multiple, independent dispersals within *Ptilotus* clades B and D from the arid Eremaean to tropical, monsoonal Northern Australia. Clade B, the most northerly clade (Fig. 8B), was estimated to have an Eremaean origin (Fig. 6), which is confirmed by reconstruction of the climatic data (Figs 12A, 13B). Two independent radiations occurred into Northern Australia (Figs 6, 13B; the *P. corymbosus* R.Br. and *P. lanatus* A.Cunn. ex Moq. species groups). This clade also includes *P. conicus*, which extends beyond Australia into monsoonal southern Indonesia. Northern Australia has a high degree of relatedness among co-occurring species, reflecting the fact that most species in these areas are descended from these two radiations within a single clade.

Shifts into southern Australia

Ptilotus clades D–F also show divergence into more temperate, southern Australia by the Middle Miocene (Figs. 11A, 14). The ancestors of these clades likely inhabited the western Eremaean (Fig. 6). The temperate species of the Euronotian and Southwest Australia cluster conspicuously in ecospace (Figs 10B, 11B), overlapping at the fringes with the Eremaean and with species occurring in the semi-arid transitional zone.

This study is the first to identify the semi-arid transitional zone of northern Southwest Australia as a biodiversity hotspot for *Ptilotus* (Fig. 7), a likely source for subsequent independent radiations into the temperate, Mediterranean climate of southern Australia (see Fig. 1A). Clade E, in particular, has an origin and strong presence in the Eremaean (Fig. 14B), but has its highest species richness and phylogenetic diversity in the semi-arid transitional zone in Southwest Australia (Figs 8E & 9E). There are clear shifts into the Mediterranean climate of near-coastal Southwest Australia in the species group that includes *P. pyramidatus*, *P. sericostachyus* (Nees) F.Muell. and *P. stirlingii* (Lindl.) F.Muell. (Figs 6 & 14B). Similar shifts within the mostly Eremaean clades D and F are also evident (Fig. 14A, C). Clade F has many common southern temperate species such as *P. manglesii* (Lindl.) F.Muell. and the most southerly species *P. spathulatus*. Nearly all of the most southerly *Ptilotus* species are perennial herbs (e.g. *P. angustifolius*, *P. beckerianus* (F.Muell.) F.Muell. ex J.M.Black, *P. erubescens* Schltdl. *P. semilanatus* (Lindl.) F.Muell. ex J.M.Black and *P. spathulatus*), suggesting that this may be an important trait facilitating shifts into temperate niches. Considering the tendency within lineages towards niche conservatism (Donoghue 2008, Edwards and Donoghue 2011), these infrequent shifts within the aervoids into the cooler and more temperate climates of southern Australia are evolutionarily significant.

Broader implications

The aervoids show a striking pattern of hypo-diverse clades in Africa and Asia contrasting with the hyper-diverse genus *Ptilotus* in mostly arid and semi-arid Australia. Our analyses suggest that the arrival of the ancestor of *Ptilotus* in an aridifying Australia was pivotal to its success, enabling early and rapid cladogenesis within the developing Eremaean due to plesiomorphic adaptation to aridity that allowed it to fill opening niche spaces. Subsequent radiations within some clades of *Ptilotus* into higher rainfall temperate and tropical regions show that the Eremaean has acted as a source of diversity. The semi-arid transitional zones, such as that in the northern Southwest Australia region, were likely integral for diversifications into these novel climatic zones.

ACKNOWLEDGEMENTS

SUPPLEMENTARY MATERIALS

The following supplementary materials are available as electronic downloads at https://www.taxonomytim.com/thesis (under Chapter 6): **S1** – A) Voucher information and GenBank accession numbers of the Sanger sequences used in the molecular datasets, and B) voucher information and GenBank accession numbers of the plastid genomes in Dataset 1; **S2** – Occurrence data of aervoid species used in the geospatial and ecological analyses; **S3** – The final dataset used in the phyloecospace analysis: midpoint values of the climatic variables for all species and the reconstructed values for the internal nodes of the aervoid phylogeny; **S4** – mrBayes blocks for the alignments of Dataset 1 used in the reconstruction of the CDS phylogeny and the constrained ITS+*matK* Sanger phylogeny; **S5** – BEAST2

input file used in the reconstruction of the dated phylogeny of Dataset 2; **S6** – CDS phylogeny of 37 species used as the topological constraint for the ITS+*matK* Sanger phylogeny; **S7** – Results of the BEAST2 analysis of Dataset 2, showing the median ages, 95% HPD intervals and posterior probability support values for each node on the topology; **S8** – Result of the Pearson's Correlation test on the 67 climatic variables from WorldClim v1.4; **S9** – Results of the ancestral state reconstruction of the 32 climatic variables on the aervoid phylogeny of Dataset 1.

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GENERAL DISCUSSION

The aim of this thesis was to investigate the taxonomy and evolution of *Ptilotus* and the other aervoid genera in Amaranthaceae by (1) resolving the phylogenetic backbone of *Ptilotus* and the aervoid genera and establishing a generic taxonomy consistent with evolutionary relationships of the taxa, (2) critically re-evaluating the taxonomy and analysing the ecological partitioning of two widespread species complexes within *Ptilotus*, and (3) elucidating the chronological and geographic origins of the aervoid genera and testing the hypothesis that the aridification of Australia has driven the diversification of *Ptilotus*.

An updated phylogeny and taxonomy of the aervoids

Part 1 of my thesis has led to an improved understanding of relationships between the aervoid genera and major clades of *Ptilotus*, and clarified important morphological characters used to establish a new generic taxonomy for the aervoids and an informal clade-based taxonomy of *Ptilotus*.

The aervoid clade has been united since it was informally recognised by Müller and Borsch (2005b), based solely on molecular phylogenetics and without identified morphological synapomorphies for the clade. Most genera included within the aervoids are either morphologically diverse (e.g. *Ptilotus* R.Br.) or very different from the other genera (e.g. *Nothosaerva* Wight and the hitherto un-sequenced *Omegandra* G.J.Leach & C.C.Towns.). The lack of a clear concept and critical analyses of morphological characters uniting this group was a leading reason for the incorrect placement of *Aerva artemisioides* Vierh. & O.Schwartz within *Aerva* Forssk.

Testing the generic assignment of Aerva artemisioides
The protologue of *A. artemisioides* incorrectly described its highly reduced flowers as pentamerous (with five sepals and stamens) and the inflorescence as a three- or seven-flowered glomerule (i.e. condensed cyme) enclosed by a bract (Schwartz 1939). This was partially corrected by Miller (1996) in the *Flora of Arabia*, who correctly described the flowers as having six sepals and four stamens. However, neither Miller (1996) or subsequent authors (e.g. Thiv *et al.* 2006) recognised that *A. artemisioides* was unlike any other species of *Aerva* in its inflorescence and floral structure. All other *Aerva* species *sensu* Miller (1996) and Thiv *et al.* (2006) have single-flowered inflorescence units (i.e. a flower enclosed by two bracteoles and bract), a pentamerous perianth, and an androecium with five stamens opposite the sepals (the four stamens of *A. artemisioides* alternate with the inner sepals).

Thiv *et al.* (2006), in a phylogenetic reconstruction of *Aerva* used to test biogeogeographic hypotheses in Arabia and East Africa, incorrectly assumed the monophyly of *Aerva* (including *A. artemisioides*) by rooting their tree on only two species of *Ptilotus*. I have corrected this error by rooting a phylogeny of the aervoids on non-aervoid outgroup species, encompassing the main clades of Amaranthaceae, which places *A. artemisioides* outside the aervoids. In combination with its unusual morphology, this placement justified the erection of the new monotypic genus *Wadithamnus* T.Hammer & R.W.Davis for *A. artemisioides*. Further study of inflorescence and floral development of *W. artemisioides* (Vierh. & O.Schwartz) T.Hammer & R.W.Davis may yield insights into the evolution of these structures within Amaranthaceae.

My aervoid phylogeny of Chapter 2, although not yet taxon-complete and missing some key genera, also resolved *Ptilotus* to be nested within *Aerva*, indicating that the latter genus, as circumscribed, is paraphyletic.

Establishing a new generic taxonomy for the aervoids

In Chapter 3, I further clarify relationships within the aervoids by resolving a phylogenetic backbone of the aervoid genera and among the major clades of *Ptilotus* using the coding DNA sequences of 41 newly sequenced plastomes. This genomic data established *Aerva s.lat.* as polyphyletic with respect to the other genera, resolved relationships between the six major clades of *Ptilotus* (i.e. clades A–F), and resolved the genus *Kelita* A.R.Bean as nested within *Ptilotus*. The plastome phylogeny placed *Omegandra* at the base of *Aerva* clade I (i.e. *A. javanica* and the two Socotran species *A. microphylla* Moq. and *A. revoluta* Balf.f.) and placed the eight species of *Aerva* clade II sister to *Ptilotus* (including *Kelita*).

The plastome phylogeny also allowed a well-supported resolution of the backbone of *Ptilotus*, revealing the relationships between all major clades. All clades recovered by Hammer *et al.* (2015) were also recovered here, with increased taxon sampling within clade B allowing recognition of two major subclades. The large clade D, previously resolved as a polytomy in Hammer *et al.* (2015), was resolved into four well-supported subclades (D1–D4) with strong backbone support. The resulting phylogeny is the most comprehensively sampled and best-resolved phylogeny for *Ptilotus*.

Several morphological characters identified for the genera and clades of *Aerva s.lat.* helped to support an updated taxonomy of the aervoids. In particular, stigma morphology was found to be diagnostic for clades and helped to segregate sister clades from one another. *Ptilotus* and *Aerva* clade II (i.e. including *A. lanata* (L.) Juss. ex Schult.) could be segregated by the latter having a bilobed stigma, while all *Ptilotus* species (i.e. including *Kelita*) have an unlobed stigma. The two Socotran species of *Aerva* were found to have an unlobed stigma, while *A. javanica* has a long and feathery bilobed stigma, quite different from any other species in the group. *Nothosaerva brachiata* and *O. kanisii* were found to have a distinctive morphology from each other and all other known species. *Nothosaerva brachiata* has an unlobed stigma, four sepals, two stamens and no staminal cup appendages, while *O. kanisii* has a minutely lobed stigma, four sepals, two stamens and staminal cup appendages. *Omegandra kanisii* uniquely has a staminal cup that does not fully encircle the ovary.

One outcome of the near-comprehensive sampling and strong resolution of the phylogeny in Chapter 3 is that it allows a new resolution of taxonomic limits among the aervoid genera. A new genus—*Paraerva* T.Hammer—has been erected for two species endemic on the Socotran Archipelago (i.e. *A. microphylla* and *A. revoluta*), drawing further attention to this biogeographically and evolutionarily important island chain off the coast of the Horn of Africa. The name *Ouret* Adan., previously rendered a synonym of *Aerva* by the nomenclatural conservation of the latter, has been resurrected for *Aerva* clade II. With these two changes, *Aerva s.str.* is monotypic and comprises only the distinctive *A. javanica*, joining two other extant monotypic genera, *Nothosaerva* and *Omegandra*. The monotypic genus *Kelita*, by contrast, is now shown to be nested within *Ptilotus*, its single species recombined as *P. uncinellus* (A.R.Bean) T.Hammer.

Part 1 of my thesis thus resulted in a better understanding of the morphology and phylogenetics of the aervoid clade, enabling an updated generic classification consistent with these new data. The aervoid clade now consists of the six genera (i.e. *Aerva s.str.*, *Nothosaerva*, *Omegandra*, *Ouret*, *Paraerva* and *Ptilotus*) and includes c. 135 species, most of which are in *Ptilotus*.

Taxonomy and ecology of two widespread species complexes within *Ptilotus*

Part 2 of my thesis clarified the taxonomy of two of the most widespread species within *Ptilotus*, *P. nobilis* (Lindl.) F.Muell. and *P. macrocephalus* (R.Br.) Poir., and provides an example of the integration of geospatial analyses of environmental variables in taxonomic research to support hypotheses of lineage separation. Evidence was also presented for the recent segregation of these diverse species based on

discrete ecological differences, indicating that edaphic and climatic niche shifts have been important in recent speciation within *Ptilotus*.

The first of these species groups includes *P. exaltatus* Nees, a previously recognised species that had been synonymised under *P. nobilis* by Bean (2008) following a molecular and morphological analysis by Lee *et al.* (2007). I critically re-evaluated this decision by carrying out a comprehensive morphological investigation of specimens across the range of both species and determined that these species could indeed be segregated based on multiple morphological characters. The geospatial analyses indicated that the two species, though broadly sympatric in parts of their ranges, occupy different parts of the landscape. The less widely distributed *P. nobilis* occurred on heavier, more sparsely vegetated soils of stony plains and tablelands, while the more widely distributed and ecologically more variable *P. exaltatus* occurred on lighter-textured soils. Consequently, I reinstated *P. exaltatus* as separate from *P. nobilis*. The morphological analysis also indicated that two subspecies of *P. nobilis* (subsp. *semilanatus* (Lindl.) A.R.Bean and subsp. *angustifolius* (Benl) Lally & W.R.Barker) are best raised to species rank. The morphologies and distributions of *P. semilanataus* (Lindl.) F.Muell. ex J.M.Black and *P. angustifolius* (Benl) T.Hammer indicate that they likely recently diverged from *P. exaltatus* and *P. nobilis*, respectively, with *P. semilanatus* occurring in semi-arid eastern Australia to the east of the range of *P. exaltatus* and *P. angustifolius* occurring in temperate South Australia, to the south of the arid *P. nobilis*.

Geospatial analyses were also used to segregate three species from the widespread *P. macrocephalus s.lat.* complex (i.e. *P. macrocephalus s.str.*, *P. psilorhachis* T.Hammer & R.W.Davis and *P. xerophilus* T.Hammer & R.W.Davis). The new species, which had not been previously recognised, were segregated on the basis of multiple morphological characters, and were found to occupy distinct climatic regions of Australia, with *P. xerophilus* throughout the Eremaean, *P. psilorhachis* in eastern Queensland and *P. macrocephalus* in south-eastern Australia (i.e. eastern New South Wales, Victoria, and south-eastern South Australia). The geographic pattern of these species, and of the clade to which they belong (*P. fusiformis* (R.Br.) Poir. and allies in clade D), indicate a shift from an ancestor in arid or semi-arid Australia into the higher rainfall monsoonal tropics and southern temperate regions. Of the species in the *P. fusiformis* species group, only *P. macrocephalus s.str.* and *P. beckerianus* (F.Muell.) F.Muell. ex J.M.Black occur in south-eastern Australia, perhaps indicating a shared evolutionary history and single radiation into temperate Australia.

While *P. macrocephalus s.str.* and *P. psilorhachis* were not included in the molecular phylogeny of *Ptilotus*, their affinities are clear, and they help shed light on the historical biogeography of the genus. In both the *P. nobilis* and *P. macrocephalus* species groups, shifts into new climatic niches appear to have accompanied speciation, indicating the likely importance of niche shifts in the diversification of the genus in Australia. This issue was addressed in Part 3 of my thesis.

Origin and climatic evolution of the aervoids

The analyses in Part 3 elucidated the chronological and geographic origins of the aervoids and provide a probable explanation for relative hyper-diversity of the aervoids in arid Australia, as *Ptilotus*, compared with the hypo-diversity in the other genera outside of arid Australia.

The chronogram and biogeographic analysis indicate that the aervoid clade likely originated in Oligocene Africa-Asia, with the ancestors of *Ptilotus* and *Omegandra* likely dispersing independently from south-eastern Asia to northern Australia. Analysis of climatic niche evolution along the aervoid phylogeny supports the hypothesis that *Ptilotus* was pre-adapted for arid or semi-arid conditions. Upon arrival in Australia in the Early Miocene, prior to the onset of severe aridity, *Ptilotus* underwent rapid

cladogenesis in Miocene Western Australia, probably filling niche spaces opening up in response to the changing climate. The ancestral range of the crowns of each major clade was estimated to occupy the Eremaean, suggesting an arid origin for all extant major clades of the genus. The ancestral range estimation is supported by an independent analysis of the evolution of climatic variables across the phylogeny (a phyloecospace analysis), which indicates the movement of the genus into the Eremaean, subsequent radiations throughout arid Australia, and shifts to neighbouring regions of monsoonal northern and temperate southern Australia. Two major hotspots for species richness and phylogenetic diversity were identified for *Ptilotus* in the Eremaean and northern Southwest phytogeographical regions of Western Australia, indicating arid and semi-arid Western Australia as diversification hotspots for the genus. The pattern of radiation from an arid lineage into monsoon-tropical and temperate-mesic biomes is an important result that has rarely been reported in the Australian literature.

Future research on *Ptilotus* and the aervoids

The aervoid species have not been thoroughly studied beyond basic taxonomy and systematics and a few preliminary studies that identified novel traits, such as the evolution of C_4 photosynthesis (Sage *et al.* 2007) and widespread apomixis (Khan *et al.* 1970) in *A. javanica*. Both *A. javanica* and *O. lanata* are of research interest for their medicinal properties (e.g. Goyal *et al.* 2011, Srinivas and Reddy 2012) and religious and cultural importance to peoples of southern Asia (e.g. Ayyanar and Ignacimuthu 2010). Apart from research into its novel ability to hyper-accumulate phosphorus (e.g. Ryan *et al.* 2009; see Appendix A), very few non-taxonomic studies have investigated aspects of the biology of the species-rich genus *Ptilotus*.

Evolution of sexual strategies

Stewart and Barlow (1976) reported gyndioecy within *Ptilotus obovatus* (Gaudich.) F.Muell. During field work for my thesis, I assessed 16 species as gynodioecious, some with very high ratios of female to hermaphrodite plants (e.g. 30:1 in a population of *P. schwartzii* (F.Muell.) Tate; unpublished data). Gynodioecy is a rare phenomenon among plant species (Godin and Demyanova 2013), and has not been well documented or rigorously studied in *Ptilotus*. Gynodioecy and a reduction in fertile stamen number appear not to be correlated, with gynodioecious species having both reduced and unreduced numbers of fertile stamens. Since Darwin (1877) there has been interest in the evolution and purpose of gynodioecy. One hypotheses is that gynodioecy may be a transitional state from hermaphroditism to full dioecy (Barrett 2002), yet a full understanding of this transition remains elusive (Spigler and Ashman 2012). A few aervoid species (e.g. *A. javanica* and *P. crispus* Benl) are truly dioecious, with female plants lacking a developed androecium and male plants lacking a developed style and stigma. As the evolution and benefit of gynodioecy remains an active research topic, the prevalence of gynodioecious and non-gynodioecious species within *Ptilotus* and the well-resolved phylogeny presented in this thesis makes it an excellent case study to investigate this sexual strategy.

Chromosome evolution

Stewart and Barlow (1976) also investigated chromosome numbers and polyploidy within *Ptilotus* and showed that the genus may have the highest base chromosome count recorded for the family (n = 27). They also reported tetraploids (n = 54) in *P. obovatus*. Other genera surveyed in Amaranthaceae *s.str.* have fewer chromosomes (e.g. n = 16 in *Aerva s.str.* and n = 13 in *Gomphrena*) (Stewart and Barlow 1976). Gene duplication through polyploidy has been correlated in other groups of plants to adaptive radiations and rapid diversifications within the angiosperms (e.g. Jiao et al. 2011, Van de Peer et al. 2017) and identified as possibly being a source of novel physiological and ecological adaptations (Ramsey 2011, te Beest et al. 2012). A full investigation of chromosomal evolution within

Amaranthaceae *s.str.* and the aervoids may shed light on the role of genome duplications in the rapid diversification of *Ptilotus*.

Evolution of floral traits and pollinator preferences

My thesis has shed light on the importance of abiotic factors, such as climate, in driving the diversification of *Ptilotus*, but biotic factors may have been equally important. Perhaps unusually in Amaranthaceae, *Ptilotus* flowers are entomophilous with a diverse range of sizes, shapes, colours, symmetries and numbers of floral parts (e.g. reduction in fertile stamen number; Hammer *et al.* 2015). Many species have nectar and are scented. Field observations indicate that they are visited by native bees, bee-flies, butterflies and nocturnal moths (e.g. Hammer *et al.* 2018). Despite its diversity of floral traits, there have been no studies of pollination biology in *Ptilotus*, and current knowledge of pollinator preferences is limited to inferences based on morphology and serendipitous observations of flower visitation. The flowers in other aervoid genera are, by comparison to most *Ptilotus* species, small, less showy and not obviously entomophilous; the reduction of the aervoid flower reaches its extreme in *Nothosaerva brachiata*, which has two anthers directly situated over the reduced style and stigma that have been observed in herbarium specimens to release pollen onto the stigma while in late bud (pers. obs.), indicating likely self-pollination in this and perhaps other species.

Given the taxonomic diversity and diversity of floral traits within *Ptilotus*, it is likely that pollinator-specific selection pressures and shifts in pollination syndromes have played an important role in the diversification of the genus, and this may contribute to explaining the disparity in species richness between the hyper-diverse *Ptilotus* and the hypo-diverse other genera. As *Ptilotus* was evolving in the emerging arid Australian communities of the Miocene, pollinator-specific selection pressures would have been encountered between competing species, spurring divergence in floral characteristics and diversifying the ecological roles filled by early clades of *Ptilotus*, perhaps contributing to its adaptive radiation (Yoder *et al.* 2010, Givnish 2015). The well-resolved phylogeny provided in this thesis and wide range of entomophilous flower morphologies in *Ptilotus* provides a useful system for studying the evolution of pollination syndromes.

The evolution of pollen morphology within the aervoids

Pollen morphology in Amaranthaceae *s.lat.* has been investigated by many previous authors. Pollen characters provide synapomorphies for clades or genera, and potentially reveal information on the evolutionary history of the group (e.g. Townsend 1993, Borsch 1998, Müller and Borsch 2005a, Flores-Olvera *et al.* 2006, Sánchez-del Pino *et al.* 2016). Borsch (1998) defined a '*Ptilotus*-type' pollen for species of *Ptilotus* and indicated that the other aervoid genera had '*Arthraerua*-type' pollen. A preliminary and unpublished investigation of 12 species of *Ptilotus* early during my PhD candidature using scanning electron microscopy revealed more morphological diversity within the genus than described by Borsch (1998). A more comprehensive and rigorous survey of pollen morphology in the aervoids may reveal synapomorphies for the six major clades of *Ptilotus*, which are currently supported solely by molecular phylogenetics and with no known morphological synapomorphies.

Ongoing taxonomic studies

In my thesis, I have established a new generic taxonomy for the aervoids and added to the knowledge of the morphology of aervoid genera. This demonstrated a clear need for full taxonomic revisions of *Aerva* and *Ouret*. Current understanding of species boundaries within *Ouret*, for instance, is very poor, as was shown in the phylogeny of Thiv *et al.* (2006), where *O. lanata* (as *A. lanata*) was polyphyletic, some specimens clustering with other, geographically proximal, species (e.g. *A. sanguinolenta* (L.) Blume and *A. leucura* Moq.). Past revisions to the taxonomy of these species have been limited in scope (e.g. Townsend 1974, 1985). A comprehensive morphological study of all taxa across their geographic ranges

and accounts of their taxonomic and nomenclature history is critically needed to help resolve specific and infraspecific boundaries within *Aerva* and *Ouret*.

Further taxonomic work is also needed within *Ptilotus*. New species continue to be found regularly and described for the genus (e.g. Hammer 2018, Hammer and Davis 2018; see Appendix B), and this is likely to continue for the near future. Additionally, a few species complexes within *Ptilotus* (e.g. the *P. manglesii* (Lindl.) F.Muell. complex) remain problematic; forthcoming taxonomic revisions will result in the clarification of more species boundaries and the description of new species.

Conclusion

The aervoids show a pattern of hypo-diverse genera sister to a larger clade of *Ouret* and the relatively hyper-diverse *Ptilotus*. The mostly arid Australian genus *Ptilotus* comprises approximately 90% of aervoid species (122 species of the 135 aervoid species currently recognised). A dozen new species have been added (as *sp. nov.* or *comb. nov.*) or reinstated to the genus as a result of this thesis and related work undertaken during my PhD candidature (see Appendix B). This hyper-diversity of *Ptilotus* within arid and semi-arid Australia is best explained by a competitive advantage in arid conditions, which enabled the genus to radiate into niche spaces opening up throughout the aridifying continent in the Miocene. This evolutionary history has allowed *Ptilotus* to become the most species-rich and widespread member of Amaranthaceae *s.str.* within Australia and one of the most species-rich genera in the family, while being almost entirely restricted to Australia. More recent evolution within *Ptilotus* provides examples of climatic shifts within multiple clades from the arid Eremaean into higher rainfall regions of monsoonal northern and temperate southern Australia. This pattern is seen in the widespread *P. nobilis* and *P. macrocephalus* species complexes. My thesis adds to our knowledge of a previously understudied and diverse clade of amaranths, and an important component of the flora of arid Australia. The well-resolved and robustly supported phylogeny will enable further studies into the evolution of sexual strategies, pollination syndromes and floral morphology in this attractive and evolutionarily important group of plants.

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APPENDIX A: PREFACE

Ptilotus is a fascinating and diverse genus that has barely been investigated for some interesting aspects of its biology. The ability of *Ptilotus* to grow well in low phosphorus (P) soils and to hyperaccumulate P when high levels of P are added to the soil has been indicated by previous studies, which have found the shoot dry matter to have concentrations of P that are toxic to other plants.

Presented here is a manuscript of the results of two experiments conducted over the course of my PhD candidature that investigate P hyperaccumulation within *Ptilotus*. In particular, a broader sampling is conducted to determined how widespread this ability is within the genus and if the ability correlates with phylogenetic relationship, ecology or morphology. *Ptilotus* species grown in high and low P conditions are also compared to native and non-native Australian species and one other Australian amaranth species *Gomphrena canescens*. Samples taken from the plants in this experiment will also be analysed using scanning electron cryomicroscopy in a forthcoming study to determine where and how P is being stored in the leaves of these species.

While this study was planned as part of my PhD thesis, it did not turn out to be an important aspect of the story of the evolution of *Ptilotus* covered by the thesis. The study is therefore presented as here as Appendix A and is not presented for examination.

Mulling over the mulla mullas: revisiting phosphorus hyperaccumulation in the Australian plant genus *Ptilotus* (Amaranthaceae)

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Abstract

- *Background and Aims* – Species in the Australian genus *Ptilotus* (Amaranthaceae) grow well in soils with either very low or very high phosphorus (P) availability; when P is abundant, they hyperaccumulate P in leaves. However, it is not known how common this trait is within the genus, if it is shared with other genera in the family, or if it correlates with the wide array of morphologies or ecologies within *Ptilotus*. This study assessed P hyperaccumulation across the morphological, ecological and phylogenetic diversity of *Ptilotus*, and a naturally co-occurring species of *Gomphrena* (Amaranthaceae).
- *Methods* – Experiment 1 tested the response of 11 species to three levels of added P (0, 50 mg kg^{-1} and 100 mg kg^{-1}), including six species of *Ptilotus* and *Gomphrena canescens*. Experiment 2 tested the response of five species, three *Ptilotus*, *Gomphrena canescens* and *Kennedia prostrata*, to two levels of added P (5 and 150 mg kg^{-1}) and two P-pulse treatments (5 and 50 mg kg^{-1}) two weeks prior to harvest.
- *Key Results* – *Ptilotus* species greatly accumulated P when grown in a high-P soil, but curtailed uptake of P from a pulse. *Ptilotus* species preferentially allocated P to leaves (reaching 73 mg g^{-1}) but did not suffer P toxicity. In contrast, *Gomphrena canescens* and *Kennedia prostrata* preferentially allocated P to stems and roots, respectively, but did suffer P toxicity.
- *Conclusions* – *Ptilotus* species accumulated high [P] in their leaves, suggesting this trait is widespread within the genus. The lack of tolerance to high [P] in *Gomphrena canescens* suggests that the mechanisms *Ptilotus* uses to tolerate high [P] are not shared with other amaranths. Further research will determine mechanisms underlying the unusual P physiology of *Ptilotus*.

Keywords: Amaranthaceae, hyperaccumulation, phosphorus, *Ptilotus*, toxicity

INTRODUCTION

Global agriculture is highly reliant on finite reserves of phosphorus (P) that are becoming increasingly scarce (Fixen and Johnston, 2012; Johnston *et al.*, 2014). To plan for the long-term stability of agriculture it is, therefore, imperative to have a greater understanding of the different mechanisms that plants have evolved to take up and store P (Lambers *et al.*, 2006). Australia is home to astonishing plant biodiversity and yet has some of the oldest and most P-impoverished soils in the world (Lambers *et al.*, 2010; Kooyman *et al.*, 2017). Previous authors have speculated on an important role of soils in influencing the diversification of plant species in Australia (e.g., Hopper, 2009; Orians and Milewski, 2007; Tsakalos *et al.*, 2018) as well as in globally analogous regions, such as the Cape region of South Africa (Schitzler *et al.*, 2011). Investigations of the adaptations among Australian species might lead to innovations in improving P efficiency in agricultural systems and enable targeted breeding programs to generate a new generation of P-efficient crops (Pang *et al.*, 2010; Veneklaas *et al.*, 2012; van de Wiel *et al.*, 2016). In addition to the development of P-efficient crops, new strategies to mitigate nutrient run-off and the eutrophication of waterways and to bioremediate P-contaminated soils may also be identified (Ye *et al.*, 2018).

A group of native Australian plants that have previously been investigated for their P-efficient strategies is the genus *Ptilotus* R.Br. (commonly mulla mulla) (Amaranthaceae). Islam *et al.* (1999) found that *Ptilotus* responded better to an increase in soil [P] and produced more biomass than co-occurring species (e.g., in families Chenopodiaceae, Poaceae and Malvaceae) in naturally low-P soil in the Pilbara region of Western Australia, where many *Ptilotus* species are abundant (Hammer *et al.*, 2018a). They concluded that P limits growth of *Ptilotus* in such environments. Glasshouse studies on *Ptilotus* species, i.e. *P. exaltatus* Nees, *P. macrocephalus* (R.Br.) Poir. and *P. polystachyus* (Gaudich.) F.Muell., have demonstrated an ability to grow well in naturally low-P soil, as well as in soil with abundant added P, to as high as 100 mg kg^{-1} (e.g., Islam *et al.*, 1999; Ryan *et al.*, 2009; Suriyagoda *et al.*, 2012, 2015). Like many other species that naturally grow in low-P environments (e.g., Proteaceae; Shane *et al.*, 2004; Shane and Lambers, 2006), *Ptilotus* has a poor ability to down-regulate the uptake of P. Unlike Proteaceae, they show no decrease in rhizosphere carboxylates (especially oxalate) with increasing P supply (Suriyagoda *et al.*, 2015). Plants grown at high levels of P stored large amounts of P in their shoots (i.e. leaves and stems), up to 40 mg g^{-1} of dry biomass in *P. polystachyus* (Ryan *et al.*, 2009) and 60 mg g^{-1} in *P. exaltatus* (Suriyagoda *et al.*, 2015). To our knowledge, no other plants accumulate P in shoots to these extraordinary levels without symptoms of toxicity, such as premature leaf senescence, chlorosis and necrosis, and stunted growth (Shane and Lambers, 2006; Ryan et al., 2009).

Ptilotus does not form mycorrhizal associations (Ryan *et al.*, 2009; Aziz *et al.*, 2015; Suriyagoda *et al.*, 2015), in common with many members of Amaranthaceae and the order Caryophyllales (Khan, 1974; Brundrett, 2003). Mycorrhizal associations generally aid soil P uptake by plants (Bolan, 1991); therefore, non-mycorrhizal plants in P-impoverished habitats require adaptations such as root exudation of carboxylates or numerous fine roots; such adaptions are present in *Ptilotus* (Ryan et al., 2009; Aziz *et al.*, 2015). Other families within Caryophyllales form associations with ectomycorrhizal fungi (Haug *et al.*, 2005; Wang and Qiu, 2006), including a unique relationship between newly discovered ectomycorrhizal fungi and neotropical members of some families closely related to Amaranthaceae (Alvarez-Manjarrez *et al.*, 2018).

All of the c. 120 species of *Ptilotus* are native to continental Australia, a few with distributions that extend to nearby islands (Hammer *et al.*, in press). The genus can be found in nearly every biome in Australia, apart from alpine regions and densely-shaded forests. It is diverse in arid regions, especially in Western Australia (Hammer *et al.*, 2018a), where annual species are locally abundant following rains. *Ptilotus* species are annual or perennial herbs or shrubs (Fig. 1) and occur in a wide range of habitats and soils, ranging from coarse sands to cracking clays (Hammer *et al.*, 2018b). Several other, distantly related, genera in Amaranthaceae (e.g., *Gomphrena* L. and *Amaranthus* L.) can likewise be found in parts of Australia (particularly in tropical and subtropical regions) but lack the diversity of *Ptilotus*. The high diversity of *Ptilotus* is thought to be the product of a recent radiation throughout the continent (Hammer *et al.*, 2015). Molecular phylogenies of *Ptilotus* have resolved several diverse clades, which correspond to morphological, ecological and geographic variation in the genus (Hammer *et al.*, in press). Ecology, and physiological adaptations, might play a particularly important role in the adaptive radiation and evolution of *Ptilotus*, as has been found in a recent study that indicated that *P. exaltatus* and *P. nobilis* (Lindl.) F.Muell., previously considered conspecific, have strong ecological and geographic partitioning, especially when soil characteristics were examined at fine scales (Hammer *et al.*, 2018b). However, the importance of hyperaccumulation of P as an adaption of *Ptilotus* is unknown. Interestingly, nutrient pulses likely play a critical role in maintaining the productivity of ecosystems with tight nutrient cycling, particularly in areas that experience drying-rewetting cycles such as in arid and semi-arid regions of Australia where *Ptilotus* is common and rainfall is highly seasonal (e.g., Lodge *et al.*, 1994; Gordon *et al.*, 2008). Perhaps, the P physiology of *Ptilotus* reflects its adaptation to this environment by enabling rapid

acquisition of P during brief periods when soil wetting enables P mineralisation (i.e. during a pulse of P). This P would then be stored in plant tissues, until needed by the plant (Ryan *et al.*, 2019; Yang *et al.*, 2017).
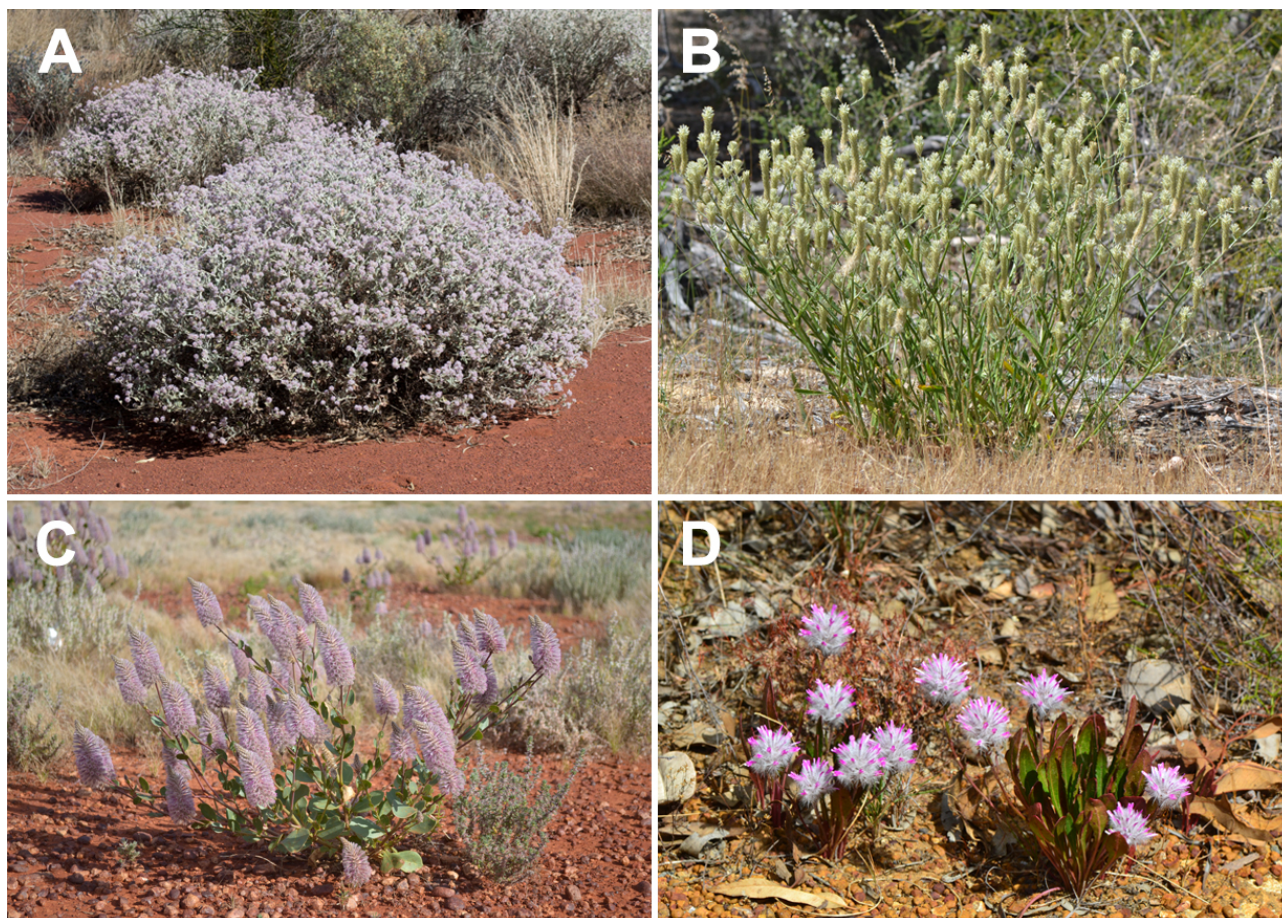
Fig. 1. *Ptilotus* species growing *in situ*: (A) *P. obovatus* (Kumarina, Western Australia), (B) *P. polystachyus* (Mindarra, Western Australia), (C) *P. exaltatus* (Mount Willoughby, South Australia) and (D) *P. manglesii* (Mindarra, Western Australia). Photographs by T. Hammer.

Despite previous studies on the P nutrition of *Ptilotus* (i.e. Islam *et al.*, 1999; Ryan *et al.*, 2009; Suriyagoda *et al.*, 2012, 2015; Aziz *et al.*, 2015), it is still unknown how widespread tolerance of high [P] is within the genus, how this correlates with interspecific ecological and morphological variation, and if it has contributed to the diversity of the genus throughout Australia (Aziz *et al.*, 2015; Hammer *et al.*, 2015; Suriyagoda *et al.*, 2015). These previous studies have not included non-*Ptilotus* species in Amaranthaceae; therefore, it is unknown if the ability to tolerate high [P] is specific to *Ptilotus*. As Islam *et al.* (1999) indicated that P may be a limiting factor to growth in their native environment, which is subject to tight nutrient cycling, the response of *Ptilotus* to a P pulse will help elucidate the role that P hyperaccumulation may have in its biology. In the present study, we revisit the P-hyperaccumulation ability of *Ptilotus* to test these unanswered questions. Specifically, we test the following hypotheses: 1) the ability to hyperaccumulate P is widespread within *Ptilotus* and does not correlate with phylogenetic clade, ecology or life history strategy; 2) the ability to tolerate high leaf [P] is widespread within *Ptilotus*, with leaf, stem and root morphologies not varying among P treatments; 3) the ability to tolerate high leaf [P] is not shared with related Australian amaranth genera; and 4) the ability of *Ptilotus* to hyperaccumulate P without toxicity symptoms will aid absorption of P following a P pulse.

MATERIALS AND METHODS

This study was conducted as two glasshouse experiments at the University of Western Australia (Crawley, Australia). The first experiment was designed to survey how common the ability to hyperaccumulate P is within the genus *Ptilotus*, and the second experiment was designed to assess the morphological responses and study P hyperaccumulation in more detail in three of the most widespread *Ptilotus* species, another species within Amaranthaceae, and a native legume known to poorly down-regulate P uptake and to show P-toxicity symptoms.

Experiment 1

Six species were grown in a glasshouse experiment from the three most diverse and widespread phylogenetic groups (i.e. clades) within *Ptilotus* (Hammer *et al.*, in press). Two groups of control species were also grown: 1) two native Australian species and 2) three non-native, cultivated legume (Fabaceae) species. The native control species were *Gomphrena canescens* R.Br. (Amaranthaceae) and *Rhodanthe chlorocephala* (Turcz.) Paul G.Wilson (Asteraceae). Both *G. canescens* and *R. chlorocephala* co-occur with *Ptilotus* species, with the former producing *en masse* floral displays alongside *Ptilotus* species in Western Australia. The cultivated control legume species were *Macroptilium atropurpureum* (DC.) Urb. (siratro), *Medicago sativa* L. (lucerne/alfalfa) and *Trifolium subterraneum* L. (subterranean clover). Fruits of the *Ptilotus* species were collected from the field in Western Australia (voucher information in Table 1).

All *Ptilotus* species have a single seed within an indehiscent, often membranous, fruit, that is enclosed by the persistent perianth (Hammer *et al.*, in press). Williams *et al.* (1989) found that poor germination of *P. exaltatus* was improved by the removal of the seed from the fruit and perianth and by removal of the seed coat. Therefore, for the following experiments, each *Ptilotus* seed was extracted from the fruit and surrounding perianth by hand, so as to not damage the embryo, and scarified to penetrate the seed coat under sterile conditions by using a scalpel to make an incision on the lateral side of the seed to avoid damaging the embryo. Seeds of the non-*Ptilotus* species were obtained from commercial sources and, to be consistent, also scarified using a scalpel to pierce the seed coat (Table 1). Prior to scarification, all seeds were surface sterilised with 70% (v/v) ethanol, and then washed.

Table 1. Species used in Experiments 1 and 2 with family, habit, native range and seed origin listed. Asterisks (*) indicate cultivated species.

Species	Family	Habit	Native Range	Seed origin	Experiment
Gomphrena canescens	Amaranthaceae	Annual herb	N Australia	Purchased from Kimseed Australia Pty Ltd.	1, 2
Kennedia prostrata	Fabaceae	Perennial herb	SW Australia	Purchased from Nindethana Seed	2
*Macroptilium atropurpureum**	Fabaceae	Perennial herb	Tropical Americas	Commercial seed supplier	1
*Medicago sativa** (cultivar: SARDI Ten)	Fabaceae	Annual herb	South-central Asia	Commercial seed supplier	1
Ptilotus exaltatus	Amaranthaceae	Annual herb	Widespread Australia	Collected (Voucher: T.Hammer, S.Dillon & K.Thiele TH20; PERTH 08735220)	1, 2
Ptilotus fusiformis	Amaranthaceae	Annual herb	N Australia	Collected (Voucher: T.Hammer, S.Dillon & K.Thiele TH17; PERTH 08735239)	1
Ptilotus manglesii	Amaranthaceae	Perennial herb	SW Australia	Collected (Voucher: T.Hammer & R.Davis TH99; PERTH 08863911)	1, 2
Ptilotus obovatus	Amaranthaceae	Shrub	Widespread Australia	Collected (Voucher: T.Hammer & S.Dillon TH52; PERTH 08743789)	1
Ptilotus polystachyus	Amaranthaceae	Annual herb	Widespread Australia	Collected (Voucher: T.Hammer & R.Davis TH64; PERTH 08619611)	2
Ptilotus rotundifolius	Amaranthaceae	Shrub	NW Australia	Collected (Voucher: T.Hammer & S.Dillon TH32; PERTH 08619964)	1
Ptilotus spathulatus	Amaranthaceae	Perennial herb	S Australia	Collected (Voucher: G.Byrne 2056; PERTH 7759134)	1
Rhodanthe chlorocephala	Asteraceae	Annual herb	Western Australia	Purchased from Kimseed Australia Pty Ltd.	1
*Trifolium subterraneum** (cultivar: Dalkeith)	Fabaceae	Annual herb	Western Europe and Mediterranean	Commercial seed supplier	1

Each pot contained 1 kg of nutrient-deficient field-collected soil locally known as 'Lancelin sand' (Uc4-22; Northcote, 1979), as used in Ryan *et al.* (2009). Analysis of the soil indicated that it contained 1 mg kg^{-1} nitrate-N, <1 mg kg^{-1} ammonium-N (Searle, 1984), <2 mg kg^{-1} bicarbonate-extractable P (Colwell method; Rayment and Higginson, 1992), 30 mg kg^{-1} bicarbonate-extractable K (Colwell method; Rayment and Higginson, 1992) and 1.05% organic carbon (chromic acid titration method; Walkley and Black, 1934), and had a pH of 5.77 (i.e., in CaCl$_2$; Rayment and Higginson, 1992). Prior to sowing, nutrient solutions were added to the soil. Macronutrient solutions were added to give a concentration of 173 mg kg^{-1} K$_2$SO$_4$, 80 mg kg^{-1} MgSO$_4$, 150 mg kg^{-1} CaCl$_2$ and 33 mg kg^{-1} (NH$_4$) NO$_3$. Micronutrient solutions were also added to give a final concentration of 0.7 mg kg^{-1} H$_3$BO$_3$, 15 mg kg^{-1} MnSO$_4$, 9 mg kg^{-1} ZnSO$_4$, 3 mg kg^{-1} CuSO$_4$, 0.2 mg kg^{-1} CoSO$_4$ and 0.30 mg kg^{-1} Na$_2$MoO$_4$. The three levels of P (as KH$_2$PO$_4$) treatment were 0, 30 and 100 mg kg^{-1} as the low-, medium- and high-P treatments, respectively. The experiment had two treatments: Species and P level. All pots were arranged in four randomised blocks, where each block represented a replication and each treatment combination was present once within each block.

The sterilised and scarified seeds of all species were germinated in Petri dishes of agar in a 25°C growth room until emergence of the radicle. Germinants were sown in pots at a depth of 1–5 mm, depending on seed size. Sowing of germinants was staggered, with the shrubs and perennial herbs being sown on 10 July 2017, and the annuals on 7 August. For the legumes, appropriate rhizobia were added to the soil surface, and watered after sowing. After one week, seedlings were thinned to the two healthiest per pot. Pots were maintained at 80% field capacity with deionized water that was applied when required. Pots were re-randomised within blocks after each watering. Pots were sprayed with fungicide on 10 August to control root-rot pathogens. Average temperatures in the glasshouse were 29°C (range 19–33°C) during the day and 24°C (19–26°C) during the night.

As the species vary in perenniality, and grow at different rates, shrubs and perennial herbs were harvested after eight weeks, and annual herbs after five weeks. Roots were washed and separated from the shoots. Fresh weights for roots and shoots were recorded. The leaves and stems were then separated, and their fresh weights recorded separately. Leaves and stems were dried in paper bags in an oven at 70°C for 72 h, and dry matter (DM) recorded. Leaf and stem samples were then ground for further analysis. For measurement of [P], leaves and stems were digested in 3:1 HNO$_3$:HClO$_4$, and P was estimated using the standard colorimetric molybdovanadophosphate method (Boltz and Lueck, 1958).

Experiment 2
An additional experiment was conducted on a subset of three *Ptilotus* species and two control species to examine the responses of each species and the storage of P in response to low- and high-P treatments and a P pulse after establishment of the plants. The chosen species of *Ptilotus*, *P. exaltatus*, *P. polystachyus* and *P. obovatus* (Gaudich.) F.Muell., are three of the most widespread and prolific in the genus, representing two distinct and species-rich phylogenetic clades, the two former species being disturbance-specialists and adapted to a wide range of edaphic conditions (Hammer *et al.*, in press; Hammer *et al.*, 2018b). As with Experiment 1, the native amaranth *G. canescens* was grown to compare with *Ptilotus*. A native legume, *Kennedia prostrata* R.Br. (Fabaceae), which is sensitive to high levels of P (Pang *et al.*, 2010), was grown as a control. Further information on these species can be found in Table 1.

Washed river sand was used in place of the 'Lancelin sand' that was used in Experiment 1 (as in Suriyagoda *et al.*, 2015). The sand was steam pasteurised at 80°C for 1 h on two consecutive days and then left to dry for two days. The sand was mixed thoroughly to homogenise and added to 1 kg pots. The pots were lined with plastic bags, which were pierced at the bottom to allow drainage. Analysis of the sterilised soil indicated that it contained the basal nutrients of < 1 mg kg^{-1} nitrate-N, < 1 mg kg^{-1}

ammonium-N (Searle, 1984), < 2 mg kg^{-1} bicarbonate-extractable P (Colwell method; Rayment and Higginson, 1992), 19.3 mg kg^{-1} bicarbonate-extractable K (Colwell method; Rayment and Higginson, 1992) and 0.1% organic carbon (chromic acid titration method; Walkley and Black, 1934), and had a pH of 6.6 (i.e, in CaCl$_2$; Rayment and Higginson, 1992). Additional nutrients were added to the pots as a nutrient solution to bring them to the same concentrations as in Experiment 1.

There were five treatments of P (as KH$_2$PO$_4$) for this experiment: four treatments initially had 5 mg P kg^{-1} (i.e. 'low-P treatment') and one treatment had 150 mg P kg^{-1} (i.e. 'high-P treatment') added before sowing of the seeds. Two of the low-P treatments were treated with a pulse of P added as a solution of KH$_2$PO$_4$ during regular watering two weeks before harvest to mimic a low-P and high-P pulse, at 5 mg kg^{-1} ('low-pulse treatment') and 50 mg kg^{-1} (i.e. 'high-pulse treatment'), respectively. One of the two remaining low-P treatments was harvested prior to the addition of the pulse (i.e. 'pre-pulse treatment'). All treatments, a total of 100 pots, were arranged in four randomized blocks, where each block represented one replication and each treatment was present within each block.

Seeds were sterilized with 70% (v/v) ethanol and washed with deionized water and scarified as for Experiment 1. As all species in this experiment germinated readily, they were directly sown into the pot at 1–5 mm depth, depending on seed size, on 27 December 2017. Appropriate rhizobia were added to the soil surface and watered after sowing to ensure nodule formation in *K. prostrata*. After germination, the seedlings were thinned to two per pot. Average temperatures in the glasshouse were 23°C (range 15–30°C) during the day and 18°C (14–25°C) during the night. An additional application of macronutrient solution (as used in Experiment 1) was added to each pot halfway through the experiment.

As *G. canescens* is a fast-growing and early-flowering annual, it was grown for a shorter period of five weeks after germination, with the plants in the pre-pulse treatment being harvested after three weeks. Plants of the remaining species were harvested eight weeks after germination, or six weeks for the pre-pulse treatment. Roots were washed and separated from the shoots. Fresh weights for roots and shoots were recorded. The leaves and stems were then separated, and their fresh weights were recorded separately. Leaf lengths, widths and thickness were measured using a ruler and calliper. Within a week of harvest, total root length and average root diameter were measured on the fresh roots using an Epson™ scanner and WinRHIZO 4.1 computer software (Regent Instruments Inc., Quebec, Canada). Leaves, stems and roots were dried in an oven at 70°C for 72 h and their dry matter (DM) recorded. Leaf, stem and root samples were then ground for further analysis. For measurement of [P], samples were digested as for Experiment 1.

Data analysis

Statistical analysis was not performed for Experiment 1 because of a lack of replication in a few species/treatment combinations due to plant deaths caused by root-rot pathogens; therefore, graphs are presented with standard deviations. For Experiment 2, statistical analysis was performed using Genstat version 9.2 (Lawes Agricultural Trust, Rothamsted Experimental Station, UK, 2007). The effect of P on each parameter for the species was examined using general two-way ANOVAs. Each ANOVA included the factors Species and P addition, as well as Block. The Species x Treatment interaction was always examined. Outliers were carefully checked, and no more than two were removed for any parameter. Normality was examined and data transformed, if appropriate. As a significant ($p < 0.05$) interaction was found for all parameters presented except one, we always present the estimated means from the interaction along with the relevant l.s.d. at $p = 0.05$. For ease of understanding, untransformed data are presented in graphs; hence, if data were transformed prior to analysis then the l.s.d. will be absent. All graphs were generated using SigmaPlot (Systat Software Inc.).

RESULTS

Experiment 1

All six *Ptilotus* species grown with added P showed accumulation of P in the leaves and stems (Fig. 2A, B). In the high-P treatment (100 mg kg^{-1} P), [P] ranged from an average of 22 to 58 mg P g^{-1} DM in leaves, and 13 to 34 mg P g^{-1} DM in stems. *Ptilotus manglesii* and *P. spathulatus* are perennial herbs that develop leaves from the apical portion of the perennial rootstock and produce flowering stems much later in their development, therefore no stems were available at harvest. *Ptilotus manglesii* showed the highest accumulation of P in the leaves, with one plant reaching 73 mg P g^{-1} DM. The other amaranth species, *G. canescens*, also accumulated P, with 42 mg P g^{-1} DM in leaves and 28 mg P g^{-1} DM in stems. In contrast, for *R. chlorocephala*, subterranean clover, siratro and lucerne, the high-P treatment showed a relatively low concentration of 9.9, 7.8, 4.1 and 3.9 mg P g^{-1} DM in the leaves and 6.3, 10.6, 7.0 and 4.7 mg P g^{-1} DM in the stems, respectively (Fig. 2A, B). The three legume species had higher [P] in the stems than in their leaves. The results indicate that the non-amaranth species were able to more strongly down-regulate their P-uptake capacity than the amaranths.

Ptilotus rotundifolius and *P. exaltatus* produced the most leaf DM of the amaranth species, and all amaranth species showed similar leaf DM in the medium- (30 mg kg^{-1} P) and high- (100 mg P) P treatments (Fig. 2C) which were greater than those in the low-P treatment (0 mg added P). Subterranean clover had a much higher leaf DM in the medium-P treatment than in the high-P treatment and produced the most leaf and stem DM of any species (Fig. 2C). Stem DM decreased in *Ptilotus* as a reaction to the high-P treatment which manifested as shorter internode lengths (Fig. 2D); this effect was not evident in *R. chlorocephala*, subterranean clover, siratro and lucerne.
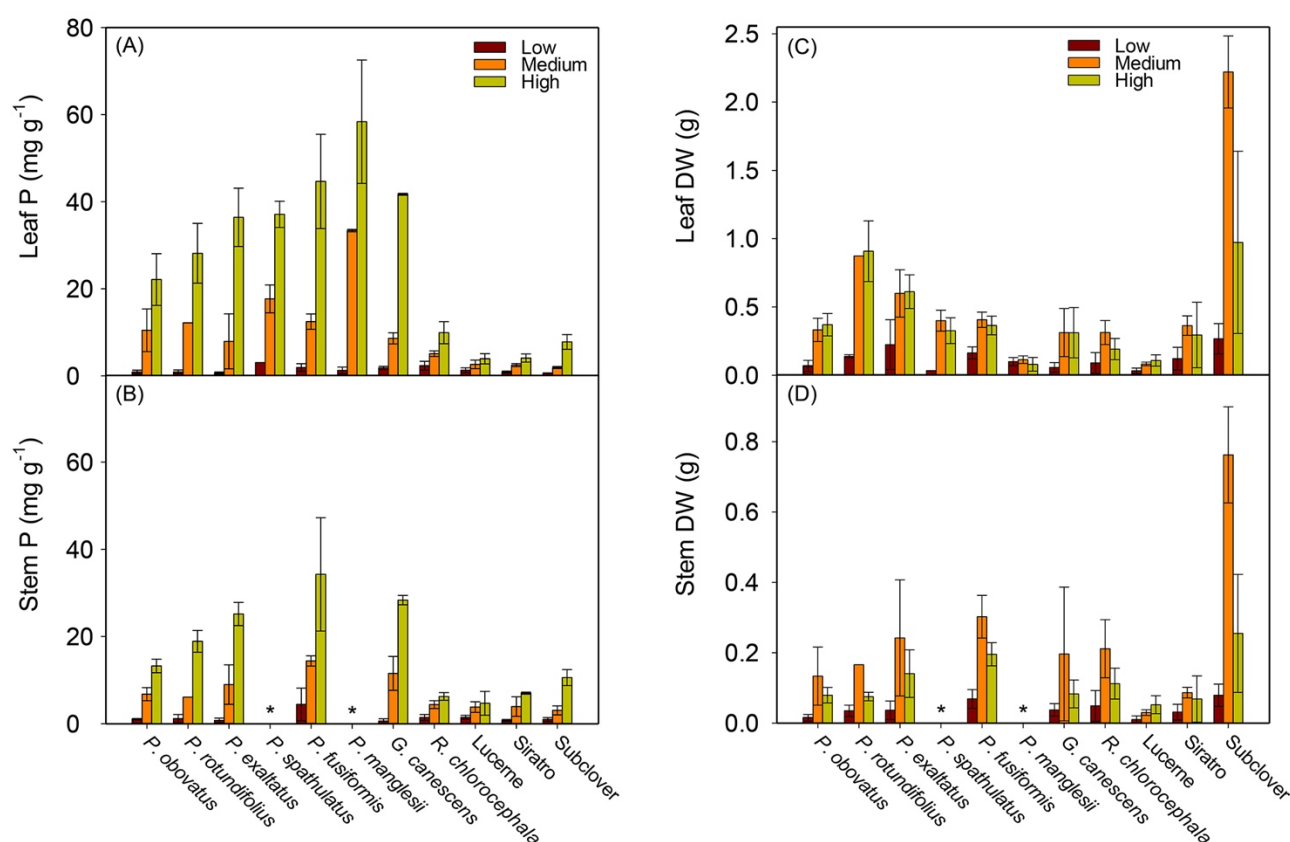
Fig. 2. Response of species in Experiment 1 to three levels of phosphorus (P) (0, 30 and 100 mg kg^{-1}, respectively): [P] in the (A) leaves and (B) stems, and dry matter (DM) of (C) leaves and (D) stems. Asterisks (*) indicate no stems produced. (mean ± 1 standard deviation, n = 1–4).

Experiment 2

The five species in Experiment 2 grew as predicted in the low-P treatment (5 mg kg^{-1} P) and responded to the addition of P in the other treatments, both positively and negatively (Fig. 3). The P accumulation in the leaves, stems and roots was affected by a two-way interaction among Species and Treatment (Table 2). In the high-P treatment (150 mg kg^{-1} P), *G. canescens* and *K. prostrata* had lower [P] in the leaves than *Ptilotus* species (albeit still elevated compared with controls from Experiment 1) (Fig. 4A), but higher [P] in the stems (Fig. 4B) and roots (Fig. 4C), respectively, than the *Ptilotus* species. As in Experiment 1, [P] in the leaves of the *Ptilotus* species was extremely high, reaching > 40 mg g^{-1} (Fig. 4A). The low-P pulse treatment (5 mg kg^{-1} P) had little impact on leaf [P]. The high-P pulse treatment (50 mg P) tended to increase biomass [P]; this was particularly marked in the roots of *K. prostrata*.
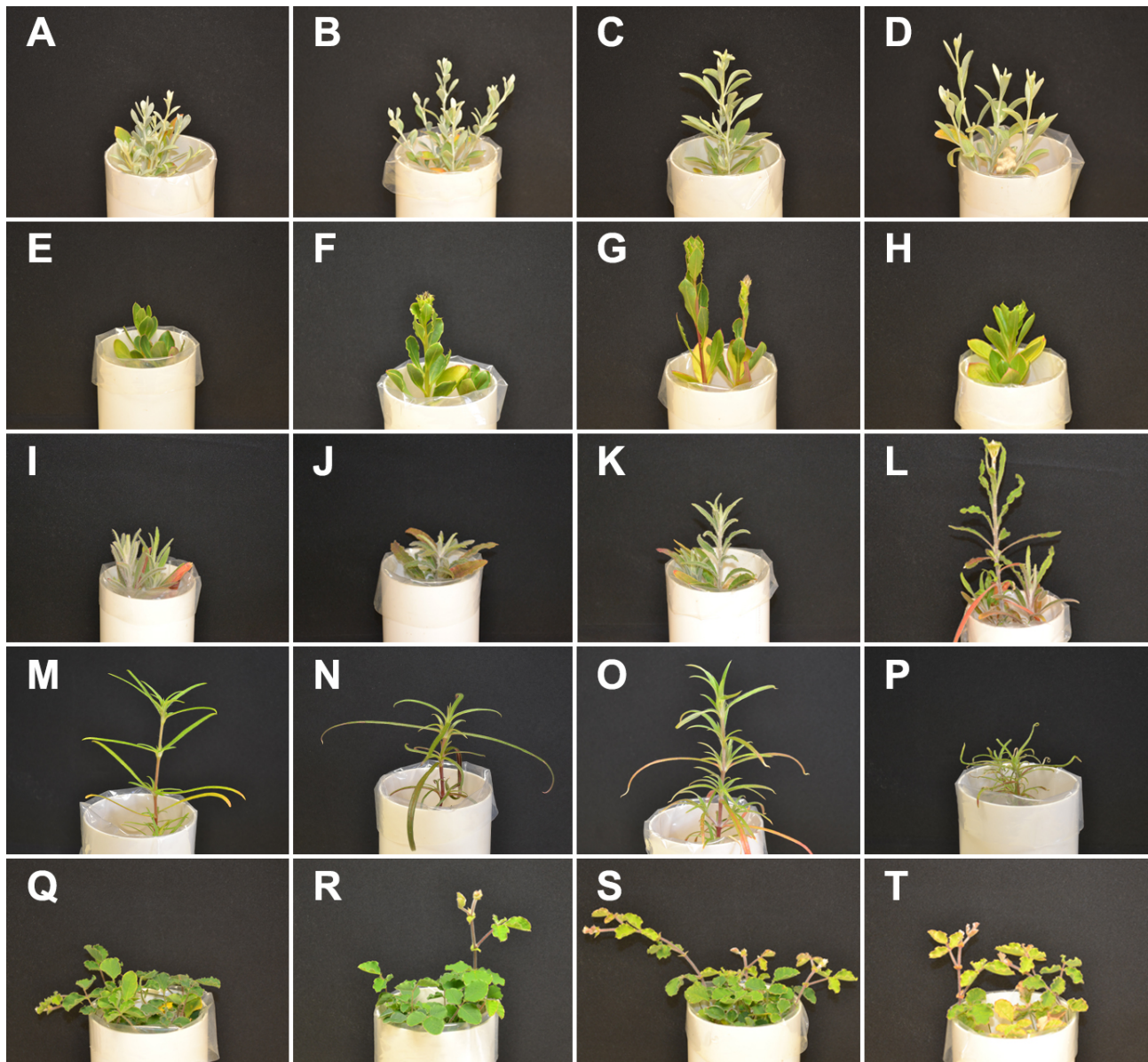
Fig. 3. Representative replicates from the treatments of each species in Experiment 2 at harvest: (A–D) *Ptilotus obovatus*, (E–H) *P. exaltatus*, (I–L) *P. polystachyus*, (M–P) *Gomphrena canescens* and (Q–T) *Kennedia prostrata*. Treatments are presented in each row, from left to right, as Low P (5 mg kg^{-1}), Low-P Pulse (5 + 5 mg kg^{-1}), High-P Pulse (5 + 50 mg kg^{-1}) and High P (150 mg kg^{-1}). Note: discolouration in *K. prostrata* at high P.

There was a two-way interaction among Species and Treatment for leaf, stem and root DM (Table 2). Leaf DM of the three *Ptilotus* species was similar across the four treatments, while *G. canescens* and *K. prostrata* showed a marked decrease in DM in the high-P treatment (Fig. 4D). Stem DM for *P. obovatus*,

P. exaltatus, *G. canescens* and *K. prostrata* was much lower in the high-P treatment, while stem DM was constant across treatments for *P. polystachyus* (Fig. 4E). An effect from the P pulse was noteworthy for the leaf and stem DM of two species; it increased in *P. exaltatus* and decreased in *G. canescens* (Fig. 4D, E). This indicates that P was limiting in the low-P treatment for *P. exaltatus*, while *G. canescens* did not respond favourably to even moderate additions of P. Root DM was lower in the high-P treatment for all species (Fig. 4F). *Gomphrena canescens* showed lower leaf, stem and root DM for all additions of P above the low-P treatment (Fig. 4D–F) and displayed the P-toxicity symptom of stunted growth (Fig. 3P). *Kennedia prostrata* also did not effectively down-regulate its P-uptake capacity and displayed symptoms of P-toxicity with discoloured leaves (Fig. 3T) and lower DM (Fig. 4D, F). Analyses of the whole plant P content (mg P plant[-1]) showed an interaction between Species and Treatment (Table 2). All *Ptilotus* species accumulated >40 mg P plant[-1] in the high-P treatment (150 mg kg^{-1} P), while *G. canescens* and *K. prostrata* accumulated lower amounts (Fig. 5). Using the pre-treatment harvest data (not presented), we calculated that the low-P pulse had a small impact on whole plant P content; the largest impact was on *P. polystachyus*, which took up an average of 2.4 mg more P than the control treatment after 5 mg of P was added. The high-P pulse had a more substantial impact on *Ptilotus* species of 5.3–8.3 mg, but had a particularly large impact on *K. prostrata*, which took up 17.9 mg of the 50 mg P added, and thereby reach a total plant P content similar to that in the high-P treatment.
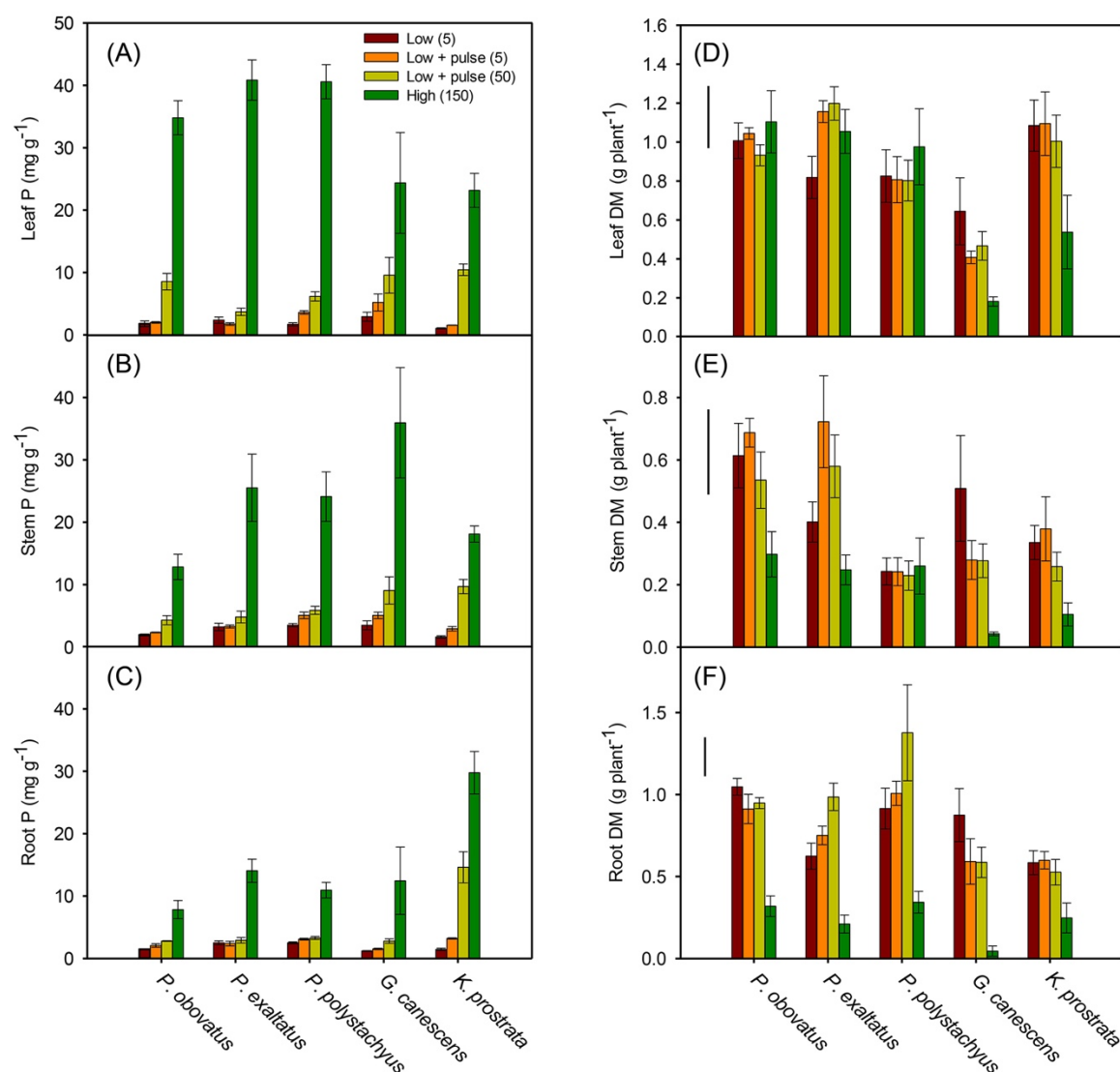
Fig. 4. Response of the five species in Experiment 2 to four levels of phosphorus (P) (5 mg P kg^{-1}, 5+5 mg P kg^{-1}, 5+50 mg P kg^{-1} and 150 mg P kg^{-1}, respectively): [P] in the (A) leaves, (B) stems and (C) roots, and dry matter (DM) per plant of the (D) leaves, (E) stems and (F) roots. Mean ± standard error (n = 4). Phosphorus concentration data (A–C) logged prior to analysis; l.s.d. indicated by vertical bar (D–F).

Table 2. Statistical outcomes of a two-way ANOVA of response variables measured in Experiment 2: significance of Species and Treatment; n.s. = not significant at $p < 0.05$.

Source of variation	Species		Treatment		Species x Treatment	
	MS[a]	p	MS	p	MS	p
Leaf P (mg g^{-1})	0.104	0.001	6.279	< 0.001	0.130	< 0.001
Stem P (mg g^{-1})	0.247	< 0.001	3.245	< 0.001	0.047	< 0.001
Root P (mg g^{-1})	0.384	< 0.001	2.959	< 0.001	0.117	< 0.001
Whole plant P (mg plant^{-1})	0.277	< 0.001	2.988	< 0.001	0.130	< 0.001
Leaf DM[b] (g plant^{-1})	1.014	< 0.001	0.075	n.s.	0.132	0.016
Stem DM (g plant^{-1})	0.300	< 0.001	0.296	< 0.001	0.049	0.033
Root DM (g plant^{-1})	0.396	< 0.001	1.652	< 0.001	0.068	0.005
Leaf % of shoot DM	673.87	< 0.001	851.98	< 0.001	60.81	0.032
Leaf water content (%)	342.78	< 0.001	26.239	0.003	14.819	0.004
Leaf thickness (mm)	0.822	< 0.001	0.0116	n.s.	0.0715	< 0.001
Leaf length (mm)	3457.37	< 0.001	761.66	< 0.001	555.85	< 0.001
% root length of 0.0–0.05 mm diameter	1561.88	< 0.001	8.01	n.s.	20.82	n.s.
% root length of 0.05–0.1 mm diameter	227.566	< 0.001	6.929	n.s.	18.926	< 0.001

[a] MS = mean square

[b] DM = dry matter
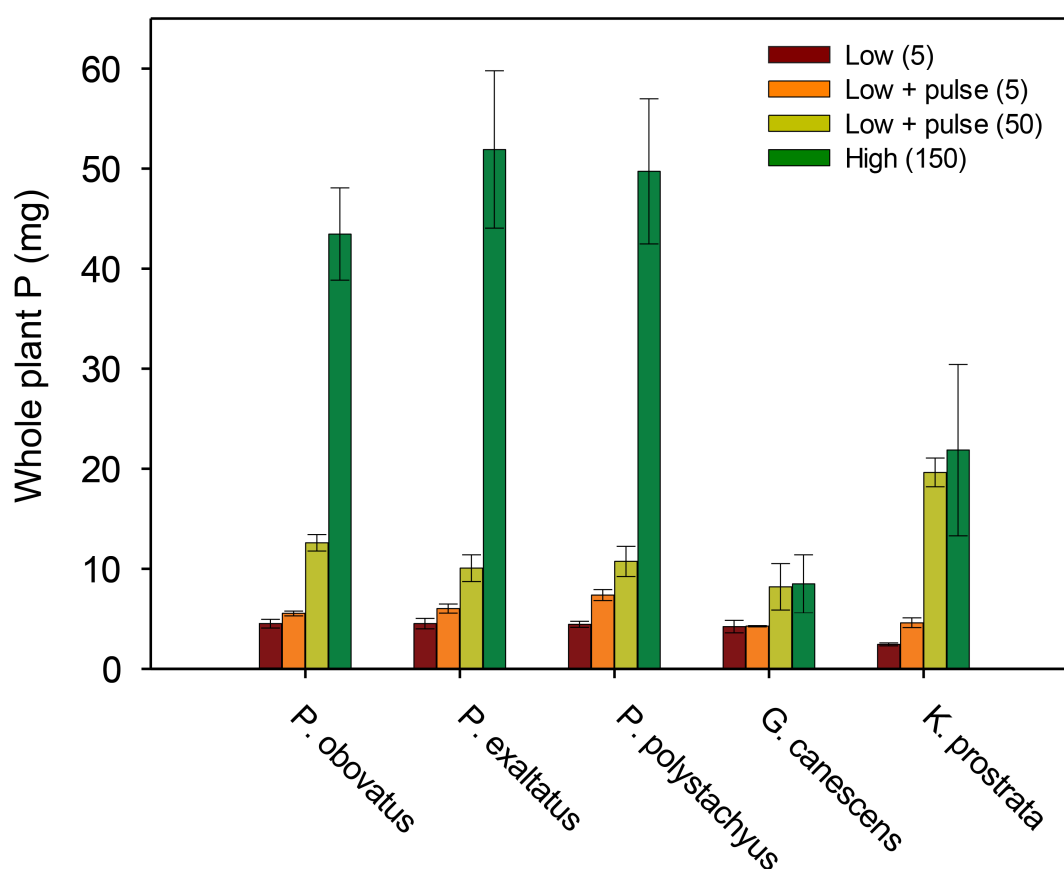
Fig. 5. Whole plant phosphorus (P) content (mg P plant^{-1}) of the five species in Experiment 2 for the four levels of P treatment (5, 5+5, 5+50 and 150 mg P kg^{-1} respectively). Mean ± standard error (n = 4). Data log-transformed prior to analysis.

Leaf percentage of shoot DM and leaf water content were affected by an interaction among Species and Treatment (Table 2). Percentage of leaf to shoot DM for *P. polystachyus* was relatively unchanged among treatments, while other species showed an increase with higher P supply (Fig. 6A). For leaf water content (as measured by the difference between fresh and dry weights), some species showed lower values in the high-P treatment, but more prominent was the much lower water content of *K. prostrata*, than that of the other species (Fig. 6B). A two-way interaction among Species and Treatment for leaf thickness and leaf length was driven by the response of *G. canescens* to the high-P treatment, because it had much thinner and shorter leaves than in the lower-P treatments (Table 2; Fig. 6C–D). Interestingly, *G. canescens* had longer leaves as a response to the P pulse treatments (Fig. 3N, O; Fig. 6D).

Figure 7 shows the proportion of total root length in the two thinnest categories of roots, that is, <0.05 mm diameter and 0.05–0.1 mm diameter. There was little effect of treatment on the distribution of root length among 15 diameter classes (Table 1; Fig 7); thus, while root DM decreased in response to high P supply, the percentage of fine roots did not change significantly with increasing P supply. However, there was a major impact of species, with *G. canescens* and *K. prostrata* showing fewer very fine roots (0.0–0.05 mm) than did the *Ptilotus* species (Fig. 7A), particularly *P. polystachyus*.
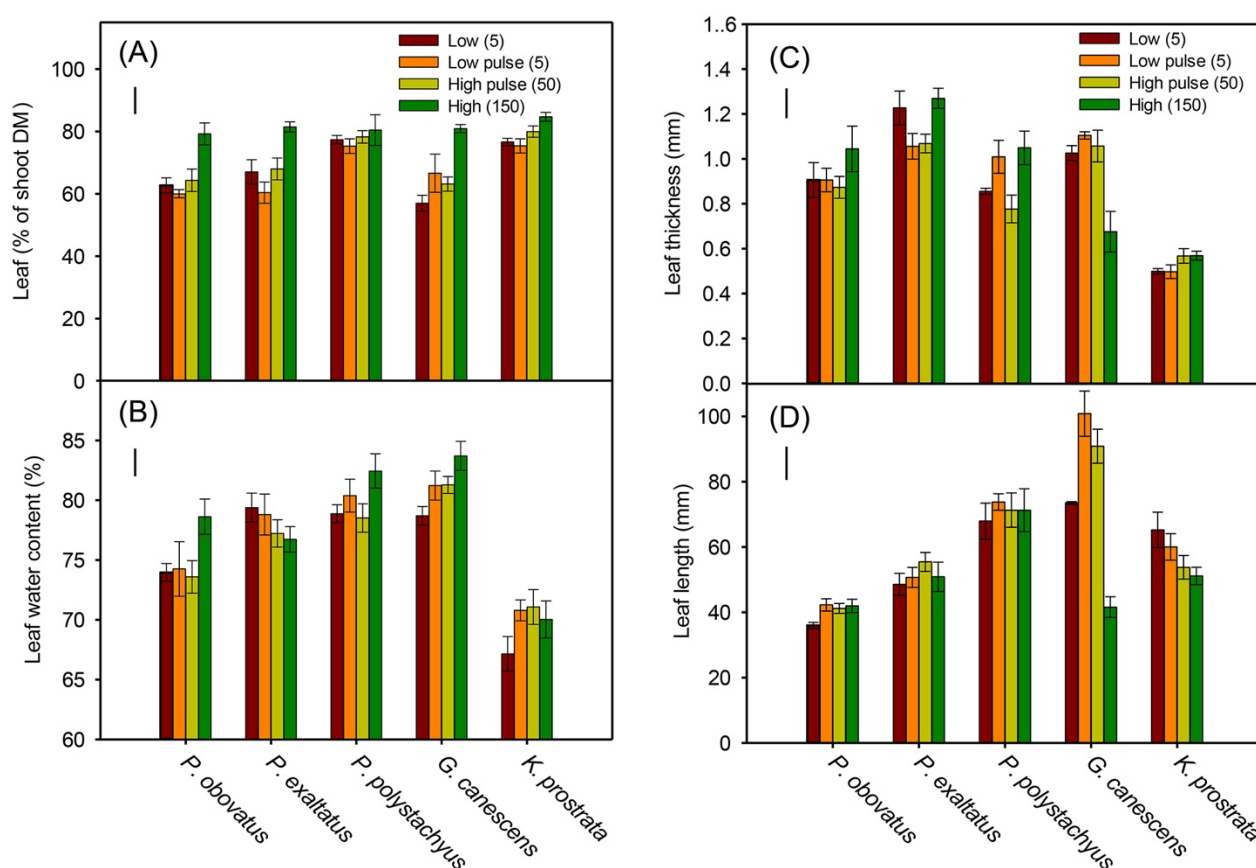
Fig. 6. Response of the five species in Experiment 2 to four levels of phosphorus (P) (5, 5+5, 5+50 and 150 mg P kg^{-1} respectively): (A) leaf % of shoot dry matter (DM), (B) leaf water content as a % of fresh weight, (C) leaf thickness in mm, and (D) leaf length in mm. Mean ± standard error (n = 4); l.s.d. indicated by vertical bar.

DISCUSSION

Our first hypothesis, that the trait to hyperaccumulate P is widespread among species of *Ptilotus* in different phylogenetic clades, ecologies and life history strategies was supported by the results of Experiment 1. Despite *Ptilotus* encompassing c. 120 morphologically variable species that occur across Australia (Hammer et al., in press), we found that all representatives that we grew from the major clades

of *Ptilotus* lack the ability to greatly down-regulate P-uptake capacity. This therefore led to the accumulation of P in their biomass. This trait was found equally among species of different ecologies, and among shrubs, perennial herbs and annual herbs. Shrubs (i.e. *P. obovatus* and *P. rotundifolius*) had slightly lower [P] than herbs, which might be attributed to slower growth or slower rates of P uptake. *Ptilotus manglesii*, one of the perennial herbs, had the highest [P] in the leaves.
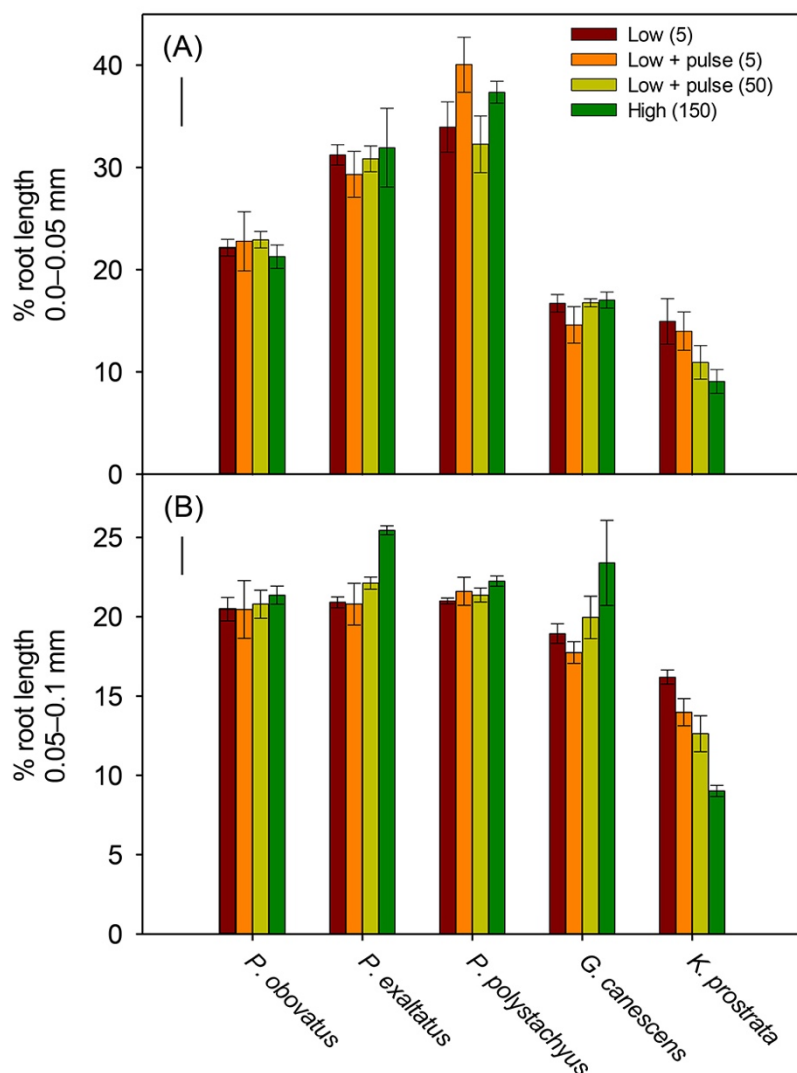
Fig. 7. Response of fine roots of the five species in Experiment 2 to four levels of phosphorus (P) (5, 5+5, 5+50 and 150 mg P kg^{-1}, respectively): % of root length for the two smallest root diameter categories, (A) <0.05 mm and (B) 0.05–0.1 mm. Mean ± standard error; l.s.d. indicated by vertical bar.

The second hypothesis, that the ability to tolerate high leaf [P] is widespread within *Ptilotus* is supported by both experiments. As with previous studies, there was no visual evidence in either experiment that *Ptilotus* species exhibited P-toxicity at 150 mg kg^{-1} P, i.e. no premature leaf senescence, chlorosis and necrosis, or stunted growth (Shane and Lambers, 2006; Ryan et al., 2009). *Ptilotus* did not have decreased leaf DM, and there was likewise no response in leaf or root morphology to the high-P treatment.

In addition to no signs of toxicity, *Ptilotus* also did not change its root morphology at high P supply. Roots of some other species (e.g., *Canavalia ensiformis* (L.) DC. and *Vigna unguiculata* (L.) Walp.) decrease their diameter in response to higher soil P availability (3.2 mM phosphate; Zobel *et al.*, 2007), while this did not occur with *Ptilotus*, which had a large percentage of roots with diameters of < 0.05 mm; this did not change with higher P levels. The innately fine roots in *Ptilotus* and *Gomphrena* may enable them to grow well in low nutrient soils, while sacrificing the ability to regulate root diameter. The fine roots of *Ptilotus* are consistent with species adapted to low-nutrient conditions (also commonly non-mycorrhizal; Oliveira *et al.*, 2014), and they are consistent with what has been found in some weedy species, including

some amaranths (e.g., Wright *et al.*, 1999). While there was no response of root morphology to P level, there was, however, a noticeable decrease in root DM in the high-P treatment, which can be attributed to the regulation of overall root growth in the presence of abundant soil P, a common response (e.g., Suriyagoda *et al.*, 2012; Suriyagoda *et al.*, 2015). In the high-P treatment, stem DM also decreased in all species, except *P. polystachyus*. Aziz et al. (2015) also found that high soil P concentrations did not affect the stem DM of *P. polystachyus*, up to 5 mM P. In Experiment 2, plants tended to have shorter internode lengths in the high-P treatment (with the exception of *P. polystachyus*), but it is unknown why stem DM differed between *P. polystachyus* and the other species.

The third hypothesis, that non-*Ptilotus* species would show symptoms of P toxicity was supported by Experiment 2. *Gomphrena canescens* and *K. prostrata* both accumulated P, but in the high-P treatment they both showed inhibited growth and discoloured and necrotic areas of the leaves. In Experiment 1, symptoms of P toxicity in *G. canescens* were not evident in the shoot DM, and yet it did hyperaccumulate P; however, stunted growth and discoloured leaves were observed. In Experiment 2, when compared with *G. canescens* and *K. prostrata*, *Ptilotus* species accumulated higher [P] in the leaves, whereas the other species showed higher concentrations in the stems and roots, respectively. This may indicate that *Ptilotus* has a mechanism that enables the leaves to tolerate very high concentrations of P that the other species lack, allowing *Ptilotus* to accumulate greater total mg P plant^{-1}. Aziz *et al.* (2015) also showed that *P. polystachyus* accumulates P primarily in leaves, but noted that highest concentrations were in mature leaves, suggesting that the plant was protecting its younger leaves. Using cryo-SEM to examine a single youngest fully emerged leaf from a plant grown under high P conditions, Aziz *et al.* (2015) found high concentrations of P in the epidermis and, in particular, in the mesophyll were accompanied by balancing cations (i.e. K and Mg, and sometimes Na and Ca) . Aziz *et al.* speculated that this might result in the precipitation of Mg salts of P in the leaves, and Ca salts of P in the mature leaves. The study did not measure concentrations outside of the cell vacuole.

Gomphrena is a genus in the same family as *Ptilotus*, yet it did not tolerate the same high levels of P as *Ptilotus* in Experiment 2. Even the addition of the low-P pulse (5 mg kg^{-1} P) caused a decrease in leaf, stem and root DM in *G. canescens*, approximately equivalent to that of the high-P pulse (50 mg kg^{-1} P). This suggests that the mechanism allowing it to tolerate high levels of P may be uniquely derived from a common ancestor of *Ptilotus* and that it may not be widely shared within the family. *Ptilotus* has the highest base chromosome count recorded for the family (n = 27), with tetraploid individuals in *P. obovatus* (n = 54), while related genera in Amaranthaceae have much lower counts, e.g., n = 16 in *Aerva* and n = 13 in *Gomphrena* (Stewart and Barlow, 1976). Polyploidy, leading to gene duplication, has been identified in other groups of plants, including the basal angiosperms, and is believed to have enabled rapid diversification events within these groups (Soltis *et al.*, 2009; Jiao *et al.*, 2011; Tank *et al.*, 2015; Van de Peer *et al.*, 2017), possibly being a source of novel physiological and ecological adaptations (Ramsey, 2011; te Beest *et al.*, 2012). It is, therefore, possible that uniquely derived genetic innovations could explain the difference in physiology between *Ptilotus* and other genera in Amaranthaceae, as well as perhaps contributing to the rapid radiation of *Ptilotus* throughout Australia (Hammer *et al.*, 2015).

The fourth hypothesis that the trait of *Ptilotus* to hyperaccumulate P without toxicity symptoms will aid in absorption of P following a pulse was not strongly supported by Experiment 2. The pre-pulse harvest showed low total plant P content, the lowest being in *K. prostrata* at 0.27 mg plant^{-1} and the highest in *G. canescens* at 1.39 mg plant^{-1}. The low-P pulse (5 mg kg^{-1} P) did not have any effect on the DM of the *Ptilotus* species, yet both *K. prostrata* and *P. polystachyus* took up approximately half of the added P. This indicates that these *Ptilotus* species will be able to make much use of P released under natural soil drying-rewetting conditions, which in some cases may release between 2 to 3 mg P kg^{-1} within 2 h after rewetting (Butterly *et al.*, 2010). The high-P pulse (50 mg kg^{-1} P) was ten times larger than the low-P

pulse, and species did respond by down-regulating their P-uptake capacity. The *Ptilotus* species did not vary significantly from each other in accumulation of the high-P pulse, although the highest accumulated was by *P. obovatus*, which took up 8.3 mg. This is in stark contrast to *K. prostrata*, which took up 17.9 mg from the high-P pulse. *Kennedia prostrata* naturally has associations with arbuscular mycorrhizal fungi (AMF) as an interface with the soil, yet Nazeri *et al.* (2014) found that when AMF were not added to the soil, there was an increase in shoot P concentration from a pulse of 143%, as opposed to 53% in the presence of AMF. Plants in our experiments were not inoculated with AMF, and this may explain the major accumulation of P by *K. prostrata* in our experiment. Previous authors have speculated that *Ptilotus* lacks adequate regulation of P uptake which is why it hyperaccumulates P when grown in soil high P concentrations (e.g., Ryan *et al.*, 2009; Suriyagoda *et al.*, 2012). Contrary to what we expected, when grown at low P concentrations and exposed to a high-P pulse, *Ptilotus* did effectively down-regulate its P-uptake capacity and did not accumulate P at high levels. It is unclear why this effective down-regulation was not expressed when *Ptilotus* was grown in high-P soil, thereby allowing *Ptilotus* to hyperaccumulate P.

In conclusion, *Ptilotus* appears unique in terms of the ability of the species within it to tolerate extremely high levels of P, leading to remarkable hyperaccumulation of P when grown in high-P soils. Future investigations aim to pinpoint where and in what forms and concentrations, P and other elements are allocated in the leaf tissues of *Ptilotus* for plants grown under low and high P conditions. This will further elucidate the mechanisms supporting the remarkable tolerance of *Ptilotus* to high levels of leaf P.

ACKNOWLEDGEMENTS

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APPENDIX B: PREFACE

The papers presented here are additional taxonomic studies on *Ptilotus* that have been published during my PhD candidature. These studies add to the knowledge of the diversity of *Ptilotus* by describing four new species, reinstating two species, synonymising three names and presenting a comprehensive identification key for the 93 species of *Ptilotus* currently recognised in Western Australia. Appendix B is not presented for examination.

B.1: Synonymisation of three Western Australian taxa with *Ptilotus* (Amaranthaceae)

> Hammer TA, Davis RW (2017) Synonymisation of three Western Australian taxa within *Ptilotus* (Amaranthaceae). *Nuytsia* **28**, 133–134.

B.2: *Ptilotus benlii* (Amaranthaceae), a new species from Western Australia

> Davis RW, Hammer TA (2017) *Ptilotus benlii* (Amaranthaceae), a new species from Western Australia. *Nuytsia* **28**, 299–302.

B.3: **The showy and the shy: reinstatement of two species from *Ptilotus gaudichaudii* (Amaranthaceae)**

> Hammer TA, Davis RW, Thiele KR (2018) The showy and the shy: reinstatement of two species from *Ptilotus gaudichaudii* (Amaranthaceae). *Australian Systematic Botany* **31**, 1–7.

B.4: *Ptilotus actinocladus* (Amaranthaceae), a new and rare species from Gascoyne bioregion, Western Australia

> Hammer TA, Davis RW (2018) *Ptilotus actinocladus* (Amaranthaceae), a new and rare species from the Gascoyne bioregion, Western Australia. *Nuytsia* **29**, 145–149.

B.5: *Ptilotus yapukaratja* (Amaranthaceae), a new species from the Gascoyne bioregion of Western Australia

> Davis RW, Hammer TA (2018) *Ptilotus yapukaratja* (Amaranthaceae), a new species from the Gascoyne bioregion of Western Australia. *Nuytsia* **29**, 157–160.

B.6: The *Ptilotus murrayi* species group: synonymisation of *P. petiolatus* under *P. murrayi* and description of the new Western Australian species *P. unguiculatus* (Amaranthaceae)

> Hammer TA (2018) The *Ptilotus murrayi* species group: synonymisation of *P. petiolatus* under *P. murrayi* and description of the new Western Australian species *P. unguiculatus* (Amaranthaceae). *Swainsona* **31**, 93–100.

B.7: A key to *Ptilotus* (Amaranthaceae) in Western Australia

> Hammer TA, Davis RW, Thiele KR (2018) A key to *Ptilotus* (Amaranthaceae) in Western Australia. *Nuytsia* **29**, 217–227.

SHORT COMMUNICATION

Synonymisation of three Western Australian taxa within *Ptilotus* (Amaranthaceae)

Ptilotus gomphrenoides F.Muell. ex Benth. is a variable species found in the Pilbara, Gascoyne, Carnarvon and Murchison bioregions of Western Australia. It currently comprises three varieties, var. *gomphrenoides*, var. *conglomeratus* (Farmar) Benl and var. *roseo-albus* (Farmar) Benl. The two latter taxa, having been formerly assigned to *P. roseo-albus* Farmar (Farmar 1905), were combined into *P. gomphrenoides* by Benl (1962), who remarked that *P. roseo-albus* could not be sustained as a distinct species and that the features Farmar used to characterise it, such as narrower and shortly pedunculate spikes and a denser indumentum on the exterior surface of the outer tepals, were common characteristics of *P. gomphrenoides*.

Benl separated the three varieties of *P. gomphrenoides* according to the degree of stem branching, whether the spikes were solitary or clustered, and spike length. These characters, however, do not withstand scrutiny with the increased number of collections now available for study. The degree of branching merges imperceptibly between all three varieties. Whether the spikes are mostly solitary or clustered varies greatly between specimens and even on individual specimens. Spike length is rarely a useful character in *Ptilotus* R.Br., as the spikes are indeterminate and their length is dependent upon environmental and seasonal factors. Recognition of var. *conglomeratus* and var. *roseo-albus*, both of which are known from only a small number of collections scattered across the range of *P. gomphrenoides*, serves to confuse the taxonomy of *Ptilotus*. We do not regard them as representing distinct lineages, but rather variation already included within *P. gomphrenoides* var. *gomphrenoides*. We therefore choose to synonymise both varieties under *P. gomphrenoides*.

Ptilotus gomphrenoides F.Muell. ex Benth., *Fl. Austral.* 5: 244 (1870). *Type*: 'Hamersley Range, N.W. coast, F. Gregory's Expedition', *F. Mueller s.n.* (*syn*: K 000357029 image!, MEL 2281815 image!).

Ptilotus gomphrenoides var. *roseo-albus* (Farmar) Benl, *Mitt. Bot. Staatssamml. München* 4: 277 (1962), *syn. nov. Ptilotus roseo-albus* Farmar var. *roseo-albus*, *Bull. Herb. Boissier sér.* 2, 5: 1090 (1905). *Type*: 'N.-W. Division, W. Australia, E. Clement, 1897' [between the Ashburton and De Grey rivers, Western Australia, 1897, *E. Clement s.n.*] (*syn*: K 000357024 image!, K 000357025 image!, K 000357026 image!, K 000357027 image!).

Ptilotus gomphrenoides var. *conglomeratus* (Farmar) Benl, *Mitt. Bot. Staatssamml. München* 4: 278 (1962), *syn. nov. Ptilotus roseo-albus* var. *conglomeratus* Farmar, *Bull. Herb. Boissier*, *sér.* 2, 5: 1090 (1905). *Type*: 'N.-W. Division, W. Australia, E. Clement, 1897' [between the Ashburton and De Grey rivers, Western Australia, 1897, *E. Clement s.n.*] (*holo*: K 000357028 image!).

Ptilotus sp. **Kennedy Range (A.P. Brown 4276)** = *Ptilotus polakii* F.Muell. subsp. *polakii*

Voucher specimen. WESTERN AUSTRALIA: 28.9 km W of Mount Sandiman Homestead, adj. N end of Kennedy Range National Park, 27 June 2006, *A.P. Brown* 4276 (PERTH!).

Notes. *Ptilotus* sp. Kennedy Range is known only from two specimens at PERTH, the voucher and a second collection from nearby (PERTH 04151321). The precise collection locality of the voucher specimen was inaccessible in 2011 during recollection attempts; however, a collection was made from *c.* 350 m away in the same habitat. This specimen (PERTH 08321086) was later identified as *P. polakii*, but could not be placed in either subsp. *polakii* or subsp. *juxtus* Lally, because of its intermediate characters. *Ptilotus polakii* subsp. *juxtus* differs from the typical subspecies in the following characters: tepal apices glabrous in uppermost 1–2 mm (vs 3–5 mm), bracts conspicuously shorter than the bracteoles (vs ± equal in length), and tepals shorter than 13 mm long (vs up to 18 mm long) (Lally 2009).

We have compared the two specimens of *P.* sp. Kennedy Range to all material of *P. polakii* housed at PERTH and conclude that they differ only in their densely hairy ovaries. The presence or absence of hairs on the ovary can be diagnostic for species of *Ptilotus*, although infraspecific variation exists in some taxa; the ovaries of *P. polakii* are usually glabrous but may have a few scattered hairs. As such, we do not think this character alone is sufficient for the recognition of *P.* sp. Kennedy Range as distinct from *P. polakii*, and recommend *P.* sp. Kennedy Range be removed from Western Australia's vascular plant census (Western Australian Herbarium 1998–). The two specimens currently filed under this name have bracts *c.* equal in length to the bracteoles, and tepals 14.5–16.5 mm long that are glabrous at the apices for 3–7 mm length, and will be included in *P. polakii* subsp. *polakii*.

Ptilotus sp. Kennedy Range is currently listed as a Priority Two taxon under Department of Parks and Wildlife Conservation Codes for Western Australian Flora (Jones 2015). As this name is being removed from the state's vascular plant census, this listing is no longer warranted.

Acknowledgements

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SHORT COMMUNICATION

Ptilotus benlii (Amaranthaceae), a new species from Western Australia

Ptilotus benlii R.W.Davis & T.Hammer, *sp. nov.*

Type: 15 km east along Northampton – Port Gregory Road from junction of Yerina Springs Road, *c.* 20 km north-west of Northampton, Western Australia, 3 October 2005, *R. Davis* 10952 (*holo*: PERTH 07200773; *iso*: CANB).

Ptilotus sp. Northampton (R. Davis 10952), Western Australian Herbarium, in *FloraBase*, https://florabase.dpaw.wa.gov.au/ [accessed 22 February 2017].

Erect *perennial herbs* to 45 cm high. *Stems* arising from an underground woody rootstock, terete, ribbed, glabrous, or rarely with very sparse, ascending, nodose hairs. *Cauline leaves* narrowly oblanceolate, 10–60 mm long, 1–4 mm wide, glabrous. *Inflorescences* terminal, spiciform, cylindrical, 20–48 mm long, (25–)28–34 mm wide, white-green. *Bracts* ovate, 5.5–7.1 mm long, 3–3.2 mm wide, transparent, glabrous; apex mucronate (mucro 0.3–0.5 mm long). *Bracteoles* broadly ovate, 5.5–7.7 mm long, 3.8–5 mm wide, transparent, with sparse, nodose hairs along midrib; apex mucronate (mucro 0.3–0.5 mm long). *Tepals* narrowly lanceolate, slightly in-rolled, 13–16 mm long, 1.1–1.8 mm wide; apex entire, green; outer surface with long, silky, nodose hairs to 5 mm long, apex glabrous; inner tepals with marginal woolly hairs at the base of the inner surface to 4 mm long. *Fertile stamens* 5; *filaments* 6.3–7 mm long, uneven, dilated towards the base, pink; *anthers* 1–1.5 mm long, 0.5–0.6 mm wide, pink. *Staminal cup* 2–2.1 mm long, symmetrical, lobed. *Staminal cup appendages* 2–2.3 mm long, 0.4–0.6 mm wide, with sparse hairs on both surfaces. *Ovary* obconical, 1.1–1.2 mm long, 1.1–1.3 mm wide, glabrous. *Stipe* 1–1.2 mm long. *Style* straight, 5.5–7.3 mm long, centrally fixed to ovary. *Stigma* capitate. *Seed* not seen. (Figure 1)

Diagnostic features. *Ptilotus benlii* may be distinguished from all other members of the genus by the following combination of characters: an erect, perennial herb; large, white-green spikes, 28–35 mm wide; five, pink stamens with staminal cup appendages.

Other specimens examined. WESTERN AUSTRALIA: track W of rail-line, 600 m S of Canna, 16 Oct. 2013, *G. Byrne* 589 (PERTH); junction of Chandler-Nungarin Road and Talgomine-Reserve Road, 8 Nov. 2014, *R. Davis* 12505 & *K.R. Thiele* (PERTH); 18 km S of Youanmi, 22 Nov. 1978, *H. Demarz* D 7256 (CANB, PERTH); Garth Kowald's Avenue, 2 km E of Mullewa Shire boundary, Tardun, 13 Oct. 2007, *J. Docherty* 453 (PERTH); Ellendale Road, 3.5 km S of Ramsay Road, E of Greenough, 5 Dec. 2005, *M. Hislop* 3550 (PERTH); northern end of Hutt Lagoon, NE of Port Gregory, 6 Nov. 2008, *G.J. Keighery* 17476 (PERTH); Lot 3157, Isseka Road East, Northampton, 25 Oct. 1999, *I.B. Shepherd* 198 (PERTH).

Phenology. Flowering from mid-spring to late spring. Fruiting from late spring to early summer.

Figure 1. *Ptilotus benlii*. A – flowering plant *in situ* showing the erect habit and green-white flowers; B – flower, showing the characteristic pink filaments and anthers. Images from *R. Davis* 10952. Photographs by R. Davis.

Distribution and habitat. *Ptilotus benlii* occurs from west of Northampton, south-east to Nungarin in the central wheat-belt, and east to the Murchison bioregion near Youanmi Station, 125 km south-east of Mt Magnet (Figure 2). It is often found growing on red or yellow clayey sands in open *Acacia* scrub with *A. rostellifera* or in open mallee woodlands.

Conservation status. *Ptilotus benlii* can be found over a wide area on a range of habitats and appears not to be under any immediate threats; however, it is under-collected to the far east and south-east of its range.

Etymology. The epithet acknowledges the significant contribution of German botanist Gerhard Benl (1910–2001) to the taxonomy of *Ptilotus* R.Br. His work on the genus spanned 40 years, during which time he described upwards of 30 species and numerous infraspecific taxa, and prepared a draft manuscript for the genus for *Flora of Australia*.

Notes. The chloroplast *matK* and nuclear ITS markers have been sequenced for *P. benlii* for a forthcoming PhD thesis (Hammer, in prep.). A preliminary phylogeny including this species has placed it as sister to *P. esquamatus* (Benth.) F.Muell., in a basal position to the '*P. drummondii* clade' (including *P. drummondii* (Moq.) F.Muell., *P. schwartzii* Tate, *P. aphyllus* Benl and *P. calostachyus* F.Muell.). *Ptilotus benlii* shares the character of staminal cup appendages with these species, but can be readily distinguished based on its larger, green-white flowering spikes and bright pink filaments and stamens. *Ptilotus esquamatus* differs from *P. benlii* in having pink spikes, bright orange staminal cup nectaries, and in lacking staminal cup appendages. All other species in this group have pink flowers, or if green, then much smaller spikes.
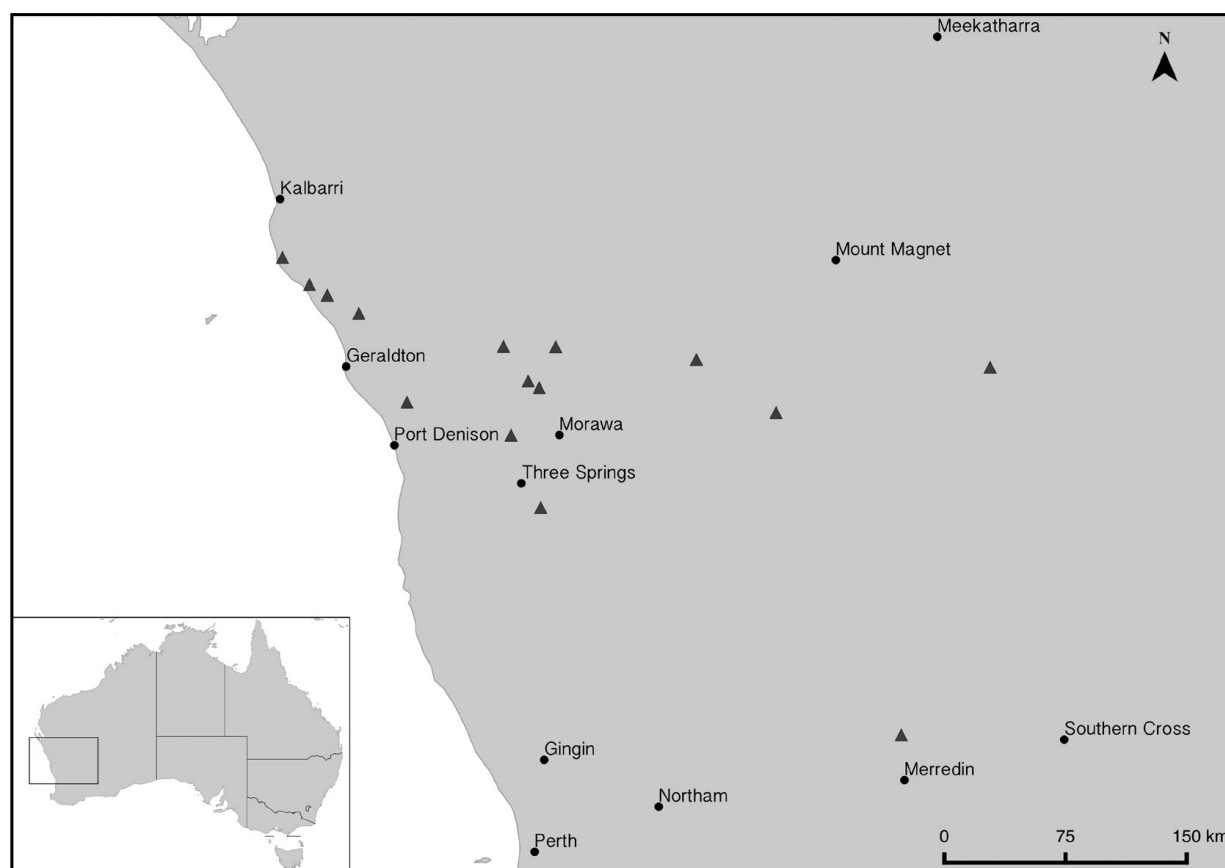
Figure 2. Distribution of *Ptilotus benlii* (▲) in Western Australia.

Acknowledgements

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The showy and the shy: reinstatement of two species from *Ptilotus gaudichaudii* (Amaranthaceae)

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Abstract. *Ptilotus* R.Br. (Amaranthaceae) is a widespread and species-rich Australian genus. One of the most common arid species, *Ptilotus gaudichaudii* (Steud.) J.M.Black (paper foxtail), currently comprises the following three subspecies: subsp. *gaudichaudii*, subsp. *eremita* (S.Moore) Lally and subsp. *parviflorus* (Benth.) Lally. In the present study, we re-evaluate the morphological basis for the recognition and status of infraspecies in *P. gaudichaudii*. Evidence from herbarium and field observations supports the reinstatement of *Ptilotus gaudichaudii* subsp. *eremita* and subsp. *parviflorus* to the rank of species as *P. eremita* (S.Moore) T.Hammer & R.W.Davis and *P. modestus* T.Hammer respectively.

Introduction

Ptilotus R.Br. (Amaranthaceae) is a diverse and widespread Australian genus comprising ~110 species, occurring in nearly all parts of Australia and being especially diverse and abundant in arid Western Australia. A common and widespread species is *P. gaudichaudii* (Steud.) J.M.Black, which is distinguished from other species by its papery yellow to green tepals that are glabrous except for very small hairs confined to the tepal midline (most species have dense hairs covering the outer tepal surface), giving it the common name paper foxtail. *Ptilotus gaudichaudii* is a member of a large clade of 20 species (informally named D3), including *P. helipteroides* (F.Muell.) F.Muell., *P. drummondii* (Moq.) F.Muell., *P. polystachyus* (Gaudich.) F.Muell. and *P. macrocephalus* (R.Br.) Poir. (Hammer *et al*. 2015).

Ptilotus gaudichaudii was first formally described in 1829 under the name *Trichinium corymbosum* Steud. The type material was collected by Charles Gaudichaud-Beaupré in 1818 at 'Baie des Chiens-Marins [Shark Bay]' in Western Australia during the Freycinet expedition. Steudel's name is an illegitimate later homonym of *T. corymbosum* (R.Br.) Spreng. and *T. gaudichaudii* Spreng. was, thus, published in 1841 as a replacement name. The combination in *Ptilotus* was made by J.M.Black in 1945. The following three subspecies are recognised: subsp. *gaudichaudii*, subsp. *eremita* (S.Moore) Lally and subsp. *parviflorus* (Benth.) Lally.

Ptilotus gaudichaudii subsp. *eremita* was first described at species rank as *Trichinium eremita* S.Moore, with the following comment (Moore 1899, p. 219):

For a time I thought this might be a very greatly reduced form of *Trichinium corymbosum* Gaudich. [=*P. gaudichaudii*], which, apart from the small size, it resembles superficially to a remarkable extent. On close examination, however, some well-pronounced differences come to light. Thus all the perianth-segments are hairy on the back, not the three outer ones alone, as is the case with *T. corymbosum*, which latter has anthers from four to ten times the size of those of *T. eremita* and longer filaments; its style also is much longer and the edge of the stigma entire, not lacerate. Moreover, though the ovary of *T. corymbosum* is said by Bentham to be glabrous, I find a fringe of long hairs attached near the top. The ovule also of *T. corymbosum* is quite different, being only half the relative width and oblong in shape, instead of broadly reniform; and this, if it be a constant character, points to a difference in the seed.

Moore clearly regarded these differences as a sufficient justification for adopting species rank. However, Benl (1959) was not convinced by Moore's arguments, considering *T. eremita* to be a transitional form within *P. gaudichaudii* and reducing it to a synonym of his *P. gaudichaudii* var. *parviflorus*. Lally (Lally and Barker 2010) subsequently reinstated it at subspecies rank, separating it from subspecies *parviflorus* on the basis of the

shorter, cream-coloured (cf. dark red) staminal filaments and a shorter style, and, presumably, also on the basis of the geographic disjunction of the two subspecies (*P. gaudichaudii* subsp. *eremita* is endemic in southern Western Australia, whereas *P. gaudichaudii* subsp. *parviflorus* occurs in southern parts of the Northern Territory and from the Flinders Ranges in South Australia through central New South Wales to central Queensland).

Ptilotus gaudichaudii subsp. *parviflorus* has a complex nomenclatural history. The entity was first formally described by von Mueller (1852) under the name *Hemisteirus psilotrichodes* F.Muell., based on a type collected from 'montibus humilioribus petraeis juxta Cudnaka' (low, rocky hills near Cudnaka (now Kanyaka), SA). Von Mueller (1864) subsequently transferred the species to *Ptilotus* as *P. hemisteirus* F.Muell. (p. 90). This name is illegitimate and nomenclaturally superfluous under Art. 52.1 of the *International Code of Nomenclature for Algae, Fungi and Plants* (McNeill *et al.* 2012), as the epithet *psilotrichodes* was available in *Ptilotus* at the time of transfer (Art. 11.4). Bentham (1870) recognised the taxon at varietal rank within the illegitimate *Trichinium corymbosum* Gaudich., as *T. corymbosum* var. *parviflorum* (p. 226). He based this varietal name on the Cudnaka type material of *Hemisteirus psilotrichodes* F.Muell. Benl (1959) transferred the taxon to *Ptilotus* as *P. gaudichaudii* var. *parviflorus* (Benth.) Benl, from where it was raised to subspecies rank by Lally (Lally and Barker 2010).

In Western Australia, *P. gaudichaudii* subsp. *gaudichaudii* and subsp. *eremita* are broadly sympatric, and field observations in Spring 2016 showed that, at some sites, they occur in mixed populations with no intermediates. This led to a thorough examination of key morphological characters of specimens from all subspecies throughout their ranges, as outlined below.

Materials and methods

Morphological examinations were carried out on specimens of the subspecies of *P. gaudichaudii* available at the Western Australian Herbarium (PERTH), the State Herbarium of South Australia (AD), the Northern Territory Herbarium at Alice Springs (NT), the Australian National Herbarium (CANB) and the Queensland Herbarium (BRI). Field observations were also made of populations in Western Australia, where two of the subspecies (subsp. *gaudichaudii* and subsp. *eremita*) co-occur. For the herbarium and fresh material, measurements and observations were made of distinguishing characters (e.g. tepal size, tepal colour, staminal filament length and colour, anther length and colour, style length, degree of tepal gape at anthesis). Locality data for all subspecies of *P. gaudichaudii* were retrieved from the Council of Heads of Australasian Herbaria's Australasian Virtual Herbarium (see http://avh.ala.org.au/, accessed 30 July 2017) and used to construct distribution maps.

Floral morphology

Field and herbarium observations show that the three subspecies of *P. gaudichaudii* currently recognised differ markedly in floral morphology at anthesis. Immediately on flower opening, the tepals in *P. gaudichaudii* subsp. *gaudichaudii* flex from the base so that they spread widely, exposing the staminal cup,

stamens, style and ovary. The flowers are protandrous. The four large, bright pink, fertile anthers are at first arranged in a ±straight line across the centre of the flower at right angles to the floral axis (Fig. 1A); the two outer stamens are longer than the two inner ones, and this aligns the anthers. At this time, the style (with unreceptive stigma) is bent downward, so that the stigma is placed well below the dehisced anthers. As the stigma becomes receptive, the style moves upward and the anthers move radially outward, eventually forming the vertices of a quadrilateral, whereas the stigma comes to the centre of the flower and occupies approximately the same position relative to the axis as previously occupied by the anthers. Native lycaenid butterflies have been observed to land and make contact with the anthers in this position while probing the staminal cup around the area of the staminode for nectar.

After anthesis, the tepals pinch inward above the ovary base, to form a tight, indurated covering around the fruit, while remaining strongly flexed and gaping widely. The papery tepals in fruit undoubtedly assist in wind dispersal of the propagule.

In *P. gaudichaudii* subsp. *parviflorus* and subsp. *eremita*, by contrast, the tepals spread only slightly from the base (more strongly only near the apex) and overlap for most of their length to form a tube, with the style and stamens included. In both subspecies, the staminal filaments are of two unequal lengths so that the stamens are didynamous (except that only three anthers are fertile in subsp. *parviflorus*). Both stamens and style remain in close proximity within the tepal tube throughout anthesis (Fig. 1B). During intensive fieldwork over multiple days, with observations being made at different times of the day and night, no insects were seen pollinating flowers of subsp. *eremita*, despite nearby subsp. *gaudichaudii* being visited by butterflies, and other nearby *Ptilotus* spp. being visited by wasps, beeflies and other insects. Given the close proximity of anthers and stigma, self-pollination may be possible.

After anthesis, the tepals pinch inward above the ovary base and indurate, at this time strongly flexing outward above the indurate portion and gaping widely as in subsp. *gaudichaudii*. Fruiting specimens of subspp. *parviflorus* and *eremita*, thus, somewhat resemble flowering specimens of subsp. *gaudichaudii*; care needs to be taken when observing herbarium specimens, to determine whether they consist of flowering or fruiting material.

In a molecular phylogeny of *Ptilotus* by Hammer *et al.* (2015), the three subspecies of *P. gaudichaudii* are highly supported (100% in all analyses) as a clade, with subsp. *eremita* being the sister taxon to a well-supported (>95% in all analyses) pairing of subsp. *gaudichaudii* and subsp. *parviflorus*. Subspecies *parviflorus* and *gaudichaudii* had more similar internal transcribed spacer (ITS; nrDNA) and *mat*K (cpDNA) sequences, despite being sampled from New South Wales and Western Australia respectively; the sampled specimen of subsp. *eremita* was collected in Western Australia ~300 km from the sample of subsp. *gaudichaudii*, yet the former is more genetically divergent than the latter when compared to subsp. *parviflorus* from New South Wales. Relationships in other clades of close, yet morphologically distinct, species (e.g. in the *P. polakii* F.Muell. clade) were unresolved on the phylogeny, supporting the notion that the subspecies of *P. gaudichaudii* are distinct lineages.
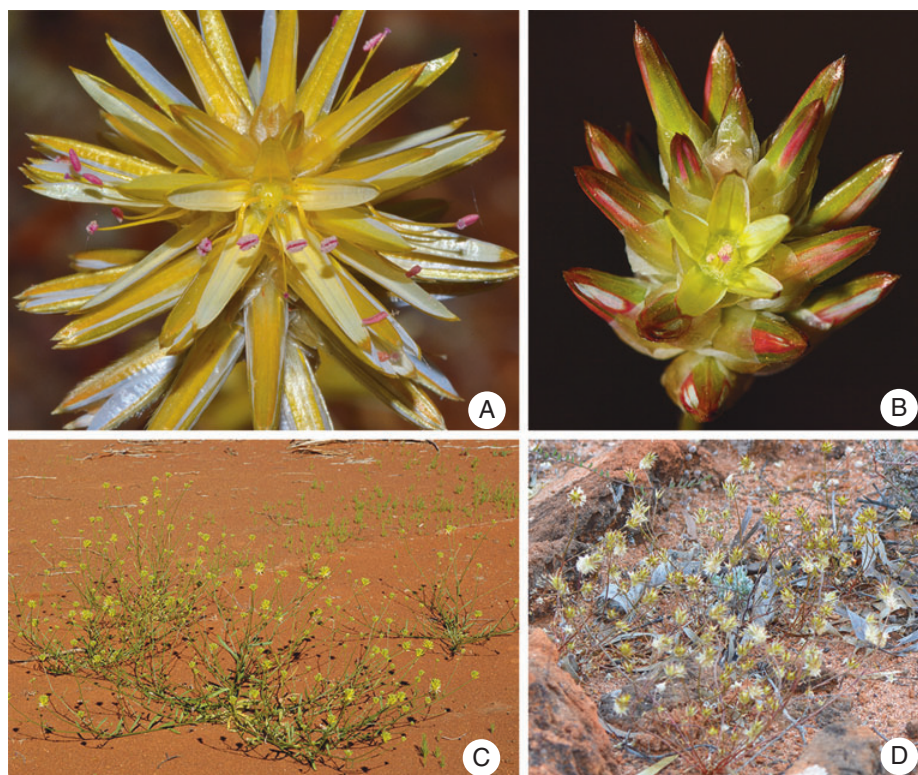
Fig. 1. Flowers at anthesis. A. *Ptilotus gaudichaudii sens. strict.* (PERTH 08863229). B. *P. eremita* (PERTH 08863237). Habit photos. C. *P. gaudichaudii sens. strict.* D. *P. eremita.* Photographs by T. A. Hammer.

Our investigations have confirmed that all three previously recognised subspecies of *Ptilotus gaudichaudii* are highly distinct and morphologically discontinuous, providing evidence that they are reproductively isolated wherever they co-occur and comprise distinct evolutionary lineages. As a result, all three are reinstated here to species rank.

Taxonomy

Ptilotus gaudichaudii (Steud.) J.M.Black, *Trans. Roy. Soc. S. Australia* 69: 309 (1945)

Trichinium corymbosum Gaudich. in H.L.C. de S.Freycinet, *Voy. Uranie* 444 (1829), *nom. illeg.*; *Trichinium gaudichaudii* Steud., *Nomencl. Bot. Ed. 2*, 2: 700 (1841).

Type: Western Australia: Shark Bay, *C.Gaudichaud s.n.* (holo: P 00609973, image!, retrieved from http://plants.jstor.org/stable/10.5555/al.ap.specimen.p00609973).

Trichinium corymbosum 'β' Nees in J.G.C.Lehmann (ed.), *Pl. Preiss.* 1: 630 (1845); *Trichinium corymbosum* var. *ramosum* Moq. in A.L.P.P. de Candolle (ed.), *Prodr.* 13(2): 292 (1849).

Type: Western Australia: 'In solo sublimosos pingui prope praedium rusticum Dom. Marrell' [near Marrell's farm, York], *L.Preiss 1368*, 30 Mar. 1840 (holo: LD 1214295, image!, retrieved from http://plants.jstor.org/stable/viewer/10.5555/al.ap.specimen.ld1214295).

Erect to decumbent annual *herbs* 15–50 cm high, 20–70 cm wide. *Stems* terete, ribbed, glabrous or glabrescent with a few scattered, appressed, nodose hairs. *Basal leaves* sessile or subsessile, narrowly lanceolate to narrowly oblanceolate, 10–30 mm long, 2–5 mm wide, mucronate, often withered at the time of flowering, glabrescent with sparse, nodose hairs. *Cauline leaves* sessile or subsessile, narrowly lanceolate to narrowly oblanceolate, 5–60 mm long, 1–3 mm wide, glabrescent or with moderately dense, nodose hairs. *Inflorescences* yellow or yellow–green, terminal, spiciform, hemispherical to ovoid (rarely shortly cylindrical), 5–25 mm long, 24–30 mm wide. *Bracts* broadly ovate, 4–5 mm long, 4.2–6 mm wide, glabrous, straw-coloured, transparent becoming whitish-opaque at margins; apex mucronate. *Bracteoles* ovate 4.5–6 mm long, 4–4.5 mm wide, glabrous, transparent becoming whitish-opaque at margins; apex mucronate. *Tepals* 12–14.5 mm long, 1.8–3 mm wide, spreading widely from near the base at anthesis, bright yellow to greenish along medial section, becoming whitish-opaque along the margins, persistent and widely gaping in fruit, fused and hardened at the base around the fruit, becoming faded, papery; abaxial surface with short hairs up to 0.4 mm long restricted to medial portion, denser, longer and silkier towards the base; adaxial surface with tufts of hairs on the margins; apex acute, glabrous. *Staminal cup* oblique, not lobed, 0.8–1.2 mm long, with crisped hairs on the interior surface, ciliate at summit. *Fertile stamens* 4; filaments yellow, persistent (becoming blackened with age), unequal, 7–10 mm long, filiform, sigmoid;

anthers pink, 1.1–1.4 mm long, 0.4–0.6 mm wide. *Staminode* 1, 3.8–5 mm long, opposite upper tepal. *Ovary* obovoid, 1–1.3 mm long, 1–1.1 mm wide, pilose or glabrous apically; stipe ~0.4 mm long. *Style* excentrically fixed on the ovary, curved, 9–11 mm long, yellow. *Stigma* capitate, pink. *Seed* round, dull light brown, ~1 mm long, ~0.9 mm wide.

Distribution and habitat

Ptilotus gaudichaudii sens. strict. is typically found in eucalypt woodlands and mulga shrublands on a variety of soils, from the western coast of Western Australia through arid South Australia and north to the southern part of the Northern Territory. Its distribution overlaps extensively with that of *P. eremita* in Western Australia, and with *P. modestus* in the Northern Territory; it is parapatric with *P. modestus* in eastern South Australia (Fig. 2).

Phenology

Flowers between April and October, with some flowering collections as late as December in the south-west of its range (e.g. PERTH 4940571).

Notes

Ptilotus gaudichaudii is most readily distinguished from the other two species described here by its longer, yellow tepals that gape widely at anthesis, by its longer staminal filaments and style, and its larger anthers (see Figs 1, 3).

Selected specimens examined

NORTHERN TERRITORY: track to Emma Bore from Umbeara Station Homestead, 25°43′07.0″S, 133°33′27.0″E, *P.Horsfall 891* (DNA A0101368); ~20 km SSW of Finke Settlement, 25°41′54.8″S, 134°15′04.6″E, *M.Kimbel 182* (DNA A0084141). 10 miles (~16.1 km) SW of Alice Springs, 23°48′54.9″S, 133°48′04.5″E, *P.Latz 2486* (DNA A0035006). SOUTH AUSTRALIA: 13 km S of Coober Pedy on Stuart Highway, 29°5′8.1″S, 134°52′7.1″E, *T.Hammer & K. Thiele TH85* (PERTH 08877890); 3 km ENE of homestead, Carriewerloo Station, 32°22′12.0″S, 137°15′00.0″E, *F.J.Badman 4369* (AD 99102351). WESTERN AUSTRALIA: E of Nalbara, Nalbara–Burnerbinmah road, 3.5 km W of Great Northern Highway, 28°37′9.5″S, 117°44′48.8″E, *R.Davis, K.Thiele & T.Hammer RD 12655* (PERTH 08839360); 8.9 km W of Boddington on Boddington–Pinjarra road, 32°50′42.6″S, 116°20′23.4″E, *R.Davis 4676* (PERTH 04912624); track, 2 km N of the Hamersley Gorge–Mount Bruce road, 7 km NW of Karijini Drive, 22°32′40.5″S, 118°1′46.2″E, *T.Hammer & S.Dillon TH 41* (PERTH 08735131); on track SE of Woolgorong Station near Paddock Gate, 27°44′33.0″S, 115°51′53.0″E, *T.Hammer & R.Davis TH57* (PERTH 08619697); 1.5 km N of Wubin parking bay on the Great Northern Highway, 29°49′35.6″S, 116°57′28.1″E, *T.Hammer, R.Davis & K.Thiele TH83* (PERTH 08863229); ~45.95 km NE (48°) of Rothsay and ~50.84 km NNW (353°) of Mount Singleton, 29°0′40.0″S, 117°14′28.7″E, 29°0′40.0″S, 117°14′28.7″E, *R.Meissner & R.Coppen 4666* (PERTH 08406235); 7 km E of Caramulla Creek,
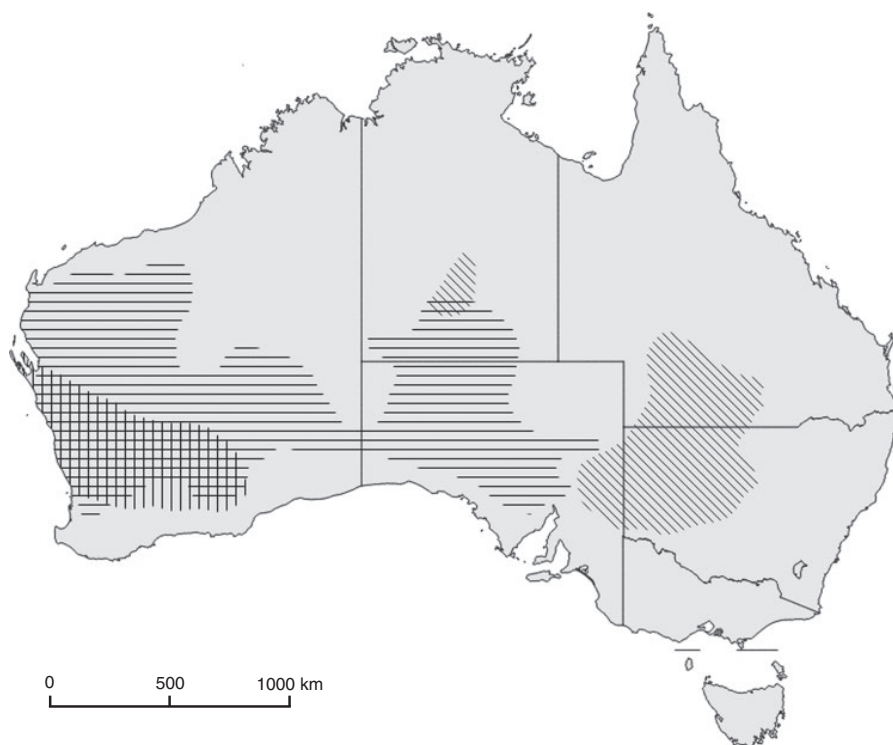
Fig. 2.   The approximate ranges of *Ptilotus gaudichaudii sens. strict.* (horizontal shading), *P. eremita* (vertical shading), and *P. modestus* (diagonal shading) based on available records from the Australasian Virtual Herbarium.

Fig. 3. Longitudinal sections of flowers in late bud from herbarium specimens. A. *Ptilotus eremita* (PERTH 08406227). B. *P. modestus* (BRI AQ696244). C. *P. gaudichaudii sens. strict.* (PERTH 08877890).

~60 km E of Newman, 23°21′48.2″S, 120°22′53.9″E, *A.R.Bean 25278* (PERTH 07462360); 20 km from Menzies along road to Leonora, 29°34′0.0″S, 121°10′0.0″E, *A.Strid 20104* (PERTH 00215821); Charles Darwin Reserve 3.5 km down South Track from Visitors' Quarters, marker for woodland is a 12-m York gum, 3 m to W of track (WPT024), 29°36′41.0″S, 116°58′3.0″E, *L.Warren 195* (PERTH 08031665).

Ptilotus modestus T.Hammer, *nom. nov.*

Hemisteirus psilotrichodes F.Muell., *Linnaea* 25: 435 (1852); *Ptilotus hemisteirus* F.Muell., *Fragm.* 4(26): 90 (1864), *nom. illeg.*, *nom. superfl.*; *Trichinium corymbosum* Gaudich. var. *parviflorum* Benth., *Fl. Austral.* 5: 226 (1870); *Ptilotus gaudichaudii* var. *parviflorus* (Benth.) Benl, *Mitt. Bot. Staatssamml. München* 3: 36 (1959); *Ptilotus gaudichaudii* subsp. *parviflorus* (Benth.) Lally, *J. Adelaide Bot. Gard.* 24: 48–49 (2010).

Type: South Australia: Cudnaka [Kanyaka], Oct. 1851, *F.Mueller s.n.* (holo: MEL *n.v.*, *fide* Lally and Barker 2010).

Erect annual *herbs* 15–25 cm high, 7–15 cm wide. *Stems* terete, ribbed, glabrescent with sparse crisped, nodose hairs. *Basal leaves* sessile or subsessile, oblanceolate, 5–30 mm long, 3–6 mm wide, glabrous or glabrescent, often withered at the time of flowering. *Cauline leaves* sessile or subsessile, linear to narrowly oblanceolate, 5–25 mm long, 1–4 mm wide, mucronate, glabrous or glabrescent, with sparse nodose hairs. *Inflorescences* green, terminal, spiciform, ovoid to hemispherical, rarely cylindrical, 12–24 mm long, 15–20 mm wide. *Bracts* broadly ovate, 3–4 mm long, 3.4–4.5 mm wide, straw-coloured and transparent with opaque, white margins, glabrous; midrib obscure; apex shortly mucronate. *Bracteoles* broadly obovate, 3.6–5 mm long, 2.6–3.8 mm wide, straw-coloured and transparent with opaque, white margins, glabrous; midrib obscure; apex shortly mucronate. *Tepals* 6–8.8 mm long, 1.2–1.8 mm wide, overlapping at anthesis for most of length to form a tube, spreading slightly towards the apex, green along medial section becoming whitish-opaque with slightly reddish tinge along the margins, persistent and widely gaping in fruit, fused and hardened at the base around the fruit, becoming faded, papery; abaxial surface with short hairs up to 0.4 mm long restricted to medial portion, denser, longer and silkier towards the base; adaxial surface with tufts of hairs on the margins; apex acute, slightly in-rolled, glabrous, sometimes reddish. *Staminal cup* oblique, not lobed, 0.3–0.4 mm long, ciliate at summit. *Fertile stamens* 3; filaments yellow becoming red after anthesis (persistent and blackened in fruit), unequal, 4.5–5.8 mm long, filiform, sigmoid; *anthers* cream to pink, 0.4–0.6 mm long, 0.3–0.5 mm wide. *Staminodes* 2, 3–4.3 mm long, filiform. *Ovary* obovoid, 0.8–1.2 mm long, 1.1–1.3 mm wide, sparsely hairy apically; stipe ~0.4 mm long. *Style* excentrically fixed on the ovary, curved, 4.5–5.5 mm long, yellow. *Stigma* capitate, pink. *Seed* round, dull light brown or straw-coloured, ~1.3 mm long, ~1.4 mm wide.

Distribution and habitat

Ptilotus modestus is found in eucalypt woodlands and mulga shrublands in red or brown sandy clay to loam soils, in

south-western Queensland, western New South Wales and eastern South Australia, and around Alice Springs, Northern Territory (Fig. 2).

Phenology

Ptilotus modestus typically flowers from May to October, with outlying records in March, April and November.

Etymology

A new name has been chosen for this taxon because we consider the earliest epithet applied to it at species rank (*psilotrichodes*) to be confusingly similar to that of a species based on a different type, namely *Ptilotus psilotrichoides* F.Muell. (=*P. capitatus* (F.Muell.) C.A.Gardner). We believe that this change is in accordance with Art. 53.3 of the *International Code of Nomenclature for Algae, Fungi and Plants* (McNeill *et al.* 2012). The only other applicable epithet, *parviflorus* (previously used at infraspecific ranks), is pre-occupied at species rank by *P. parviflorus* (Lindl.) F.Muell. (=*P. obovatus* (Gaudich.) F.Muell.). We, therefore, chose the name *Ptilotus modestus* (the epithet being Latin for modest) for this species, in reference to the flowers that are smaller, less colourful and less widely gaping than the more showy flowers of *P. gaudichaudii*.

Notes

Ptilotus modestus is, in many respects, similar to *P. eremita*, including in the tepals usually not conspicuously gaping at anthesis, with both species being markedly different from *P. gaudichaudii* in this respect (Figs 1, 3).

Ptilotus modestus consistently has three fertile stamens, as opposed to the four fertile stamens found in *P. eremita* and *P. gaudichaudii sens. strict. Ptilotus modestus* is the only taxon within Clade D3 (Hammer *et al.* 2015) to have three fertile stamens, with all other members of the clade having four or five fertile stamens. A reduction of fertile stamens (from 5 to as few as 1) is a common character separating species in *Ptilotus* (Hammer *et al.* 2015), but fertile stamen number is usually consistent among infraspecies. The staminal filaments of *P. gaudichaudii* subsp. *parviflorus* were described by Lally (Lally and Barker 2010) as being dark red to purple. This character was not consistently observed in our study. Flowers in bud had filaments that were cream-coloured (like those in *P. eremita*) to pinkish, darkening noticeably as the flowers aged. This darkening of the staminal filaments of older flowers has also been observed in specimens of *P. gaudichaudii sens. strict.* (e.g. PERTH 05100399). The present study confirmed the observation by Lally (Lally and Barker 2010) that *P. modestus* has consistently longer styles and staminal filaments than has *P. eremita*.

Selected specimens examined

NEW SOUTH WALES: Arumpo Station near mining site, S along fence, N of Euston, 33°50′49.3″S, 142°48′58.8″E, *R.G.Coveny, G.Chapple, P.G.Kodela, H.McPherson 18806* (AD 118644). NORTHERN TERRITORY: 17 miles (~27.4 km) N of Alice Springs, 23°27′07.4″S, 133°52′03.8″E, *D.J.Nelson 1786* (AD 96932290). SOUTH AUSTRALIA: Danggali Conservation Park, 100 m along track to NW, 5 km N of turn-off to Mulga Dam, 33°09′46″S,

140°54′22″E, *D.D.Cunningham & B.R.Moore 613* (AD 99317011). QUEENSLAND: 16 km from Jundah towards Winton, 24°46′10″S, 142°55′37″E, *A.R.Bean 22477* (BRI AQ696244); Mariala National Park 120 km WNW of Charleville, 26°4′47″S, 114°4′20″E, *R.Johnson RJMA06* (BRI AQ649069); 48 km W of Hungerford, 28°59′35″S, 143°58′33″E, *G.N.Batianoff & D.Butler 0509171* (BRI AQ748410); 7 km W of Tomoo, SW of Mitchell, 27°4′29″S, 147°18′38″E, *A.R.Bean 24370* (BRI AQ618423); Quilpie–Jundah road, S of Lake Thunda, 25°33′52″S, 143°22′59″E, *J.Silcock JLS651* (BRI AQ832640); 50 km W of Charleville, 26°37′26″S, 145°48′37″E, *G.N.Batianoff & D.Butler 0509292* (BRI AQ748409).

Ptilotus eremita (S.Moore) T.Hammer & R.W.Davis, *comb. nov.*

Trichinium eremita S.Moore, *J. Linn Soc., Bot.* 34: 218 (1899); *Ptilotus gaudichaudii* subsp. *eremita* (S.Moore) Lally, *J. Adelaide Bot. Gard.* 24: 48 (2010).

Type: Western Australia: 'Ad Gibraltar florebat mens. Sept.' [Goldfields near Gibraltar], Sept. 1895, *S.Moore s.n.* (holo: BM 000895588, image!, retrieved from http://plants.jstor.org/stable/ 10.5555/al.ap.specimen.bm000895588; iso: K 000356905, image!, retrieved from http://apps.kew.org/herbcat/getImage. do?imageBarcode=K000356905).

Erect annual *herbs* 3–20 cm high, 5–15 cm wide. *Stems* terete, ribbed, glabrescent, with sparse nodose hairs. *Basal leaves* sessile or subsessile, oblanceolate, 5–20 mm long, 5–8 mm wide, glabrous or glabrescent, often withered at time of flowering. *Cauline leaves* sessile or subsessile, linear to narrowly oblanceolate, 5–38 mm long, 1–4 mm wide, mucronate, glabrous or glabrescent with sparse nodose hairs. *Inflorescences* green, terminal, spiciform, hemispherical to ovoid (rarely shortly cylindrical), 12–25 mm long, 16–21 mm wide. *Bracts* ovate, 3.8–4.5 mm long, 3.2–3.6 mm wide, straw-coloured and transparent with opaque, white margins, glabrous; midrib obscure; apex shortly mucronate. *Bracteoles* obovate, 4.2–5 mm long, 3.4–3.7 mm wide, straw-coloured and transparent with opaque, white margins, glabrous; midrib obscure; apex shortly mucronate. *Tepals* 5–8.1 mm long, 1.6–2.1 mm wide, overlapping at anthesis for most of length to form a tube, spreading slightly towards the apex, green along medial section, becoming whitish-opaque along the margins with a slightly reddish tinge, persistent and widely gaping in fruit, fused and hardened at the base around the fruit, becoming faded, papery; abaxial surface with short hairs up to 0.4 mm long restricted to medial portion, denser, longer and silkier towards the base; adaxial surface with tufts of hairs on the margins; apex acute, slightly in-rolled, glabrous, sometimes reddish. *Staminal cup* symmetrical, not lobed, 0.2–0.3 mm long, with crisped hairs on inner surface. *Fertile stamens* 4; filaments cream-coloured, unequal, 2.4–2.6 mm long, filiform, persistent; *anthers* cream-coloured, 0.3–0.5 mm long, 0.3–0.4 mm wide, often at the same level as the stigma. *Staminode* 1, 1.4–1.6 mm long, opposite upper tepal. *Ovary* obovoid 0.6–0.8 mm long, 0.7–0.8 mm wide, sparsely hairy apically; stipe 0.1–0.3 mm long. *Style* excentrically fixed on the ovary, curved, 1.9–4 mm long, cream-coloured. *Stigma* capitate, pink. *Seed* round, dull light brown or straw-coloured, ~1.5 mm long, ~1.4 mm wide.

Distribution and habitat

Ptilotus eremita commonly occurs in eucalypt woodlands and mulga shrublands or coastal scrublands in Western Australia, from Bernier Island and around Shark Bay to southeast of Norseman, nearly its entire range overlapping with *P. gaudichaudii* (see Fig. 2). It has been found at field sites with *P. gaudichaudii* (e.g. 29°49′35.6″S, 116°57′28.1″E and 28°37′9.5″S, 117°44′48.8″E) without any apparent intermediates.

Phenology

Flowers between June and October.

Notes

Ptilotus eremita can be easily distinguished from the largely sympatric *P. gaudichaudii sens. strict.* by its shorter tepals, style and staminal filaments and smaller anthers (Fig. 3). The tepals are green, usually with a reddish tinge around the margins and towards the apex, contrasting with the typically bright yellow tepals of *P. gaudichaudii sens. strict*. Its flowers are morphologically similar to those of *P. modestus* in that the tepals do not spread widely at anthesis but remain erect and overlap to form a tube; it differs in having four fertile stamens and shorter stamens, staminode and style.

Selected specimens examined

WESTERN AUSTRALIA: E of Nalbara, Nalbara–Burnerbinmah road, 3.5 km W of Great Northern Highway, 28°37′9.5″S, 117°44′48.8″E, *R.Davis, K.Thiele & T.Hammer RD 12656* (PERTH 08839379); 1.5 km N of Wubin parking bay on the Great Northern Highway, 29°49′35.6″S, 116°57′28.1″E, *T.Hammer, R.Davis & K.Thiele TH82* (PERTH 08863237); Goomalling cemetery near railroad, 31°16′46.8″S, 116°50′11.8″E, *T.Hammer, R.Davis & F.Nge TH96* (PERTH 08863083); ~5.92 km NW (325°) of Rothsay and ~13.58 km E (90°) of Karara Homestead, 29°14′23.2″S, 116°51′3.2″E, *R.Meissner & R.Coppen 4665* (PERTH 08406219); ~15.37 km NNE (32°) of Rothsay and ~46.23 km NW (316°) of Mount Singleton, 29°9′58.9″S, 116°58′11.6″E, *R.Meissner & R.Coppen 4664* (PERTH 08406227); E side of Clough's Bar track 4.8km along track from junction with Useless Loop Road, 26°15′31.0″S, 113°21′39.0″E, *A.Markey 1658* (PERTH 05252148); approximately 77 km W of Paynes Find, 29°4′24.7″S, 116°55′24.1″E, *A.Markey & S. Dillon 3676* (PERTH 07377045); N of Goldfields Road between Hines Hill North Road and Neening Road, 31°29′27.0″S, 118°5′6.0″E, *M.Hislop, M.Griffiths WW 139-39* (PERTH 06977588); W of hospital site, Bernier Island, 24°56′59.0″S, 113°07′41.0″E, *R.D.Royce 5987* (PERTH 217573).

Key to the species of the *Ptilotus gaudichaudii* clade

1. Perianth 12–15 mm long; tepals yellow to yellow–green, gaping widely at anthesis; style 9–11 mm long; anthers >1 mm long ....
.... *Ptilotus gaudichaudii*

Perianth 5–9 mm long; tepals green and often with reddish margins, slightly spreading at anthesis and forming a tube; style 2–6 mm long; anthers <0.7 mm long 2
2. Fertile stamens 3; filaments of fertile stamens 4–6 mm long; staminodes 2, >3 mm long; style >4 mm long; central and eastern Australia..................
....*Ptilotus modestus*

Fertile stamens 4; filaments of fertile stamens 2–3 mm long; staminode 1, <2 mm long; style <4 mm long; Western Australia....................................
.... *Ptilotus eremita*

Conflicts of interest

The authors declare that they have no conflicts of interest.

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SHORT COMMUNICATION

Ptilotus actinocladus (Amaranthaceae), a new and rare species from the Gascoyne bioregion, Western Australia

Ptilotus R.Br. (Amaranthaceae) is a genus of approximately 110 species, all of which are native to continental Australia and with most of the diversity occurring in Western Australia (Bean 2008; Hammer *et al.* 2015). During the construction of a comprehensive identification key to the genus for *KeyBase* (available at http://keybase.rbg.vic.gov.au/keys/show/6609), specimens identified as *P. pseudohelipteroides* Benl in Western Australia were found to be inconsistent with the morphology of *P. pseudohelipteroides* from eastern Australia, leading to the erection of the phrase name *P.* sp. Doolgunna (D. Edinger 4419). This short communication presents the result of an investigation into the taxonomic status of that phrase name, the new species *P. actinocladus* T.Hammer & R.W.Davis.

Ptilotus actinocladus T.Hammer & R.W.Davis, *sp. nov.*

Type: Doolgunna Station, Western Australia [precise locality withheld for conservation reasons], 5 August 2003, *G. Byrne* 307 (*holo*: PERTH 06592813).

Ptilotus sp. Doolgunna (D. Edinger 4419), Western Australian Herbarium, in *FloraBase*, https://florabase.dpaw.wa.gov.au/ [accessed 25 October 2017].

Prostrate *annual herbs* to 10 cm high, 25 cm wide. *Stems* terete, ribbed, glabrescent, with a persistent tuft of nodose hairs at the base of leaves and buds; a central stem ascending to *c.* 2(–10) cm long, particularly when young, with radiating prostrate lateral stems to 18 cm long, becoming numerous with age. *Basal leaves* not seen. *Cauline leaves* linear-lanceolate to oblanceolate, 4–22 mm long, 0.5–3 mm wide, glabrescent or with sparse nodose hairs; apex mucronate, mucro 0.25–0.50 mm long. *Inflorescences* terminal, spiciform, globular to cylindrical, 5–15 mm long, 11–14 mm wide, pink. *Bracts* ovate, 3.0–3.3 mm long, 1.4–1.8 mm wide, transparent, glabrous; apex mucronate, mucro 0.2–0.3 mm long. *Bracteoles* broadly ovate, 3.0–3.5 mm long, 1.9–2.0 mm wide, transparent, glabrous; apex mucronate, mucro 0.1–0.2 mm long. *Outer tepals* narrowly lanceolate, 4.5–4.9 mm long, 0.9–1.2 mm wide; apex margins in-rolled, truncate to shortly tapering, serrated; outer surface with long, silky, nodose hairs to 1.5 mm long, apex glabrous. *Inner tepals* narrowly lanceolate, 4.1–4.7 mm long, 0.5–0.8 mm wide; apex margins in-rolled, acute, not serrated; outer surface with long, silky, nodose hairs to 1.5 mm long, apex glabrous. *Fertile stamens* 4; *filaments* 1.4–1.6 mm long, uneven, dilated towards the base; *anthers* 0.4–0.5 mm long, 0.15–0.20 mm wide, pink. *Staminode* 2.3–2.5 mm long, sinuate. *Staminal cup* 0.3–0.5 mm long, oblique, lobed. *Staminal cup appendages* alternating with staminal filaments, 0.5–0.6 mm long, 0.2–0.3 mm wide, transparent, with sparse hairs on both surfaces; apex truncate, serrate; those appendages adjacent to the staminode are basally adnate to it, with acute apices. *Ovary* obconical, 1.2–1.5 mm long, 0.8–1.0 mm wide, apically villous; stipe 0.1–0.2 mm long. *Style* slightly curved, 1.1–1.3 mm long, slightly excentric on the ovary apex. *Stigma* capitate. *Seed* round, light brown, *c.* 1.5 mm long, *c.* 0.9 mm wide. (Figures 1A, 2A)

Figure 1. Comparative morphology of plants *in situ*, highlighting differences in habit and leaf form. A – *Ptilotus actinocladus* (*G. Byrne* 2759); B – *P. pseudohelipteroides* (*T. Hammer & K. Thiele* TH 91); C – *P. helipteroides* (*R. Davis, T. Hammer & B. Anderson* RD 12266). Photographs by G. Byrne (A) and T. Hammer (B, C).

Diagnostic features. *Ptilotus actinocladus* may be distinguished from all other members of the genus by the following combination of characters: a prostrate annual herb with glabrescent stems and leaves, glabrous and translucent bracts, pink flowers, tepals <10 mm long, 4 fertile stamens, 1 staminode 2.3–2.5 mm long, staminal cup appendages (pseudostaminodes) 0.5–0.6 mm long, an excentric style placed on the ovary summit, and an apically hairy ovary.

Other specimens examined. WESTERN AUSTRALIA: [localities withheld for conservation reasons] 14 July 2007, *G. Byrne* 2759 (PERTH); 13 July 2004, *D. Edinger* 4419 (PERTH); 15 July 2004, *D. Edinger* 4423 (PERTH); 4 Nov. 1965, *D.W. Goodall* 3267 (PERTH); 24 Aug. 1973, *E. Wittwer* 1137 (PERTH).

Phenology. Flowering collections have been made from July to November.

Distribution and habitat. *Ptilotus actinocladus* has been collected from Doolgunna Station, Woodlands Station and Belele Station in Western Australia (Figure 3), with only a single collection known from each of the latter two locations, from 1973 and 1965, respectively. The habitat has been described as flat, seasonally inundated plains with sparse vegetation.

Figure 2. Longitudinal section of dissected flowers in late bud. A – *Ptilotus actinocladus* (*G. Byrne* 2759); B – *P. pseudohelipteroides* (*T. Hammer & K. Thiele* TH 91); C – *P. helipteroides* (*R. Meissner & Y. Caruso* 561). Scale bar = 1 mm.

Conservation status. *Ptilotus actinocladus* is listed by Smith and Jones (2018) as Priority One under Conservation Codes for Western Australian Flora, under the name *P.* sp. Doolgunna (D. Edinger 4419). Given that this species is only represented in recent specimens from near Doolgunna Station, we consider this species to be rare and of conservation concern.

Etymology. The epithet derives from the Greek *aktis* (a ray or beam) and *klados* (a branch or stem), referring to the radiating, prostrate flowering stems, sometimes becoming numerous, which are characteristic of this species (Figure 1A).

Taxonomic notes. The new species can be easily distinguished from *P. helipteroides* (F.Muell.) F.Muell., with which it overlaps in distribution, by its distinctly radiating, prostrate flowering stems, whereas *P. helipteroides* is erect (rarely decumbent) and can reach >60 cm high (Figure 1). The habit of *P. pseudohelipteroides* is bushy (quite noticeable on many specimens), and it has a persistent indumentum covering the stems and leaves; the flowering stems are often closely clustered. This is quite different from *P. actinocladus*, in which all flowering stems (except the young apical shoot) are prostrate (flush with the ground). *Ptilotus actinocladus* can be further distinguished from *P. pseudohelipteroides* by the shape of the staminal cup appendages, which are basally adnate (rising
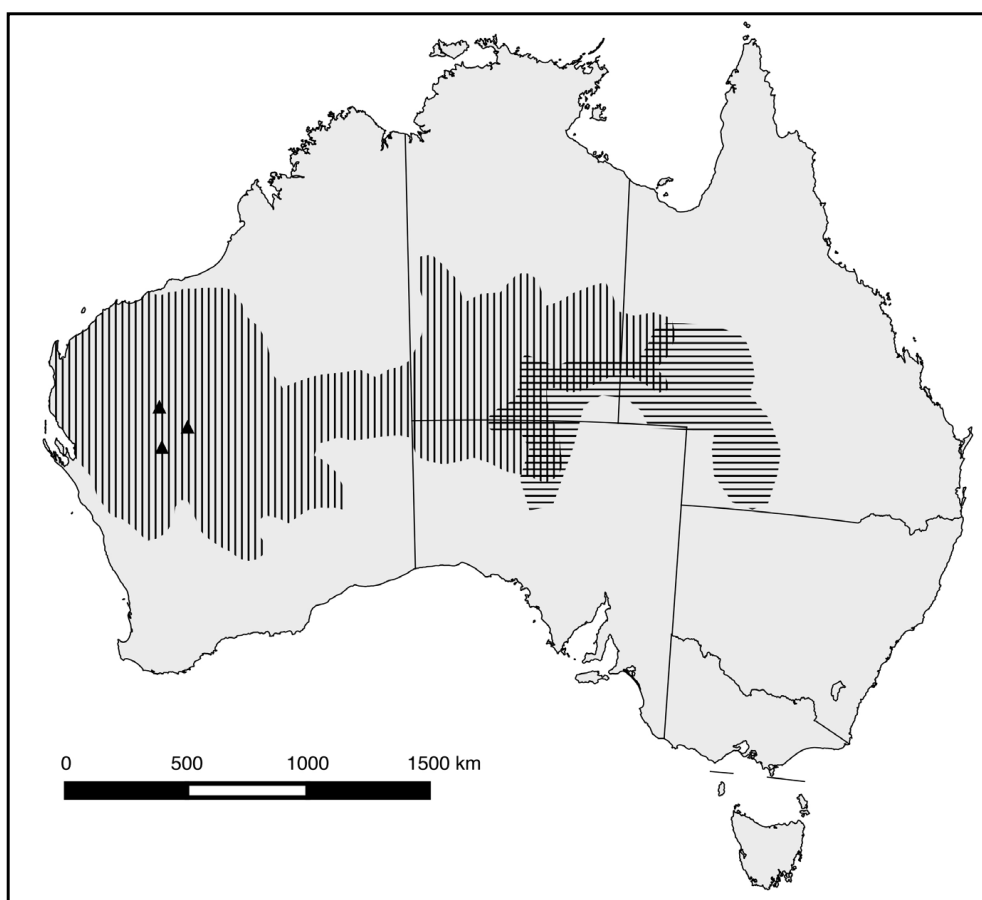
Figure 3. The distributions of *Ptilotus actinocladus* (triangles) and *P. helipteroides* (vertical shading) and *P. pseudohelipteroides* (horizontal shading) as inferred from records retrieved from the Australasian Virtual Herbarium (http://avh.chah.org.au/).

above the rest of the staminal cup) to the staminode and have acute apices in both *P. actinocladus* and *P. helipteroides*. In *P. pseudohelipteroides*, the staminal cup appendages are not adnate to the base of the staminode and have a rounded, or sometimes flattened, shape (see Benl 1959). *Ptilotus helipteroides* can be distinguished from both *P. actinocladus* and *P. pseudohelipteroides* by having larger anthers and longer staminal filaments, staminode, style and tepals than either of the two other species, which are similar in the sizes of the floral parts (Figure 2).

The type of *P. actinocladus* (PERTH 06592813) has been previously sequenced for the phylogeny of the genus as *P. pseudohelipteroides* (GenBank accessions: KP875954 for ITS and KP875857 for *matK*; Hammer *et al.* 2015). In that phylogeny, this species was placed as sister to *P. helipteroides*. The true *P. pseudohelipteroides* (from central and eastern Australia; see Figure 3) was not included, but presumably it would sit with the other two on the molecular phylogeny. These species together with *P. gaudichaudii* (Steud.) J.M.Black, *P. eremita* (S.Moore) T.Hammer & R.W.Davis (previously *P. gaudichaudii* subsp. *eremita* (S.Moore) Lally) and *P. modestus* T.Hammer (previously *P. gaudichaudii* subsp. *parviflorus* (Benth.) Lally) form a highly supported clade (>99% in all analyses; Hammer *et al.* 2015). This clade shares a noteworthy synapomorphy in the development of the fruit. After anthesis, the persistent tepals, enclosing the ovary and seed, harden considerably and pinch inward above the ovary base to form a tight, indurated covering around the fruit, and the tepal apicies flex outward and gape widely. The hardened tepal bases may provide some limited protection for the seed, while the gaping, papery, persistent tepals most likely aid in wind dispersal (see Hammer *et al.* 2018).

Ptilotus gaudichaudii, *P. eremita* and *P. modestus* can be easily distinguished from the new species by lacking staminal cup appendages and having green or yellow tepals with abaxial hairs restricted to the midline.

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SHORT COMMUNICATION

Ptilotus yapukaratja (Amaranthaceae), a new species from the Gascoyne bioregion of Western Australia

Ptilotus yapukaratja R.W.Davis & T.Hammer, *sp. nov.*

Type: Lorna Glen, Western Australia, 16 June 2017, *K. Millet* 346 (*holo*: PERTH 08904618; *iso*: CANB, MEL).

Low, compact, woody, perennial *shrubs* to 30 cm high, 35 cm wide. *Stems* terete, glabrescent, with moderately dense, ascending, sub-verticillate hairs to 0.3 mm long. *Basal leaves* absent. *Cauline leaves* scattered, incurved, narrowly oblanceolate, boat-shaped in cross section, fasciculate at dwarf stem shoots, 4–10 mm long, 0.9–1.5mm wide, with ascending, sparse, stiff, sub-verticillate hairs to 0.2 mm long; apex mucronate. *Inflorescences* spiciform, terminal, solitary, pink, ovoid, 20–32 mm long, 28–32 mm diam., 15–25 flowers per inflorescence. *Bracts* translucent, pink along midrib, 5.7–6.7 mm long, ovate, with verticillate hairs becoming glabrous towards margins; midrib prominent. *Bracteoles* translucent, pink along midrib, 4.8–5.9 mm long, broadly ovate, with verticillate hairs along central portion; midrib prominent. *Flowers* curved slightly upwards. *Outer tepals* pink fading white towards the base, narrowly oblanceolate, concave, flattening towards the apex, 13–14 mm long; outer surface hairy except at the apex, with dense, appressed to slightly spreading, sub-verticillate hairs to 3 mm long; inner surface glabrous; apex shortly tapering, entire. *Inner tepals* pink fading white towards base, narrowly oblanceolate, concave, 12–13 mm long; outer surface hairy except at the apex with dense, appressed to ascending, sub-verticillate hairs to 2 mm long; inner surface glabrous except for a basal tuft of tangled sub-verticillate hairs on the margins; apex centrally folded, attenuate, entire. *Staminal cup* symmetrical, 1.2–1.5 mm long, with sub-verticillate hairs to 1 mm long. *Stamens* 2; filaments glabrous, straight, dilating towards base, 6.5–7.8 mm long; *anthers* 0.5–0.7 mm long. *Staminodes* 3, 0.9–1.1 mm long. *Ovary* ellipsoid, gibbous, 2.9–3.1 mm long, 1.6–1.8 mm wide, with a row of verticillate hairs across the summit; *stipe* terete, 0.7–0.9 mm long. *Style* straight to slightly sinuate, excentrically fixed to ovary, 7.7–8 mm long, with verticillate hairs on the basal portion. *Seed* glossy, brown, 1.9–2.1 mm long. (Figure 1)

Other specimens examined. WESTERN AUSTRALIA: [localities withheld for conservation reasons] 22 Oct. 2013, *M. Griffiths & S. Cherriman s.n.* (PERTH); 17 Feb. 2016, *J. Jackson & V. Jackson* 330 (PERTH); 17 Feb. 2016, *J. Jackson & V. Jackson* 331 (PERTH).

Diagnostic features. Ptilotus yapukaratja can be distinguished from all other *Ptilotus* R.Br. species by the following combination of characters: a rigid habit, glabrous incurved leaves, bracts longer than bracteoles, two fertile stamens, an excentrically placed style on the ovary, and a hairy ovary.

Phenology. The new species is only known from two flowering and fruiting collections made from the same locality, one made in October and the other in June. This would suggest flowering times are in response to random rain events.

Figure 1. *Ptilotus yapukaratja*. A – plant *in situ*, showing habit and habitat; B – a close-up showing an inflorescence with an open flower. Images by K. Millet from *K. Millet* 346.

Distribution and habitat. Currently only known from north of Lorna Glen Station, where it is found at the base of breakaways on shallow rocky slopes in open scrub on brown clayey-sandy soils.

Conservation status. To be listed as Priority One under Conservation Codes for Western Australian Flora (M. Smith pers. comm.). *Ptilotus yapukaratja* is only known from the one remote location north of Lorna Glen Station.

Etymology. The epithet derives from the Matuwa words *yapu* (rock) and *karatja* (belonging to), referring to the rocky habitat where the species occurs (see Figure 1A).

Affinities. The new species is clearly aligned with the *P. parvifolius* (F.Muell.) F.Muell. complex (subclade D2 in Hammer *et al.* 2015), and it is morphologically most similar to *P. rigidus* Lally and *P. daphne* Lally (see Lally 2009). It differs from *P. rigidus* in having narrowly oblanceolate leaves 4–10 mm long (*cf.* narrowly obovate, 2.5–5.5 mm long) and longer bracts (5.7–6.7 mm long vs 4.5–5.2 mm long); it varies from *P. daphne* in having much longer bracts (5.7–6.7 mm long vs 3–4.5 mm long), many more flowers per inflorescence (15–25 vs 7–10), pink tepals fading white towards base (*cf.* purple throughout), and a row of hairs along the ovary summit (*cf.* hairy ovary throughout). It could also be confused with the morphologically similar *P. polakii* F.Muell. subsp. *polakii*; however, it differs from this taxon in having incurved leaves and an ellipsoid, gibbous and hairy ovary (*cf.* straight leaves and an obovoid, glabrous ovary).

Ptilotus yapukaratja has had its ITS (nrDNA) and *matK* (cpDNA) markers sequenced for a forthcoming PhD thesis on the molecular systematics of the genus (Hammer in prep.), and is most similar in its nucleotide sequence to *P. disparilis* Lally and *P. fasciculatus* W.Fitzg. (which are also members of subclade D2). Unlike the new species, *P. fasciculatus* is a prostrate perennial herb that occurs on the margins of salt lakes in the Geraldton Sandplains, Avon Wheatbelt and Mallee bioregions of Western Australia. *Ptilotus disparilis* differs from the new species in having tepals less than 6.5 mm long with dense, wavy hairs at the apex and is endemic to South Australia (Lally 2008). Also included in a forthcoming molecular study were *P. rigidus* and *P. daphne*, which were resolved in the same clade as the new species, but despite their morphological similarity, they were found to be more varied in the nucleotide sequence (in ITS and *matK*) than the new species. This suggests that the new species can be distinguished in both morphological and molecular characters.

Acknowledgements

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The *Ptilotus murrayi* species group: synonymisation of *P. petiolatus* under *P. murrayi* and description of the new Western Australian species *P. unguiculatus* (Amaranthaceae)

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Abstract: The *Ptilotus murrayi* F.Muell. species group encompasses the morphologically similar *P. murrayi*, *P. gomphrenoides* F.Muell. ex Benth. and *P. petiolatus* Farmar. These three species overlap in distribution in Western Australia, where they are easily confused with one another. A critical examination of specimens shows that there are no consistent morphological characters to justify the separation of *P. murrayi* and *P. petiolatus*, and therefore the latter is formally synonymised under the former. *Ptilotus* sp. Edaggee Station (T.E.H.Aplin 3208) is found to be a distinctive new species and described as *Ptilotus unguiculatus* T.Hammer. Updated descriptions are given for *P. murrayi* and *P. gomphrenoides*.

Keywords: Amaranthaceae, Australia, new species, *Ptilotus*, taxonomy

Introduction

Ptilotus R.Br. (Amaranthaceae) is a genus of c. 120 species native to Australia, mostly occurring in arid or semi-arid regions of Australia, with the highest diversity in Western Australia (Hammer *et al.* 2015). A recent molecular phylogeny by Hammer *et al.* (2015) resolved several molecular clades within the genus, including the large clade informally named 'clade B'. Within clade B, *Ptilotus murrayi* F.Muell. and *P. gomphrenoides* F.Muell. ex Benth. were resolved as sister species. The morphologically similar *P. petiolatus* Farmar was not included in the phylogeny (Hammer *et al.* 2015). Together these species comprise the *P. murrayi* species group.

Ptilotus murrayi was described by Mueller (1863) based on a type from Will's Creek (the Diamantina River, incorrectly noted in the protologue as 'Cooper's Creek') near the border of South Australia and Queensland, collected by J. Murray on the Howitt expedition (Fig. 1A). Specimens of *P. murrayi* from Western Australia were not available to Mueller, but he did mention having seen similar specimens from Western Australia that had pedunculate inflorescences:

Vidi etiam specimina manca plantae persimilis ad montes Hamersley Range sub expeditione Francisci Gregorii collecta, spicis pedunculatis praecipue divellenda.

<div align="right">Mueller (1863: 145)</div>

Bentham (1870) formally described *P. gomphrenoides* based on these pedunculate specimens from Gregory's

expedition in the Hamersley Range (Fig. 1B). Farmar (1905) erected *P. petiolatus* from specimens collected by E. Clement in north-western Western Australia in 1897. He separated *P. petiolatus* from *P. murrayi* on the basis that the former has inflorescences with acute apices, shorter staminal filaments and larger anthers:

[…] *quod flores* P. murrayi *simillimus sed spicis acutis filamentis brevioribus antheris majoribus differt*

<div align="right">Farmar (1905: 1089)</div>

He went on to say that *P. murrayi* was incompletely known, and that only a few fragments ('an inch in length') were available at K, however he felt confident that these differences were sufficient to erect the new species.

Farmar (1905) also erected *P. roseo-albus* Farmar within this species group, differing from *P. gomphrenoides* in having shortly pedunculate spikes (as opposed to long-pedunculate) and a denser abaxial sepal indumentum. Within *P. roseo-albus* he described var. *conglomeratus* Farmar, which differed from the typical variety in having cylindrical inflorescences in clusters, as opposed to conical and loosely arranged inflorescences. The two varieties of *P. roseo-albus* were recombined under *P. gomphrenoides* by Benl (1962) as *P. gomphrenoides* var. *roseo-albus* (Farmar) Benl and var. *conglomeratus* (Farmar) Benl, stating that the characters that Farmar used were common characteristics of *P. gomphrenoides*. Hammer & Davis (2017) recently synonymised these varieties under the typical *P. gomphrenoides*, as these varieties were found to intergrade imperceptibly into one another. Black (1923) described *P. murrayi* var. *major* J.M.Black from the Diamantina River, South

Fig. 1. Comparative morphology of flowering inflorescences. **A** *Ptilotus murrayi*; **B** *P. gomphrenoides*. Photos: A K.R. Thiele; B R. Davis.

Australia, on the basis of its larger leaves, longer stems and inflorescences, and slightly longer sepals. This variety was synonymised by Bean (2008), who recognized that the characters attributed to *P. murrayi* var. *major* were within the typical variability of var. *murrayi*. In a manuscript by Benl (unpubl.), *P. petiolatus* subsp. *limbatus* Benl MS was included as a manuscript name based on a specimen from Edaggee Station, Western Australia (Fig. 2), which he primarily differentiated from subsp. *petiolatus* by the new subspecies having clawed sepals. While the new taxon was never formalised, it was included under the phrase name *Ptilotus* sp. Edaggee Station (T.E.H.Aplin 3208) at the Western Australian Herbarium awaiting evaluation of its taxonomic status.

The geographic distributions of taxa in the *P. murrayi* species group overlap significantly in Western Australia, and this, coupled with the strong morphological similarities, has led to some confusion between them. *Ptilotus murrayi* has a disjunct distribution, occurring in Western Australia from Carnarvon, Gascoyne, Murchison, Pilbara and Dampierland (Interim Biogeographic Regionalisation for Australia, IBRA; Department of the Environment 2018) regions and in south-western Queensland and north-eastern South Australia in the Mitchell Grass Downs and Channel Country IBRA regions. It has not been recorded from the Northern Territory or north-western South Australia (Fig. 3A). *Ptilotus petiolatus* and *P. murrayi* are sympatric throughout the range of the former. *Ptilotus petiolatus* is known from 15 specimens, ten from Dampierland and five from the Pilbara IBRA regions. *Ptilotus gomphrenoides* is also almost entirely sympatric with *P. murrayi* in Western Australia, occurring in the Carnarvon, Gascoyne, Pilbara, and northern Murchison bioregions (Fig. 3B). *Ptilotus* sp. Edaggee Station (T.E.H.Aplin 3208) is known from a single collection on Edaggee Station, approximately 70 km SSE of Carnarvon, Western Australia and 75 km SW of the southwestern-most record of *P. murrayi*, which occurs near the Gascoyne River (Fig. 3B).

During the construction of an online identification key to all species in *Ptilotus* for the *Flowering Plants of Australia* project on *KeyBase* (available at http://keybase.rbg.vic.gov.au/keys/show/6609), *P. murrayi*, *P. gomphrenoides* and *P. petiolatus* were found to be difficult to discriminate due to unclear morphological boundaries, which was also noted by Burbidge (unpubl.) in a key constructed for *Ptilotus*. A thorough examination of the morphology of specimens for each species was conducted to critically evaluate the species boundaries within the group. This showed that specimens of *P. murrayi* and *P. petiolatus* could not be reliably separated, including those that Farmar had originally used to erect *P. petiolatus*. As a result, the later name of *P. petiolatus* is here formally synonymised under *P. murrayi*. *Ptilotus* sp. Edaggee Station (T.E.H.Aplin 3208) is also assessed and found to be a very distinctive species. The morphological differences between the two accepted species in the *P. murrayi* species group (i.e. *P. murrayi* and *P. gomphrenoides*) are clarified and descriptions are given for both.

Material and methods

This study was based on the examination of dried specimens of *P. gomphrenoides*, *P. murrayi*, *P. petiolatus* and *P.* sp. Edaggee Station (T.E.H.Aplin 3208) housed at PERTH and specimens of *P. murrayi* on loan from BRI and AD, covering the geographic range of all species. Scans of type specimens were assessed through *JSTOR Global Plants* (https://plants.jstor.org/). The specimens were critically evaluated irrespective of current determinations and matched to the protologues and type specimens, and when necessary, specimens were re-determined as a result of this study. Care was taken to evaluate the specimens based on their likely natural posture in the field, and when available, label data were used to infer the natural habit of the specimens. A distribution map of the species was produced using georeferenced records retrieved from the *Australasian Virtual Herbarium* (https://avh.chah.org.au/). The terminology used in this paper for the perianth of *Ptilotus* is different from what is conventionally used within Australia. The term 'sepal' is used here in place of 'tepal', as recent studies support the calycine origin of the uniseriate perianth for all Caryophyllales, the corolla having been lost (see Ronse De Craene 2013; Vrijdaghs *et al.* 2014).

Results and discussion

The examination revealed that *P.* sp. Edaggee Station (T.E.H.Aplin 3208) is clearly a distinctive species within the genus. Benl (unpubl.) intended to describe this taxon as a subspecies of *P. petiolatus*. While it is understandable that he believed the two entities were related, given their superficial similarities (e.g. habit, leaf size and shape, inflorescence size and shape; Fig. 2), *P.* sp. Edaggee Station (T.E.H.Aplin 3208) is significantly different from *P. petiolatus* in important floral characters. It may not be closely related to the *P. murrayi* species group. The sepals of *P.* sp. Edaggee Station (T.E.H.Aplin 3208) are conspicuously clawed (i.e. narrow at the base and dilated toward the apex), and more so in the outer sepals, where the dilated apex is 0.7–0.9 mm wider than the base. *Ptilotus petiolatus*, *P. murrayi* and *P. gomphrenoides* do not have clawed sepals. In other *Ptilotus* species, this trait is consistent within a species and is often shared between closely related species. Within clade B, several species groups possess clawed sepals, such as the species group including *P. latifolius* R.Br. and *P. chamaecladus* Diels and the species group including *P. conicus* R.Br. and *P. corymbosus* R.Br. *Ptilotus* sp. Edaggee Station (T.E.H.Aplin 3208) also differs from *P. petiolatus*, by possessing crisped nodose hairs on the inner margins of the sepals, which often become tangled and obscure the ovary and staminal cup. Sepals in *Ptilotus petiolatus*, *P. murrayi* and *P. gomphrenoides* are adaxially glabrous (see Fig. 1). *Ptilotus* sp. Edaggee Station (T.E.H.Aplin 3208) has longer staminal filaments (0.7–0.9 mm) and style (0.6–0.8 mm) than

P. petiolatus (0.3–0.6 and 0.3–0.5 mm, respectively), which ensure the anthers and stigma are raised above these obscuring hairs. Furthermore, the bracteoles of *P.* sp. Edaggee Station are much longer and wider than the bracts (by c. 1 mm), while the bracts and bracteoles of the other species are similar in size. These differences are sufficient to recognise *P.* sp. Edaggee Station (T.E.H.Aplin 3208) as a distinct species, and therefore *P. unguiculatus* T.Hammer is erected to accommodate it (see below).

Ptilotus murrayi and *P. petiolatus* can both be segregated from *P. gomphrenoides* in Western Australia by the latter species having pedunculate inflorescences; peduncles are short but present on some specimens (e.g. those previously included under *P. roseo-albus*). *Ptilotus gomphrenoides* could additionally be segregated from the other two species by examination of the midrib region of the sepals (i.e. the thickened prominent midrib flanked by two lateral veins that is clearly differentiated from the scarious region surrounding it). All *Ptilotus* species have five sepals that are arranged with two outer sepals and three inner sepals. Sometimes the outer and inner sepals are morphologically similar, but in the *P. murrayi* species group they are conspicuously different. The outer sepals in the *P. murrayi* species group are conspicuously different to the inner sepals in that they enclose the inner sepals and possess larger and more conspicuous scarious margin, particularly towards the apex. The midrib region and margin of the sepal usually vary in colour in these species, being either dark pink or green around the midrib and pink on the margin (Fig. 1). The outer sepal has a prominent and concaved midrib (thus allowing it to tightly enclose the inner sepals in bud), but the midrib region is much narrower and less starkly coloured. *Ptilotus gomphrenoides* has a much wider sepal midrib region (0.5–0.8 mm), which is usually pink to dark pink, whereas the *P. murrayi* and *P. petiolatus* consistently have a narrow (0.2–0.3 mm) and green sepal midrib region. The outer sepals in *P. gomphrenoides* have a longer scarious margin above the midrib (0.8–1.5 mm) and rounded apices (Fig. 1B), whereas *P. murrayi* and *P. petiolatus* have a shorter scarious margin above the midrib (0.5–1.0 mm) and more acute apices (Fig. 1A). Using the characters outlined above, specimens of *P. gomphrenoides* could be confidently segregated from *P. murrayi* and *P. petiolatus*.

The differences Farmar stated as separating *P. petiolatus* and *P. murrayi* do not withstand scrutiny with the specimens currently available. All collections of *P. petiolatus* have inflorescences with an acute apex, while collections of *P. murrayi* can have either an acute or truncated apex; it is likely that Farmar had only access to specimens of *P. murrayi* with the latter character. Farmar indicated that staminal filament length and anther length were both important in splitting *P. petiolatus* from *P. murrayi*. This study found, however, that throughout the geographic ranges of both species, they overlap in staminal filament length (0.3–0.6 mm) and anther length

(0.3–0.5 mm). Benl (1979) suggested that *P. petiolatus* could be identified based on its unusually short style, but *P. murrayi* and *P. petiolatus* were found to have the same range of style length (0.3–0.5 mm) with the specimens currently available.

Benl (1971) used leaf size to split *P. murrayi* from *P. petiolatus* and *P. gomphrenoides* in his key. Leaf size in these species imperceptibly intergrades, and is not correlated with geography and any other characters, rendering the use of this as a distinguishing character ineffectual. Benl (1971) furthermore noted a difference in phyllotaxis of *P. petiolatus* and *P. gomphrenoides*, considering the former to be subopposite ('annähernd gegenständig', p. 172) and the latter to be alternate. However, leaf arrangement in these species is not consistent across all specimens available. Each of the three species has specimens that have an alternate leaf arrangement, but some specimens can be found to have an apparent opposite or subopposite arrangement.

Bentham (1870) described *P. gomphrenoides* as 'apparently erect' and *P. murrayi* as 'apparently prostrate' (pp. 243–244). All three species were found to have prostrate specimens (this character being more common in *P. murrayi* than the other species), as well as specimens described on labels as ascending or erect. Habit could not be correlated with other characters (e.g. leaf size or the size of floral parts), to reliably discriminate between the species. Several specimens in *P. gomphrenoides* were found to exhibit the characters attributed by Farmar (1905) and Benl (1971) to *P. petiolatus*, such as slightly smaller flowers, leaves and an overall more petite habit (e.g. *A.C.Beauglehole 48962*), which may indicate that these characters attributed to *P. petiolatus* by previous authors are within the normal range variation for both *P. gomphrenoides* and *P. murrayi*. Furthermore, no differences between the habitats of the three species could be discerned, all typically occurring on flat floodplains or on the fringes of ephemeral wetlands, creeks or rivers, with clayey red or brown soils.

Given that there are no consistent characters with which to separate *P. petiolatus* from *P. murrayi*, the former encompassing the normal variation from throughout the range of *P. murrayi* (including in specimens available from eastern Australia), *P. petiolatus* is here synonymised under *P. murrayi*. No significant differences were found between the specimens of *P. murrayi* from Western Australia and eastern Australia, leaving the question open as to the significance of the large geographic disjunction in the records between Western Australia and eastern Australia (see Fig. 3A), which may be more appropriately investigated with an in-depth molecular study. Full descriptions for the modern concepts of *P. murrayi* and *P. gomphrenoides* are given below. A key to all accepted species of *Ptilotus* in Western Australia, including those described here, is currently under review by the journal *Nuytsia* and is expected to be forthcoming.

Taxonomy

Ptilotus unguiculatus T.Hammer, *sp. nov.*

Type: Edaggee Station, Western Australia [precise locality withheld for conservation reasons], 3 July 1970, *T.E.H.Aplin 3208* (holo: PERTH 04073819!, **Fig. 2**; iso: M 0241497 image!).

Ptilotus sp. Edaggee Station (T.E.H.Aplin 3208) Western Australian Herbarium, in FloraBase, https://florabase. dpaw.wa.gov.au/ [accessed: 9 May 2018].

Decumbent *annual herb* to 8 cm high, 20 cm wide. *Stems* terete, ribbed, with dense nodose hairs on young growth and the hairs becoming sparse with age. *Basal leaves* not seen. *Cauline leaves* alternate, lanceolate to ovate, entire, 8–30 mm long, 5–11 mm wide, glabrous; base narrowly attenuate or petiolate, 5–10 mm long; apex mucronate, mucro c. 0.1 mm long. *Inflorescences* solitary or in clusters of 2–3, terminal, sessile, spiciform, conical, 5–12 mm long, 7–8 mm wide, white, subtended by leaves; apex acute. *Bracts* broadly ovate, 1.8–1.9 mm long, 1.4–1.5 mm wide, translucent, glabrous; midrib obscure; apex acute. *Bracteoles* broadly ovate, 3.0–3.1 mm long, 2.4–2.5 mm wide, translucent, glabrous; midrib obscure; apex acute. *Outer sepals* spathulate, strongly clawed, 3.5–3.6 mm long, 0.4–0.5 mm wide at base, 1.1–1.4 mm wide at dilated apex; midrib region prominent, green, 1.5–2.5 mm long, 0.1–0.3 mm wide; basal outer surface with long, wavy, nodose hairs, not exceeding sepal apex; inner surface glabrous apart from crisped nodose hairs on margins becoming dense and tangled within; apex acute, the scarious margin 1.0–1.2 mm long, glabrous, translucent, serrate, in-rolled. *Inner sepals* narrowly spathulate, clawed, 2.5–3.1 mm long, 0.3–0.4 mm wide at base, 0.6–0.8 mm wide at the dilated apex; midrib region prominent, green, 1.7–2.2 mm long, 0.2–0.3 mm wide; basal outer surface with long, wavy, nodose hairs at base, becoming densely woolly with age, not exceeding sepal apex; inner surface glabrous apart from crisped nodose hairs on margins becoming dense and tangled within; apex acute, the scarious margin 0.3–0.7 mm long, glabrous, translucent, serrate, in-rolled. *Fertile stamens* 5; *filaments* 0.7–0.9 mm long; *anthers* 0.4–0.5 mm long, 0.2–0.3 mm wide, yellow. *Staminal cup* 0.3–0.4 mm long, unlobed. *Ovary* globular, 0.6–0.7 mm long, 0.6–0.8 mm wide, glabrous. *Style* straight, 0.6–0.7 mm long, centrally placed on the ovary apex. *Stigma* unlobed, capitate. *Fruit* not seen. *Seed* not seen.

Diagnostic characters. *Ptilotus unguiculatus* can be distinguished from other members of the genus by the following combination of characters: decumbent habit, dense pubescence on young stems, glabrous and petiolate leaves, sessile and terminal inflorescences (subtended by leaves), bracts that are c. 1 mm shorter and narrower than bracteoles, conspicuously clawed (dilated at the apex) sepals, crisped nodose hairs on the abaxial margin of the sepals that obscure the ovary, base of sepal adaxial surface with long silky nodose hairs that do not exceed the sepal apices, five fertile stamens, staminal cup appendages absent, a central style centrally placed on the ovary apex, and a glabrous ovary.

Fig. 2. Scan of *Ptilotus* sp. Edaggee Station (T.E.H.Alpin 3208), the proposed holotype of *P. unguiculatus* (PERTH 04073819). Insert: Close-up of inflorescence.

Phenology. The only examined specimen was collected as flowering, but not fruiting, in early July. It is likely that this species has a similar flowering and fruiting time to other annual *Ptilotus* species in region, which is typically from June to September.

Distribution and habitat. *Ptilotus unguiculatus* is currently known only from Edaggee Station, in the Carnarvon bioregion of Western Australia, where it was collected from a disturbed area. No habitat description was included with the specimen. Inspection of the

locality from aerial satellite images indicates it to be a plain with open shrubs.

Conservation status. *Ptilotus unguiculatus* is listed as Priority 1 under Conservation Codes for Western Australian Flora, under the name *P.* sp. Edaggee Station (Smith & Jones 2018). It is only known from the type specimens, collected in 1970 from a disturbed area on Edaggee Station, Western Australia.

Etymology. The specific epithet is from the Latin *unguiculatus* (having claws), referring to the conspicuously clawed sepals of the new species.

Notes. This new species may be potentially confused with *P. villosiflorus* F.Muell., which is also a small herb with greenish-white flowers, but which differs by typically occurring on coastal dunes, having sepals that are not clawed and abaxial sepal hairs that exceed the sepal apex. *Ptilotus latifolius* and *P. chamaecladus* are species closely related to *P. villosiflorus* that do have clawed sepals. *Ptilotus latifolius* is a large rounded, upright herb or subshrub with tangled branches that has bracteoles that are longer than the sepals. Both *P. chamaecladus* and *P. latifolius* have staminal cup lobes alternating with the stamens, while the new species has an unlobed staminal cup. The fruit of *P. villosiflorus*, *P. chamaecladus* and *P. latifolius* is hard and indehiscent, while those of the *P. murrayi* species group are membranous and irregularly dehiscent. Due to the similarity of the new species to the *P. latifolius* species group and despite Benl (unpubl.) considering the taxon to be closely allied with the *P. murrayi* species group, I could not confidently place the new species in either group. The difference in the fruit between these two groups is an important diagnostic character, but there was no fruit available for *P. unguiculatus*.

Ptilotus murrayi F.Muell.

Fragm. 3: 145 (1863). — **Type:** 'From the flooded tracts of Will's Creek [=Diamantina River], beyond desert' [state unknown], 1861, *J.Murray s.n.* (syn.: BM 000895594 image!, K 000357020 image!, MEL 2235243 image!, MEL 2235244 image!, PERTH 01558226!).

Ptilotus murrayi var. *major* J.M.Black, *Trans. & Proc. Roy. Soc. S. Austral.* 47: 368 (1923). — **Type:** 'Between Herrgott [=Marree] and Innamincka', South Australia, Jun. 1916, *R.Cockburn s.n.* (holo.: AD 97747822A image!).

Ptilotus petiolatus Farmar, *Bull. Herb. Boissier* ser. 2, 5: 1089 (1905), syn. nov. — **Type:** 'North West Australia, Between the Ashburton and De Grey rivers', Western Australia, [purchased] Aug. 1900, *E.Clement s.n.* (syn.: K 000349177 image!, K 000349178 image!, K 000349179 image!).

Prostrate mat-forming or decumbent *annual herb* 5–20 cm high, 20–100 cm wide. *Stems* terete, ribbed, glabrous. *Basal leaves* not seen. *Cauline leaves* alternate or subopposite, narrowly lanceolate to broadly lanceolate or spathulate, entire, 5–30 mm long, 3–9 mm wide, glabrous; base narrowly attenuate or petiolate, 5–10 mm long; apex mucronate, mucro c. 0.1 mm long. *Inflorescences* solitary or in clusters, axillary or terminal, sessile, spiciform, cylindrical, 5–30 mm long, 4–8 mm wide, white; apex acute or truncate. *Bracts* ovate, 1.0–1.8 mm long, 0.8–1.1 mm wide, translucent, glabrous; midrib obscure; apex rounded. *Bracteoles* ovate, 1.5–2.1 mm long, 0.7–1.0 mm wide, translucent, glabrous; midrib obscure; apex acute. *Outer sepals* narrowly lanceolate, 2.0–3.3 mm long, 0.4–0.7 mm wide; midrib region prominent, green, 1.5–2.5 mm long, 0.1–0.3 mm wide; basal outer surface with long, wavy, nodose hairs at base, becoming densely woolly with age; inner surface glabrous; apex acute, scarious margin 0.5–1.0 mm long, glabrous, white. *Inner sepals* lanceolate, 1.8–2.8 mm long, 0.3–0.7 mm wide; midrib region prominent, green, 1.7–2.2 mm long, 0.2–0.3 mm wide; basal outer surface with long, wavy, nodose hairs at base, becoming densely woolly with age; inner surface glabrous; apex acute, scarious margin 0.3–0.7 mm long, glabrous, pink. *Fertile stamens* 5; *filaments* 0.3–0.6 mm long; *anthers* 0.3–0.5 mm long, 0.1–0.2 mm wide, yellow. *Staminal cup* 0.3–0.5 mm long, unlobed. *Ovary* globular, 0.7–1 mm long, 0.5–0.9 mm wide, glabrous. *Style* straight, 0.3–0.5 mm long, centrally placed on the ovary apex. *Stigma* unlobed, capitate. *Fruit* smooth, membranous, irregularly dehiscent. *Seed* round, black, glossy, c. 0.9 mm long, c. 0.7 mm wide. **Fig. 1A.**

Diagnostic characters. *Ptilotus murrayi* may be distinguished from all other members of the genus by the following combination of characters: a prostrate or decumbent annual herb with glabrous stems and leaves, sessile axillary inflorescences, sepals not clawed, outer sepals with acute apices, a glabrous adaxial sepal surface, densely woolly hairs on the base of the abaxial sepal surface, 5 fertile stamens, staminal cup appendages absent, a central style centrally placed on the ovary apex, and a glabrous ovary.

Phenology. *Ptilotus murrayi* typically flowers from April to August throughout its range, extending into late October in Queensland and South Australia.

Distribution and habitat. *Ptilotus murrayi* occurs in the Carnarvon, Gascoyne, Murchison, Pilbara and Dampierland IBRA regions in Western Australia, and in south-western Queensland and north-eastern South Australia in Mitchell Grass Downs and Channel Country IBRA regions, with several outlying collections in the Simpson-Strzelecki Dunefields (Fig. 3A). The typical habitat is a flat floodplain or on the fringes of ephemeral wetlands, billabongs or rivers with silty or more commonly clayey red or brown soils.

Conservation status. *Ptilotus murrayi* is not listed as of conservation concern in any state in which it occurs.

Selected specimens examined
QUEENSLAND: 5.6 km W of turnoff to Birdsville along Windorah to Bedourie road, 27 June 2010, *D.Halford QM116* (BRI); Elizabeth Springs Conservation Park,

24 Oct. 2010, *R.Moore 1304* (BRI); Carlo, 16 June 2010, *J.Silcock JLS263* (BRI); 18 km E of Mount Leonard Station Homestead, 9 June 2010, *G.P.Turnpin GPT1333* (BRI).

SOUTH AUSTRALIA: Cordillo Downs on track near S. boundary c. 1 km S of Bluebush Dam, 6 May 2011, *D.C.Bickerton, P.J.Lang & D.J.Duval 76* (AD).

WESTERN AUSTRALIA: Liveringa Station near Fitzroy River, 20 Apr. 1985, *T.E.H.Aplin 179* (PERTH); track behind station to Yanarrie River within 200 m of homestead Yanrey Station, 6 May 2004, *G.Byrne 943* (PERTH); Maitland Road, Bullgarra Cell, Karratha, 20 Aug. 1986, *K.Glennon 239* (PERTH); on the side of North West Coastal Highway on a mound in a recently burnt flood plain, 2 km W of Yule River, 17 Aug. 2015, *T.Hammer, S.Dillon & K.Thiele TH 25* (PERTH); c. 800 m ENE of Balmoral Caravan Park, Balmoral Road, Nickol, Karratha, 14 Apr. 2011, *V.Long VLK tall-11* (PERTH); c. 20 km E of Nanutarra Station homestead, c. 200 km W of Paraburdoo, 15 May 1997, *A.A.Mitchell 4735* (PERTH); 8.7 km NNW of Whim Creek, Mallina Station, Pilbara, 19 Apr. 2006, *S.van Leeuwen PBS 5816* (PERTH).

Ptilotus gomphrenoides F.Muell. ex Benth.

Fl. Austral. 5: 244 (1870). — **Type:** 'Hamersley Range, N.W. coast, F. Gregory's Expedition', *F.Mueller s.n.* (syn: K 000357029 image!, MEL 2281815 image!).

Ptilotus roseo-albus Farmar var. *roseo-albus*, *Bull. Herb. Boissier* ser. 2, 5: 1090 (1905). — *Ptilotus gomphrenoides* var. *roseo-albus* (Farmar) Benl, *Mitt. Bot. Staatssamml. München* 4: 277 (1962). — **Type:** 'N.-W. Division, W. Australia, E. Clement, 1897' [between the Ashburton and De Grey rivers, Western Australia, 1897, *E.Clement s.n.*] (syn: K 000357024 image!, K 000357025 image!, K 000357026 image!, K 000357027 image!).

Ptilotus roseo-albus var. *conglomeratus* Farmar, *Bull. Herb. Boissier* ser. 2, 5: 1090 (1905). — *Ptilotus gomphrenoides* var. *conglomeratus* (Farmar) Benl, *Mitt. Bot. Staatssamml. München* 4: 278 (1962). — **Type:** 'N.-W. Division, W. Australia, E. Clement, 1897' [between the Ashburton and De Grey rivers, Western Australia, 1897, *E.Clement s.n.*] (holo: K 000357028 image!).

Erect, decumbent or less-commonly prostrate *annual herb* (2–) 10–30 cm high, 35–100 cm wide. *Stems* terete, ribbed, glabrous or with sparse nodose hairs. *Basal leaves* not seen. *Cauline leaves* alternate, narrowly lanceolate to broadly lanceolate, entire, 5–30 mm long, 1–5 mm wide, glabrous; base narrowly attenuate or petiolate, 3–10 mm long; apex mucronate, mucro 0.1–0.2 mm long. *Inflorescences* solitary or in clusters, axillary or terminal, pedunculate (peduncle 1–10 mm long) or rarely sessile, spiciform, cylindrical, 4–20 mm long, 6–7 mm wide, pinkish white; apex acute or truncate. *Bracts* ovate, 1.1–1.4 mm long, 0.8–0.9 mm wide, translucent, glabrous; midrib obscure; apex rounded. *Bracteoles* ovate, 1.2–1.5 mm long, 0.8–0.9 mm wide, translucent, glabrous; midrib faint or obscure; apex acute. *Outer sepals* narrowly lanceolate, 2.0–3.0 mm long, 0.5–0.8 mm wide; midrib region prominent, pink or rarely greenish, 1.2–1.5 mm long, 0.4–0.8 mm wide; basal outer surface with long, wavy, nodose hairs at base, becoming densely woolly with age; inner surface glabrous; apex rounded, scarious
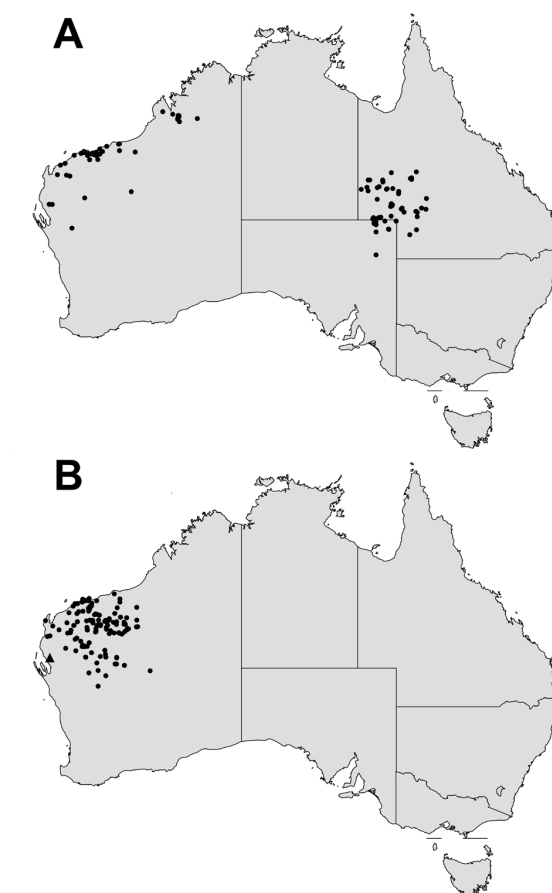
Fig. 3. Occurrence of herbarium records. **A** *Ptilotus murrayi*; **B** *P. gomphrenoides* (circles) and *P. unguiculatus* (triangle).

margins 0.8–1.5 mm long, glabrous, white. *Inner sepals* lanceolate to broadly lanceolate, 1.8–2.5 mm long, 0.7–1.2 mm wide; midrib region prominent, pink or rarely green, 1.0–1.5 mm long, 0.5–0.8 mm wide; basal outer surface with long, wavy, nodose hairs at base, becoming densely woolly with age; inner surface glabrous; apex rounded, scarious margin 0.5–0.8 mm long, glabrous, pink. *Fertile stamens* 5; *filaments* 0.3–0.7 mm long; *anthers* 0.2–0.4 mm long, 0.2–0.3 mm wide, yellow or pink. *Staminal cup* 0.1–0.2 mm long, unlobed. *Ovary* globular, 0.6–0.7 mm long, 0.5–0.7 mm wide, glabrous. *Style* straight, 0.2–0.4 mm long, centrally placed on the ovary apex. *Stigma* unlobed, capitate. *Fruit* smooth, membranous, irregularly dehiscent. *Seed* round, black, glossy, c. 0.8 mm long, c. 0.7 mm wide. **Fig. 1B.**

Diagnostic characters. *Ptilotus gomphrenoides* can be distinguished from other species in the genus by the following combination of characters: an erect or decumbent annual herb with glabrous stems and leaves, pedunculate axillary or terminal inflorescences, sepals not clawed, outer sepals with rounded apices, a glabrous adaxial sepal surface, densely woolly hairs on the base of the abaxial sepal surface, 5 fertile stamens, staminal cup

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appendages absent, a central style centrally placed on the ovary apex, and a glabrous ovary.

Phenology. *Ptilotus gomphrenoides* flowers from April to September, with a few outlying specimens having been collected from October to January.

Distribution and habitat. *Ptilotus gomphrenoides* occurs in the Carnarvon, Gascoyne, Pilbara and northern Murchison IBRA regions of Western Australia (Fig. 3B). The typical habitat is flat seasonally-inundated floodplains, riverbanks or creek lines with clayey red or brown soils.

Conservation status. *Ptilotus gomphrenoides* is not listed as of conservation concern in Western Australia.

Selected specimens examined
WESTERN AUSTRALIA: Callina Creek c. 40 km WSW of Marble Bar, 30 Apr. 2006, *A.Bean 25214* (PERTH); Chichester Ranges, 20 July 2011, *S.Chalwell 379* (PERTH); Turn off to Python Pool near Tom Price railway road, 16 July 2013, *R.Davis 12261* (PERTH); 1.1 km S along fenceline from Mount Brockman road and 22 km W of Hamersley Station homestead, 24 Sep. 2006, *D.Halford Q9265* (PERTH); Nammuldi/Silvergrass lease area near Mt Brockman, WNW of Tom Price, 28 Aug.–7 Sep. 1998, *M.Maier s.n.* (PERTH 05986389); N of Catho Well mesa, Mount Stuart Station, West Pilbara Iron project area, 7 Aug. 2008, *K.McMaster LCH 25882* (PERTH); 14 miles S of Nullagine, Sep. 1971, *R.Mirrington 710919* (PERTH); Gascoyne River crossing at Yinnetharra, Western Australia, 14 Aug. 2002, *S.Patrick 4291* (PERTH); Karijini National Park, 900 m NNW of the summit of Mt Hyogo and 13.3 km SW of Mt Bruce and 31.1 km NNE of Mt Bennett, Hamersley Range, 24 Aug. 1995, *S.van Leeuwen 2053* (PERTH); E side of track, 5.3 km N of Ripon Hill Road on track of abandoned Braeside Station Homestead, 10.5 km W of Pulgorah Cone, 119.9 km ESE of Marble Bar, Warrawagine Station, Pilbara, 22 Apr. 2004, *S.van Leeuwen et al. PBS 7047* (PERTH).

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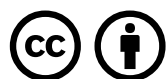
SHORT COMMUNICATION

A key to *Ptilotus* (Amaranthaceae) in Western Australia

Ptilotus R.Br. (Amaranthaceae) is a genus of approximately 120 species, all of which are native to continental Australia and with most of the diversity occurring in Western Australia (Hammer *et al.* 2015). The key presented here for 96 Western Australian taxa is a continuation of on-going work to produce an Australia-wide key for *Ptilotus* by the authors, which was originally presented on *KeyBase* (available at http://keybase.rbg.vic.gov.au/keys/show/6609, accessed 16 August 2018; previously mentioned in Hammer & Davis 2018). The Western Australian key was constructed by examining specimens lodged at the Western Australian Herbarium (PERTH) and includes all of the 93 named species now recognised (i.e. excluding *P. petiolatus* Farmar and including *P. unguiculatus* T.Hammer; see Hammer 2018). The subspecies of *P. polakii* F.Muell., *P. sericostachyus* (Nees) F.Muell. and *P. stirlingii* (Lindl.) F.Muell. are also included in the key. However, the infraspecific taxa of *P. drummondii* (Moq.) F.Muell., *P. obovatus* (Gaudich.) F.Muell. and *P. schwartzii* (F.Muell.) Tate, currently recognised on the plant census for Western Australia, were excluded pending on-going studies into their taxonomic status. Also excluded from the key are the phrase names *P.* sp. Beaufort River (G.J. Keighery 16554), *P.* sp. Mt Narryer (A.S. George 17484) and *P.* sp. Porongorup (R. Davis 10805), which are in need of further study.

As new species are discovered (e.g. Davis & Hammer 2018; Hammer & Davis 2018) and new evidence is found to change existing taxonomic concepts (e.g. Hammer *et al.* 2018a, 2018b), there will no doubt be future revisions needed to this Western Australian key. An interactive version of it is available on *KeyBase* (http://keybase.rbg.vic.gov.au/keys/show/6627, accessed 8 August 2018) as part of the *Flowering plants of Western Australia* project.

Notes on distinctive characters

We use 'sepal' in this key instead of the traditionally used 'tepal' to describe the uniseriate perianth of *Ptilotus* (for more information, see Hammer 2018). All species of *Ptilotus* have five sepals, with two outer enclosing three inner in bud. Inner and outer sepals may differ conspicuously in morphology, or may be almost indistinguishable in fully open flowers. The term 'clawed' refers to the base of the sepal being conspicuously narrower than the dilated apex. Enclosing the base of the solitary flower are two opposite bracteoles (i.e. the prophylls), which in *Ptilotus* are membranous and can be translucent or opaque and hairy or glabrous. At the base of the bracteoles is a single bract, which is alternate to the two bracteoles (for more information on this flowering arrangement, see Acosta *et al.* 2009). The morphology of the bract and the bracteoles is often diagnostic.

Early steps in the key include the placement of the style on the ovary summit, and the number of fertile stamens. The style is usually either clearly central or clearly excentric (see Figure 4 in Hammer *et al.* 2015); where this is ambiguous or where we have found infraspecific variation, we have included the species in both sections of the key. The androecium of *Ptilotus* is 5-merous, usually comprising a distinct androecial cup with stamens or staminodes opposite the sepals (Figure 1A, B). Commonly, one or more stamens are infertile and reduced to staminodes. The number of fertile stamens is consistent within species and across geographic ranges (e.g. Hammer *et al.* 2018b), with the exception of *P. manglesii*
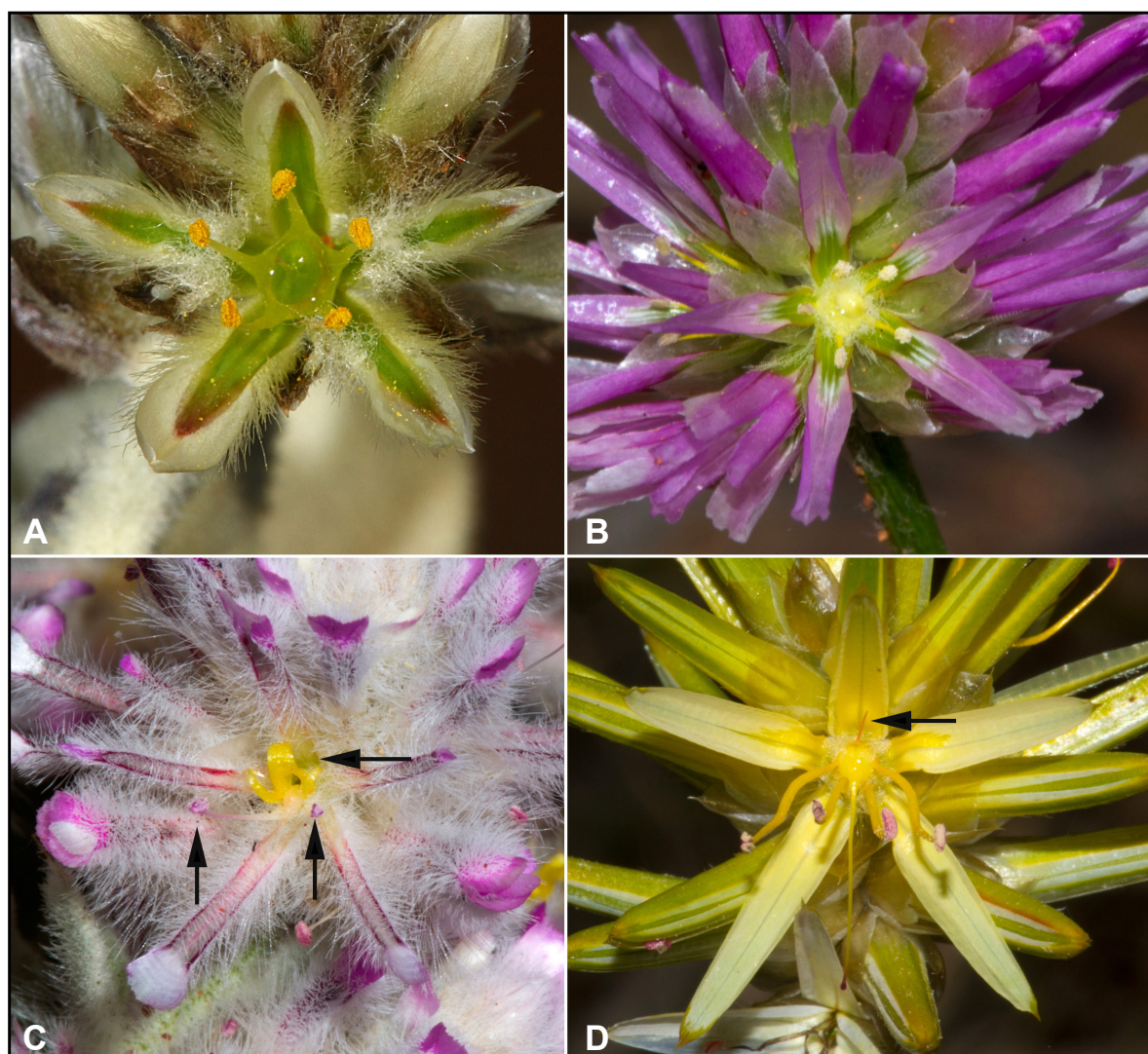
Figure 1. Morphology of the androecium in *Ptilotus*. A – five fertile stamens opposite sepals in *P. luteolus* (Benl & H.Eichler) R.W.Davis; B – five fertile stamens opposite sepals in *P. grandiflorus* F.Muell.; C – reduced stamen number to two with three showy staminodes in *P. appendiculatus* Benl; D – reduced stamen number to four with an inconspicuous staminode in *P. gaudichaudii* (Steud.) J.M.Black. Horizontal arrows indicate staminodes (C, D) and vertical arrows indicate fertile stamens (C). Photographs by R. Davis (A) and T. Hammer (B–D).

(Lindl.) F.Muell., which may have three to five fertile stamens in different flowers on an individual plant. Infertile stamens (staminodes) may be showy (sometimes flattened and coloured; Figure 1C) or may comprise a reduced filament that appears as an inconspicuous appendage on the staminal cup (Figure 1D). In some species a staminode may be so reduced that it appears as just a minute projection on the staminal cup or, rarely, may appear completely absent. In addition, some species have small appendages (previously called 'pseudostaminodes') that project from the staminal cup and alternate with the stamens or staminodes.

Many species of *Ptilotus* are gynodioecious, i.e. populations comprise a mix of male-sterile (i.e. functionally female) and bisexual plants (Stewart & Barlow 1972; Hammer *et al.* 2018a; Figure 2). Bisexual plants have a fully developed androecium comprising one to five fertile stamens and up to four staminodes as described above. In male-sterile plants, all of the fertile stamens fail to fully form, leaving reduced appendages with clearly non-functional anthers (Figure 2B). While gynodioecy is common in the genus, it has not been adequately surveyed between and within species. In some species

Figure 2. Variation of stamens in the gynodioecious *P. obovatus*, showing flowers of a bisexual individual on the left and a female individual on the right. Arrows indicate fertile (A) and poorly-developed (B) anthers. Photographs by R. Davis (A) and K. Thiele (B).

(e.g. *P. obovatus* and *P. schwartzii*) the ratio of male-sterile to bisexual plants may be very high; in others, occasional male-sterile individuals may be found in populations that are mostly bisexual (e.g. *P. exaltatus* Nees; Hammer *et al.* 2018a). Male-sterile individuals can be identified using this key, but care should be taken in couplet 3 to discriminate stamens (with poorly developed anthers) from staminodes (which lack anthers). Stamens with anthers in such individuals are counted as fertile in this couplet, despite being functionally sterile. One species (*P. crispus* Benl) is truly dioecious, with all individuals either female and lacking an androecium, or male with the ovary reduced and lacking a style.

Indumentum in all species comprises multi-cellular hairs, ranging from simple to branching, i.e. verticillate or dendritic (see Hammer *et al.* 2015, 2017 for discussions and figures). Simple hairs are described as nodose when the nodes between hairs are swollen and septate when they are not. Verticillate hairs have distinct whorls of side-branches at the nodes; in dendritic hairs, the side-branches do not form distinct whorls.

Key to *Ptilotus* in Western Australia

Taxa marked with an asterisk appear more than once in the key.

1.   Sepals < 1.6 mm long; flowers unisexual, the males lacking styles, the females lacking sterile stamens ....**P. crispus**

1:   Sepals 2–50 mm long; flowers bisexual, or functionally female with sterile stamens

  2.   Style excentrically placed on the ovary summit

    3.   Fertile stamens 1 or 2

      4.   Ovary glabrous

        5.   Stems herbaceous

          6.   Fertile stamen 1..**P. alexandri**

          6:   Fertile stamens 2

7.   Adaxial surface of inner sepals with a basal tuft of hairs

    8.   Leaves thick, semi-succulent to succulent; flowers green; sepals with short, appressed hairs on abaxial surface.. **P. chortophytus**

    8:   Leaves flat, not semi-succulent or succulent; flowers pink to purple; sepals with long, spreading hairs on abaxial surface

        9.   Plants decumbent; bracts 1.8–4.5 mm long; bracteoles 3–5 mm long...**P. stirlingii** subsp. **stirlingii**

        9:   Plants prostrate; bracts 5.3–6 mm long; bracteoles 5.4–6.3 mm long..**P. stirlingii** subsp. **australis**

7:   Adaxial surface of inner sepals ± glabrous

    10.   Staminodes conspicuous, *c.* 2 mm long.. **P. halophilus**

    10:   Staminodes minute or absent

        11.   Bracts 6.5–7 mm long .... **P. sericostachyus** subsp. **roseus**

        11:   Bracts 4–5.7 mm long .... **P. sericostachyus** subsp. **sericostachyus**

  5:   Stems woody or basally woody

    12.   Leaves petiolate

        13.   Glabrous portion of outer sepal apex 3–5 mm long; bracts ± equal in length to bracteoles; style 6–10 mm long .... **P. polakii** subsp. **polakii**

        13:   Glabrous portion of outer sepal apex 1–2 mm long; bracts shorter than bracteoles; style 4–5.5 mm long.. **P. polakii** subsp. **juxtus**

    12.   Leaves sessile or subsessile

        14.   Stems erect or ascending; leaves not crowded at the base of the stem.............................**P. beardii**

        14:   Stems prostrate, mat-forming; leaves crowded at the base of the stem

            15.   Sepals < 7 mm long, apex not rounded (presumed extinct) .... **P. caespitulosus**

            15:   Sepals > 8 mm long, apex rounded..**P. fasciculatus**

4:   Ovary hairy

  16.   Stems herbaceous

    17.   Inner sepals with a prominent basal tuft of hairs inside

        18.   Leaves with persistent verticillate hairs on adaxial surface to 2 mm long...**P. andersonii**

        18:   Leaves glabrous or glabrescent

            19.   Flowers pink; sepal apex rounded and dilated; lower portion of sepals densely hairy ....**P. chippendalei**

            19:   Flowers creamish green; sepal apex acute, not dilated; lower portion of sepals glabrous...**P. seminudus**

    17:   Inner sepals without basal tuft of hairs inside

        20.   Bracts longer than bracteoles .... **P. blackii**

        20:   Bracts shorter than bracteoles

    21. Leaves with persistent villous indumentum ....**P. appendiculatus**

    21: Leaves mostly glabrous .... **P. axillaris**

  16: Stems woody or basally woody

    22. Bracts shorter than bracteoles

     23. Stems divaricately branching .... **P. lazaridis**

     23: Stems with no pattern of branching, i.e. not divaricate

      24. Leaves > 3 mm wide, not in fascicles

       25. Leaves with persistent verticillate hairs .... **P. kenneallyanus***

       25: Leaves glabrous.. **P. stipitatus**

      24: Leaves < 1.6 mm wide, in fascicles

       26. Stems hairy; sepals 15–19 mm long... **P. daphne**

       26: Stems glabrous; sepals 11–13 mm long ....**P. rigidus**

    22: Bracts longer than bracteoles

     27. Leaves glabrous or glabrescent, incurved, semi-succulent ....**P. yapukaratja**

     27: Leaves with persistent verticillate hairs, not as above ....**P. kenneallyanus***

3: Fertile stamens 3–5

 28. Ovary glabrous

  29. Stems prostrate or decumbent

  30. Annuals

   31. Outer sepals at least 10 mm longer than inner sepals .... **P. crosslandii**

   31: Outer and inner sepals similar in size

    32. Sepals glabrous, apex truncate-serrate...**P. grandiflorus***

    32: Sepals with straight hairs on abaxial surface, apex acute....................................**P. procumbens**

  30: Perennials

   33. One stamen modified into a conspicuous staminode to 17 mm long..........................**P. declinatus**

   33: Stamens all fertile or staminodes short and inconspicuous

    34. Sepals with hairs exceeding the apex ....**P. symonii**

    34: Sepals with hairs not exceeding the apex

     35. Inflorescences becoming long-cylindrical; basal leaves distinctly spathulate.. **P. spathulatus**

     35: Inflorescences mostly ovoid; basal leaves oblanceolate

      36. Plants single-stemmed ....**P. clivicola**

      36: Plants multi-stemmed

       37. Sepals white to green; bracts prominently sickle-shaped ....**P. falcatus**

       37: Sepals pink; bracts not sickle-shaped..**P. manglesii***

  29: Stems erect

   38. Leaves with hairs obscuring surface

39. Staminodes absent or obscure .... **P. eriotrichus**

39: Staminodes prominent and coloured

40. Staminodes pink; sepals 12–20 mm long .... **P. sessilifolius**

40: Staminodes yellow; sepals 6.5–9.5 mm long.. **P. incanus**

38: Leaves glabrous or with sparse hairs not obscuring the surface

41. Stems woody or basally woody, divaricately branching .... **P. divaricatus**

41: Stems herbaceous, not divaricately branching

42. Small herb < 8 cm tall; basal rosette of spathulate leaves .... **P. pyramidatus**

42: Herbs > 8 cm tall; leaves not as above

43. Sepals with hairs restricted to midrib of abaxial surface .... **P. gaudichaudii***

43: Sepals with hairs not restricted to midrib of abaxial surface

44. Bracts opaque; fertile stamens 3

45. Flowers purple to pink; sepals gaping widely at anthesis, straight;
ovary obscured by a plug of woolly hairs at the base of the sepals ....**P. exaltatus***

45: Flowers creamish green, rarely with a pale pink flush; sepals not
gaping widely at anthesis, ± falcately down-curved; ovary not
obscured, the hairs at the base of the sepals ± erect, not forming a
woolly plug...**P. nobilis***

44: Bracts translucent; fertile stamens 4 or 5

46. Sepal abaxial surface glabrous apart from basal hairs; flowers pink;
fertile stamens 5 ....**P. grandiflorus***

46: Sepal abaxial surface hairy apart from apex; flowers cream to green;
fertile stamens 4

47. Inflorescences 30–60 mm wide; flowers not opening broadly,
radially symmetric; old flowers not appressed to rachis.................... **P. macrocephalus***

47: Inflorescences 18–28 mm wide; flowers opening broadly,
bilaterally symmetric; old flowers appressed to rachis.........................**P. polystachyus***

28: Ovary hairy

48. Perennials

49. Inflorescences interrupted...**P. distans**

49: Inflorescences not interrupted

50. Stems prostrate or decumbent

51. Bracts dark, opaque; sepal apex glabrous for 4–8 mm, truncate-serrate ....**P. manglesii***

51: Bracts translucent; sepal apex hairy, acute ....**P. holosericeus**

50: Stems erect

52. Stems woody or basally woody (when young); sepals without densely
woolly indumentum on the adaxial surface; staminodes 2, flattened,
yellow and showy ....**P. obovatus**

52: Stems herbaceous; sepals with densely woolly indumentum on the adaxial
surface; staminodes 2, filiform, not showy, obscured by woolly sepal hairs..........**P. exaltatus***

48: Annuals

53. Inner sepals adaxially glabrous

54. Outer sepals much longer than inner...**P. trichocephalus**

54: Outer and inner sepals subequal

55. Sepals with hairs restricted to midrib on abaxial surface

56. Sepals 10–15 mm long; anthers > 0.9 mm long..**P. gaudichaudii***

56: Sepals 6–9 mm long; anthers < 0.6 mm long...**P. eremita**

55: Sepals with hairs not restricted to midrib on abaxial surface

57. Sepal apex truncate-serrate; flowers orange to yellow .... **P. carlsonii**

57: Sepal apex acute; flowers creamish green, sometimes with pale pinkish
tinge

58. Stems prostrate, sprawling; sepals < 6 mm long...**P. aervoides***

58: Stems erect or ascending; sepals > 8 mm long

59. Ovary with a distinct coma of hairs ....**P. fusiformis**

59: Ovary without a distinct coma of hairs

60. Inflorescences 30–60 mm wide; flowers not opening broadly,
radially symmetric; old flowers not appressed to rachis.................... **P. macrocephalus***

60: Inflorescences 18–28 mm wide; flowers opening broadly,
bilaterally symmetric; old flowers appressed to rachis

61. Pedicel (i.e. the stalk attaching the bract and flowering unit to the
rachis) after abscission squat, with a prominent disc at apex;
ovary gibbous; staminal cup with sparse, short hairs..........................**P. polystachyus***

61: Pedicel (i.e. the stalk attaching the bract and flowering unit to the
rachis) after abscission slender, with a reduced disc at apex;
ovary not gibbous; staminal cup with copious, long, silky hairs ....**P. giganteus**

53: Inner sepals adaxially hairy or with row of hairs on inwardly folding margins

62. Inflorescences interrupted ....**P. tetrandrus**

62: Inflorescences not interrupted

63. Bracts glabrous, translucent

64. Flowers green; sepals > 15 mm long; staminode inconspicuous ....**P. macrocephalus***

64: Flowers pink; sepals < 10 mm long; staminode conspicuous

65. Sepals > 6 mm long; staminode > 3 mm long; anthers > 0.6 mm long.......**P. helipteroides***

65: Sepals < 5.5 mm long; staminode < 2.6 mm long; anthers < 0.6 mm
long ....**P. actinocladus**

63: Bracts hairy, opaque

66. Sepals < 10 mm long...**P. carinatus**

66: Sepals > 16 mm long

67. Flowers purple to pink; sepals gaping widely at anthesis, straight;
ovary obscured by a plug of woolly hairs at the base of the sepals...................**P. exaltatus***

       67: Flowers creamish green, rarely with pale pink flush; sepals not gaping
            widely at anthesis, ± falcately down-curved; ovary not obscured, the
            hairs at the base of the sepals ± erect, not forming a woolly plug.........................**P. nobilis***

2: Style centrally placed on the ovary summit

  68. Leaves persistently hairy

    69. Stems herbaceous

      70. Bracts and bracteoles longer than sepals...**P. decipiens***

      70: Bracts and bracteoles shorter than sepals

        71. Inflorescences nodding; flowers creamish green

          72. Bracteoles glabrous; leaves to 45 mm long with velvety indumentum.......................**P. gardneri**

          72: Bracteoles pilose; leaves to 90 mm long with villous indumentum...........................**P. clementii**

        71: Inflorescences not nodding; flowers pink ....**P. helipteroides***

    69: Stems woody or basally woody

      73. Ovary glabrous

        74. Abaxial sepal hairs woolly, clearly exceeding apex ....**P. albidus**

        74: Abaxial sepal hairs not woolly, not exceeding apex

          75. Leaves > 30 mm wide

            76. Sepals and staminal filaments pink; inflorescences > 30 mm wide....................**P. rotundifolius**

            76: Sepals creamish green; staminal filaments white; inflorescences < 21 mm
              wide... **P. marduguru**

          75: Leaves < 20 mm wide

            77. Leaves narrowly oblanceolate to narrowly elliptical...**P. wilsonii**

            77: Leaves ovate to spathulate

              78. Stems and leaves with yellow, villous indumentum of long, branching hairs ....**P. luteolus**

              78: Stems and leaves with grey-green, tomentose indumentum of short,
                branching hairs ....**P. astrolasius**

      73: Ovary hairy

        79. Stamens longer than sepals...**P. helichrysoides**

        79: Stamens shorter than sepals

          80. Flowers interrupted, in solitary, terminal spikes ....**P. royceanus**

          80: Flowers densely arranged, in terminal panicles ....**P. mollis**

  68: Leaves glabrous or glabrescent, or mature plants leafless

    81. Stems erect

      82. Staminal cup appendages present

      83. Bracteoles longer than sepals...**P. latifolius**

      83: Bracteoles shorter than sepals

        84. Sepals green; inflorescences > 24 mm wide... **P. benlii**

        84: Sepals pink to pale pink or whitish; inflorescences < 20 mm wide

85. Stems leafless at maturity .... **P. aphyllus**

85: Stems with leaves at maturity

  86. Erect, slender herbs; taproot fleshy; inflorescences cylindrical .... **P. calostachyus**

  86: Rounded herbs or subshrubs (stems basally woody); taproot woody; inflorescences globular to ovoid

    87. Stems becoming woody, branching; leaves narrow-linear, sparse .... **P. schwartzii**

    87: Stems herbaceous, simple; leaves lanceolate to spathulate, basally crowded.. **P. drummondii***

82: Staminal cup appendages absent

 88. Inner sepals glabrous on adaxial surface

 89. Stems herbaceous

  90. Sepal abaxial surface glabrescent, hairs concentrated at apex............................**P. decalvatus**

  90: Sepal abaxial surface with persistent hairs at base or throughout

   91. Sepals woolly at base of abaxial surface, glabrous at apex........................**P. gomphrenoides***

   91: Sepals villous throughout length of abaxial surface... **P. lanatus**

 89: Stems woody

  92. Style ± straight.. **P. arthrolasius**

  92: Style conspicuously sigmoid ....**P. chrysocomus**

 88: Inner sepals with hairs on adaxial surface or on inner margins

 93. Inner sepals clawed

  94. Inflorescence units in corymb- or umbel-like clusters...

   95. Inflorescence units hemispherical to ovoid, in loose corymbs.......................... **P. corymbosus**

   95: Inflorescence units obovoid, in dense umbel-like clusters............................**P. johnstonianus**

  94: Flowers not clustered as above

   96. Staminal filaments narrowly ligulate, dilated into a disc under anthers................... **P. conicus**

   96: Staminal filaments not as above

    97. Bracts and bracteoles with an awn-like apex

     98. Staminal cup with divergent, forked appendages.. **P. capitatus**

     98: Staminal cup without appendages.. **P. spicatus**

    97: Bracts and bracteoles without awn-like apex .... **P. mitchellii***

 93: Inner sepals not clawed

  99. Abaxial sepal hairs exceeding sepal apex

   100. Bracteoles as long as sepals ....**P. villosiflorus**

   100: Bracteoles at most half the length of sepals ....**P. subspinescens**

  99: Abaxial sepal hairs not exceeding sepal apex

   101. Inflorescences nodding; staminal cup long and tube-like, 8–11 mm long .... **P. auriculifolius**

   101: Inflorescences not nodding; staminal cup short, < 1.5 mm long

102. Bract apex aristate...**P. decipiens***

102: Bract apex not aristate ....**P. mitchellii***

81: Stems prostrate or decumbent

103. Inner sepals hairy adaxially

104. Sepal apex acute

105. Plants mat-forming; stems prostrate, persistently stellate-hairy ....**P. roei**

105: Plants not mat-forming; stems decumbent or erect, glabrous or glabrescent

106. Staminal cup appendages present ....**P. drummondii***

106: Staminal cup appendages absent

107. Sepals conspicuously clawed ....**P. unguiculatus***

107: Sepals not clawed..**P. esquamatus**

104: Sepal apex truncate-serrate

108. Sepals 3.5–4.5 mm long, sparsely silky-hairy abaxially ....**P. exiliflorus**

108: Sepals 5–7.7 mm long, densely woolly abaxially ....**P. humilis**

103: Inner sepals glabrous adaxially

109. Ovary villous-woolly at summit ....**P. aervoides***

109: Ovary glabrous

110. Sepals not clawed

111. Inflorescences ± pedunculate; outer sepals with a rounded apex; inner
sepal midrib region 0.5–0.8 mm wide ....**P. gomphrenoides***

111: Inflorescences sessile; outer sepals acute; inner sepal midrib region
0.2–0.3 mm wide ....**P. murrayi**

110: Sepals conspicuously clawed

112. Staminal cup appendages present ....**P. chamaecladus**

112: Staminal cup appendages absent ....**P. unguiculatus***

Acknowledgements

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