

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

Timothy A. Hammer,¹ Xiao Zhong,² Catherine Colas des Francs-Small,² Paul G. Nevill,³ Ian D. Small² & Kevin R. Thiele¹

¹ School of Biological Sciences, Faculty of Science, The University of Western Australia, Crawley, Western Australia, 6009, Australia

² ARC Centre of Excellence in Plant Energy Biology, The University of Western Australia, Crawley, Western Australia, 6009, Australia

³ ARC Centre for Mine Site Restoration, School of Molecular and Life Sciences, Curtin University, Bentley, Western Australia, 6102, Australia

Address for correspondence: Timothy A. Hammer, timothy.hammer@research.uwa.edu.au

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 *Oureta* 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.

Keywords Amaranthaceae; Caryophyllales; new genus; nomenclature; phylogenomics; *Ptilotus*; taxonomy

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.

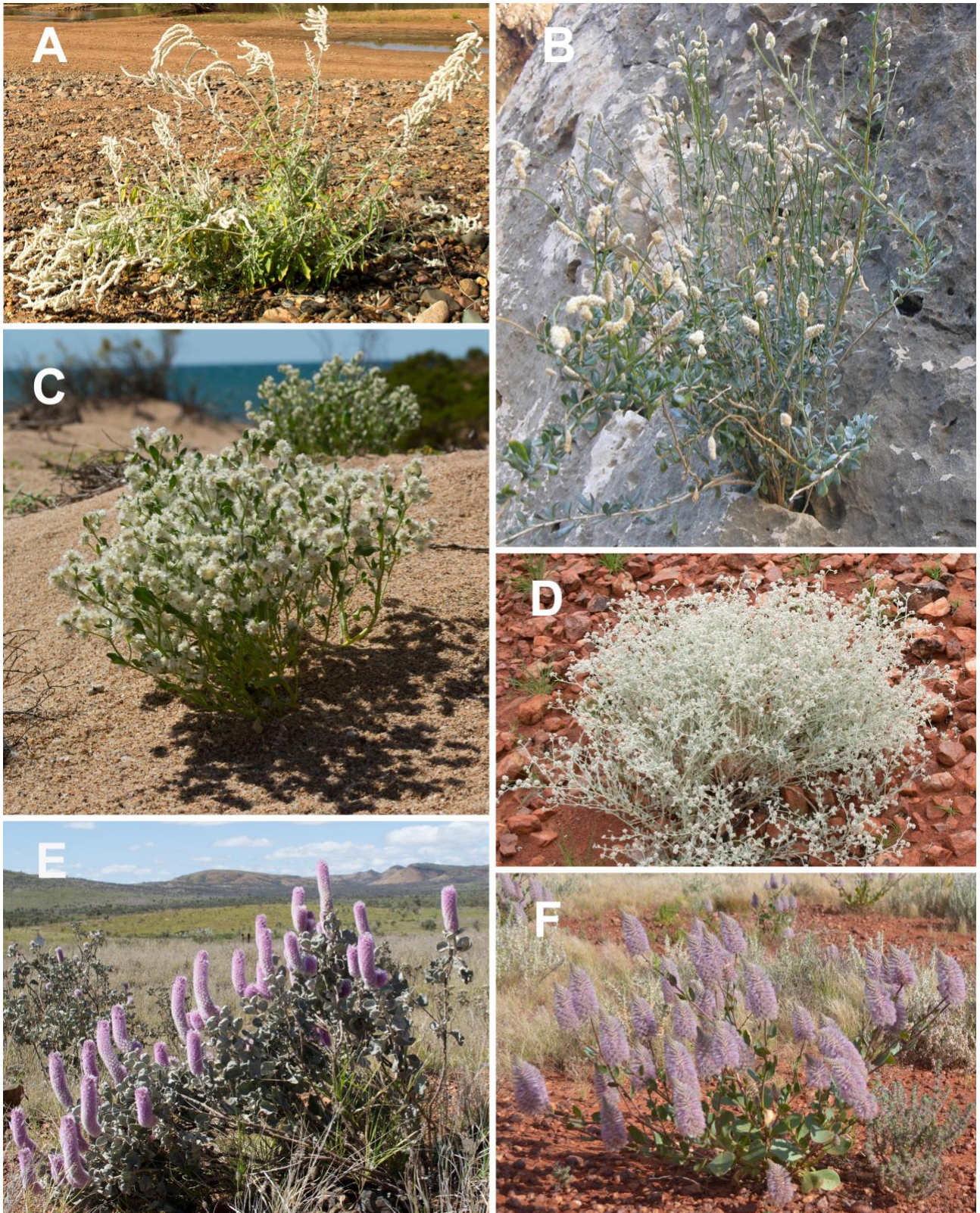


Fig. 1. Species representing major clades within the aervoids. **A**, *Aerva javanica*; **B**, *A. microphylla*; **C**, *Ptilotus villosiflorus*; **D**, *P. astrolasius*; **E**, *P. rotundifolius*; **F**, *P. exaltatus*. — Photos taken by T. Hammer in Western Australia (A, C–E) and South Australia (F) 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 and have indicated that most infra-familial taxa of previous authors (including Amaranthoideae, Amarantheae and Aervinae) are paraphyletic (e.g., Kadereit & al., 2003; Müller & Borsch, 2005; Sage & al., 2007). 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 *Aerva 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 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 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 (Kato & 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 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), representing all major clades of aroids, 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) that 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.

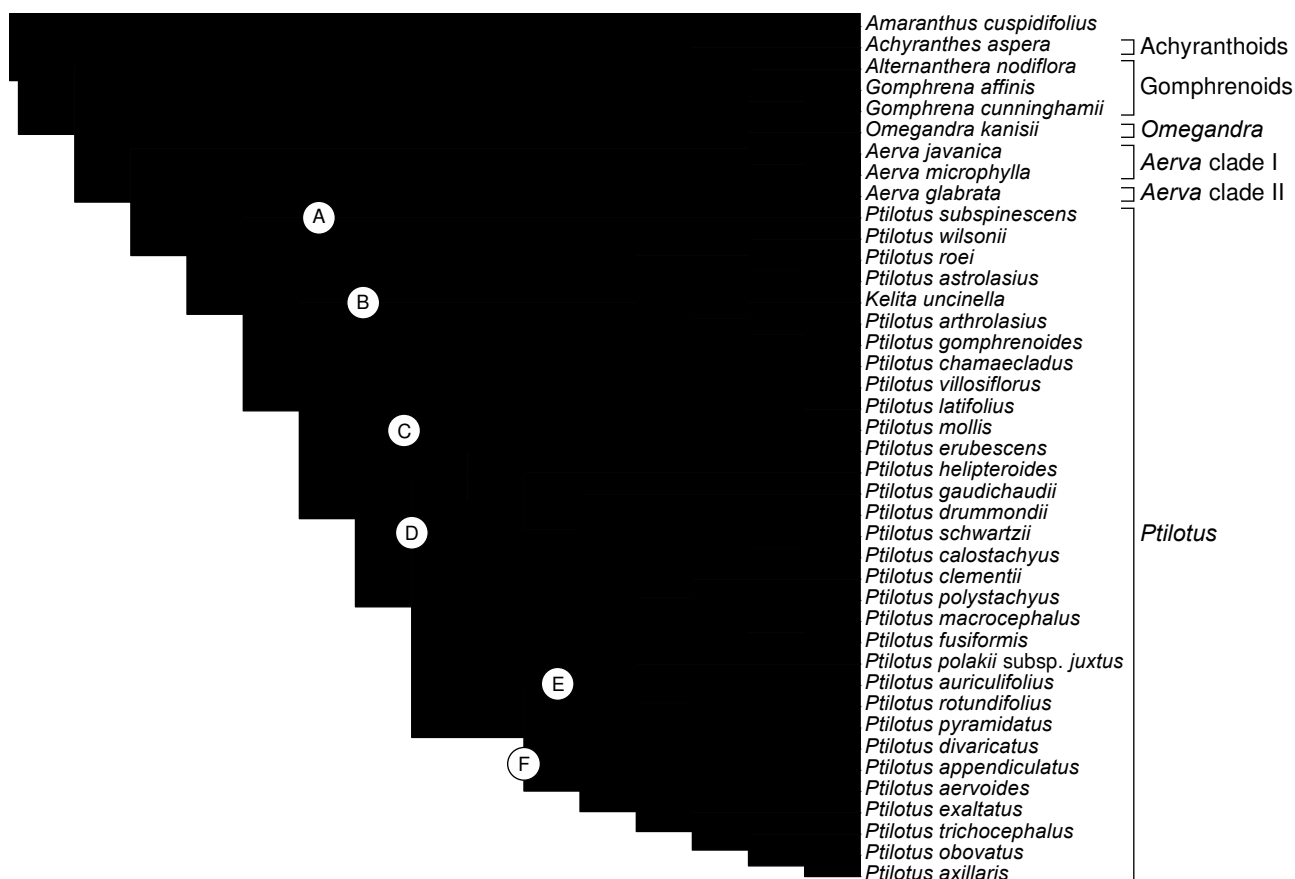


Fig. 2. The MrBayes 50% majority consensus tree of the CDS dataset with support values above the branches (BI posterior probabilities/ML bootstrap percentages). Asterisks (*) indicate maximum support values (1.00 or 100% respectively). Major clades outside of *Ptilotus* indicated on the right and major clades within *Ptilotus* (A–F) are indicated on the branches.

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.

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 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 position 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. *juxta* 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.

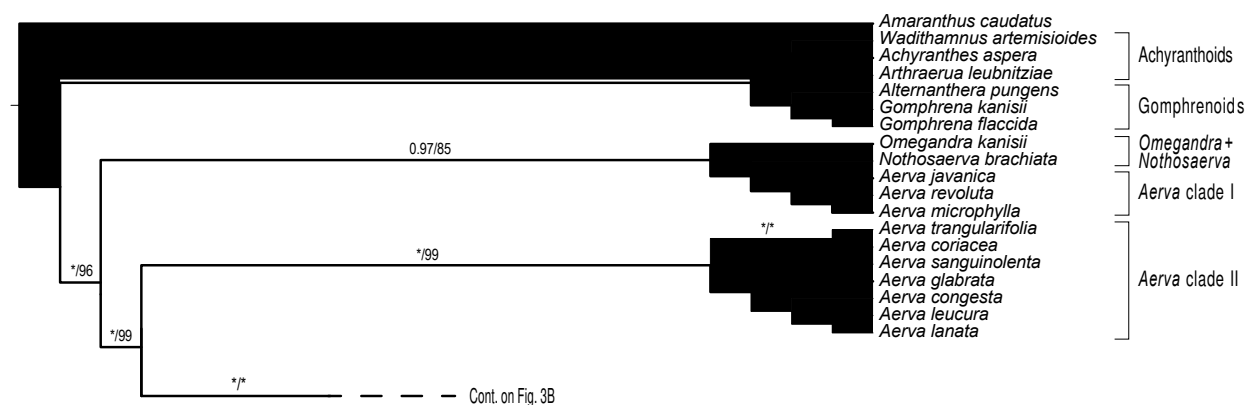
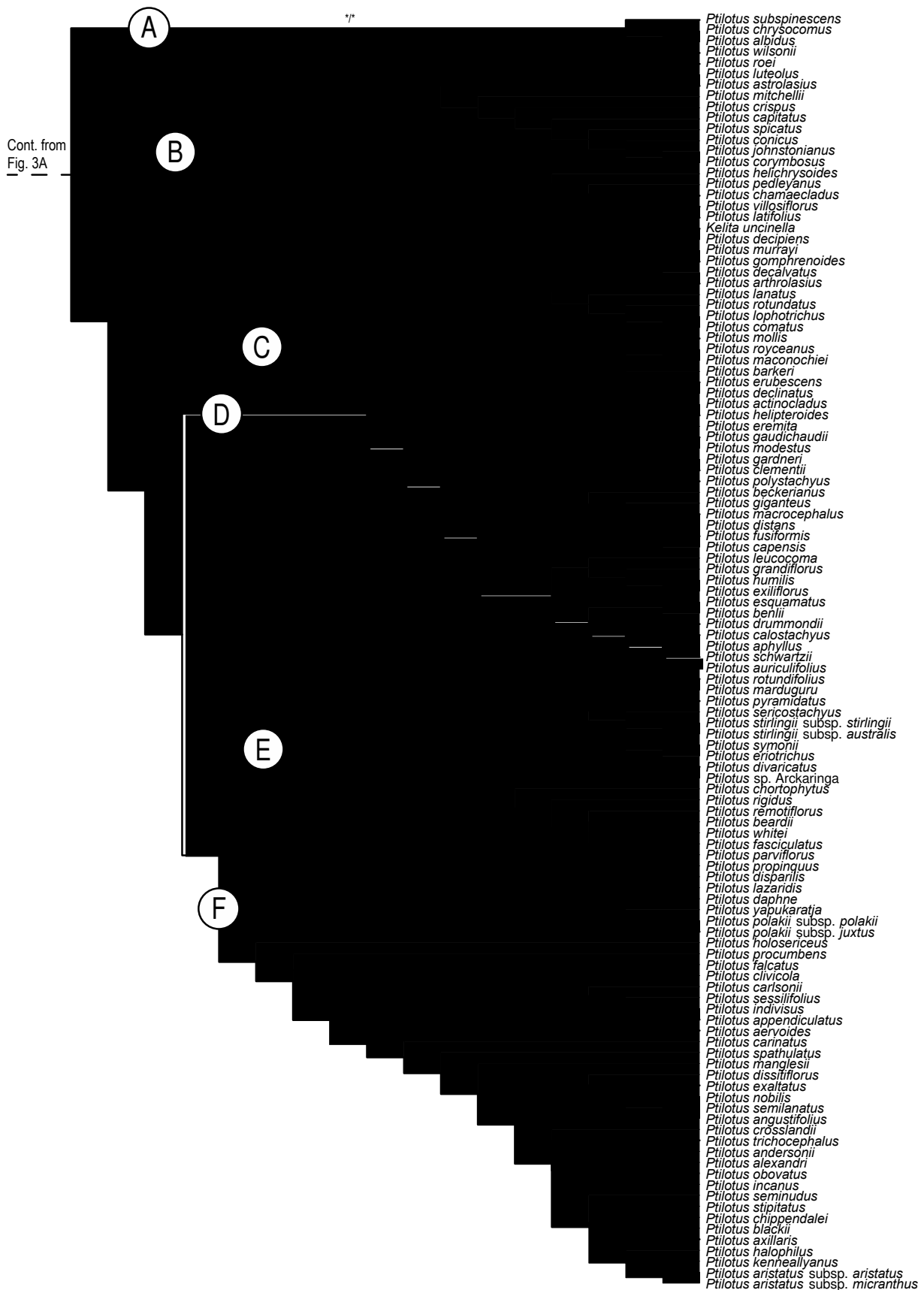


Fig. 3. The MrBayes 50% majority consensus tree of the Sanger ITS+*matK* dataset with support values above branches (BI posterior probabilities/ML bootstrap percentages). **A.** The whole tree with *Ptilotus* collapsed and major clades indicated on the right; **B** (next page), The expanded tree of *Ptilotus* 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.



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. 4D,E), 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 uncinuate 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 uncinuate sepal apices; this is an autapomorphy within the aervoids, and hence uninformative of relationships.

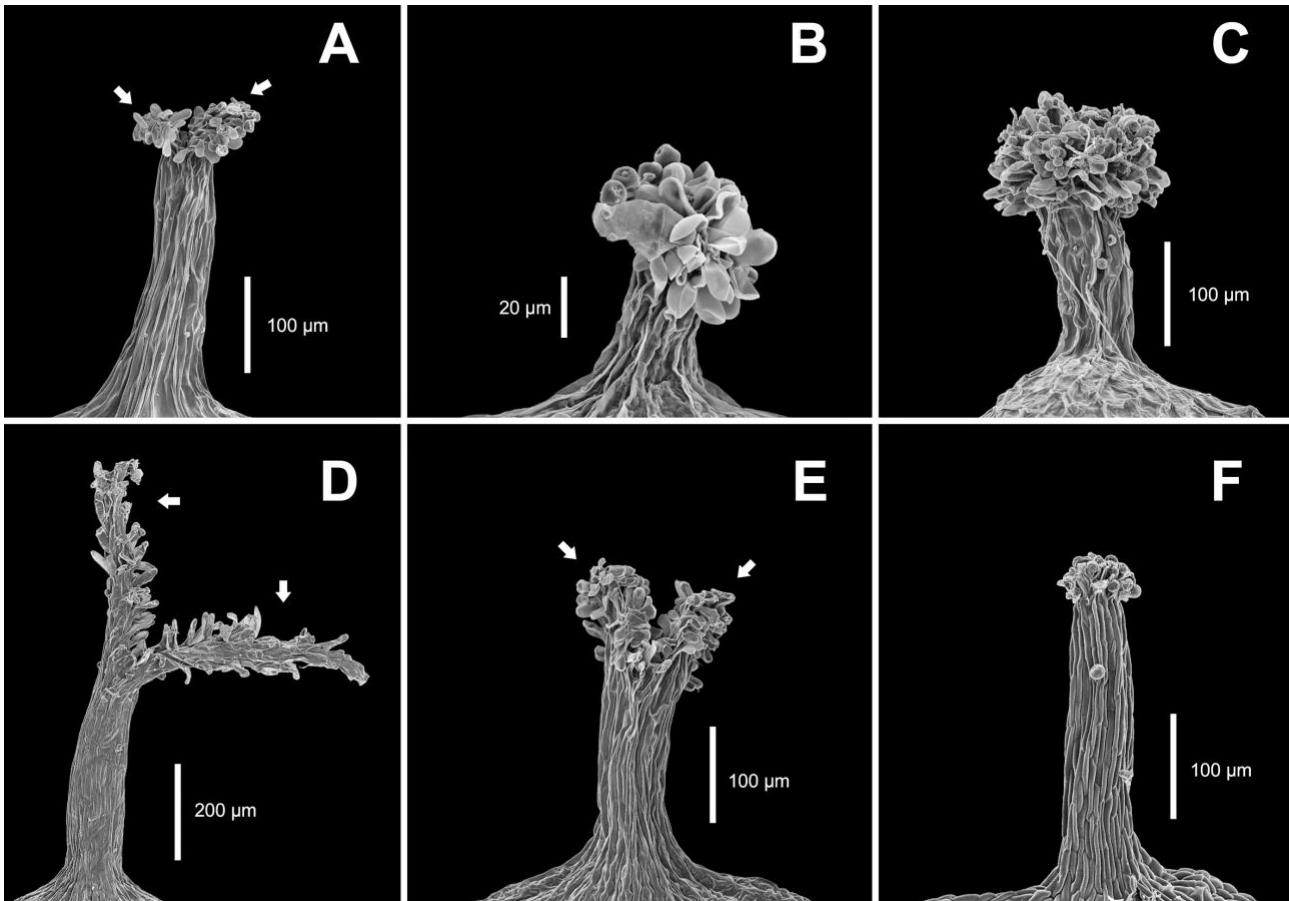


Fig. 4. SEM images of style and stigma morphology in the various clades resolved in the aervoids. **A**, *Omegandra kanisii* (BRI AQ0766448); **B**, *Nothosaerva brachiata* (E 00732828); **C**, *Aerva revoluta* (E 00161902); **D**, *A. javanica* (♀; PERTH 08743924); **E**, *A. lanata* (♀; E 00732832); **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 uncinately apiculate sepals, *P. decipiens* has straight-aristate bracteoles and sepals. Sepals of both species have conspicuous midribs. The uncinately apiculate sepals 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). Uncinately apiculate sepals, 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 paraphyly 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	<i>Omegandra</i>	<i>Nothosaerva</i>	<i>Paraerva</i>	<i>Aerva</i> s.str.	<i>Ouret</i>	<i>Ptilotus</i>
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 Iamónico

& 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). Iamónico & 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. Amaranth.: 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 Iamónico & 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 Iamónico 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 Iamónico 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.rbv.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 Iamónico (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

TAH devised the project with supervision by KRT and IDS. PGN and IDS provided genome sequences and funding for analysis. TAH gathered data, performed the analyses, and interpreted the results. XZ and CCdF aided in gathering and analysis of the genomic data. TAH wrote all sections of the manuscript and produced the figures. XZ and CCdF wrote parts of the methods section. The taxonomy section was written by TAH and KRT. The draft manuscript was revised by PGN, IDS and KRT.

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Appendix 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*
leubnitziae 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* (Farmer)
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* Schldl., 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 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* (Farmer) 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* Schldtl., 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. *juxta* 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. 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, Abraha area, 6 March 1986, *Sheded* 3254 (E: E00161900). ASIA. Iran, Kerman, Esfandagheh to Jiroft, 25 km W to Jiroft (Sabzvaran), 11 June 1977, *Assadi & al.* 1893 (E: E00161898); United Arab Emirates, Abu Dhabi, Wadi Mubarras 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. *juxta* 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).