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Source: Australian Systematic Botany, 35(1) : 1-18

Published By: CSIRO Publishing

URL: https://doi.org/10.1071/SB21005

RESEARCH PAPER
https://doi.org/10.1071/SB21005

Australian Systematic Botany

Resolution of the *Eremophila tietkensii* (Scrophulariaceae) species complex based on congruence between morphological and molecular pattern analyses

Amy L. Curtis[A,B,*], Pauline F. Grierson[B], Jacqueline Batley[B], Jeremy Naaykens[C], Rachael M. Fowler[D], Anita Severn-Ellis[B] and Kevin R. Thiele[A,B]

For full list of author affiliations and declarations see end of paper

***Correspondence to:**
Amy L. Curtis
Western Australian Herbarium, Biodiversity and Conservation Science, Department of Biodiversity, Conservation and Attractions, Locked Bag 104, Bentley Delivery Centre, WA 6983, Australia
Email: amy.curtis@dbca.wa.gov.au

Handling Editor:
David Williams

Received: 12 February 2021
Accepted: 17 November 2021
Published: 2 March 2022

Cite this:
Curtis AL *et al.* (2022)
Australian Systematic Botany
35(1), 1–18. doi:10.1071/SB21005

ABSTRACT

Eremophila R.Br. comprises at least 238 species endemic to Australia, with many more having not yet been formally described. Three putative new taxa, namely, *E.* sp. Hamersley Range (K. Walker KW 136), *E.* sp. Calvert Range (A. A. Burbidge 738) and *E.* sp. Rudall River (P. G. Wilson 10512), were segregated from a broadly defined *E. tietkensii* F.Muell. & Tate by J. Hurter at the Western Australian Herbarium in 2012. Both *E.* sp. Hamersley Range and *E.* sp. Rudall River are listed as being of conservation concern in Western Australia, the former occurring in the Pilbara region in areas of prospective interest for mining development. We sought to determine whether these phrase-named entities should be formally described as new species, using multivariate analyses of morphometric and molecular data derived from specimens in the Western Australia Herbarium. *Eremophila* sp. Rudall River could not be adequately separated from *E. tietkensii* by either morphological or molecular data, and is here included within that species. By contrast, *E.* sp. Hamersley Range and *E.* sp. Calvert Range are clearly morphologically and genetically distinct. We thus describe them here as the new species *E. naaykensii* A.L.Curtis & K.R.Thiele and *E. hurteri* A.L.Curtis & K.R.Thiele. The recognition of these taxa will help inform their conservation prioritisation and subsequent management.

Keywords: congruence, ddRADseq, *Eremophila*, herbarium sampling, morphology, Pilbara, taxonomy, Western Australia.

Introduction

Eremophila R.Br. (Scrophulariaceae) comprises ~238 species and ~58 subspecies of perennial shrubs endemic to arid and semi-arid Australia (Chinnock 2007*a*). Species diversity is highest in Western Australia (WA), where more than 90% of *Eremophila* species occur (Chinnock 2007*a*). Many putative new taxa within *Eremophila* still require formal delimitation and description (see e.g. Brown and Buirchell 2011; Chinnock and Doley 2011; Edginton 2015; Brown and Davis 2016; Buirchell and Brown 2016). Approximately half of these putative taxa are found in WA, and many are listed under federal and state level legislation as rare or threatened (Western Australian Herbarium's FloraBase, see https://florabase.dpaw.wa.gov.au/, accessed 10 February 2021). Reducing uncertainty of these unresolved taxa of *Eremophila* is thus important for determining management actions to protect potentially rare and endangered species. More generally, accurate circumscription and naming of taxa is fundamental to effective conservation and sustainable management (Burgman *et al.* 2000; Wege *et al.* 2015) and to formulate policies to protect genetic and ecological diversity, understand evolutionary processes, and mitigate risks from land-use change and development (Coates *et al.* 2014; Oliveira *et al.* 2017).

Eremophila tietkensii was described by von Mueller and Tate (1890) from specimens collected on the 1889 expedition of William Tietkens in the Northern Territory

(NT; Chinnock 2007*b*). *E. tietkensii* was considered by Ewart and Jarrett (1928) to be a variety of *E. latrobei*. However, Chinnock (2007*b*), after examining the type and resolving some nomenclatural confusion, reinstated *E. tietkensii* at the species level. Chinnock regarded *E. tietkensii* as widespread and distributed from the Cape Range and Carnarvon biogeographic region of WA to the NT border region (Chinnock 2007*b*; Western Australian Herbarium's FloraBase, see https://florabase.dpaw.wa.gov.au/). Substantial variation in leaf morphology across its geographic range led him to suggest that recognition of subspecies may be warranted.

In 2012, three putative taxa occurring within the Pilbara bioregion and adjacent Great Sandy and Little Sandy Desert bioregions in north-west WA were segregated from *E. tietkensii* and given the phrase names *E.* sp. Hamersley Range (K. Walker KW 136), *E.* sp. Rudall River (P. G. Wilson 10512), and *E.* sp. Calvert Range (A. A. Burbidge 738; Western Australian Herbarium's FloraBase, see https://florabase.dpaw.wa.gov.au/; Fig. 1). Of these, *E.* sp. Hamersley Range was listed as Priority 3 and *E.* sp. Rudall River as Priority 2 (Western Australian Herbarium's FloraBase, see https://florabase.dpaw.wa.gov.au/). The third phrase-named taxon, *E.* sp. Calvert Range, was not conservation-listed despite being represented by fewer specimens collected from a more limited geographic range than the other two.

Eremophila sp. Hamersley Range is currently known from the Hamersley subregion of the Pilbara bioregion of north-western Australia and occurs across a geographic range of ~200 km from south of Paraburdoo to north-west of Newman (Western Australian Herbarium's FloraBase, see https://florabase.dpaw.wa.gov.au/). This taxon was segregated on the basis of having one to four flowers per axil, with pedicels 2.5–3 times the length of the flowers, and the ovary being ribbed and with a dense covering of glandular and eglandular hairs (Western Australian Herbarium's FloraBase, see https://florabase.dpaw.wa.gov.au/). *Eremophila* sp. Hamersley Range has been recorded as favouring high parts of the landscape such as breakaways and upper

Fig. 1. (*a*) *Eremophila tietkensii*. Photograph: R. Fowler. (*b*) *E.* sp. Rudall River. Photograph: J. Hurter. (*c*) *E.* sp. Calvert Range. Photograph: A. Brown. (*d*) *E.* sp. Hamersley Range. Photograph: J. Naaykens.

hill slopes (Department of Mines and Petroleum 2016). *Eremophila* sp. Rudall River is known from across a ~400-km range from the east of the Pilbara bioregion to the Great Sandy Desert around the Rudall and Oakover Rivers (Western Australian Herbarium's FloraBase, see https://florabase.dpaw.wa.gov.au/). It occurs on quartzitic scree slopes (Office of the Environmental Protection Authority 2014), and is characterised by persistent leaf bases, coriaceous, ovate leaves, and up to three flowers per axil (Western Australian Herbarium's FloraBase, see https://florabase.dpaw.wa.gov.au/). The third putative taxon, *E.* sp. Calvert Range, is poorly known from only three specimens from an area ~100 km north-west to ~50 km south of Lake Disappointment in the Little Sandy Desert bioregion (Western Australian Herbarium's FloraBase, see https://florabase.dpaw.wa.gov.au/). It was segregated from the other taxa on the basis of having sericeous eglandular hairs on the ovary, the leaf surface being strumose, and having sepals fused at base. Despite its apparently limited range, *E.* sp. Calvert Range was not conservation-listed, partly owing to the remoteness of its range and the likelihood that it has been under-collected. *Eremophila tietkensii sens. str.* (i.e. not including the phrase-named entities) is morphologically variable and overlaps in geographic range and leaf morphology with each of the other taxa (Fig. 1). There has been ongoing confusion in identifications within the *E. tietkensii* complex, with some specimens (e.g. PERTH 08731535, PERTH 08957274, PERTH 06017142, PERTH 06023983, PERTH 06570496, PERTH 06653561) being reassigned from *E. tietkensii* to *E.* sp. Hamersley Range and *vice versa* (Western Australian Herbarium's FloraBase, see https://florabase.dpaw.wa.gov.au/).

Phylogenetic relationships among the taxa in the *E. tietkensii* species complex remain largely unknown. A recent molecular phylogenetic study of *Eremophila* based on chloroplast, mitochondrial and nuclear rDNA (Fowler 2018) included three samples of *E. tietkensii* and one sample of *E.* sp. Hamersley Range; this study concluded that *E. tietkensii* was non-monophyletic. However, the clade in which these samples were placed (which contained representatives from *Eremophila* sections *Eremophila*, *Eremeaea* Chinnock, and *Pulchrisepelae* Chinnock) is poorly resolved overall, with low support values on most nodes (Fowler 2018). Given these uncertainties, the objective of this study was to determine, using a combined morphological and molecular approach, whether *E.* sp. Hamersley Range, *E.* sp. Rudall River and *E.* sp. Calvert Range should be formalised as new taxa or synonymised under *E. tietkensii*.

Materials and methods

Characterisation and quantification of morphometric traits

Morphological measurements of all specimens within the *E. tietkensii* species complex held at the Western Australian Herbarium were assessed. Preliminary examination of herbarium specimens indicated that some *E. tietkensii* specimens had been misidentified and were likely to belong, instead, to one of the putative taxa (confirmed with herbarium experts); these specimens were re-determined accordingly. Morphological characters of 67 specimens of *E. tietkensii*, 18 of *E.* sp. Hamersley Range, 14 of *E.* sp. Rudall River and 6 of *E.* sp. Calvert Range were measured or assessed quantitatively and qualitatively (Table 1).

Leaf surface trichomes were examined using compound and scanning electron microscopy (SEM) to determine their morphology and anatomy. No discernible differences were found between abaxial and adaxial trichomes; so, data from both leaf surfaces were combined. For light microscopy (LM), leaf samples of ~5 × 5 mm were taken from herbarium specimens and placed on a glass slide with sufficient water to rehydrate. The sample was left to soften for 1 min to aid in removal of trichomes, which were scraped onto the slide with forceps and examined under a compound light microscope. For SEM, leaf samples of ~2 × 2 mm were taken from herbarium specimens, coated with gold using a JEOL Smart Coater sputter coater, and imaged using a Philips 505 Scanning Electron Microscope at the Western Australian Herbarium.

Measurements of vegetative, floral and fruit characters were made by hand with a ruler to the nearest 0.5 mm. For all quantitative characters, three measurements per individual were averaged, with ratios calculated before averaging. For leaf characters, the three largest fully developed leaves on each specimen were chosen. Of the 23 characters measured or scored, only those that were assessable on most specimens were included to ensure the number of individuals retained in the final dataset was maximised. The final dataset contained seven morphological characters: L1, L4, L5, L9, L10, L11, and O3 (Table 1). Leaf length (L1) and ratio (shape) characters (L4 and L5) were included, while leaf width (L2) and distance to widest point (L3) were excluded so as to remove logical auto-correlations between the ratios and their base measurements. The same reasoning was used to include petiole width (L9) and ratio of petiole length:width (L10) while excluding petiole length (L8). The apex shapes (L6 and L7) were excluded because they were highly variable within individual on specimens and could not be adequately scored. While potentially informative, comparison of sepal size and indumentum across all specimens was problematic because sepals in members of the *E. tietkensii* complex are accrescent, enlarging significantly during and after flowering, and glabrescent. Consequently, sepal length, width and indumentum were excluded. Density of glandular and eglandular hairs on the ovary, another potentially useful character, was not able to be used because of an insufficient number of specimens with ovaries at a comparable phenological stage; indumentum density was found to vary greatly as the ovary matured. Using mostly vegetative characters meant that a higher

Table 1. Traits measured for morphometric ordination and classification analyses, and description of character states scored for each trait.

Character	State	Type	Code
Leaves			
Average length (mm)	Largest, mature leaves	Continuous	*L1*
Average width (mm)	Largest, mature leaves	Continuous	*L2*
Average distance from leaf axil to widest point of leaf blade (mm)	Largest, mature leaves	Continuous	*L3*
Average leaf length:width		Ratio	*L4*
Average distance from leaf axil to widest point of leaf blade:length		Ratio	*L5*
Apex shape	(0) acute, (1) acuminate, (2) attenuate, (3) mucronate	Multistate	*L6*
Apex	(0) not recurved, (1) recurved		*L7*
Average petiole length (mm)	Largest, mature leaves	Continuous	*L8*
Average petiole width (mm)	Largest, mature leaves	Continuous	*L9*
Average petiole length:width		Ratio	*L10*
Trichomes, abaxial and adaxial	(0) completely septate, (1) half septate with apical cell longer than cells in bottom half	Binary	*L11*
Pedicels			
Average length (mm)	Corolla present, mid-flowering	Continuous	*P1*
Sepals			
Average length (mm)	Corolla present, mid-flowering	Continuous	*S1*
Average width (mm)	Corolla present, mid-flowering	Continuous	*S2*
Average length (mm)	Fruiting	Continuous	*S3*
Average width (mm)	Fruiting		
Density of hairs on margins	(0) very sparse, (1) sparse, (2) moderate, (3) dense	Multistate	*S4*
Apex shape	(0) obtuse, (1) rounded, (2) acute (3) retuse	Multistate	*S5*
Habit			
Plant height (m)	Herbarium specimen sheet	Continuous	*H1*
Plant width (m)	Herbarium specimen sheet	Continuous	*H2*
Ovary			
Density of glandular hairs	(0) absent, (1) sparse, (2) moderate [30% surface area covered], (3) dense [>30% covered]	Multistate	*O1*
Density of eglandular hairs	(0) absent, (1) sparse, (2) moderate [30% surface area covered], (3) dense [>30% covered]	Multistate	*O2*
Sericeous yellow eglandular trichomes	(0) absent, (1) present	Binary	*O3*
Texture	(0) not ribbed, (1) ribbed	Binary	*O4*

proportion of herbarium specimens could be included in analyses; this approach also had the advantage that plant identification can be made in the absence of flowers.

The dataset was analysed using non-metric multi-dimensional scaling (nMDS), principal coordinate analysis (PCoA) ordinations, and unweighted pair-group method with

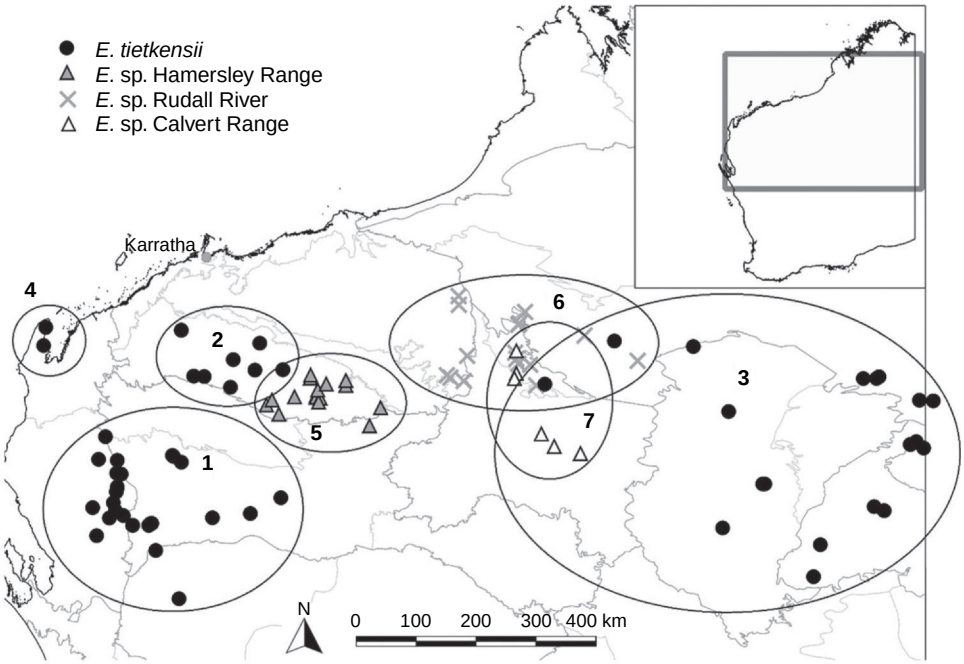

Fig. 2. Collection locations of all PERTH voucher specimens of *Eremophila tietkensii, E.* sp. Hamersley Range, *E.* sp. Rudall River and *E.* sp. Calvert Range in WA. Specimens were assigned to *a priori* groups numbered 1–7.

Table 2. Total number of *Eremophila* specimens used in morphometric and molecular analyses.

Morphometric analysis		Molecular analysis					
Species	Herb. specimens	Morphometrics	Herb. specimens	Additional material	DNA extraction	Sequenced	SNP analysis
E. sp. Hamersley Range	18	18	18	1	19	18	15
E. tietkensii	67	63	67	6	73	54	36
E. sp. Rudall River	14	14	14	1	14	11	10
E. sp. Calvert Range	6	6	6	–	6	6	6
Total	105	101	105	8	109	86	67

Herb., herbaceous.

arithmetic mean (UPGMA) classification in PRIMER (ver. 6.1, see https://www.primer-e.com/; Clarke and Gorley 2006). Differences within and among recovered groups were analysed using ANOSIM, which generates an R statistic with a theoretical range from 0 (indicates a random distribution) to 1 (perfect separation of groups; Clarke and Gorley 2006).

Specimens were assigned to broad geographic groups (Fig. 2) to enable assessment of geographic structuring or partitioning within the widespread *E. tietkensii* complex.

Molecular analysis

In total, 101 herbarium specimens were sampled for a ddRADSeq molecular analysis. Four specimens from the Western Australian Herbarium collections of *E. tietkensii* and the phrase-named taxa were excluded because of insufficient material or poor quality (Table 2). Additional non-vouchered silica-dried material of each species, except *E.* sp. Calvert Range, was provided by B. Buirchell for inclusion in the molecular analysis. As the purpose of this study was to determine species boundaries rather than produce a phylogeny or test evolutionary hypotheses or hierarchies, an outgroup was not included.

Genomic DNA was extracted using the Qiagen DNEasy Plant Minikit (Qiagen, Hilden, Germany) following the manufacturer's protocol modified in the following ways: at Step 2, 1.35 mL of Buffer AP1, in addition to 1 μL of dithiothreitol for every 1 mL of Buffer AP1, and 4 μL of RNase A was

added to each tube containing 80 mg of plant material, vortexed, then samples were incubated for 30 min at 65°C; and at Step 11, 40 µL of Buffer AE heated to 65°C was added to each spin column filter for elution and then incubated at room temperature (15–25°C) for 10 min.

DNA concentration was quantified with a Qubit dsDNA BR Assay Kit and a Qubit 3.0 Fluorometer (Invitrogen, Carlsbad, CA, USA). Fragment lengths were checked with a LabChip GX Touch 24 Nucleic Acid Analyser using the HT DNA gDNA reagents (Perkin and Elmer, Waltham, MA, USA). Of the 109 samples, 86 were of suitable quality to be used for library preparation.

Library preparation followed the procedure in Severn-Ellis *et al.* (2020). DNA extracts were diluted to 300-ng concentration per sample with nuclease-free water and digested with 5 units of HpyCH4IV restriction enzyme (New England Biotechnologies (NEB), Ipswich, MA, USA), and 5 U of HinfI restriction enzyme (NEB) per sample and 3 µL of the master mix was added to each sample. Barcoded and common adapters designed to complement the pairs of restriction enzyme overhangs were prepared as described by Peterson *et al.* (2012). Ligated fragments were cleaned of excess, unligated adapters, and fragments within the range of 250–800 base pairs were selected using Ampure XP beads (Beckman Coulter, Brea, CA, USA) and enriched by polymerase chain reaction (PCR) amplification by using Phusion Hot-Start High-Fidelity Polymerase Master Mix (Thermo Fisher Scientific, Waltham, MA, USA).

Pooled libraries were cleaned (Ampure XP beads), quantified with Qubit and visualised and quality-checked using the LabChip GX Touch 24 with HT DNA HiSens Dual Protocol Reagents. Molarity of the final library was calculated and 50–100 µL of the ddRAD bead cleaned library was diluted to a final concentration of 10–20 nM by using normalisation buffer. The pooled ddRAD libraries were sequenced (2 × 150-bp reads) across two Illumina HiSeq X lanes at the Kinghorn Centre for Clinical Genomics (KCCG) Sequencing Laboratory in Darlinghurst, New South Wales, Australia.

Sequence data analysis

De novo assembly of RAD loci and single-nucleotide polymorphism (SNP) calling was performed following the bioinformatics workflow described in Severn-Ellis *et al.* (2020, see https://github.com/ascheben/RAD_analysis_workflow/). Pooled data were de-multiplexed using Stacks (ver. 2.53, see https://catchenlab.life.illinois.edu/stacks/) process_radtags (Catchen *et al.* 2013), with barcode rescue (-r), quality filtering (-q, -c) and RAD tag checks (–renz_1 HpyCH4IV –renz_2 HinfI). Low-quality reads were discarded and reads were trimmed (144 bp) with Trimmomatic (ver. 0.39, see http://www.usadellab.org/cms/index.php?page=trimmomatic; Bolger *et al.* 2014). Quality checks using FastQC (ver. 0.11.9, see https://www.bioinformatics.babraham.ac.uk/projects/fastqc/; Andrews 2010) were conducted, followed by a preliminary diversity assessment based on pairwise distances using Mashtree (ver. 0.37, see https://metacpan.org/dist/Mashtree; Katz *et al.* 2019), whereafter individuals with extremely low genotyping rates were removed. *De novo* assembly of RAD loci and SNP calling was performed using Stacks (ver. 2.53; Catchen *et al.* 2013). For high levels of intraspecific divergence, it has been recommended that n = M in the Stacks software (Paris *et al.* 2017), and was considered appropriate for the present study. Stacks parameters of M-3, m-3, and n-3 were chosen following experimentation with different parameter settings (Paris *et al.* 2017) rendering the highest number of polymorphic loci in 80% of the populations studied.

Further filtering with VCFtools (ver. 0.1.16, see https://vcftools.github.io/; Danecek *et al.* 2011) was executed to remove indels and retain only biallelic, high-quality SNPs. The minor allele frequency (MAF) filters rare alleles, and the threshold was set at 0.05. The filters that remove the most SNPs are usually depth (−minDP) and the missingness (max-missing) filters. For heterozygous samples, read depths ≥5 have been suggested to avoid undercalling of heterozygous genotypes (Maruki and Lynch 2017; Bilton *et al.* 2018). Depth and missingness filters were fine-tuned to find a balance between the quantity and quality of SNPs. Read depths of ≥3 (DP3) and ≥5 (DP5) were evaluated and a read depth of 5 (MAF = 0.05) was chosen. Missingness was maintained at 90% across samples (max-missing = 0.8). Finally, individuals with low genotyping rates (missingness >50%) based on the filtered SNPs were removed.

A principal component analysis (PCA) was conducted using the gdsfmt, SNPRelate, gridExtra, and ggrepel packages in R (RStudio, Inc., Boston, MA, USA) to visualise genetic diversity using detected SNPs and to assess whether samples formed clusters congruent with the results of the morphometric analyses.

Results

Key morphometric differences among taxa

Examination of abaxial and adaxial leaf trichomes by using both SEM and LM showed that trichomes of *Eremophila* sp. Hamersley Range were substantially different from those of the other three taxa in the complex (Fig. 3*a*). Trichomes in *E.* sp. Hamersley Range were distinctly septate with six to eight cells of approximately equal length, with the terminal cell with a rounded tip (Fig. 3*a–b*, 4*f–i*). By contrast, *E. tietkensii* trichomes had four to five cells per trichome, with the terminal cell comprising half to one-third of the total length of the trichome and being attenuate at the apex (Fig. 3*c–d*, 4*a–e*). Trichomes in *E.* sp. Rudall River (Fig. 3*e–f*, 4*j–k*) and *E.* sp. Calvert Range (Fig. 3*g–h*, 4*l–m*) were similar to those of *E. tietkensii* but were slightly longer (up to 350 µm in *E.* sp. Rudall River and 300 µm in *E.* sp. Calvert Range, compared with 250 µm in *E.* sp. Hamersley Range

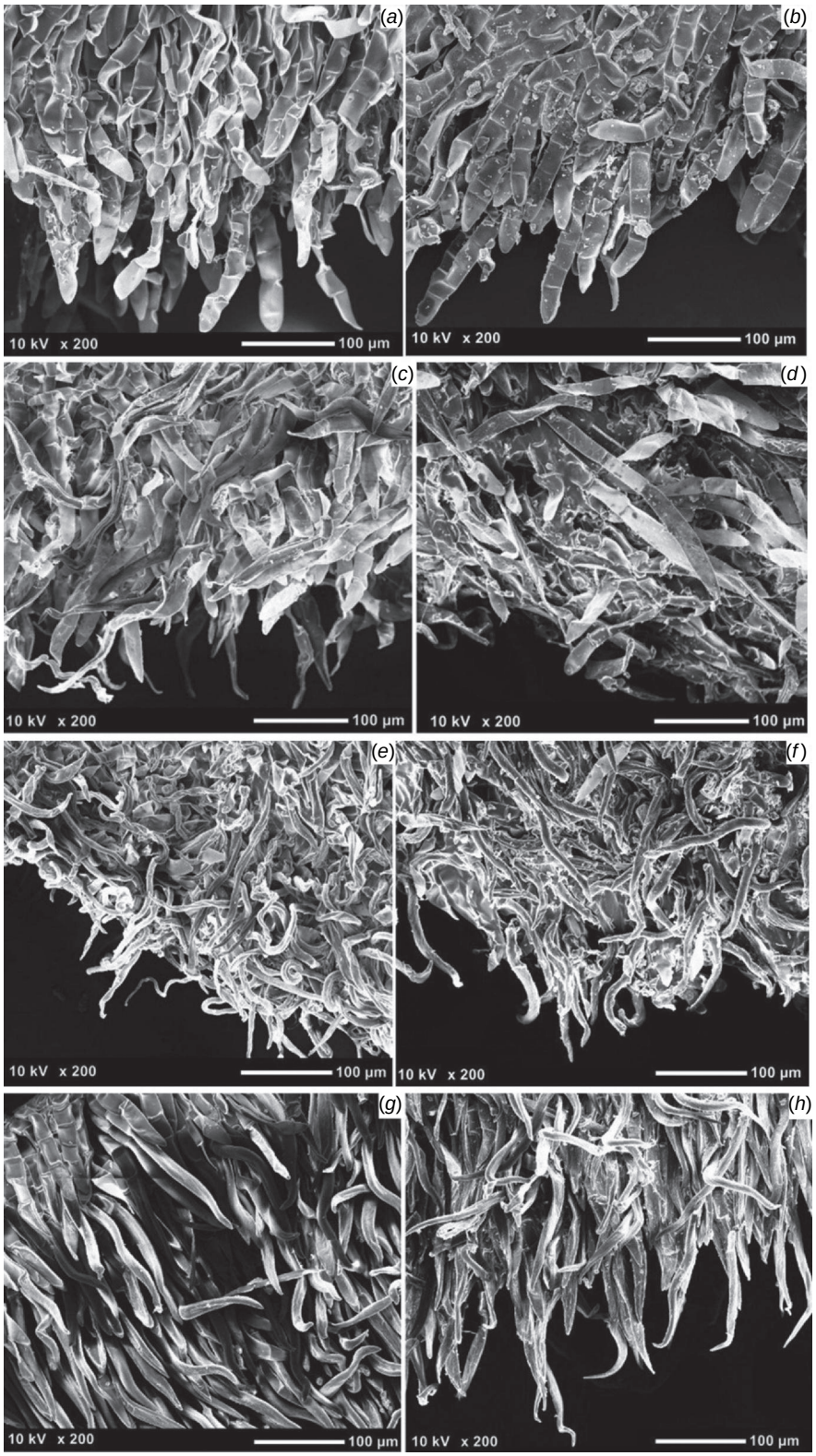

Fig. 3. Scanning electron microscopy (SEM) images of adaxial trichomes on leaves of *Eremophila* sp. Hamersley Range: (*a*) PERTH 03557464, (*b*) PERTH 09105972; *E. tietkensii*: (*c*) PERTH 03856275, (*d*) PERTH 03899918; *E.* sp. Rudall River: (e) PERTH 03878759, (*f*) PERTH 04201159; and *E.* sp. Calvert Range: (*g*) PERTH 03878570, (*h*) PERTH 07512821.

and *E. tietkensii*) and narrower (12–25 µm wide in *E.* sp. Rudall River and 10–22 µm wide in *E.* sp. Calvert Range, compared with 20–30 µm in *E.* sp. Hamersley Range and *E. tietkensii*), and tended to have slightly longer terminal cells. Overall, *E.* sp. Hamersley Range was the only taxon that could be distinguished on the basis of trichomes; the remaining three taxa differed only slightly and were highly variable.

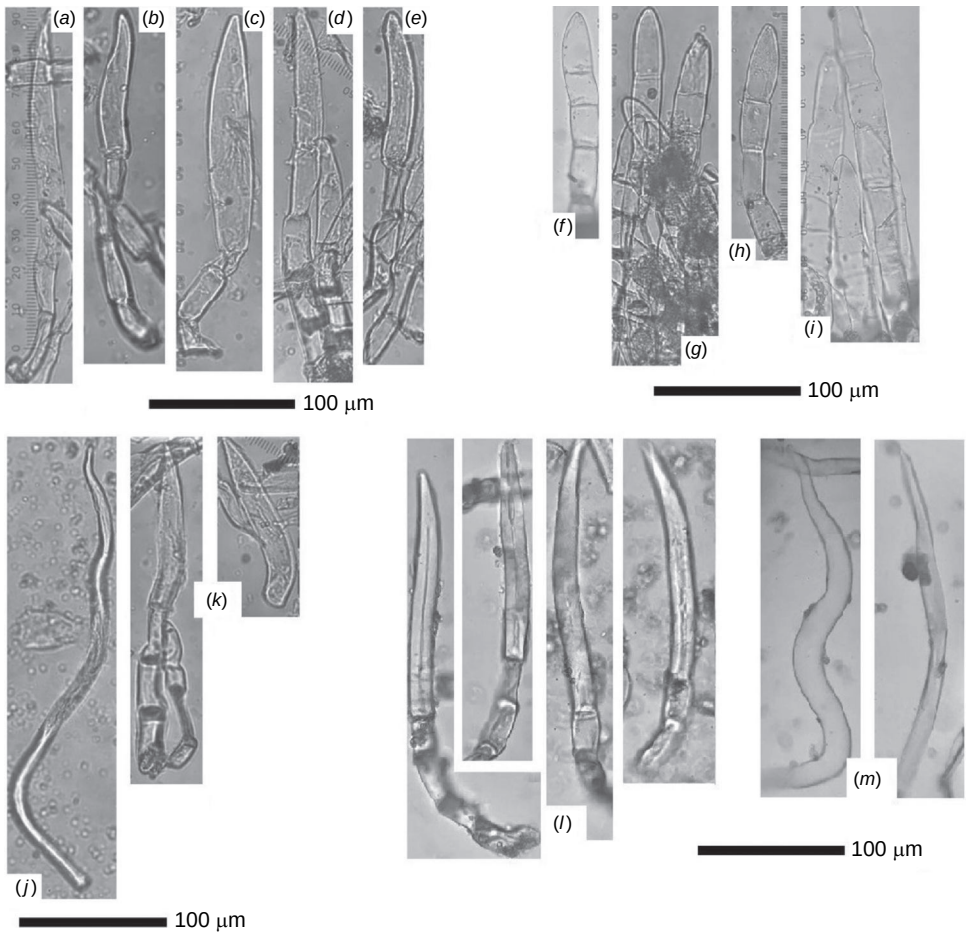

Fig. 4. Trichomes under compound microscope at 400× of *Eremophila tietkensii*: (*a*) PERTH 08316899, (*b*) PERTH 03881202, (*c*) PERTH 06752519, (*d*) PERTH 03851281, (e) PERTH 07324545; *E.* sp. Hamersley Range: (*f*) PERTH 06653537, (*g*) PERTH 0653561, (*h*) PERTH 06017142, (*i*) PERTH 8521; *E.* sp. Rudall River: (*j*) PERTH 08305447, (*k*) PERTH 03878740; and *E.* sp. Calvert Range: (*l*) PERTH 07765886, (*m*) PERTH 07512821.

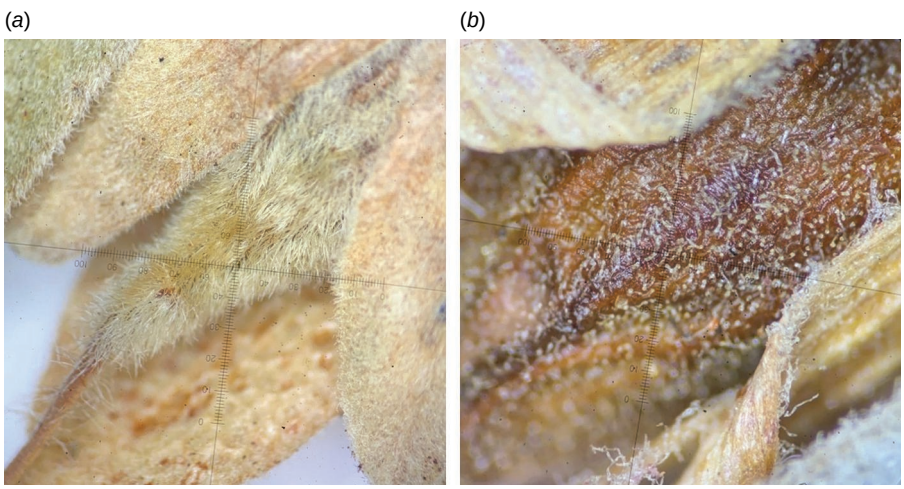

Fig. 5. Ovary indumentum of (*a*) *Eremophila* sp. Calvert Range, showing sericeous eglandular trichomes; and (*b*) *E.* Hamersley Range, showing glandular and eglandular trichomes.

Eremophila sp. Calvert Range had a sericeous eglandular indumentum on the ovary that is distinct from that of the other three species (Fig. 5*a*). Ovaries in *Eremophila* sp. Hamersley Range had a sparse to moderate covering of glandular and eglandular hairs (Fig. 5*b*); those in *E.* sp. Rudall River mostly lacked eglandular hairs and had a

moderate covering of glandular hairs, and, in *E. tietkensii*, ovaries mostly lacked eglandular hairs and had a moderate to dense covering of glandular hairs.

Morphometric groupings of taxa

Non-metric multidimensional scaling (nMDS) and UPGMA clustering showed that *Eremophila* sp. Hamersley Range and *E.* sp. Calvert Range are morphologically distinct from *E. tietkensii* and *E.* sp. Rudall River; however, each taxon is morphologically closer to *E. tietkensii* than to each other (Fig. 6*a*). Whereas, overall, *E. tietkensii* and *E.* sp. Rudall River formed somewhat separate groups, some *E. tietkensii* individuals were morphologically more similar to *E.* sp. Rudall River than they were to other *E. tietkensii* specimens. *Eremophila tietkensii* showed high levels of morphological

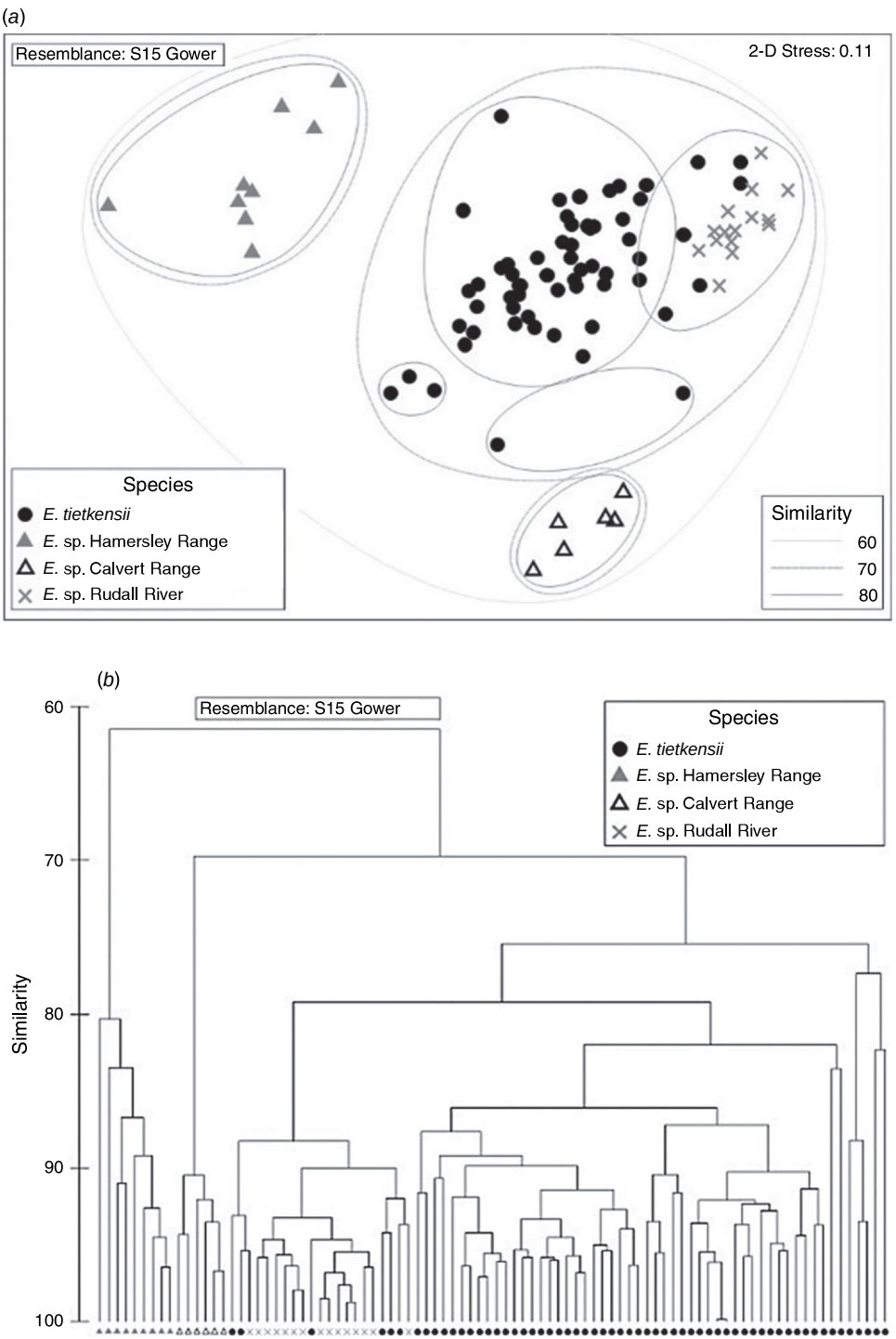

Fig. 6. (*a*) Non-metric multi-dimensional scaling (nMDS) ordination of all individuals on the basis of selected traits (Table 1) overlain with groupings from cluster analysis (*b*); and (*b*) UPGMA classification of all individuals on the basis of selected traits (Table 1).

variability, with several individuals forming groups away from the rest of *E. tietkensii* when an 80% similarity threshold based on the UPGMA classification was applied (Fig. 6*b*). *Eremophila* sp. Hamersley Range and *E.* sp. Calvert Range each clustered as one group both at the 70 and 80% similarity threshold (Fig. 6*a*). *Eremophila* sp. Hamersley Range and *E.* sp. Calvert Range formed distinct groups in the UPGMA classification (Fig. 6*b*). *Eremophila* sp. Rudall River and *E. tietkensii* formed weakly separated groups, with several individuals of *E. tietkensii* not being sufficiently distinguished from *E.* sp. Rudall River to join the larger *E. tietkensii* group (Fig. 6*b*).

Pairwise ANOSIM tests between *Eremophila* sp. Hamersley Range and *E.* sp. Calvert Range and between *E.* sp. Calvert Range and *E.* sp. Rudall River returned an R statistic of 1, indicating a complete separation of groups on the basis of morphological characters (Table 3). Comparisons of *Eremophila tietkensii* and *E.* sp. Hamersley Range ($R = 0.96$, $P < 0.001$), and *E. tietkensii* and *E.* sp. Calvert Range ($R = 0.88$, $P < 0.001$), also showed high separation between groups (Table 3). The weakest separation of groups was between *E. tietkensii* and *E.* sp. Rudall River ($R = 0.5$, $P < 0.001$), as observed in the ordination (Fig. 6*a*). When *E. tietkensii* and *E.* sp. Rudall River were analysed separately from the rest of the complex, the strength of separation of groups increased only slightly ($R = 0.52$, $P < 0.001$, Table 3).

When considering only *Eremophila tietkensii* and *E.* sp. Rudall River, the *E.* sp. Rudall River group was more similar to the larger *E. tietkensii* group at the 60% similarity threshold than five *E. tietkensii* individuals (PERTH 08317178, PERTH 08332215, PERTH 06752519, PERTH 03975320, PERTH 08316937; Fig. 7). At the 80% similarity threshold, two *E.* sp. Rudall River specimens were more similar to *E. tietkensii* than to each other. Individuals from the same geographic location did not cluster together, indicating that morphological characters were not correlated with geographical location (Fig. 7). Geographic Groups 1 and 3 clustered together at the 70% similarity threshold despite Group 3 being ~1000 km east of Group 1.

The five *Eremophila tietkensii* individuals that clustered with *E.* sp. Rudall River had unusually short and wide leaves. One specimen (PERTH 08332215) occurred on the border of the *E.* sp. Rudall River population, and in the classification and ordination based on morphology was grouped with *E.* sp. Rudall River; it may have been misidentified initially. One specimen (PERTH 03975320) was the southernmost occurring *E. tietkensii* individual, ~625 km from the nearest *E.* sp. Rudall River populations. Two specimens (PERTH 08316937 and PERTH 08317178) had a more compact habit than did the other three. They were collected 7 years apart and located ~1 km from each other, but ~500 km away from the nearest *E.* sp. Rudall River populations. One specimen (PERTH 06752519) occurred even closer to the *E. tietkensii* geographic Group 1, some ~585 km from the nearest *E.* sp. Rudall River populations. These four specimens are unlikely to be misidentifications of *E.* sp. Rudall River; even if some specimens were reassigned from *E. tietkensii* to *E.* sp. Rudall River and *vice versa*, the morphological separation between these two entities was found to be weak.

No clear structure was found in the principal coordinate analysis (PCoA) performed on *Eremophila tietkensii* only. Axis 1 (PCo1) explained 54.9% of the variation and was correlated mainly with mean leaf and petiole length. Axis 2 (PCo2) explained 19.6% of the variation and was correlated with the distance to widest point:length ratio (Fig. 8). Despite the wide geographic range of the samples, there was no clear separation into geographically distinct morphotypes.

Table 3. ANOSIM R statistic and *P*-values of global and pairwise tests comparing *Eremophila* taxa on the basis of the morphometric dataset using 999 permutations.

Test and taxon		R statistic	*P*-value
Pairwise test between taxa			
E. sp. Calvert Range	*E. tietkensii*	0.88	0.001
E. sp. Calvert Range	*E.* sp. Hamersley Range	1	0.001
E. sp. Calvert Range	*E.* sp. Rudall River	1	0.001
E. tietkensii	*E.* sp. Hamersley Range	0.96	0.001
E. tietkensii	*E.* sp. Rudall River	0.50	0.001
E. sp. Hamersley Range	*E.* sp. Rudall River	1	0.001
Global test		0.78	0.001
Global test and pairwise test between taxa			
E. tietkensii	*E.* sp. Rudall River	0.521	0.001

Molecular analysis

Dried and carefully preserved herbarium material was found to be a generally excellent source of DNA material for ddRADSeq analysis. There was no clear correlation between the age of herbarium specimen and the quality or quantity of DNA suitable for use in the molecular analysis following demultiplexing and trimming (Supplementary Fig. S1). The two oldest samples that passed filtering and quality checks and were used in the SNP analysis were collected in 1941.

A total of 1 851 822 978 sequence reads were generated, with 1 712 241 716 remaining following demultiplexing. After trimming, 1 511 865 102 reads remained. The total number of variant sites before filtering was 14 164 739. After removing samples with >90% SNPs missing, 67 of 78 individuals remained, and after filters were applied, 39 396 variant sites remained. Finally, when a single SNP from each ddRAD locus based on the distance between SNPs

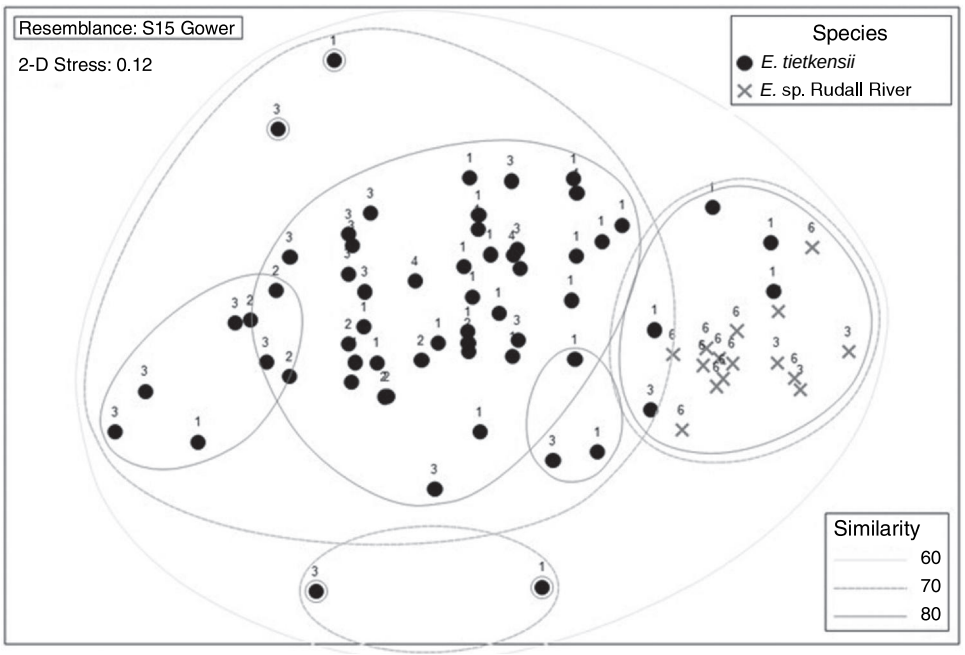

Fig. 7. Non-metric multi-dimensional scaling (nMDS) ordination of *Eremophila tietkensii* and *E.* sp. Rudall River individuals with pedicels present overlain with groupings from cluster analysis. Numbers indicate geographic areas of individuals as shown in Fig. 2.

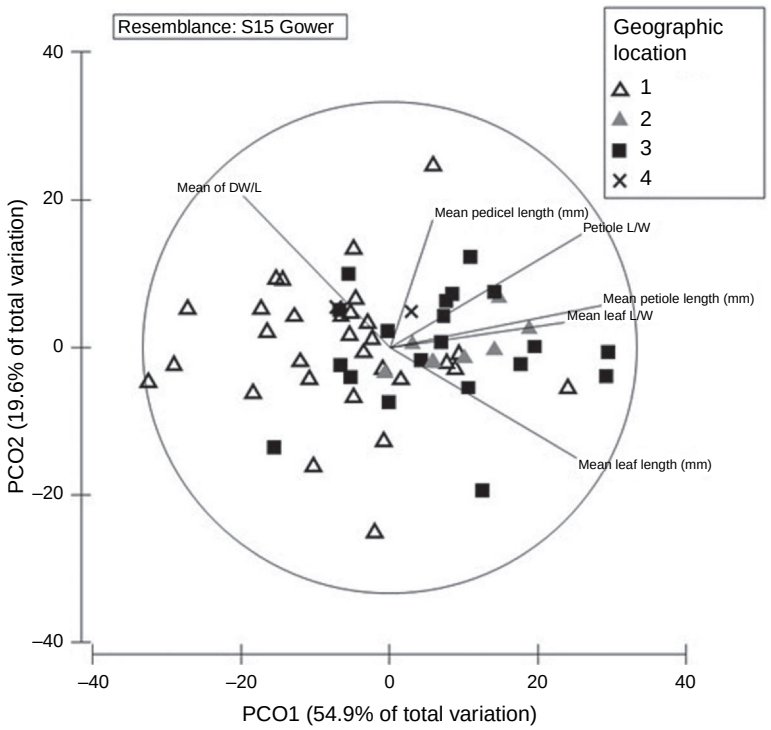

Fig. 8. Principal coordinate analysis (PCoA) for six traits of all *Eremophila tietkensii* individuals. Numbers represent geographic groups as per Fig. 2.

was randomly kept and samples with over 50% missing SNPs were removed, all 67 individuals remained, with a total of 7032 SNPs.

The first two components of the PCA based on SNPs explained 46.5% of the variation (Fig. 9). The broad geographic groupings within *Eremophila tietkensii* were recovered on the PCA ordination, except that *E.* sp. Rudall River sat between and mixed with *E. tietkensii* geographic Groups 2 and 3 and was not genetically divergent from them (Fig. 9). The *E. tietkensii* specimen from Exmouth (Group 4)

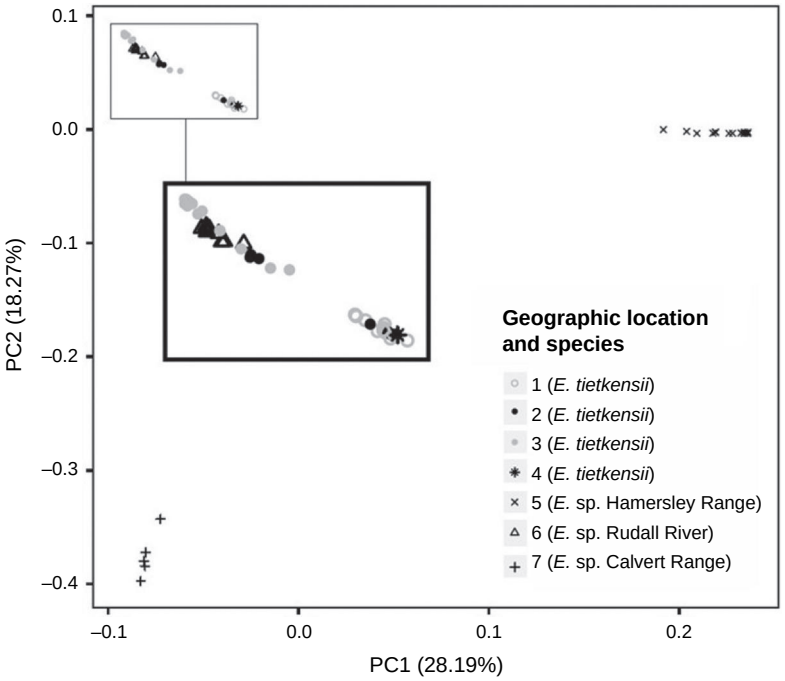

Fig. 9. Principal component analysis of detected SNPs of *Eremophila tietkensii, E.* sp. Hamersley Range, *E.* sp. Calvert Range, and *E.* sp. Rudall River. Inset shows positions of *E. tietkensii* and *E.* sp. Rudall River specimens.

clustered with geographic Groups 1 and 2 and did not appear to be genetically differentiated. *Eremophila tietkensii* Group 2 clustered with Groups 1 and 3, despite Group 3 being very geographically distant. *Eremophila* sp. Hamersley Range and *E.* sp. Calvert Range were strongly genetically segregated from each other and from the rest of *E. tietkensii* and *E.* sp. Rudall River, despite their close geographical proximity to all groups (Fig. 9).

Discussion

Congruence between morphology and molecular patterns of variation

There is a high degree of congruence between patterns of morphological and molecular variation in the *Eremophila tietkensii* species complex. Morphological variation resolved three groups, comprising *E.* sp. Hamersley Range, *E.* sp. Calvert Range and *E. tietkensii* (including *E.* sp. Rudall River). The same three groups were strongly resolved in the SNP analysis. *Eremophila* sp. Rudall River could not be adequately segregated from *E. tietkensii* in either the morphological or molecular analysis and resolved between *E. tietkensii* geographic Groups 2 and 3 either genetically or geographically. While the morphometric analysis did not resolve geographic structuring in morphology in *E. tietkensii*, there was distinct geographical patterning between the *a priori* geographic groups on the basis of the molecular analysis (Fig. 9). The genetic patterning was not strong enough to indicate that *E. tietkensii* could be reasonably taxonomically split on the basis of geography; the spread of molecular variation across its range was slightly but not substantially greater than for the other taxa, indicating that gene flow, altough restricted, is sufficient to have prevented allopatric speciation. The lack of consistency in morphological types of *E. tietkensii* within geographic groups, in conjunction with the genetic structuring among geographic groups, suggests that differences in morphology may be the result of phenotypic plasticity. Detailed analysis of substrate, habitat and underlying geology may explain differences in phenotypic variation and drivers of species distributions, because these factors have been used to explain variation and distribution in other *Eremophila* species (Coates *et al.* 2014).

Eremophila sp. Hamersley Range was genetically highly divergent from *E. tietkensii* despite its close geographic proximity. Although this analysis was unable to estimate time since divergence our results suggest that these two taxa have been genetically isolated for a long period of time. A growing number of studies of evolutionary history and distribution patterns of Pilbara biota are showing similar patterns of divergence between species (e.g. Anderson *et al.* 2016). Since the formation of the Great Sandy and Gibson deserts <1 million years ago, the Pilbara region has been separated from similar landforms by extensive sand plains (Pepper *et al.* 2013*b*). Species ranges contracted and expanded throughout this time, including those of *Acacia* spp. (Ladiges *et al.* 2006), *Triodia* spp. (Anderson *et al.* 2016), reptiles (Doughty *et al.* 2011; Pepper *et al.* 2013*a*), beetles (Guthrie *et al.* 2010) and spiders (Durrant *et al.* 2010). Owing to the topographical heterogeneity of the

Pilbara compared with surrounding low-lying plains, localised areas within the region were likely to be more climatically stable, acting as refugia for species that could not otherwise persist in the arid conditions outside of these environments (Byrne *et al.* 2016, 2017). These processes may have contributed to the high level of genetic divergence between *E.* sp. Hamersley Range and *E. tietkensii*.

The resolution of the *Eremophila tietkensii* complex provided here will allow the taxa to be included in future phylogenetic studies where reconstruction of patterns and timing of divergence among lineages can be inferred, providing insights into the evolutionary history of species in north-western Australia. These results will also assist in biological surveys, because these species can now be accurately discriminated, and population studies can be undertaken. We also note here the largely untapped potential of herbarium material for ddRADSeq molecular analyses for such studies. DNA extraction from herbarium material is often considered to be a supplement to field sampling (Beck and Semple 2015). We found that the quality of DNA obtained for molecular analyses was excellent in carefully stored specimens and not strongly correlated with specimen age. This observation is also consistent with those of recent similar studies elsewhere (Bakker *et al.* 2016; Forrest *et al.* 2019; Joyce *et al.* 2021). Overall, we have demonstrated that even without additional sampling, species delimitation can be achieved in an accurate, efficient and cost-effective manner by using ddRADseq to sample the genomes of herbarium specimens.

Taxonomy

Taxonomic implications

The congruence between morphological and molecular patterns of variation, together with discriminatory characters, allows the recognition of *Eremophila* sp. Hamersley Range and *E.* sp. Calvert Range as morphologically and genetically distinct from *E. tietkensii*. These taxa occur in close geographic proximity and are likely to have the opportunity to interbreed. Their clear genetic and morphological separation indicates that they do not do so. This, in turn, indicates that they are likely to comprise independently evolving metapopulation lineages without significant gene exchange. Therefore, we believe that they should be recognised as distinct species under the unified species concept (de Queiroz 2007). By contrast, the similar morphology and lack of clear genetic separation between *E. tietkensii* and *E.* sp. Rudall River indicates that the latter cannot be recognised at species rank and should be absorbed back into *E. tietkensii*.

Accordingly, below we formally name and describe as new species *Eremophila naaykensii* A.L.Curtis & K.R.Thiele and *E. hurteri* A.L.Curtis & K.R.Thiele, and circumscribe *E. tietkensii* to include specimens currently phrase-named as *E.* sp. Rudall River.

Key to species of *Eremophila* section *Eremophila* (amended from Chinnock 2007*a*)

1. Leaves linear to linear-oblanceolate or lanceolate.....2
   Leaves ovate to obovate or oblanceolate.....8
2. Sepals separated at base.....115. *E. oppositifolia*
   Sepals imbricate at base.....3
3. Outside surface of sepals glabrous; branches and leaves green, pseudoglabrous, with hairs usually completely obscured by resin.....117. *E. cryptothrix*
   Outside surface of sepals pubescent; branches and leaves grey to grey-green, hairs obvious.....4
4. Sepals <6.5 mm long; corolla 5.5–7.5 mm long (Qld).....114. *E. arbuscula*
   Sepals and corolla >7 mm long (WA).....5
5. Leaves linear-oblanceolate, channelled, <3 mm wide, very prominently tuberculate.....122. *E. mirabilis*
   Leaves lanceolate to oblanceolate, flattened, >7.5 mm wide or if narrower, not prominently tuberculate.....6
6. Flowers 1–4 per axil; corolla lilac, white, yellow, pale blue, pink or mauve, anthers included.....6a
   Flowers 1 per axil; corolla cream or pink; anthers usually extending beyond throat.....7

6a. Leaf indumentum comprising simple, uniseriate hairs, the terminal cell much longer than the others and usually attenuate.....6b
   Leaf indumentum comprising simple, uniseriate hairs that are evenly septate, the terminal cell no longer than the others and with a bluntly rounded tip.....*E. naaykensii*

6b. Ovary densely glandular-puberulous with scattered or numerous longer eglandular hairs, style glabrous or with a few scattered, simple, spreading, short eglandular hairs.....*E. tietkensii*
   Ovary densely sericeous with yellow, simple, eglandular hairs; style with sparse, long spreading, eglandular hairs for most of its length.....*E. hurteri*

7. Hairs on vegetative parts matted, posterior sepal oblong to oblanceolate, broadly acute.....124. *E. macmilliana*
   Hairs on vegetative parts not matted, posterior sepal similar to anterior pair, widely ovate to suborbicular or sepals subequal, oblanceolate, obtuse.....123. *E. platycalyx*
8. Corolla white or cream sometimes tinged bluish-green on lobes.....9
   Corolla pale blue, blue, pale lilac, violet, pale mauve, pink or white-tinged lilac.....11
9. Fruit glabrous; corolla white.....116 *E. reticulata*
   Fruit pubescent; corolla cream sometimes tinged bluish-green.....10
10. Leaves very widely depressed, ovate, obtuse, very rigid; ovary/fruit with eglandular hairs.....120. *E. rigida*
    Leaves obovate, acute, flexible; ovary/fruit with eglandular hairs.....123. *E. platycalyx*
11. Leaves subopposite to opposite, widely depressed ovate, flabellate or spathulate (SA; NT).....118. *E. rotundifolia*
    Leaves alternate or irregularly opposite, ovate, spathulate or oblanceolate (WA).....12
12. Branches sulcate; sepals unequal, outer 3 broader than inner pair, oblanceolate to obovate; flowers 1, rarely 2, per axil.....119. *E. spathulata*
    Branches non-sulcate; sepals subequal, elliptic to oblanceolate; flowers 2–4 per axil.....121. *E. tietkensii*

Eremophila tietkensii F.Muell & Tate, *Trans. Proc. & Rep. Roy. Soc. S. Australia* 8: 109 (1890)

Type: Laura Vale, Northern Territory, [June] 1889, *W. H. Tietkens s.n.* (holo: MEL 82820).

Eremophila latrobei var. tietkensii (F.Muell. & Tate) Ewart & P.H. Jarrett [see https://id.biodiversity.org.au/name/apni/114129/api/apni-format], *Proc. Roy. Soc. Victoria* 40: 87 (1928) [see https://id.biodiversity.org.au/instance/apni/548054].

Eremophila pachomai Paczkowska & A.R.Chapman, *W. Austral. Fl. Descr. Cat.* 339 (2000), *nom. inval.* [manuscript name; no Latin description or diagnosis provided or referenced]

Eremophila sp. Rudall River (P. G. Wilson 10512), Western Australian Herbarium: L. J. Biggs & C. M. Parker, *Nuytsia* 23:504 (2013).

Rounded to flat-topped shrub 0.6–2(−3) m tall, aromatic. Young *stems* covered in a persistent, fine, grey to yellowish, appressed tomentum of simple hairs, obscurely tuberculate beneath the indumentum; older stems with grey to very pale grey, slightly fissured bark, at first with prominently raised and knob-like persistent leaf bases. *Leaves* scattered, pale greyish-green or grey, petiolate; petioles (2.5–)5–10(–16) mm long; lamina ovate to lanceolate, (19–)32–57(–91) × (4–)10–21(–32) mm, smooth; indumentum dense, very short, appressed, white to grey, velutinous, often matted-resinous, comprising simple, uniseriate hairs, the terminal cell much longer than the others and usually attenuate; margins entire; apex acute, attenuate, or mucronate. *Flowers* 2–4 per axil, pedicellate; pedicels (5–)10–14(–25) mm long, with indumentum as for stems. *Sepals* 5, imbricate, subequal, elliptic to oblanceolate, broadly acute to obtuse with a mucro, (7–)10–13(–18) × (1.5–)3–5(–7) mm, pinkish-purple to mauve, maroon or red, pubescent with ±appressed, tangled hairs, the margins more densely so, enlarging after flowering and then glabrescent and with prominent veins. *Corolla* 22–28 mm long, pale blue, blue, pale lilac to pale mauve, white tinged lilac, mauve or pink; outer surface of lobes and tube with scattered eglandular hairs particularly near the margins, often almost glabrous; mid-inner tube with moderate density of eglandular hairs. *Stamens* 4, included; filaments with long eglandular hairs towards base, glabrous above; anthers glabrous. *Ovary* ovoid-oblong, densely glandular-puberulous with scattered or numerous longer eglandular hairs; style glabrous or with a few scattered, simple, spreading, short eglandular hairs. *Fruit* dry, woody, ovoid-conical, ±beaked, ribbed, 6–7 × 3–4.5 mm; exocarp adhering to endocarp, glandular-puberulous but usually with some longer eglandular hairs, occasionally resinous; endocarp vertically ribbed, splitting into four segments towards apex.

Distribution and habitat

Occurs from Exmouth on the western coast of WA in the Carnarvon IBRA bioregion (Thackway and Cresswell 1995) to just over the NT border in the east, throughout the Pilbara, Gascoyne, Little Sandy Desert, Great Sandy Desert, Gibson Desert and Central Ranges IBRA bioregions, and down to the Murchison bioregion to the south. Occurs on a range of substrates and landscape positions, including red−brown sand, silty loam, skeletal loam over ironstone, rocky quartz, gravel, laterite, dolerite, and limestone on flats, undulating plains, saline clay plains, plateaus, gully slopes, valley floors, creeklines, scree slopes, and outcrops (Western Australian Herbarium's FloraBase, see https://florabase.dpaw.wa.gov.au/).

Phenology

Flowers in late winter to at least mid-spring, with fruits maturing from early spring onward.

Conservation status

Eremophila tietkensii is widespread in WA, including in several national parks and nature reserves, and is not considered to be under threat. It is known in the NT only from regions close to the WA border between Lakes Mackay and Neale.

Notes

Eremophila tietkensii is a widespread and morphologically variable species. It differs from *E. naaykensii* in having shorter pedicels (shorter than or similar in length to the flowers cf. usually longer than the flowers) among less dense leaf clusters at branch apices, and having leaf indumentum trichomes with an elongated terminal cell, and from *E. hurteri* in its glandular-puberulous cf. densely silky ovary indumentum.

Plants from the vicinity of the Rudall and Oakover Rivers have leaves that are generally shorter and more ovate than is typical, and these populations were previously segregated as *Eremophila* sp. Rudall River. However, the differences in leaf shape are continuous and highly variable. Some plants distant from the Rudall-Oakover area have equally small leaves, while some plants from within that area have longer, more lanceolate leaves. Two disjunct, far-western collections from the Cape Range are typical for the species.

Selected specimens examined

WESTERN AUSTRALIA. 17 km on Exmouth Road, Exmouth, *Anonymous* D 11305 (PERTH 03856208); 25.4 km NW of Cobra on the Gifford Creek Road, Upper Gascoyne, *R. J. Chinnock* 6888 (PERTH 08316937); The Gap, 1.4 km N of the turnoff to Christmas Pool, Paterson Range, *R. J. Chinnock* 6965 (PERTH 08317186); Mu Hills, Ngaanyatjarraku, *R. J. Chinnock* 8002 (PERTH 08669945); 12.5 km N of Towrana, *R. J. Chinnock* 8002 (PERTH 08316945); 21.4 km N of Gascoyne Junction, *R. J. Chinnock* 3796 (PERTH 08316899); Yalthalla Creek near Mount Rica, Hamersley Range, Ashburton, *C. A. Gardner* 6420 (PERTH 03856275); 20 km WSW Parngurr, Little Sandy Desert, *P. K. Latz* 17817 (PERTH 08305447); Rudall River district, ~500 km S of Broome, *P. G. Wilson* 10512 (PERTH 03878740).

Eremophila naaykensii A.L. Curtis & K. R. Thiele, sp. nov.

Eremophila sp. Hamersley Range (K. Walker KW 136) Western Australian Herbarium: L. J. Biggs & C. M. Parker, *Nuytsia* 23: 504 (2013).

Type: Hamersley Range (specifically Hancock Range) within mining tenement E-47/1329-I neighbouring Mining Area C, within Juna Downs Street, ~103 km WNW of Newman townsite, 5 km E of Great Northern Highway, Western Australia, 21 Feb. 2018, *C. van den Bergh CV Opp 18* (holo: PERTH 09105972!).

Rounded to obconical shrubs or small trees 1–2.5(–3.5) m tall, aromatic. Young *stems* clothed in a persistent, fine, grey to yellowish, appressed tomentum of simple hairs, obscurely tuberculate beneath the indumentum; older stems with grey to very pale grey, slightly fissured bark, at first with prominently raised and knob-like persistent leaf bases. *Leaves* scattered but tending to be clustered towards the stem apices, pale green or grey-blue, petiolate; petioles (6–)9–13(–18) mm long; lamina lanceolate, (37–)55–71.5(–89) × (5–)7.5–12(–15) mm, smooth; indumentum dense, very short, appressed, white to grey, velutinous, often matted-resinous, comprising simple, uniseriate hairs that are evenly septate, the terminal cell no longer than the others and with a bluntly rounded tip; margins entire; apex attenuate. *Flowers* (1)2–4 per axil, appearing clustered in the dense, terminal leaf clusters, pedicellate; pedicels (20–)28–33.5(–40) mm long and ±sigmoidal, with indumentum as for stems. *Sepals* 5, imbricate, subequal, elliptic to oblanceolate, broadly acute to obtuse, sometimes mucronate, (7–)8–10(–14) mm × (2.5–)3–5(–6), yellowish, greenish, red or purple-tinged in flower (likely to be colouring further after anthesis), pubescent with ±appressed, tangled hairs, the margins more densely so, enlarging after flowering and then glabrescent and with prominent veins. *Corolla* 20–28 mm long, cream, pale blue, lilac, yellow, pink or purple sometimes with spots on upper lobe, the throat and inside of tube pale yellow to cream; outer surface of lobes and tube with scattered eglandular hairs particularly near the margins, often almost glabrous; mid-inner tube with moderately dense eglandular hairs. *Stamens* 4, included; filaments with long eglandular hairs towards base, glabrous above; anthers glabrous. *Ovary* sparsely to moderate pubescent with glandular and eglandular hairs, ribbed; style with short, patent, eglandular hairs for most of its length. Mature *fruits* not seen.

Distribution and habitat

Endemic in the Pilbara IBRA bioregion (Thackway and Cresswell 1995). Current records indicate a geographic range of ~200 km from west to east in the southern half of the central to eastern portions of the Hamersley Ranges, occurring from the vicinity of Paraburdoo east to north-west of Newman (Western Australian Herbarium's FloraBase, see https://florabase.dpaw.wa.gov.au/). Generally found in rocky ranges of the Hamerseley Plateau, often high in the landscape on the tops of ironstone ranges, breakaways and on upper slopes, often in and around rocky gullies and gorges, associated with low open *Eucalyptus leucophloia* and *Corymbia ferriticola* woodlands with mixed *Acacia aneura sens. lat.* and *Acacia* spp. open shrublands and tall shrublands.

Phenology

Flowers in late winter to at least mid-spring, often seasonally dependent, with fruits maturing from early spring onward.

Conservation status

Eremophila naaykensii is currently known from six populations and is listed as a Priority Three species under the Conservation Codes for Western Australian flora, under the name *E.* sp. Hamersley Range (K. Walker KW 136; Western Australian Herbarium's FloraBase, see https://florabase.dpaw.wa.gov.au/).

Etymology

Named in honour of Jeremy Naaykens, Senior Advisor Riparian Ecology and Botany at Rio Tinto Australia. Jeremy has contributed much to our knowledge of the flora of the Pilbara region, and has collected specimens from most known populations of *Eremophila naaykensii*. His enthusiasm for the species often led to his disappearance up rocky ravines and gorges to collect specimens when other more pressing work was required.

Notes

Eremophila naaykensii was previously included in *E. tietkensii*, from which it can be distinguished by the presence of evenly septate hairs with rounded tips on the adaxial and abaxial leaf blades, leaves that are densely clustered at the stem apices (not terminally clustered in *E. tietkensii*), and pedicels longer than the flowers (generally the same length as the flower in *E. tietkensii*). It almost certainly belongs in the clade of *Eremophila* that contains sections *Eremaeae, Pulchrisepalae, Eremophila* and *Eriocalyx* Benth. (Fowler 2018). However, phylogenetic relationships within this clade are poorly resolved with low support, and the precise phylogenetic relationships of *E. naaykensii* are currently unknown.

Selected specimens examined

WESTERN AUSTRALIA. [precise localities withheld for conservation reasons] *J. Bull & J. Waters* ONS PH 62.04 (PERTH 09126120); *S. Reiffer & H. Ajduk* WPT 1-TS (PERTH 08772088); *S. van Leeuwen* 3723 (PERTH 06023983); *S. van Leeuwen* 3828 (PERTH 06110134); *S. van Leeuwen* 4074 (PERTH 06017339); *M. E. Trudgen* MET 17478 (PERTH 06653561).

Eremophila hurteri A.L.Curtis & K.R.Thiele sp. nov.

Type: base of Calvert Range (campsite), Calvert Range, WA, 7 August 2000, *A. A. Burbidge* 738 (*holo*: PERTH 07512821!).

Eremophila sp. Calvert Range (A. A. Burbidge 738) Western Australian Herbarium: L. J. Biggs & C. M. Parker, *Nuytsia* 23: 504 (2013).

Intricate flat-topped shrubs 1–1.5 m tall, aromatic. Young *stems* with indumentum of short, woolly, usually yellowish, sometimes grey, hairs, sometimes appearing sericeous, obscurely tuberculate beneath the indumentum; older stems grey to dark brown, scarcely fissured, often distinctly tuberculate, at first with prominently raised and knob-like persistent leaf bases. *Leaves* scattered, silvery, petiolate; petioles (7–)8–10(–11) mm long, decurrent; lamina lanceolate, (45–)50–69.5(–84.5) × (9–)9.5–12.5(–14) mm, finely strumose; indumentum dense, very short, white to grey, woolly, often matted-resinous, comprising simple, uniseriate hairs, the terminal cell much longer than the others and attenuate; margins entire; apex attenuate. *Flowers* 1 or 2 per axil, pedicellate; pedicels (4.5–)9–13(–15) mm long, straight to curved, with indumentum as for stems. *Sepals* 5, imbricate, subequal, elliptic to oblanceolate, broadly acute to obtuse, sometimes mucronulate, 7–9 × 2–3.5 mm in flower, yellow in bud, turning white or pink or mauve at anthesis, densely short-tomentose with ± silky hairs, enlarging after flowering and then glabrescent and with prominent veins. *Corolla* 20–28 mm long, white to pale purple or mauve; outer surface of lobes and tube with scattered eglandular hairs particularly near the margins, often almost glabrous; mid-inner tube lanate with eglandular hairs. *Stamens* 4, included; filaments with woolly eglandular hairs towards base, glabrous above; anthers glabrous. *Ovary* densely sericeous with yellow, simple, eglandular hairs; style with sparse, long spreading, eglandular hairs for most of its length. *Mature fruits* not seen.

Distribution and habitat

Endemic in the Little Sandy Desert IBRA bioregion (Thackway and Cresswell 1995). Current records indicate a geographic range of ~220 km from north to south either side of Lake Disappointment (Western Australian Herbarium's FloraBase, see https://florabase.dpaw.wa.gov.au/). Occurs on sandstone ranges, rocky scree slopes and stony plains at the bases of low ranges.

Phenology

Flowers in late winter to at least mid-spring, with fruits maturing from early spring onward.

Conservation status

Eremophila hurteri is currently known from six populations. It is not currently listed under the Conservation Codes for Western Australian flora (Western Australian Herbarium's FloraBase, see https://florabase.dpaw.wa.gov.au/).

Etymology

Named in honour of Johan Hurter, ecologist and botanist at EcoRex Environmental Consulting and previously the Rio Tinto Identification Botanist at the Western Australian Herbarium. Johan first suggested that there may be multiple species within *Eremophila tietkensii*, and segregated *E. hurteri* (as *E.* sp. Calvert Range), *E. naaykensii* (as *E.* sp. Hamersley Range) and *E.* sp. Rudall River.

Notes

Eremophila sp. Calvert Range was previously included in *E. tietkensii,* from which it can be distinguished by an indumentum of yellow, sericeous, simple, eglandular hairs on the ovary (simple eglandular and glandular hairs in *E. tietkensii*) and by the strumose leaf surfaces (not strumose in *E. tietkensii*). It almost certainly belongs in the clade of *Eremophila* that contains sections *Eremaeae, Pulchrisepalae, Eremophila* and *Eriocalyx* (Fowler 2018). However, phylogenetic relationships within this clade are poorly resolved with low support, and the precise phylogenetic relationships of *E. hurteri* are currently unknown.

Other specimens examined

WESTERN AUSTRALIA. At base of Durba Hills, Wiluna, *A. A. Burbidge* 733 (PERTH 07765886); Rudall River Region, East Pilbara, *R. P Hart* 571 (PERTH 01226991); 4.5 km Sth Parngurr, Little Sandy Desert, *P. K. Latz* 17825 (PERTH 08305382); 28 Aug. 2004, *W. P. Muir* WPM 1046 (PERTH 08609942); 40 km S of Rudall River, ~500 km S of Broome, East Pilbara, *P. G. Wilson* 10540 (PERTH 03878570).

Supplementary material

Supplementary material is available online.

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Data availability. The data that support this study are available in GenBank at https://www.ncbi.nlm.nih.gov/bioproject/765188.

Conflicts of interest. Kevin R. Thiele is an Associate Editor for *Australian Systematic Botany*. Despite this relationship, he did not at any stage have Associate Editor-level access to this manuscript while in peer review, as is the standard practice when handling manuscripts submitted by an editor to this journal. *Australian Systematic Botany* encourages its editors to publish in the journal and they are kept totally separate from the decision-making process for their manuscripts. The authors declare that they have no further conflicts of interest.

Declaration of funding. This project was funded in part by Rio Tinto.

Acknowledgements. We thank the Curator and staff at the Western Australian Herbarium for access to equipment and specimens and for providing material for both morphometric and molecular work, particularly Steve Dillon for his careful initial observations on trichome morphology in this group, and for assistance with SEM. The Batley Laboratory at the University of Western Australia provided access to resources for the molecular work. Thanks go to Bevan Buirchell for providing additional field samples, and Johan Hurter for photographs and for the initial groundwork that led to this project. Finally, we thank the reviewers whose comments improved an earlier version of this paper.

Author affiliations

[A]Western Australian Herbarium, Biodiversity and Conservation Science, Department of Biodiversity, Conservation and Attractions, Locked Bag 104, Bentley Delivery Centre, WA 6983, Australia.

[B]School of Biological Sciences, Faculty of Science, The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia.

[C]Rio Tinto Iron Ore, 152–158 St Georges Terrace, Perth, WA 6000, Australia.

[D]School of BioSciences, The University of Melbourne, Parkville, Vic. 3010, Australia.