Biodiversity and Conservation Science

Genetic assessment of *Aluta quadrata* across the Western Range

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Executive Summary

Knowledge of genetic diversity and structure is important for the management of rare and restricted species, particularly where they may be impacted by anthropogenic activities or industrial operations. Because genetic diversity underpins the ability of populations to persist and adapt to environmental changes or disturbance, genetic assessments of restricted species can provide an indication of the current health and future stability of populations (Ellstrand & Elam 1993), inform the design of strategies for population augmentation and translocation (Bischoff *et al.* 2010), and provide a basis for defining management units within a species (Palsbøll *et al.* 2006). Recent developments in genomic technologies have provided cost-effective opportunities for high resolution estimation of genetic diversity and structuring to inform conservation management.

This exemplar study, which is a collaboration between Rio Tinto and Biodiversity and Conservation Science at the Department of Biodiversity, Conservation and Attractions, expands on a previous study that investigated genetic diversity and structuring of *Aluta quadrata* across the three disjunct populations of the species' restricted distribution on the southern edge of the Hamersley Range (Byrne *et al.* 2016). The aim of the current study was to apply extensive sampling and genomic data to investigate genetic variation within the largest of these sites, Western Range. In addition, the genomic data was used to determine the collection locality of a specimen housed in the WA Herbarium that is recorded as being collected from Mt Boggola, some 60-70 km south of the species' known distribution. In doing so, 232 plants were sampled across the Western Range and included for dartseq analysis, along with samples previously collected from the species' full range, as well as seven samples from the WA Herbarium.

The range-wide patterns of genomic diversity and structure detected were consistent with those found by Byrne *et al.* (2016); confirming the strong genetic distinction of the three locations where *A. quadrata* is found. This re-iterates the need for these locations to be considered separate management units. This dataset also proved useful in correctly assigning five herbarium specimens to their attributed collection sites in Howie's Hole and Western Range. This provides high confidence in the assignment of the Mt Boggola specimen, which fell within the range of variation seen across the Western Range. Therefore, the data provide evidence that this specimen was originally collected from the Western Range and not from Mt Boggola.

Within the Western Range, individuals showed a clinal pattern of genetic variation, rather than discrete genetic clusters or widespread homogeneity. This indicates that gene flow is not widespread across the 5 km of sampled range but is restricted by distance. Where the species is relatively continuous, this allows for stepping-stone dispersal to maintain connectivity across the range but dispersal may be interrupted by larger gaps in distribution. We therefore consider the Western Range to represent a single meta-population connected in an isolation-by-distance manner across the east-west axis of the range. Together, the results of this study

will be useful to informing future management actions for the conservation of *Aluta quadrata*. For the Western Range in particular, in line with the recommendations of Byrne *et al.* (2016), any rehabilitation activities should use seed sourced only from the Western Range.

Background

Knowledge of genetic diversity and structure is important for the management of rare and restricted species, particularly where they may be impacted by anthropogenic activities or industrial operations. Genetic diversity underpins the ability of populations to persist and adapt to environmental changes or disturbance, therefore genetic assessments of restricted species can provide an indication of the current health and future stability of populations (Ellstrand & Elam 1993), inform the design of strategies for population augmentation and translocation (Bischoff *et al.* 2010), and provide a basis for defining management units within a species (Palsbøll *et al.* 2006).

Recent developments in genomic technologies have provided cost-effective opportunities for improved estimation of genetic diversity and structuring to inform conservation management. Reduced representation sequencing methods, such as *DArTseq* (Kilian *et al.* 2012), provide many thousands of genome-wide SNP markers that allow for high resolution, individual-based analyses, such that highly precise estimates of genetic diversity and differentiation can be obtained without subjective groupings of individuals into populations (Allendorf *et al.* 2010) and from smaller sample sizes than those required for traditional population genetic approaches (Nazareno *et al*. 2017). Secondly, genomic approaches typically work with short sequence fragments, permitting the use of potentially degraded DNA extracted from museum and herbarium collections (e.g. Beck & Semple 2015).

Aluta quadrata is a rare species, restricted to a banded ironstone formation that extends east and west of Paraburdoo, on the southern edge of the Hamersley Range in the Pilbara region. A previous study using microsatellite markers assessed genetic diversity across the full geographic range of the species, which is limited to three disjunct locations; Howie's Hole, the Western Range and Pirraburdoo Creek (Byrne *et al.* 2016). The study found that each of the three locations were sufficiently genetically distinct to represent separate management units and that diversity was lowest in Pirraburdoo Creek, which harbours the smallest population (Byrne *et al.* 2016). Following this study, further requirements have arisen regarding a more detailed assessment of genetic diversity within the largest population at Western Range. In addition, there is a query regarding a specimen of *A. quadrata* (PERTH 05724767) housed in the WA Herbarium that is purportedly from Mt Boggola, 60-70 km south of the currently known distribution. This could indicate a fourth location for the species, or simply an error in the original collection notes. This research project expands on the previous study to respond to these recent enquiries.

Objectives

Our primary aim is to use genomic data for a high resolution investigation of genetic diversity and structure of *A. quadrata* across the 5 km length of the Western Range. In addition, we aim to determine the collection locality of the herbarium specimen allegedly collected from Mt Boggola. Completion of these aims required extensive sampling of *A. quadrata* across the Western Range, sampling of herbarium specimens and the inclusion of samples from Howie's Hole and Pirraburdoo Creek to put the genomic data for the Western Range into context and test all possible source locations for the Mt Boggola specimen.

Methods

Aluta quadrata occurs in three geographically discrete locations, with a total extent of occurrence of approximately 75 km^2. The species is more abundant at the Western Range and Howie's Hole, with a number of populations or meta-populations across each range, while the fewer plants located at Pirraburdoo Creek are considered to constitute a single population (Byrne *et al.* 2016). Figure 1A presents a map of this distribution, as well as the locations of the eight populations sampled across the three locations in 2012. Figure 1B shows the additional sampling of 232 samples from 29 sites across the Western Range by Woodman Environmental for Rio Tinto in October 2018.

In addition to the fresh leaf material collected from the Western Range, a subset of the samples collected in 2012 were included in this study. Dried leaf material was taken from collections stored at DBCA; eight samples from each of the eight populations collected across the Western Range, Pirraburdoo and Howie's Hole locations (Byrne *et al.* 2016). Dried leaf material was also taken from seven specimens vouchered in the WA Herbarium collection; four from Howie's Hole, two from the Western Range and the single specimen allegedly collected from Mt Boggola. The remaining 11 specimens in the herbarium collection were in poor condition and not suitable for successful DNA extraction. A complete list of all samples included for genetic analysis in this study is provided in Appendix 1.

DNA was extracted from all fresh leaf samples using a simple CTAB protocol (Doyle and Doyle 1987), modified by the addition of 0.1% sodium sulphite (Byrne *et al.* 2001) and 1% PVP (polyvinylpyrrolodine) to the extraction buffer. Due to sample degradation in the older leaf material, DNA extractions from all dried leaf samples were undertaken using the Invisorb DNA Plant HTS 96 Kit (Stratec Molecular, Germany). All DNA samples were sent to Diversity Arrays Technology Pty Ltd (DArT, Canberra, Australia) for DArTseq™ analysis as per Sansaloni *et al.* (2010). Briefly, library preparation involved DNA digestion using two restriction enzymes, *PstI*

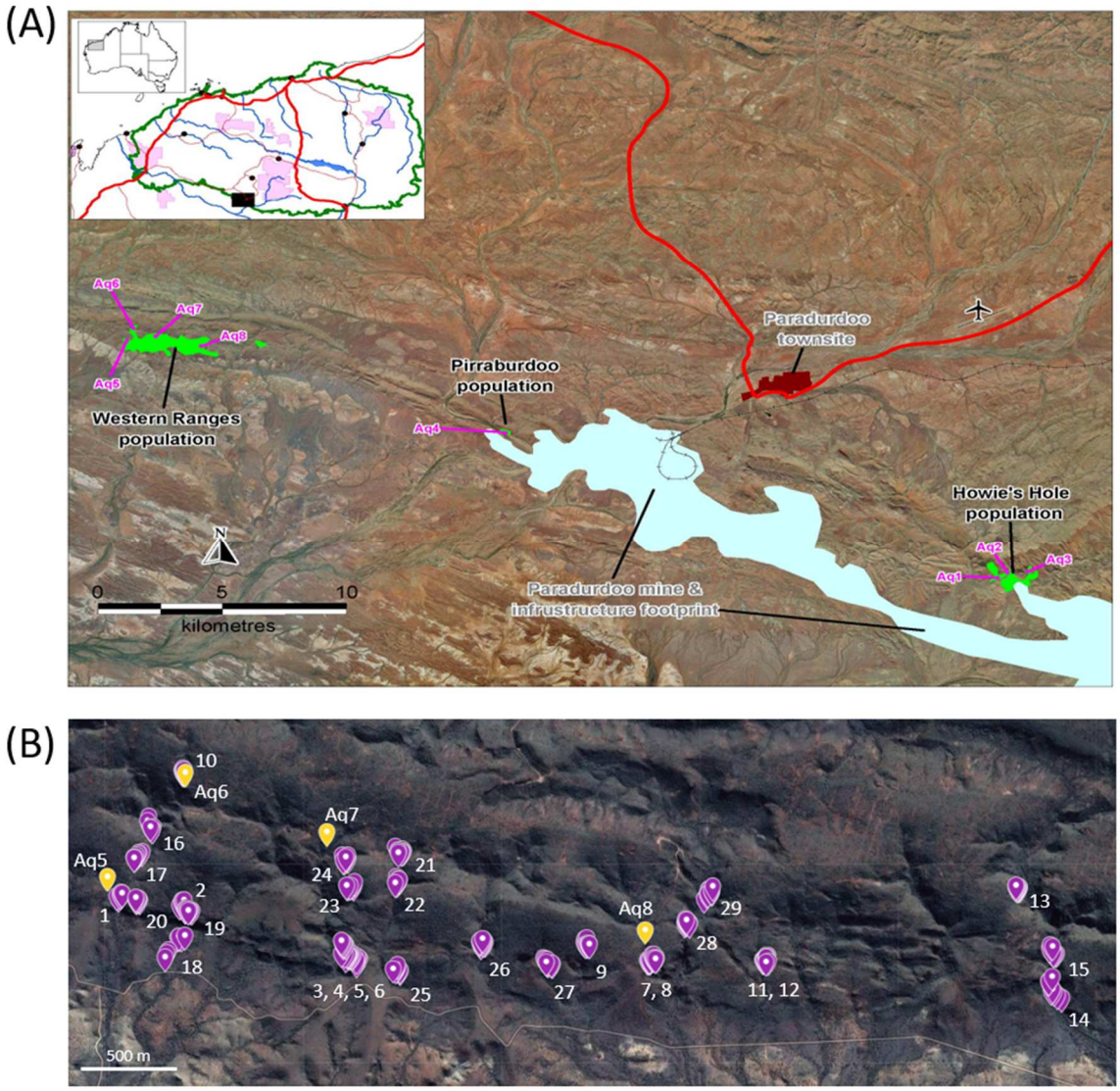

Figure 1 The distribution of *Aluta quadrata* and sampling sites for genetic analyses. A) indicates the sampling for the 2012 study across the full geographic range and B) indicates the sampling undertaken within the Western Range in 2012 (yellow) and 2018 (purple).

and *HpaII*, and fragment ligation with uniquely barcoded adaptors. Following PCR and quantification, the samples were standardised and pooled for sequencing in the HiSeq 2500 (Illumina). Read assembly, quality control and SNP calling were undertaken by DArT using their proprietary DArTsoft14 software. This pipeline uses technical replicates for a measure of genotyping reproducibility and Mendelian inheritance patterns to filter sequencing errors and paralogous regions. The DArT pipeline produced 21,628 SNP loci with 20.85% missing data for further analysis.

We used the *dartR* package v1.1.6 (Gruber *et al.* 2018) in *R* (R Core Development Team, 2008) for further quality control filtering to retain only loci with: reproducibility >0.95, call rate >0.95, minor allele frequency >0.02 and heterozygosity values between 0.02-0.95. To avoid linkage we removed secondary loci by reducing the dataset to a single SNP per fragment

based on the 'best' method. These filtering steps produced a dataset of 4,757 high quality SNP loci with just 1.26% missing data. Analyses of population structure often rely on assumptions of neutrality so as a final filtering step we used BAYPASS v2.1 (Gautier 2015) to identify any loci that represent outliers from neutral expectations and may be influenced by selection. The *simulate.baypass* function was applied in *R* to simulate allele count data and calibrate the genetic differentiation parameter, *XtX*, to generate thresholds for identifying outlier loci in the empirical dataset. We applied extended running parameters (-*nval*=100000, -*burnin*=10000, -*npilot*=30, -*pilotlength*=2000) and ran the model three times. Across the three runs, 87 loci were consistently identified as outliers at the 1% threshold and removed from further analysis, resulting in a dataset of 4,670 putatively neutral loci.

The complete dataset was analysed for a relative measure of genome-wide population structuring across the full geographic range of *A. quadrata*, to assess consistency between the two marker types and to confirm the identities of the herbarium specimens. Genetic relationships among all individuals was assessed with principal coordinates analysis (PCoA) using the '*adegenet*' package v.2.0.1 (Jombart 2008) in *R*. Genetic differentiation among the subpopulations detected was assessed by pairwise F_{ST}, estimated using GENEPOP (Raymond & Rousset 1995), while genetic diversity parameters (allelic richness, heterozygosity, percentage polymorphic loci and the inbreeding coefficient) were estimated using the *hierfstat* package v.0.04-22 (Goudet 2005) in *R*.

To further investigate genetic structuring and diversity within the Western Range, we subset the data to remove all samples from Howie's Hole, Pirraburdoo and the WA Herbarium. This led to 124 loci being monomorphic and these were removed from the dataset, leaving 4,546 polymorphic loci for Western Range-specific analyses. The 232 Western Range samples were collected in 29 groups of eight samples to represent populations across the range, however, many of these groups overlapped geographically or were less than 500 m apart and therefore cannot be treated as discrete populations in these analyses. For this reason, analyses of population structure were undertaken at the individual level to identify any subpopulation structure and genetic diversity was estimated for the resulting subpopulations detected.

Several methods were used to thoroughly investigate genetic structuring within the Western Range. First, the Bayesian analysis implemented in STRUCTURE (Pritchard *et al.* 2000) was used to detect *K* genetic clusters, without any priors regarding population identity or geographic location. Assuming correlated frequencies, we applied a 100,000 burnin period and 100,000 Markov chain Monte Carlo replications to test *K* values from one to eight, with five iterations for each value of *K*. The method of Evanno *et al.* (2005) was used to determine the most likely value of *K*. Because discrete clustering patterns can be confounded with patterns of isolation-by-distance (Meirmans 2012), we also used a spatially explicit alternative, implemented in TESS v2.3.1 (Durand *et al.* 2009). TESS assumes spatial autocorrelation in the data and accounts for the geographic distribution of samples in the clustering model. The analysis was run using the CAR admixture model, performing 100 iterations for each *K* value up to eight, with 50,000 sweeps, a burnin of 10,000 and the default

spatial interaction parameter. The most likely value(s) of K was determined by stabilisation in the deviance information criterion across K_{max}. Finally, genetic structure within the Western Range was also assessed using PCoA because this multivariate method does not rely on any evolutionary models and is therefore free of the assumptions made by FASTSTRUCTURE and TESS (Jombart *et al.* 2009). Finally, isolation-by-distance was assessed using GENALEX (Peakall & Smouse 2006) to test for a correlation between genetic distance and geographic distance among individuals across the Western Range.

Genetic diversity and differentiation were estimated for each of the subpopulations detected across the Western Range, using *hierfstat* and GENEPOP, as above. To account for the substantial difference in sample size between the subpopulations found, these analyses were run with a random subset of the data that matched sample sizes.

Results

Principal coordinates analysis indicated three distinct genetic clusters across the full range of *Aluta quadrata*, corresponding with each of the discrete geographic locations sampled, Howie's Hole, Pirraburdoo Creek and Western Range. The primary axis separated all three clusters, while the second axis further separated the Pirraburdoo cluster from the other two (Figure 2). Regarding the herbarium specimens, one Western Range DNA sample (the oldest of all the specimens) failed to meet read quality standards and was excluded from the PCoA. The remaining six specimens were sequenced with sufficient read quality, including the alleged Mt Boggola specimen, although there was significantly more missing data in this sample (the second oldest of the specimens) than the others. In the PCoA, the four herbarium specimens attributed to Howie's Hole were associated with the correct genetic cluster, as was the remaining Western Range specimen. The alleged Mt Boggola specimen was associated with the Western Range but on the edge of the range of variation for this cluster. Estimates of genetic differentiation among the three genetic clusters were high ($F_{ST\ HOW\text{-}PIRR}$=0.196, $F_{ST\ HOW\text{-}WRA}$=0.145, $F_{ST\ PIRR\text{-}WRA}$=0.170), with a global F_{ST} value of 0.153.

Genetic diversity was moderate across the species' range, with allelic richness, heterozygosity and the percentage of polymorphic loci being highest in the Western Range cluster and lowest in the Pirraburdoo cluster (Table 1). The inbreeding coefficients were slightly elevated in all three locations, suggesting some level of inbreeding.

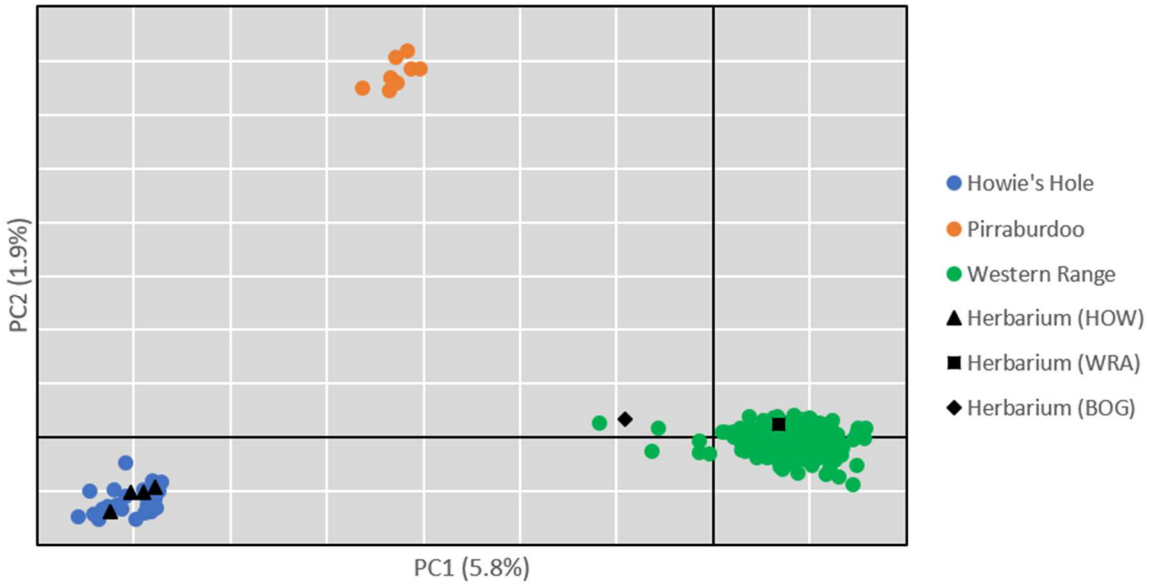

Figure 2 Principal coordinates analysis (PCoA) of the relationships among samples of *Aluta quadrata* from Howie's Hole, Pirraburdoo and the Western Range to form three distinct genetic clusters. Black symbols indicate the assignment of six specimens from the WA herbarium putatively sourced from: triangle=Howie's Hole, square=Western Range and diamond= Mt Boggola.

Table 1 Diversity estimates for the three genetic clusters detected across the full geographic distribution of *Aluta quadrata*.

Cluster /Location	Population Code	*%P*	A_R	H_E	F_{IS}
Howie's Hole	HOW	76.72	1.232 ± 0.003	0.233 ± 0.003	0.113 ± 0.005
Pirraburdoo Creek	PIRR	54.37	1.187 ± 0.003	0.189 ± 0.003	0.088 ± 0.007
Western Range	WRA	97.69	1.243 ± 0.002	0.243 ± 0.002	0.087 ± 0.002
Mean ± SE		76.26 ± 12.508	1.221 ± 0.017	0.222 ± 0.017	0.096 ± 0.008

Within the Western Range, all three clustering analyses indicated a signal of clinal genetic variation (Figure 3). The STRUCTURE and TESS analyses both found the most likely number of clusters was two, with a clinal pattern of individual assignment between the eastern and western ends of the range. This was supported by the primary axis of the PCoA, gradually separating individuals along a west-east axis. The second axis showed further clinal variation that is interrupted slightly to distinguish the eastern-most individuals (sites 13, 14 and 15)

from the rest of the range. Finally, there was a significant correlation between geographic and genetic distance across the Western Range (r = 0.06, p <0.001).

To explore the significance of the distinction of the eastern sites 13, 14 and 15 in the PCoA, we split them from the dataset to form two subpopulations and found low genetic differentiation (F_{ST} = 0.029) between them. Genetic diversity was slightly higher across all measures in the main part of the range, relative to the smaller eastern subpopulation (Table 2).

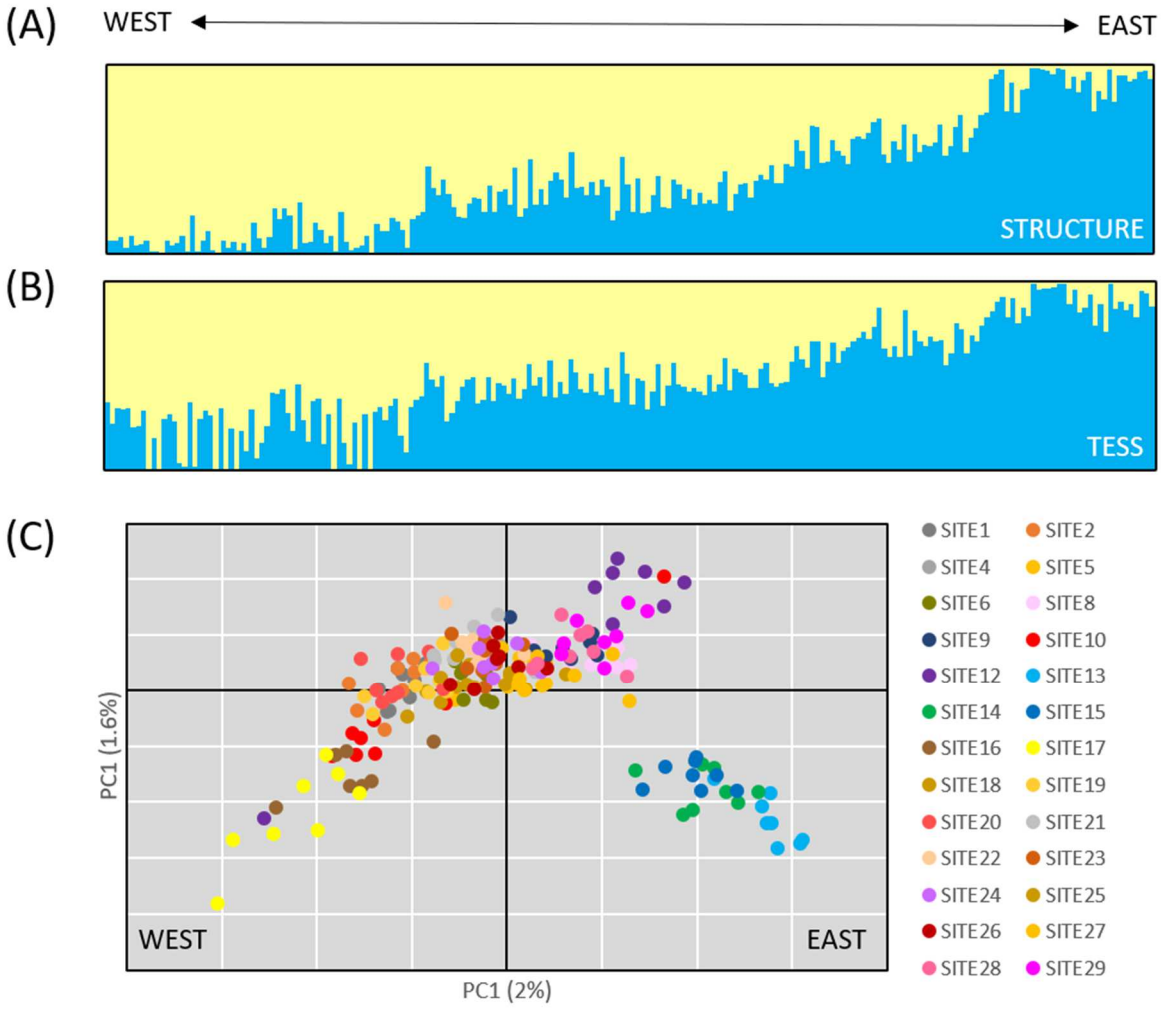

Figure 3 Results of clustering analyses among individuals within the Western Range: (A) the STRUCTURE analysis and (B) the TESS analysis, in both cases K=2 and the assignment of each individual to each genetic cluster is represented by the coloured proportions in each vertical bar. Individuals are ordered by increasing longitude to highlight the east-west cline in cluster assignment. (C) the principal coordinates analysis, coloured by the sites sampled to highlight the east-west cline across both axes.

Table 2 Diversity estimates for two subpopulations of *Aluta quadrata* across the Western Range.

Cluster /Location	Sites	%*P*	A_R	H_E	F_{IS}
Western	1-12, 16-29	89.66	1.867 ± 0.004	0.253 ± 0.003	0.061 ± 0.004
Eastern	13, 14, 15	84.07	1.815 ± 0.005	0.242 ± 0.003	0.068 ± 0.004
Mean ± SE		86.87 ± 2.80	1.841 ± 0.026	0.247 ± 0.005	0.065 ± 0.003

Discussion

The range-wide patterns of genomic diversity and structure found in this study are consistent with those found in the 2012 study; confirming the strong genetic distinction of the three locations where *A. quadrata* is found, such that gene flow between locations must be very minimal (Byrne *et al.* 2016). This re-assessment was necessary to provide context for the diversity and differentiation found within the Western Range; however, it also provides reassurance as to the robust dataset produced by the previous study, which used an older genetic technology. Due to the use of different markers, the genetic diversity estimates between the two studies cannot be directly compared. Nevertheless, these data also show similar patterns across the range; diversity was highest in the two larger locations and lowest in Pirraburdoo. This is consistent with theoretical expectations, where smaller populations are likely to have reduced genetic diversity due to the effects of genetic drift, inbreeding and possibly selection in narrower environmental conditions (Ellstrand & Elam 1993). In addition, this study found indications of inbreeding across all three locations, which were also detected in the previous study. We, therefore, reiterate that these three locations should be considered separate management units.

The correct assignment of the herbarium specimens collected from Howie's Hole and the Western Range demonstrates the ability to use older leaf material to correctly identify the source location of herbarium specimens. This provides high confidence in the assignment of the specimen with questionable source information from Mt Boggola. This specimen was associated with the Western Range cluster in the PCoA, and although it falls at the edge of this cluster, it remains within the range of variation seen across the Western Range. The edge-placement is likely attributed to the higher level of missing data in this specimen, which is an unavoidable consequence of DNA degradation in older leaf material. Nevertheless, the three genetic clusters are clearly distinct so there is no ambiguity to suggest that the specimen could be from Howie's Hole or Pirraburdoo. Moreover, using the level of differentiation among these three broader locations as a guide, it is also highly unlikely that the specimen is from Mt Boggola, some 60-70 km south of the species' range, because it would then be expected to show equal or higher differentiation than that among the three known locations, which are just 10-30 km apart. Therefore, despite the missing data, our data provide clear evidence that this specimen was originally collected from the Western Range and not Mt Boggola. This is

consistent with ground-truthing reports that could not find *A. quadrata* in the Mt Boggola region (Biota 2007). We will contact the WA Herbarium to update its database.

Within the Western Range, individuals showed a clinal pattern of genetic variation, rather than discrete genetic clusters or widespread homogeneity. This clinal variation is a robust result, as detected by multiple, independent analyses with differing evolutionary assumptions. This improves on the 2012 study, which found low differentiation among the four populations sampled across the Western Range (Byrne *et al.* 2016), because the more extensive sampling effort and analysis at the individual level, in conjunction with the greater power of genomic markers, provided much higher resolution to detect the clinal pattern. This result indicates that gene flow is not widespread across the 5 km of sampled range but is restricted by distance. Where the species is relatively continuous, this allows for stepping-stone dispersal to maintain connectivity across the range but dispersal may be interrupted by larger gaps in distribution. This likely accounts for the differentiation of the easternmost sites 13, 14 and 15 in the PCoA, which are geographically separated from the rest of the range by a gap of approximately 1.5 km. However, in comparison to the levels of differentiation among the Western Range, Pirraburdoo and Howie's Hole, the differentiation between the main Western Range and these slightly disjunct sites is very low. The clinal pattern of gene flow is consistent with the likely means of dispersal for the species, pollen movement by insects. For example, bees have been found to forage on average <1 km and typically show a leptokurtic pattern of movement (Hagler *et al*. 2011) so occasional longer distance pollen movements likely prevent growing differentiation between distribution gaps across the range. We therefore consider the Western Range to represent a single meta-population connected in an isolation-by-distance manner across the east-west axis of the range.

The results of this study will be useful for informing future management actions for the conservation of *Aluta quadrata*. For the Western Range in particular, in line with the recommendations of Byrne *et al.* (2016), any rehabilitation activities should use seed sourced only from the Western Range.

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APPENDIX 1 Sampling details for all *Aluta quadrata* specimens included for genetic analysis in this study

Location	Population Code	Latitude	Longitude	Year Collected	Source Material
Western Range	POP01-01	-23.183130	117.41156	2018	Fresh leaves
Western Range	POP01-02	-23.183202	117.41159	2018	Fresh leaves
Western Range	POP01-03	-23.183048	117.41169	2018	Fresh leaves
Western Range	POP01-04	-23.183130	117.41148	2018	Fresh leaves
Western Range	POP01-05	-23.183067	117.41164	2018	Fresh leaves
Western Range	POP01-06	-23.183094	117.41167	2018	Fresh leaves
Western Range	POP01-07	-23.182967	117.41171	2018	Fresh leaves
Western Range	POP01-08	-23.182994	117.41172	2018	Fresh leaves
Western Range	POP02-01	-23.183546	117.41501	2018	Fresh leaves
Western Range	POP02-02	-23.183609	117.41496	2018	Fresh leaves
Western Range	POP02-03	-23.183536	117.41508	2018	Fresh leaves
Western Range	POP02-04	-23.183545	117.41516	2018	Fresh leaves
Western Range	POP02-05	-23.183590	117.41521	2018	Fresh leaves
Western Range	POP02-06	-23.183374	117.41509	2018	Fresh leaves
Western Range	POP02-07	-23.183401	117.41510	2018	Fresh leaves
Western Range	POP02-08	-23.183374	117.41517	2018	Fresh leaves
Western Range	POP03-01	-23.186338	117.42490	2018	Fresh leaves
Western Range	POP03-02	-23.186320	117.42485	2018	Fresh leaves
Western Range	POP03-03	-23.186256	117.42484	2018	Fresh leaves
Western Range	POP03-04	-23.186303	117.42415	2018	Fresh leaves
Western Range	POP03-05	-23.186266	117.42460	2018	Fresh leaves
Western Range	POP03-06	-23.186293	117.42468	2018	Fresh leaves
Western Range	POP03-07	-23.186555	117.42476	2018	Fresh leaves
Western Range	POP03-08	-23.186383	117.42477	2018	Fresh leaves
Western Range	POP04-01	-23.186122	117.42457	2018	Fresh leaves
Western Range	POP04-02	-23.186167	117.42445	2018	Fresh leaves
Western Range	POP04-03	-23.186176	117.42450	2018	Fresh leaves
Western Range	POP04-04	-23.186140	117.42439	2018	Fresh leaves
Western Range	POP04-05	-23.185969	117.42438	2018	Fresh leaves
Western Range	POP04-06	-23.186095	117.42440	2018	Fresh leaves
Western Range	POP04-07	-23.186104	117.42433	2018	Fresh leaves
Western Range	POP04-08	-23.186131	117.42434	2018	Fresh leaves
Western Range	POP05-01	-23.186167	117.42434	2018	Fresh leaves
Western Range	POP05-02	-23.186149	117.42438	2018	Fresh leaves
Western Range	POP05-03	-23.186104	117.42434	2018	Fresh leaves
Western Range	POP05-04	-23.186086	117.42432	2018	Fresh leaves
Western Range	POP05-05	-23.186095	117.42427	2018	Fresh leaves
Western Range	POP05-06	-23.186086	117.42422	2018	Fresh leaves
Western Range	POP05-07	-23.186096	117.42412	2018	Fresh leaves
Western Range	POP05-08	-23.186069	117.42395	2018	Fresh leaves
Western Range	POP06-01	-23.185572	117.42408	2018	Fresh leaves
Western Range	POP06-02	-23.185599	117.42414	2018	Fresh leaves

Western Range	POP06-03	-23.185608	117.42402	2018	Fresh leaves
Western Range	POP06-04	-23.185554	117.42408	2018	Fresh leaves
Western Range	POP06-05	-23.185509	117.42406	2018	Fresh leaves
Western Range	POP06-06	-23.185527	117.42406	2018	Fresh leaves
Western Range	POP06-07	-23.185491	117.42397	2018	Fresh leaves
Western Range	POP06-08	-23.185437	117.42395	2018	Fresh leaves
Western Range	POP07-01	-23.186375	117.44091	2018	Fresh leaves
Western Range	POP07-02	-23.186348	117.44097	2018	Fresh leaves
Western Range	POP07-03	-23.186411	117.44090	2018	Fresh leaves
Western Range	POP07-04	-23.186384	117.44093	2018	Fresh leaves
Western Range	POP07-05	-23.186357	117.44098	2018	Fresh leaves
Western Range	POP07-06	-23.186429	117.44104	2018	Fresh leaves
Western Range	POP07-07	-23.186374	117.44104	2018	Fresh leaves
Western Range	POP07-08	-23.186366	117.44096	2018	Fresh leaves
Western Range	POP08-01	-23.186266	117.44114	2018	Fresh leaves
Western Range	POP08-02	-23.186211	117.44120	2018	Fresh leaves
Western Range	POP08-03	-23.186148	117.44114	2018	Fresh leaves
Western Range	POP08-04	-23.186148	117.44122	2018	Fresh leaves
Western Range	POP08-05	-23.186229	117.44130	2018	Fresh leaves
Western Range	POP08-06	-23.186292	117.44134	2018	Fresh leaves
Western Range	POP08-07	-23.186275	117.44123	2018	Fresh leaves
Western Range	POP08-08	-23.186338	117.44131	2018	Fresh leaves
Western Range	POP09-01	-23.185508	117.43736	2018	Fresh leaves
Western Range	POP09-02	-23.185355	117.43742	2018	Fresh leaves
Western Range	POP09-03	-23.185264	117.43750	2018	Fresh leaves
Western Range	POP09-04	-23.185589	117.43747	2018	Fresh leaves
Western Range	POP09-05	-23.185535	117.43754	2018	Fresh leaves
Western Range	POP09-06	-23.185553	117.43760	2018	Fresh leaves
Western Range	POP09-07	-23.185553	117.43761	2018	Fresh leaves
Western Range	POP09-08	-23.185589	117.43766	2018	Fresh leaves
Western Range	POP10-01	-23.176816	117.41514	2018	Fresh leaves
Western Range	POP10-02	-23.176780	117.41512	2018	Fresh leaves
Western Range	POP10-03	-23.176671	117.41517	2018	Fresh leaves
Western Range	POP10-04	-23.176725	117.41510	2018	Fresh leaves
Western Range	POP10-05	-23.176698	117.41507	2018	Fresh leaves
Western Range	POP10-06	-23.176599	117.41513	2018	Fresh leaves
Western Range	POP10-07	-23.176644	117.41513	2018	Fresh leaves
Western Range	POP10-08	-23.176653	117.41519	2018	Fresh leaves
Western Range	POP11-01	-23.186366	117.44729	2018	Fresh leaves
Western Range	POP11-02	-23.186384	117.44734	2018	Fresh leaves
Western Range	POP11-03	-23.186347	117.44742	2018	Fresh leaves
Western Range	POP11-04	-23.186357	117.44729	2018	Fresh leaves
Western Range	POP11-05	-23.186357	117.44727	2018	Fresh leaves
Western Range	POP11-06	-23.186320	117.44736	2018	Fresh leaves
Western Range	POP11-07	-23.186339	117.44731	2018	Fresh leaves
Western Range	POP11-08	-23.186266	117.44740	2018	Fresh leaves

Western Range	POP12-01	-23.186175	117.44753	2018	Fresh leaves
Western Range	POP12-02	-23.186166	117.44752	2018	Fresh leaves
Western Range	POP12-03	-23.186158	117.44745	2018	Fresh leaves
Western Range	POP12-04	-23.186203	117.44747	2018	Fresh leaves
Western Range	POP12-05	-23.186203	117.44745	2018	Fresh leaves
Western Range	POP12-06	-23.186230	117.44746	2018	Fresh leaves
Western Range	POP12-07	-23.186446	117.44758	2018	Fresh leaves
Western Range	POP12-08	-23.186501	117.44747	2018	Fresh leaves
Western Range	POP13-01	-23.182739	117.46147	2018	Fresh leaves
Western Range	POP13-02	-23.182712	117.46143	2018	Fresh leaves
Western Range	POP13-03	-23.182667	117.46143	2018	Fresh leaves
Western Range	POP13-04	-23.182685	117.46144	2018	Fresh leaves
Western Range	POP13-05	-23.182730	117.46142	2018	Fresh leaves
Western Range	POP13-06	-23.182721	117.46137	2018	Fresh leaves
Western Range	POP13-07	-23.182712	117.46131	2018	Fresh leaves
Western Range	POP13-08	-23.182622	117.46133	2018	Fresh leaves
Western Range	POP14-01	-23.188287	117.46383	2018	Fresh leaves
Western Range	POP14-02	-23.188161	117.46357	2018	Fresh leaves
Western Range	POP14-03	-23.187999	117.46342	2018	Fresh leaves
Western Range	POP14-04	-23.187937	117.46326	2018	Fresh leaves
Western Range	POP14-05	-23.187855	117.46332	2018	Fresh leaves
Western Range	POP14-06	-23.187467	117.46313	2018	Fresh leaves
Western Range	POP14-07	-23.187476	117.46313	2018	Fresh leaves
Western Range	POP14-08	-23.187250	117.46329	2018	Fresh leaves
Western Range	POP15-01	-23.186057	117.46354	2018	Fresh leaves
Western Range	POP15-02	-23.185958	117.46327	2018	Fresh leaves
Western Range	POP15-03	-23.186039	117.46344	2018	Fresh leaves
Western Range	POP15-04	-23.185904	117.46327	2018	Fresh leaves
Western Range	POP15-05	-23.186030	117.46338	2018	Fresh leaves
Western Range	POP15-06	-23.185805	117.46317	2018	Fresh leaves
Western Range	POP15-07	-23.185633	117.46321	2018	Fresh leaves
Western Range	POP15-08	-23.185687	117.46331	2018	Fresh leaves
Western Range	POP16-01	-23.179404	117.41325	2018	Fresh leaves
Western Range	POP16-02	-23.179079	117.41326	2018	Fresh leaves
Western Range	POP16-03	-23.179621	117.41334	2018	Fresh leaves
Western Range	POP16-04	-23.179260	117.41317	2018	Fresh leaves
Western Range	POP16-05	-23.179584	117.41345	2018	Fresh leaves
Western Range	POP16-06	-23.179693	117.41344	2018	Fresh leaves
Western Range	POP16-07	-23.179729	117.41343	2018	Fresh leaves
Western Range	POP16-08	-23.179630	117.41331	2018	Fresh leaves
Western Range	POP17-01	-23.180941	117.41271	2018	Fresh leaves
Western Range	POP17-02	-23.180868	117.41288	2018	Fresh leaves
Western Range	POP17-03	-23.180941	117.41268	2018	Fresh leaves
Western Range	POP17-04	-23.180950	117.41263	2018	Fresh leaves
Western Range	POP17-05	-23.181068	117.41258	2018	Fresh leaves
Western Range	POP17-06	-23.181141	117.41244	2018	Fresh leaves

Western Range	POP17-07	-23.181140	117.41247	2018	Fresh leaves
Western Range	POP17-08	-23.181186	117.41244	2018	Fresh leaves
Western Range	POP18-01	-23.185280	117.41485	2018	Fresh leaves
Western Range	POP18-02	-23.185199	117.41498	2018	Fresh leaves
Western Range	POP18-03	-23.185208	117.41503	2018	Fresh leaves
Western Range	POP18-04	-23.185163	117.41490	2018	Fresh leaves
Western Range	POP18-05	-23.185135	117.41521	2018	Fresh leaves
Western Range	POP18-06	-23.185878	117.41433	2018	Fresh leaves
Western Range	POP18-07	-23.186014	117.41423	2018	Fresh leaves
Western Range	POP18-08	-23.186249	117.41416	2018	Fresh leaves
Western Range	POP19-01	-23.183843	117.41525	2018	Fresh leaves
Western Range	POP19-02	-23.183887	117.41559	2018	Fresh leaves
Western Range	POP19-03	-23.183798	117.41528	2018	Fresh leaves
Western Range	POP19-04	-23.183842	117.41553	2018	Fresh leaves
Western Range	POP19-05	-23.183807	117.41538	2018	Fresh leaves
Western Range	POP19-06	-23.183842	117.41549	2018	Fresh leaves
Western Range	POP19-07	-23.183816	117.41539	2018	Fresh leaves
Western Range	POP19-08	-23.183861	117.41544	2018	Fresh leaves
Western Range	POP20-01	-23.183299	117.41269	2018	Fresh leaves
Western Range	POP20-02	-23.183272	117.41271	2018	Fresh leaves
Western Range	POP20-03	-23.183371	117.41273	2018	Fresh leaves
Western Range	POP20-04	-23.183299	117.41270	2018	Fresh leaves
Western Range	POP20-05	-23.183380	117.41257	2018	Fresh leaves
Western Range	POP20-06	-23.183254	117.41265	2018	Fresh leaves
Western Range	POP20-07	-23.183317	117.41253	2018	Fresh leaves
Western Range	POP20-08	-23.183191	117.41253	2018	Fresh leaves
Western Range	POP21-01	-23.180596	117.42692	2018	Fresh leaves
Western Range	POP21-02	-23.180839	117.42735	2018	Fresh leaves
Western Range	POP21-03	-23.180767	117.42724	2018	Fresh leaves
Western Range	POP21-04	-23.180839	117.42730	2018	Fresh leaves
Western Range	POP21-05	-23.180794	117.42708	2018	Fresh leaves
Western Range	POP21-06	-23.180875	117.42723	2018	Fresh leaves
Western Range	POP21-07	-23.180840	117.42706	2018	Fresh leaves
Western Range	POP21-08	-23.180939	117.42709	2018	Fresh leaves
Western Range	POP22-01	-23.182159	117.42700	2018	Fresh leaves
Western Range	POP22-02	-23.182429	117.42703	2018	Fresh leaves
Western Range	POP22-03	-23.182249	117.42705	2018	Fresh leaves
Western Range	POP22-04	-23.182357	117.42711	2018	Fresh leaves
Western Range	POP22-05	-23.182366	117.42698	2018	Fresh leaves
Western Range	POP22-06	-23.182457	117.42696	2018	Fresh leaves
Western Range	POP22-07	-23.182294	117.42710	2018	Fresh leaves
Western Range	POP22-08	-23.182475	117.42689	2018	Fresh leaves
Western Range	POP23-01	-23.182653	117.42460	2018	Fresh leaves
Western Range	POP23-02	-23.182563	117.42449	2018	Fresh leaves
Western Range	POP23-03	-23.182563	117.42453	2018	Fresh leaves
Western Range	POP23-04	-23.182617	117.42435	2018	Fresh leaves

Western Range	POP23-05	-23.182436	117.42462	2018	Fresh leaves
Western Range	POP23-06	-23.182645	117.42426	2018	Fresh leaves
Western Range	POP23-07	-23.182626	117.42442	2018	Fresh leaves
Western Range	POP23-08	-23.182618	117.42418	2018	Fresh leaves
Western Range	POP24-01	-23.181254	117.42427	2018	Fresh leaves
Western Range	POP24-02	-23.181137	117.42407	2018	Fresh leaves
Western Range	POP24-03	-23.181290	117.42412	2018	Fresh leaves
Western Range	POP24-04	-23.181083	117.42399	2018	Fresh leaves
Western Range	POP24-05	-23.181344	117.42407	2018	Fresh leaves
Western Range	POP24-06	-23.181073	117.42424	2018	Fresh leaves
Western Range	POP24-07	-23.181263	117.42404	2018	Fresh leaves
Western Range	POP24-08	-23.181155	117.42413	2018	Fresh leaves
Western Range	POP25-01	-23.186973	117.42715	2018	Fresh leaves
Western Range	POP25-02	-23.186838	117.42698	2018	Fresh leaves
Western Range	POP25-03	-23.186882	117.42714	2018	Fresh leaves
Western Range	POP25-04	-23.186847	117.42699	2018	Fresh leaves
Western Range	POP25-05	-23.186774	117.42704	2018	Fresh leaves
Western Range	POP25-06	-23.186865	117.42694	2018	Fresh leaves
Western Range	POP25-07	-23.186639	117.42703	2018	Fresh leaves
Western Range	POP25-08	-23.186856	117.42678	2018	Fresh leaves
Western Range	POP26-01	-23.185488	117.43186	2018	Fresh leaves
Western Range	POP26-02	-23.185606	117.43166	2018	Fresh leaves
Western Range	POP26-03	-23.185479	117.43179	2018	Fresh leaves
Western Range	POP26-04	-23.185479	117.43156	2018	Fresh leaves
Western Range	POP26-05	-23.185515	117.43177	2018	Fresh leaves
Western Range	POP26-06	-23.185416	117.43156	2018	Fresh leaves
Western Range	POP26-07	-23.185379	117.43181	2018	Fresh leaves
Western Range	POP26-08	-23.185316	117.43176	2018	Fresh leaves
Western Range	POP27-01	-23.186544	117.43535	2018	Fresh leaves
Western Range	POP27-02	-23.186291	117.43514	2018	Fresh leaves
Western Range	POP27-03	-23.186526	117.43532	2018	Fresh leaves
Western Range	POP27-04	-23.186336	117.43520	2018	Fresh leaves
Western Range	POP27-05	-23.186606	117.43559	2018	Fresh leaves
Western Range	POP27-06	-23.186381	117.43531	2018	Fresh leaves
Western Range	POP27-07	-23.186570	117.43556	2018	Fresh leaves
Western Range	POP27-08	-23.186472	117.43528	2018	Fresh leaves
Western Range	POP28-01	-23.184563	117.44293	2018	Fresh leaves
Western Range	POP28-02	-23.184436	117.44297	2018	Fresh leaves
Western Range	POP28-03	-23.184445	117.44303	2018	Fresh leaves
Western Range	POP28-04	-23.184454	117.44301	2018	Fresh leaves
Western Range	POP28-05	-23.184489	117.44321	2018	Fresh leaves
Western Range	POP28-06	-23.184309	117.44300	2018	Fresh leaves
Western Range	POP28-07	-23.184535	117.44321	2018	Fresh leaves
Western Range	POP28-08	-23.184354	117.44302	2018	Fresh leaves
Western Range	POP29-01	-23.183304	117.44404	2018	Fresh leaves
Western Range	POP29-02	-23.182770	117.44429	2018	Fresh leaves

Western Range	POP29-03	-23.183059	117.44419	2018	Fresh leaves
Western Range	POP29-04	-23.182824	117.44427	2018	Fresh leaves
Western Range	POP29-05	-23.183014	117.44423	2018	Fresh leaves
Western Range	POP29-06	-23.182941	117.44443	2018	Fresh leaves
Western Range	POP29-07	-23.182860	117.44441	2018	Fresh leaves
Western Range	POP29-08	-23.182643	117.44449	2018	Fresh leaves
Howie's Hole	Aq-1-01	-23.284556	117.75458	2012	Dried leaves
Howie's Hole	Aq-1-03	-23.284556	117.75458	2012	Dried leaves
Howie's Hole	Aq-1-08	-23.284556	117.75458	2012	Dried leaves
Howie's Hole	Aq-1-11	-23.284556	117.75458	2012	Dried leaves
Howie's Hole	Aq-1-14	-23.284556	117.75458	2012	Dried leaves
Howie's Hole	Aq-1-17	-23.284556	117.75458	2012	Dried leaves
Howie's Hole	Aq-1-20	-23.284556	117.75458	2012	Dried leaves
Howie's Hole	Aq-1-24	-23.284556	117.75458	2012	Dried leaves
Howie's Hole	Aq-2-01	-23.282667	117.75781	2012	Dried leaves
Howie's Hole	Aq-2-03	-23.282667	117.75781	2012	Dried leaves
Howie's Hole	Aq-2-08	-23.282667	117.75781	2012	Dried leaves
Howie's Hole	Aq-2-11	-23.282667	117.75781	2012	Dried leaves
Howie's Hole	Aq-2-14	-23.282667	117.75781	2012	Dried leaves
Howie's Hole	Aq-2-17	-23.282667	117.75781	2012	Dried leaves
Howie's Hole	Aq-2-20	-23.282667	117.75781	2012	Dried leaves
Howie's Hole	Aq-2-24	-23.282667	117.75781	2012	Dried leaves
Howie's Hole	Aq-3-01	-23.281972	117.76511	2012	Dried leaves
Howie's Hole	Aq-3-03	-23.281972	117.76511	2012	Dried leaves
Howie's Hole	Aq-3-08	-23.281972	117.76511	2012	Dried leaves
Howie's Hole	Aq-3-11	-23.281972	117.76511	2012	Dried leaves
Howie's Hole	Aq-3-14	-23.281972	117.76511	2012	Dried leaves
Howie's Hole	Aq-3-17	-23.281972	117.76511	2012	Dried leaves
Howie's Hole	Aq-3-20	-23.281972	117.76511	2012	Dried leaves
Howie's Hole	Aq-3-24	-23.281972	117.76511	2012	Dried leaves
Pirraburdoo	Aq-4-01	-23.222194	117.56053	2012	Dried leaves
Pirraburdoo	Aq-4-03	-23.222194	117.56053	2012	Dried leaves
Pirraburdoo	Aq-4-08	-23.222194	117.56053	2012	Dried leaves
Pirraburdoo	Aq-4-11	-23.222194	117.56053	2012	Dried leaves
Pirraburdoo	Aq-4-14	-23.222194	117.56053	2012	Dried leaves
Pirraburdoo	Aq-4-17	-23.222194	117.56053	2012	Dried leaves
Pirraburdoo	Aq-4-20	-23.222194	117.56053	2012	Dried leaves
Pirraburdoo	Aq-4-24	-23.222194	117.56053	2012	Dried leaves
Western Range	Aq-5-01	-23.182167	117.41092	2012	Dried leaves
Western Range	Aq-5-03	-23.182167	117.41092	2012	Dried leaves
Western Range	Aq-5-08	-23.182167	117.41092	2012	Dried leaves
Western Range	Aq-5-11	-23.182167	117.41092	2012	Dried leaves
Western Range	Aq-5-14	-23.182167	117.41092	2012	Dried leaves
Western Range	Aq-5-17	-23.182167	117.41092	2012	Dried leaves
Western Range	Aq-5-20	-23.182167	117.41092	2012	Dried leaves
Western Range	Aq-5-24	-23.182167	117.41092	2012	Dried leaves

Western Range	Aq-6-01	-23.176806	117.41528	2012	Dried leaves
Western Range	Aq-6-03	-23.176806	117.41528	2012	Dried leaves
Western Range	Aq-6-08	-23.176806	117.41528	2012	Dried leaves
Western Range	Aq-6-11	-23.176806	117.41528	2012	Dried leaves
Western Range	Aq-6-14	-23.176806	117.41528	2012	Dried leaves
Western Range	Aq-6-17	-23.176806	117.41528	2012	Dried leaves
Western Range	Aq-6-20	-23.176806	117.41528	2012	Dried leaves
Western Range	Aq-6-24	-23.176806	117.41528	2012	Dried leaves
Western Range	Aq-7-01	-23.179889	117.42314	2012	Dried leaves
Western Range	Aq-7-03	-23.179889	117.42314	2012	Dried leaves
Western Range	Aq-7-08	-23.179889	117.42314	2012	Dried leaves
Western Range	Aq-7-11	-23.179889	117.42314	2012	Dried leaves
Western Range	Aq-7-14	-23.179889	117.42314	2012	Dried leaves
Western Range	Aq-7-17	-23.179889	117.42314	2012	Dried leaves
Western Range	Aq-7-20	-23.179889	117.42314	2012	Dried leaves
Western Range	Aq-7-24	-23.179889	117.42314	2012	Dried leaves
Western Range	Aq-8-01	-23.184861	117.44075	2012	Dried leaves
Western Range	Aq-8-03	-23.184861	117.44075	2012	Dried leaves
Western Range	Aq-8-08	-23.184861	117.44075	2012	Dried leaves
Western Range	Aq-8-11	-23.184861	117.44075	2012	Dried leaves
Western Range	Aq-8-14	-23.184861	117.44075	2012	Dried leaves
Western Range	Aq-8-17	-23.184861	117.44075	2012	Dried leaves
Western Range	Aq-8-20	-23.184861	117.44075	2012	Dried leaves
Western Range	Aq-8-24	-23.184861	117.44075	2012	Dried leaves
Howie's Hole	HERB-01	-23.282663	117.75799	2001	Dried leaves (PERTH 06414141)[H]
Western Range	HERB-02	-23.187694	117.44746	2009	Dried leaves (PERTH 08605483)[H]
Western Range	HERB-03	-23.358333	117.44167	1985	Dried leaves (PERTH 05643287)[H]
Howie's Hole	HERB-04	-23.284167	117.75639	2006	Dried leaves (PERTH 08230080)[H]
Howie's Hole	HERB-05	-23.282806	117.76652	2007	Dried leaves (PERTH 07759568)[H]
Howie's Hole	HERB-06	-23.280472	117.75032	2007	Dried leaves (PERTH 07759517)[H]
Mt Boggola	HERB-07	-23.796667	117.58944	1992	Dried leaves (PERTH 05724767)[H]

H: indicates specimen ID from the Western Australian Herbarium