Population survey for *Triodia veniciae* in the East Pilbara, June 2017

A report to Roy Hill Iron Ore

September 2017

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Summary

Scope

Kings Park and Botanic Garden were contracted in May 2017 by Roy Hill Holdings Pty Ltd to conduct a survey in the eastern Chichester Range for a poorly known and putatively rare 'spinifex' grass, *Triodia veniciae*, in order to:

- Identify the substrate and habitat range of *Triodia veniciae* populations.
- Survey the geographic extent of potentially suitable habitat for populations of *T. veniciae*.
- Clarify the taxonomy status of any ambiguous populations using genetic techniques.
- Estimate the population size of identified locations.
- Recommend an appropriate priority status for *T. veniciae*, based on the above findings.

The survey was conducted 22-27 June, 2017. A total of 157 sites containing (or expected to contain) *Triodia* were surveyed, focusing on 63 sites of shale substrate on the Chichester Range, matching the substrate from the three previously known locations.

Main findings

Triodia veniciae was present at 27 shale patches across a 140 km range along the Chichester Range from Roy Hill Station in the east to Mulga Downs in the west. Since some of these patches were in close proximity to each other, patches less than 2 km apart were grouped into a single "population", resulting in a total of 14 populations for the purpose of this study. *Triodia veniciae* plants were always found on shale, but less than half of all shale sites visited (27 of 63, 43.9%) had *T. veniciae* present. A visual estimate of *T. veniciae* population size was made, based on a detailed count of clumps in one population. Combined, the patches visited in this survey were estimated to contain at least 144,500 plants of *Triodia veniciae*. Additional survey effort would undoubtedly uncover additional populations.

Preliminary genetic investigations of populations of *T. veniciae* from across the newly delimited distribution demonstrate that majority of populations are diploid (with rare triploid plants), with a single tetraploid population located about 70 km north-west of Roy Hill Mine along the Roy Hill Rail line. The western-most populations located on Mulga Downs have an unusual genome size that is close to that expected for triploids, but investigation of seed endosperm ploidy excludes the triploid possibility; these plants are most likely of tetraploid origin followed by genome reduction, suggesting significant divergence from the eastern populations. DNA sequence data also indicates genetic divergence between the eastern and western populations.

Given these considerations, a conservation status of Priority Three is recommended for *T. veniciae*, which will be independently evaluated by the Threatened Species Branch of the Western Australian Dept. of Biodiversity, Conservation, and Attractions (DBCA) during the manuscript review prior to formal publication of the name *Triodia veniciae*.

Introduction

Triodia veniciae M.D. Barrett *(manuscript name, Barrett (2018) - publication in review)* is a recently discovered species, first recognised during a *Triodia* survey undertaken for Roy Hill Holdings Pty Ltd (Roy Hill) in August 2016. In that survey, *Triodia veniciae* had been located at three sites, two close to the Roy Hill Mine Accommodation Village, and one along the Roy Hill Rail Corridor north-west of the Mine. The locations and initial discussion of *Triodia veniciae* are detailed in the subsequent report: (Barrett, Krauss & Grierson 2016), referred to therein as "*Triodia* sp. Roy Hill (M.D.Barrett MDB 5412)".

In May 2016, the Botanic Gardens and Parks Authority (BGPA) were commissioned by Roy Hill to undertake a survey for "*Triodia* sp. Roy Hill", in order that its conservation status could be evaluated and appropriate priority status assigned. The paper providing a formal description and naming of *Triodia veniciae* MS has been submitted for publication in the WA Herbarium journal *Nuytsia*, and is currently in review. *Triodia veniciae* has been treated in the Pilbara spinifex identification app SPIKEY (Barrett et al. 2017), and differences to other *Triodia* species can be found there.

The scope of the survey

- Identify the substrate and habitat range of *T. veniciae* populations.
- Survey the geographic extent of potentially suitable habitat for populations of *T. veniciae*.
- Estimate the population size of identified locations.

During the survey it became apparent that genetic confirmation of the identity of some populations was necessary, and so preliminary genetic analysis of sequence divergence between populations was included.

Methods

Study Area

The area surveyed for *Triodia veniciae* study lies along the eastern Chichester Range on the north side of the Fortescue valley in the eastern Pilbara, between Mulga Downs station in the west to Balfour Downs station in the east.

In addition to the wide-scale survey, we also estimated the extent of *T. veniciae* within the bounds of the Roy Hill Iron Ore mine tenement, which is located approximately 280 kilometres (km) south of Port Hedland and 110 km north of Newman in the Pilbara Region of Western Australia. The Roy Hill mine is located on Mining Lease areas M46/518, M46/519, L47/346 and L46/104.

Habitat matching and site selection

The three known sites of *T. veniciae* that had been located in 2016 shared a similar substrate and landscape position, all being on shale slopes on the northern margin of the main Marra Mamba ridgeline of the eastern Chichester Range, immediately to the north of the Fortescue Valley. These shale deposits are geologically older, and in lower strata, than the Marra Mamba Banded Iron Formation (BIF) deposits, and due to the general southward dip slope of the eastern Chichester Range, the shale deposits mostly lie on the northern margin of the eastern Chichester Range. The exposed shale deposits typically have a paler orange colour than iron ore or loam substrates. In addition, the vegetation on shale substrates is less dense than on most other rock types in the region. Consequently, candidate sites could be readily identified using the imagery available on

Google Earth®, by their paler and less vegetated nature, and slope topography. On and immediately adjacent to the Roy Hill mine tenement , high resolution aerial imagery captured for Roy Hill was used to identify target sites more accurately than was possible with Google Earth imagery. Additional sites with similar slope and rockiness but different geologies were also identified and surveyed in order to establish whether *Triodia veniciae* was also present on substrates other than shale.

Distribution and population definition
Between 22-27 May 2017, a total of 157 sites were visited, including 63 candidate shale sites. The *Triodia* species present at each site was recorded, and when *T. veniciae* was present, an estimate of the population size was made. Populations were localized and patchy, mostly following changes in topography and substrate, but sometimes apparently suitable habitat was only partially occupied. *Triodia veniciae* populations were geo-located at one or more points per site to confirm exact points of occurrence, generally near the center of a continuous patch. Even at a local scale (hundreds of metres), population boundaries could not be exhaustively mapped due to time constraints on the survey. Additional patches are to be expected within 100-1000 m around many of the identified geo-referenced points.

Following the survey, identified points of occurrence were assessed for grouping into populations. There is no agreed definition of a "population", nor a single practical working definition in use in Western Australia. Instead practical working definitions are typically developed taking into account species distribution, patchiness and disjunction, reproductive and regeneration strategies, pollination syndrome, and dispersal abilities of pollen and seed (S. van Leeuwen, pers. comm.). Regeneration behavior of *T. veniciae* is not known, as no recently burnt sites containing *T. veniciae* were located. As far as can be estimated from available data, *Triodia veniciae* is likely to be predominantly outcrossing, wind-dispersed, with an unknown pollen dispersal distance. However population structure in several range restricted species of *Triodia* suggest that the majority of pollen movement may be more limited than might initially be assumed, on the scale of 1-10 km. As a conservative measure, for the purpose of this study, occurrence points were grouped at two levels:

(1) A 'population' is defined as a patch or group of patches located within **2 km** of each other.
(2) A 'patch' is defined as a ±continuous patch of *T. veniciae* plants, at least **100 m** distant from the nearest patch (i.e. patches < 100 m distant were aggregated into a single patch).

Thus each **population**, as defined here, typically consists of a disjunct arrangement ('metapopulation') of localized **patches** of *T. veniciae*.

Population size estimates
Although *Triodia veniciae* populations were always of limited extent, plants were typically quite numerous (>1000) at each location, and consequently it was impossible to conduct an accurate census and cover all sites in the time available. A single site of defined area, hereafter referred to as the 'reference site' was therefore counted semi-accurately (see method below), and population size of other sites estimated visually based on the relative density and extent compared to the reference site. The population census derived in this way is likely to be an underestimate in most cases, since (1) visual estimates of the area of a sloping and undulating area will likely underestimate the actual area, and (2) it is likely that many "clumps" are actually several individuals. Clumps often grow in lines or patchy tufts that may represent a single individual, or may

be composed of several discrete individuals. It is impossible to conclusively demonstrate individual boundaries without very extensive genetic fingerprinting, so the best proxy method for census is a count of "clumps", recognizing that clump boundaries are impossible to determine in many cases.

A single site (the 'reference site') covered in a typical monoculture of *T. veniciae* (population 4, patch 1 in Table S1) was boundary mapped using a Trimble GeoExplorer differential GPS (Digital Mapping Solutions, Perth, WA, Australia) and area calculated in EarthPoint tolls for Google Earth (http://www.earthpoint.us/shapes.aspx). The precise count of *T. veniciae* in this patch estimated 3232 clumps in 1549 m^2 (2.1 clumps per m^2), although there are some limitations on accuracy of clump counts compared to genetic individuals as described above. This density estimate is probably similar to most of the sites visited, although a few locations had considerably sparser clumps, or were less dense due to the shale area being dominated by other species, especially *T. epactia*.

Since there was insufficient time to boundary-map all populations visited, an estimation of number of clumps in other populations was done visually at the time of visitation, and only approximate centre-points of each population was recorded.

Morphological identification of Triodia veniciae
Triodia identifications for this study followed the most recent Pilbara treatment, in the identification app SPIKEY (Barrett et al. 2017). The critical features of *T. veniciae* compared with other local Triodia species are:

- Resinous foliage.
- Epistomatous leaves (lacking stomatal grooves on the lateral-abaxial surface).
- Narrowly lanceolate glumes with L:W ratio >4 [<4 in *T. epactia*].
- Lemma lobes narrowly acute and 2.1-3.1 mm long [lemma lobes >4 mm long in the previously-confused species *T. melvillei* and *T. pisoliticola*].

The character of hairy leaf sheath surfaces noted in SPIKEY was found to be absent or variable in western-most populations, and is therefore not a diagnostic character, although it does hold as a diagnostic feature in eastern populations around the Roy Hill mine site.
Two additional characters that proved useful in the field are:

- Strong aromatic scent that, with experience, is immediately distinguishable from the only other co-occurring resinous species, *T. epactia* (which has a weaker and nondescript scent).
- Narrow leaf sheaths < 6 mm wide near the apex (usually > 6 mm wide in T. epactia).

Flow cytometry
Genome size estimates were carried out using flow cytometry following the procedures outlined in Barrett et al. (2016). A minimum of ten plants from each of eight populations (five collected for this survey, and three previously described in Barrett et al 2016 as Triodia sp. Roy Hill) were screened and the amount of DNA present in individual nuclei stained with propidium iodide (PI) measured using a flow cytometer following the methodology described in Barrett et al. (2016).

At each site, sampled plants were at least 10 m apart to minimize the possibility of repeat sampling from large clonal clumps. Sampled locations are shown in Figure 4, and

locations listed in Table S2. Samples were stored at 4 °C, and kept refrigerated for 1-4 weeks until flow cytometry was performed.

DNA sequencing
DNA was obtained from single samples from each of ten *T. veniciae* populations, across the full range of the species, including three from the western Mulga Downs populations, and seven from the eastern Roy Hill mining tenements and Roy Hill Rail line.

The Internal Transcribed Spacers 1 and 2 region of nuclear ribosomal DNA (ITS) were sequenced using standard DNA extraction, polymerase chain reaction (PCR) and DNA sequencing methodology, as described in Anderson et al. (2016). The resulting sequences were aligned against a larger database of *Triodia* samples, and phylogenetic trees constructed using the Maximum-Likelihood algorithm implemented in RAxML (Stamatakis, 2006).

Results and discussion

Survey locations
Triodia veniciae plants were always found to be growing on shale (Figure 1,2), and rarely on areas containing shale partly covered by a thin veneer of ironstone gravel eroded from upslope. Of 157 sites surveyed, 63 had shale substrate, and 27 of these contained *Triodia veniciae* (Figure 3, 4).

Triodia veniciae was located across 140 km along the northern margin of the Chichester Range (Figure 3), from Roy Hill Station in the east, and Mulga Downs in the west. Of the total 63 shale sites visited, 27 (42.9%) contained *Triodia veniciae* (including the three previously-known sites). A total of 36 sites were shale, or mixed shale-conglomerate or shale-ironstone gravel, but lacked *Triodia veniciae*. An additional 94 positions visited had substrates other than shale, and always lacked *Triodia veniciae*.

Shale Plant Communities
The shale sites were typically monocultures of *T. veniciae*, but sometimes with a few other species such as *Triodia epactia*, *Goodenia cusackiana*, or *Newcastelia* sp. Hamersley Range and *Senna* spp. were also present. Shale sites lacking *T. veniciae* were usually dominated by *Triodia epactia*.

Figure 1: *Triodia veniciae* clumps and shale substrate.

Figure 2: Typical *Triodia veniciae* habit on shale hill-slopes. The low rounded hill on the left is entirely covered by *Triodia veniciae*. The larger hill to the right has a hard layer of ironstone at the summit, and is covered by other species of *Triodia* (*T. brizoides*, *T. scintillans*). *Triodia veniciae* plants occur on shale on the lower slopes only, up to about half the height of the hill, and is absent from the gullies. The surrounding plain is covered by other *Triodia* species (*T. epactia*, *T. longiceps*, *T. wiseana*).

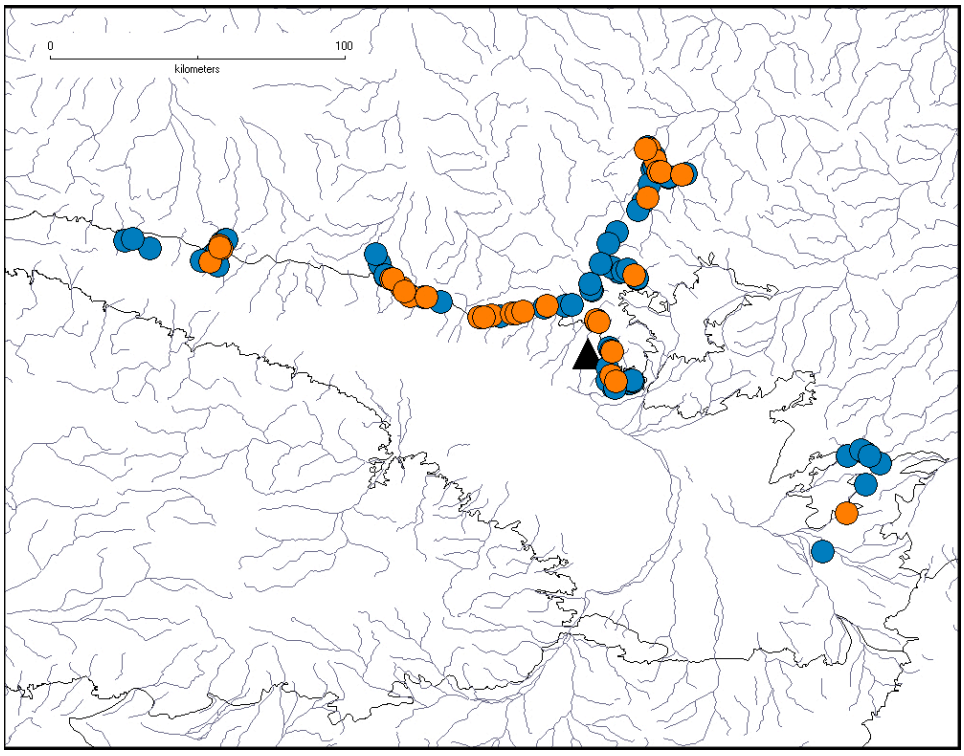

Figure 3: Map of surveyed locations with shale substrates indicated (orange). The position of the Roy Hill Mine Services Area is indicated (black triangle). Background map indicates drainage lines, and the position of the Fortescue and Hamersley Bioregions.

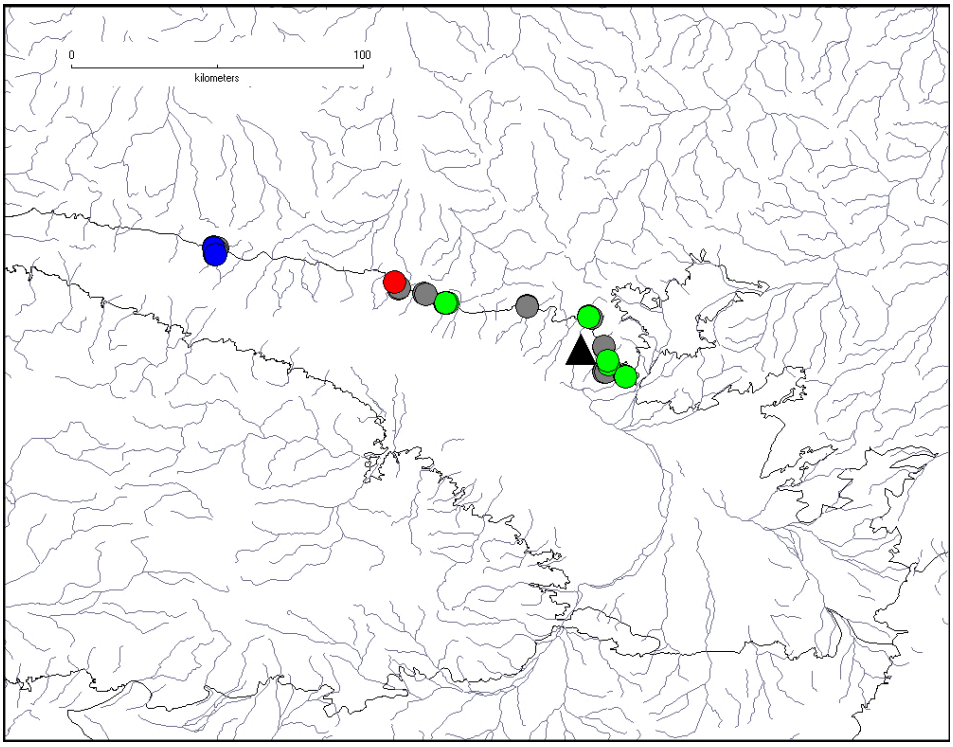

Figure 4: Map of *Triodia veniciae* locations (circles). The populations surveyed for ploidy level are coded by ploidy: diploid (green), tetraploid (red) or reduced-tetraploid (blue); grey circles indicate populations not sampled for ploidy. The position of the Roy Hill Mine Services Area is indicated (black triangle). Background map indicates drainage lines, and the position of the Fortescue and Hamersley Bioregions.

Population Census

The 27 recorded *T. veniciae* patches (at least 100 m apart), were aggregated into 14 populations (at least 2 km apart) for the purposes of this survey. Across all sites and populations visited a conservative estimate of at least 144,500 *Triodia veniciae* clumps was made. This is likely to be an underestimate of the populations visited, due to the difficulty of rapidly estimating such large numbers of plants. The full population extent was traversed in only a few cases, and usually there was additional suitable habitat nearby with very high probability of occurrence that was not visually inspected. Furthermore, several additional sites could not be accessed for this study (including 47 sites on the Roy Hill mining tenement), that are highly likely to harbour *T. veniciae*. Consequently the actual number of plants of *T. veniciae* its whole distribution is expected to be 3-10 times the number estimated here.

Of the 14 populations of *Triodia veniciae* recognised here, four were within the Roy Hill Iron Ore mine tenements, containing an estimated 22,800 plants (15.8% of the estimated total surveyed *T. venciae*). A total of 47 additional locations within the Roy Hill Tenements, located in between confirmed populations, were identified from aerial imagery as suitable shale habitat, and are expected to harbor *T. veniciae* (Table S3). Assuming near-complete occupancy of these un-surveyed locations, a conservative estimate of 3,000 plants per un-surveyed site suggests an additional 141,000 or more plants may exist on the Roy Hill mining tenements. A full survey of *T. veniciae* on Roy Hill tenements would therefore almost certainly significantly increase the number of plants, as would more extensive survey in areas outside the Roy Hill tenements.

Populations of *T. veniciae* immediately to the east and north of the Roy Hill mine site lie outside the Roy Hill tenements, and will presumably remain un-impacted by mining operations.

Other than the relatively dense series of populations on and near the Roy Hill tenements, the most numerous and densest populations of *T. veniciae* known lie along the Roy Hill Rail line. Although the rail line represents a somewhat random transect through the area, the intersection of many *T. veniciae* populations is probably not a coincidence. The position of the Roy Hill rail line runs along the north side of the main Chichester Range ridge line, and the shale deposits that lie conformably below the Marra Mamba deposits of the Chichester Range are best exposed on the northern margin of the Chichester Range ridge line, so the rail and *T. veniciae* habitat are therefore more closely co-located than might be expected by chance.

Genetic variation 1: flow cytometry

Three ploidy cytotypes were located in *Triodia veniciae*. Plants in the eastern part of the distribution, from Roy Hill Station to c. 50 km along the Roy Hill Rail line (west of Marble Bar Road), were composed of diploid plants, with very rare triploid individuals. A single population on the Roy Hill Rail corridor about 65 km along the Roy Hill Rail line (west of Marble Bar Road) was composed only of tetraploid plants. Populations near the Great Northern Highway on Mulga Downs were composed only of plants with approximate "triploid" genome size (see below).

Three dominant genome sizes were located, all separated spatially. The single tetraploid population was located not far from diploid populations, and the genome size is an almost exact double of the diploid content, suggesting that this tetraploid is of recent origin.

In contrast, the western Mulga Downs populations had a genome size significantly different from either the diploid or tetraploid origin, and the best current explanation of the evidence is that it is a tetraploid lineage that has lost considerable amounts of the duplicated DNA, i.e. is well along the path toward diploidization. It is possible that this genomic rearrangement has progressed to a point of infertility with other populations, but this remains to be tested.

In the Mulga Downs populations, the unusual genome size close to an expected "triploid" value suggested that these populations might represent an apomictic triploid race. To test this idea, three seed (all that could be located from plants at the time of collection) were also processed for flow cytometry, by chopping entire seed in 500 uL of buffer and processing as for leaves. Grass grains (caryopses) consist of a small embryo that will eventually grow into a parent plant, and the rest of the grain is filled with endosperm, a storage tissue that is digested on germination. Sexually reproducing seed should produce an embryo peak with DNA content matching a parent plant (resulting from fertilization of a haploid egg cell with a haploid pollen nucleus, n+n=2n), and an endosperm DNA content 1.5 times higher than the embryo (e.g. in a diploid plant resulting from fertilization of a diploid cell with a haploid pollen nucleus, 2n+n=3n). Apomictic seeds, on the other hand, should have higher embryo-endosperm ratios (expected to have an endosperm:embryo ploidy ratio of 2, 2.5 or 3, depending on whether fertilization occurs and ploidy of the fusing cells (Dobes et al, 2013). The Mulga Downs seeds all had observed embryo-endosperm ratios of 1.51, almost exactly the expected ratio for endosperm derived by normal sexual recombination, and hence plants in this population are not triploid (since a ratio of 1.5 times a triploid embryo would imply a 4.5-ploid endosperm, and fractional genome content is not possible). The most likely explanation for the observed genome size in the Mulga Downs populations is that it is a tetraploid that become substantially diploidized over time with the loss of nearly a quarter of the total duplicated gene content, a process observed in many polyploids (Wendel, 2015). Such a process of genome reduction will ultimately result in reproductive isolation from both the original diploid genotype and its polyploids.

Genetic variation 1: ITS sequence variation
Three substitutions were uniformly different between all three Mulga Downs samples, and all seven "eastern" *T. veniciae* samples. This level of divergence in ITS sequences is unusual within geographically close populations of the same species, but is insufficient to indicate a distinct taxon without additional supporting evidence, such as morphological differences.

Taxonomic evaluation of differences between populations
We found distinct differences in genomic DNA content, moderate differences in ITS sequences, and no consistent differences in morphology between eastern and western populations. Consequently we consider the Mulga Downs populations to represent a variant of *T. veniciae*, although the genomic content and ITS difference suggest the existence of significant intra-specific divergence between eastern and western populations. With future work, including survey of intervening areas, it is likely that these two areas could be treated as independent management units.

An additional *Triodia* entity from shale in the Hamersley Range north West of Tom Price is morphologically close to *T. veniciae*, but ITS sequences instead place it close to *T. karijini* MS, and *T. pisoliticola* MS which also occur along the Hamersley Range system (Barrett et al 2017), and it was therefore excluded from further consideration.

Factors affecting the conservation status of Triodia veniciae
Relevant factors in determining conservation status are:

- Habitat specialization, and distribution of habitat.
- The total number of individuals.
- The number of populations (population in part by genetic connectivity via seed and pollen dispersal).
- Population size and connectivity.
- Genetic structure within the species.
- Threatening processes.

Triodia veniciae was shown in this survey to be highly habitat selective and restricted to shale hill-slopes of the eastern Chichester Range. These habitats, and *T. veniciae* populations, are small and disjunct, albeit scattered, along a 140 km band of the Chichester Range. The extent of *Triodia veniciae* may extend a few tens of km to the east or west of the range described here, but aerial imagery shows that shale geology becomes rare and widely separated or absent beyond this zone, and attempts to find additional patches further west on Mulga Downs, and to the east on Balfour Downs were unsuccessful. Past surveys of disjunct shale geology in the Hamersley Range, and in the Chichester Range north of Nullagine, and west of Mulga Downs, have failed to locate any plants of *T. veniciae*. Consequently it is considered that the extent of *T. veniciae* has been established within ~20 km by this survey.

An estimated minimum total of 144,500 plants of T. veniciae was found, distributed in 27 patches (patches defined as being discrete and at least 100 m apart), which were aggregated for the purpose of this survey into 14 populations using a "minimum 2 km separation" rule. These numbers will certainly be an underestimate, since populations away from the Roy Hill rail line could not be accessed. Reports of "*Triodia melvillei*" from both the nearby Christmas Creek and Cloudbreak tenements (Mattiske Consulting, 2005; ENV Australia, 2010) almost certainly refer to *Triodia veniciae*, however no specimens from those areas are available for confirmation. *Triodia melvillei* is not known from north of the Fortescue River.

Both genome size estimates and ITS sequence data suggest that there are two genetic units within *T. veniciae*, with those on Mulga Downs distinct from populations further east. However, it is not known whether additional populations occur in the intervening area, and whether any disjunction or intergradation occurs, due to the limitations of this survey.

The threatening processes potentially affecting *T. veniciae* are:

- Habitat destruction
- Processes associated with small and restricted populations.

It is not known whether any *T. veniciae* populations have yet been impacted by iron ore extraction along the eastern Chichester Range, however it is likely that future activities will do so. Five of the known populations (populations 2,3,4 and 6 in Table S1) occur on the Roy Hill tenements. The confirmed populations on Roy Hill tenements are estimated to contain about 22,800 plants (15.3%) of the total 144,500 plants estimated for *T. veniciae*. The number of plants on Roy Hill tenements will certainly be higher due to the likely existence of and 47 additional predicted patches (many of these less than 1 km distant from each other, so not meeting the 2 km population requirement as above), however the overall percentage of total *T. veniciae* plants is probably only a little higher than 15%, since additional populations are also expected to occur away from Roy Hill tenements (although with decreasing frequency away from the Roy Hill / Christmas Creek area). Loss of a significant proportion of the populations on Roy Hill tenements through clearing for mineral extraction would affect a significant proportion of the total number of plants and populations of the species, however many additional populations would remain undisturbed outside the leases. Cumulative effects of population loss of *T. veniciae* when incorporating losses from other nearby mines may however be more significant.

Along the Roy Hill rail line, several locations were found at which the substrate appeared to be suitable for *T. veniciae*, but no plants were detected. These populations are on relatively low hills on the northern margin of the Chichester range, close to the northern distribution limit of the species. Consequently it is possible that fluctuating climate and marginal habitat have led to the localized extinction of *T. veniciae*. The proximity of a solitary tetraploid population could possibly be evidence of past population isolation and demographic fluctuation such that the ploidy level could become fixed away from the dominant diploids. If these hypotheses are correct, then extreme drought periods may pose a long-term threat to some *T. veniciae* populations, especially those on small hills, on low slope, or isolated from other populations (and harder to re-colonize). Nevertheless the risk of widespread extinction of *T. veniciae* from such processes is low in the short to medium term.

Recommendations on conservation status of Triodia veniciae

Considering that:

- At least 14 populations of *T. veniciae* exist (with a minimum separation of 2 km);
- Populations are disjunct and have small spatial area;
- Populations form dense, monocultures, with plant numbers estimated to be > 140,000 clumps;
- There is evidence of genetic structure (east and west - possibly with additional diploid and tetraploid forms in the eastern group) within the species, lowering the overall security if genetically divergent populations are considered of additional conservation significance;
- At least some populations are predicted to be impacted by mineral extraction;
- While some indication of the potential impact by Roy Hill operations has been obtained, the extent to which the nearby Christmas Creek and Cloudbreak

operations have or will impact on populations of *T. veniciae* is completely unknown;
- The populations most likely to be impacted are of the most widespread "eastern diploid" form:

a conservation status of Priority Three is recommended for *T. veniciae*.

The requirements for a Priority Three species, as described by the Priority codes for Western Australian flora (*https://www.dpaw.wa.gov.au/plants-and-animals/threatened-species-and-communities*) is:
"Priority Three: Species are known from several locations, and the species does not appear to be under immediate threat, or is known from a few but widespread locations with either large populations or large areas of un-surveyed and apparently suitable habitat, most of which is not under imminent threat. Species may be included if they are comparatively well known from several locations but do not meet survey requirements, and known threatening processes exist that could affect them. Such species are in need of further study."

However, additional genetic survey is recommended for *Triodia veniciae*, especially the status and relationships of the Mulga Downs populations. Should the Mulga Downs populations be recognised at intra-specific rank, or otherwise be treated as a separate conservation unit, it is likely to attract a Priority One status.

Given these considerations, a conservation status of Priority Three will be recommended for *T. veniciae*, which will be independently evaluated by the Threatened Species Branch of the Western Australian Dept. of Biodiversity, Conservation, and Attractions (DBCA) during the manuscript review prior to formal publication of the name *Triodia veniciae*.

Supplementary Tables *(Excel spreadsheet "Barrett_2017_Triodia_veniciae_Survey_supplementary_tables.xlsx")*

Supplementary Table S1. Locations of ground-truthed *T. veniciae* populations (often including several patches within 1 km of each other), patches (at least 100 m distant from other patches), and reference points (within patches).

Supplementary Table S2. Location of samples used to assess ploidy in *T. veniciae*.

Supplementary Table S3. Additional likely patches of *T. veniciae* on Roy Hill tenements, identified from aerial imagery.

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