Chromosome Counts for taxa in the *Galium aparine* species complex (*G. aparine* and *G. spurium*) from six locations in south-west Western Australia

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Executive Summary

Chromosome counts were carried out to determine the identification of taxa from the *Galium aparine* complex (*G. aparine* and *G. spurium*) collected from four sites in the Bridgetown area. In two of the sites two different morphological forms (large and small) were identified and it was postulated that these might represent occurrences of two different taxa at the same location. Previous cytological studies indicate that *G. aparine* is usually 2n=66 while *G. spurium* is 2n = 20. The chromosome counts on material from both forms and the four sites were uniformly 2n = 66. These results confirm that the forms found at all four sites are *G. aparine* and not *G spurium*.

Background

Galium aparine (Cleavers) and *Galium spurium* (False Cleavers) have been recorded from Western Australia with the Western Australian Herbarium records indicating nine collections of *G. aparine* and 15 collections of *G. spurium* each scattered across the southwest of the State. *Gallium aparine* occurs usually in moist, shaded habitats, whereas *G. spurium* favours sunnier drier habitats. *Gallium aparine* may be confused with *G. spurium* which is closely related and although they can be distinguished by certain morphological characteristics (Malik and Vanden Born 1988) differences are often cryptic with each species showing significant morphological variation depending upon the conditions under which the species are growing and the habitat in which they occur.

Gallium aparine is common in temperate zones on all continents, but is restricted to higher altitudes in the tropics (Holm et al., 1977). In Europe, it occurs from Portugal in the west to Russia in the east, and from the UK in the north to Italy in the south. It occurs in Alaska, extending across the wheat belt of Canada and throughout the USA. It is also found in Argentina, Chile and Uruguay and in Asia it extends from Pakistan to China and is found in Japan, Australia and New Zealand. *Galium spurium* is also widespread across Europe, Asia, Africa and Canada, and is naturalized in Australia.

In Australia *Gallium aparine* is particularly common in the wetter temperate regions of the south east being widespread and common in New South Wales, the ACT, Victoria, Tasmania and south-eastern South Australia. It is also recorded from south-west Western Australia and in the cooler districts of south-east Queensland. *Gallium spurium* is common in more arid areas found in South Australia, eastern NSW and eastern Victoria and also occurs more occasionally in south-western Western Australia.

Holm *et al*. (1991) described *G. aparine* as a serious or principal weed in 10 countries. Worldwide it has been reported as a weed of 19 crops in 31 countries (Holm et al., 1977). Although it commonly occurs in vegetable crops, beets, pastures, vineyards and plantation crops, it is most troublesome in cereals and canola (Brassica napas), where it may cause

significant yield reductions and interfere with harvesting. *Galium spurium* is also known as a significant weed in Europe and Canada.

In Canada *G. aparine* and *G spurium* are both considered to be major weeds. They are described as having several characteristics that predispose them toward weediness (Malik and Vanden Born, 1988). These include rapid seedling development, spiny fruits that increase dispersal and crop contamination, and seedling emergence throughout the growing season, which helps the plants escape herbicides and mechanical removal. In prairie regions of Canada *Galium spurium* is regarded as the more weedy and aggressive of the two species and is considered better adapted to growing conditions in these areas (Malik and Vanden Born,1988). Currently in Western Australia *G. aparine* is a declared weed while *G. spurium* is not declared and is not considered a significant threat.

A key distinguishing feature for these two species is there chromosome number. The primary chromosome number for *G. aparine* is 2n =66 (Moore 1975; Malik and Vanden Born, 1988) and it is assumed to be a hexaploid although deviations of 2n =64 have been noted from localities in Europe. In contrast the chromosome number of *G. spurium* is uniformly 2n = 20 with the same chromosome counts from disparate locations across a significant part of its range.

Objectives

The study aimed to utilise chromosome count information to determine the identification of taxa from the *Galium aparine* complex collected from four sites in the Bridgetown area. These sites are described in the following section. In two of the sites two different morphological forms (large and small) were identified and it was postulated that these might represent occurrences of two different taxa at the same location.

Population sampling

The Site descriptions are taken from Reeves and Webber (2013).

Site 1. is located along the roadside adjacent to the Warren River in an area with 80% shade cover along the road and waterway. *Galium* sp was found actively growing and flowering, and in early stages of seed production. Seed from two forms (small and large) was collected from this site.

Site 2. is located in an old bluegum plantation that has been re-planted. The area is in full sun and is heavily infested with wild oats, wild radish, variegated thistle and erodium. *Galium* sp. was growing along the edge of disturbed areas such as roadside in loam with high gravel content. Plants were flowering and in the early stages of seed production.

Site3. is on a road verge and had two distinct forms of *Galium* growing together. Reeves and Webber (2013) suggest that two species may be present with one similar to the form at Site 2 in terms of form and habit, colouration and stage of flowering. The other form was smaller

and more compact with dark green colouration compared to the “bright” green at Site 2. It also had more compact leaf whorls and flowers were concentrated at the apical tip rather than along the stem

Site 4. is 500m along the road from site 3. It was a heavily infested roadside with a *Galium* sp. that appeared to be the same form as the smaller, more compact form at Site 3.

Methods

Previously-collected seeds from the four locations in the Bridgetown area were germinated in a controlled temperature room, and roots were harvested after 5 days. Roots were treated with 0.1% colchicine for 2–3 h, fixed overnight in 3:1 100% ethanol: glacial acetic acid and stored at 4°C in 70% alcohol. The roots were stained using the Feulgen technique (Darlington & La Cour, 1970) with 3 minutes hydrolysis in 1N HCl at 60°C and stained with Leuco-Basic Fuchsin in the dark for 1 h. Stained tips were squashed in aceto-orcein and viewed at 1000x under oil immersion. Chromosomes were counted in cells with suitable mitotic metaphase and prophase spreads. At least four root tip squashes were prepared for each form from each site and chromosomes were counted from at least four cells per squash.

Results and Discussion

Chromosome counts for six different seed collections from the four sites is summarised as follows:

Site	Form (taxon)	Diploid Chromosome Number
1	Large	2n = 66
1	Small	2n = 66
2	Large	2n = 66
3	Large	2n = 66
3	Small	2n = 66
4	Small	2n = 66

The findings from this study show that both forms (small and Large), from the four different sites, have the same chromosome number of 2n= 66 and are therefore *G. aparine* not *G. spurium*. The location of these sites in the Bridgetown area, a relatively mesic part of the southwest, is consistent with habitat and climate that would be expected to favour *G. aparine.* It is therefore interesting to note that Herbarium collections of both *G. aparine* and *G. spurium* have been made from this area and also from the much drier parts of the southwest. Given the similarity in morphology it is possible that specimens identified as *G spurium* in more mesic parts are actually *G. aparine* and specimens identified as *G aparine* in more arid parts are *G. spurium*. It is recommended that all specimens of both taxa in the Herbarium be re-assessed to confirm that they have been assigned to the correct species.

Further chromosome counts from known sites of both species might also be useful in assisting the taxonomic identification of material.

References

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