

## *Cortaderia* spp. in New Zealand: patterns of genetic variation in two widespread invasive species

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**Abstract:** *Cortaderia selloana* (pampas grass) and *C. jubata* (purple pampas grass) are both invasive in New Zealand. *Cortaderia selloana* is found throughout most of the country, whereas *C. jubata* is restricted to the North Island and the northern South Island. We examine the genetic variation present in each of the species, and compare this to the findings of an earlier study that analysed the variation in invasive *C. jubata* plants from New Zealand. *Cortaderia selloana* is comprised of two main genetic groupings in New Zealand, one of which is more common than the other. The distribution of these two genetic groupings is discussed with respect to introduction history. *Cortaderia jubata* has considerably more genetic variation than expected, higher than estimated by a previous work. We discuss these results in the context of *Cortaderia* invasion in New Zealand and how this may impact on the design of a biocontrol programme.

**Keywords:** *Cortaderia*; genetics; invasion; microsatellites; weed

### Introduction

*Cortaderia* Stapf (Gramineae) is a genus of large perennial tussocks originating in South America. It is regarded as being made up of 19 species, although due to the lack of a comprehensive recent revision, the actual number of species in the genus is uncertain (Linder et al. 2010). Within the genus there are some species that have become invasive outside of their native range, particularly in New Zealand, the United States and South Africa (Robinson 1984; Ahmad et al. 2006; Edgar & Connor 2010). Two *Cortaderia* species are invasive in New Zealand, *C. selloana* (Schult. & Schult. f.) Asch. & Graebn. and *C. jubata* (Lemoine) Stapf (Edgar & Connor 2010). Until recently (Linder et al. 2010), seven native species of large tussock were regarded also as being in *Cortaderia* (since moved to a new genus, *Austroderia*). The two species differ in their breeding system, with *C. selloana* being gynodioecious, albeit with very low seed set in hermaphrodites making it functionally almost dioecious, whereas *C. jubata* is an autonomous apomict (produces seeds via parthenogenesis without the need for pollination).

*Cortaderia selloana* (pampas grass;  $2n=8X=72$ ) was first introduced into New Zealand about 1876 as a fodder species (Knowles & Ecroyd 1985), and has subsequently been used as both an ornamental and shelter species (Grimmet 1935). *Cortaderia selloana* was sold and grown extensively through the 1930s and 40s, and was still being evaluated as a valuable species in the 1950s (Jacques 1957; Knowles & Ecroyd 1985). During this time several 'strains' were developed by taking vegetative clones from existing plants, collecting seed, or even from experimental crosses. While some 'strains' or varieties were given names, the origins of most were unclear. The use of *C. selloana* as a fodder decreased sharply in the 1950s as land improvement became more common and better yielding pasture species were discovered. By the 1980s, *C. selloana* was recognised as being invasive, especially in plantation forestry, and by the early 2000s was having considerable impacts on primary productivity and the environment as a weed (Harris & Skilton 2007).

*Cortaderia jubata* (jubata grass;  $2n = 12X = 108$ ) is naturalised in New Zealand also from South America, and is a problem weed of forestry areas. *Cortaderia jubata* is an escape from ornamental plantings, first recorded in the 1960s as *C. atacamensis* (Knowles & Ecroyd 1985). Unlike *C. selloana*, as an apomict it has only female inflorescences, and is often identified by the purple hue of the florets, although this character is not completely reliable.

The cost of control of *C. selloana* and *C. jubata* in New Zealand is substantial at \$4000–\$6000 per hectare on the conservation estate, and it is also a considerable problem weed of roadsides, proving difficult to control with herbicides (Popay et al. 2003). Due to the cost and the widespread nature of the two species, a feasibility study on the biocontrol of *C. selloana* and *C. jubata* in New Zealand has been undertaken. Part of this study involved characterising the genetic variation present in introduced *Cortaderia* in New Zealand, to inform the range of material that would need to be searched for in the native range to allow accurate bio-prospecting for control agents. An earlier study by Okada et al. (2009) showed that some New Zealand individuals of *C. jubata* are similar to plants found in Ecuador and Peru, although the origin or amount of variation of *C. selloana* in New Zealand has not been assessed. What is clear is that *C. jubata* has undergone a strong founder effect during naturalisation, with most of the introduced range consisting of a single genetic clone. *Cortaderia selloana* may have undergone a similar process during naturalisation, although it is unknown how severe an effect this would have had on genetic variation due to it reproducing sexually and having multiple introductions. This study aims to determine whether a wider range of *C. jubata* samples from New Zealand has similar levels of genetic variation to those included in the Okada et al. (2009) study from both New Zealand and California, and whether *C. selloana* has also undergone a strong founder event during naturalisation in New Zealand. As these species differ substantially in breeding system, but have both been successful invaders in New Zealand, it is of interest to determine whether we see strong differences in the genetic variation of the invading populations.

Materials and methods

Plant material and DNA extraction

Leaf samples of 582 invasive *Cortaderia* samples were sourced from sites across the two main islands of New Zealand (Fig. 1, Table S1 in Supplementary Material). All samples were dried in silica gel immediately after sampling or collected from the Allan Herbarium (CHR) in Lincoln, New Zealand. The samples collected cover most of the known range of *Cortaderia* in New Zealand, especially for *C. jubata*. For *C. selloana* our sampling is more comprehensive in the North Island, where it is more commonly invasive; in the South Island it is more often restricted to plantings and shelter belts.

DNA was extracted using either an iNtRON plant DNA extraction kit (iNtRON Biotechnology, Seongnam, South Korea) or the Maxwell® 16 Tissue DNA purification kit (Promega Corporation, Madison, USA) following the manufacturers' instructions. DNA quality and quantity were determined using a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, Kentucky, USA).

Polymerase Chain Reaction amplification and microsatellite genotyping

Eight microsatellite primers amplifying nine polymorphic microsatellite loci published for *C. selloana* and *C. jubata* were used for genotyping (Ahmad et al. 2006; Okada et al. 2009) (Table 1). Polymerase chain reaction (PCR) conditions were optimised and the forward primers were labelled at the 5' end with different fluorescent dyes (6-FAM, NED or HEX) to allow multiplexing for genotyping.

Polymerase chain reactions were performed with final concentrations of 1 U of iTaq DNA polymerase (iNtRON Biotechnology), 1 × PCR buffer, 1.5 mM MgCl<sub>2</sub>, 40 µg/mL BSA (NEB), 208 µM dNTP-Mix, 167 nM 5'-labelled forward primer, 167 nM reverse primer, and 10–50 ng of DNA in a total reaction volume of 15 µL, adjusted with filtered (0.22 µm) and autoclaved Millipore H<sub>2</sub>O. Thermocycling was conducted on Bioer GenePro thermocyclers: 94°C for 4 min, 35 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 45 s, and a 72°C final extension period for 20 min. Ten µL were used to view the amplification products on 2.5% agarose gels.

Samples (1 µL) of the resulting amplified DNAs were prepared by adding 9 µL of Hi-Di formamide (Applied Biosystems, Carlsbad, California, USA) and 1 µL of ROX-labelled size standard (Applied Biosystems), before being separated on an ABI3730 Genetic Analyzer (Applied Biosystems).

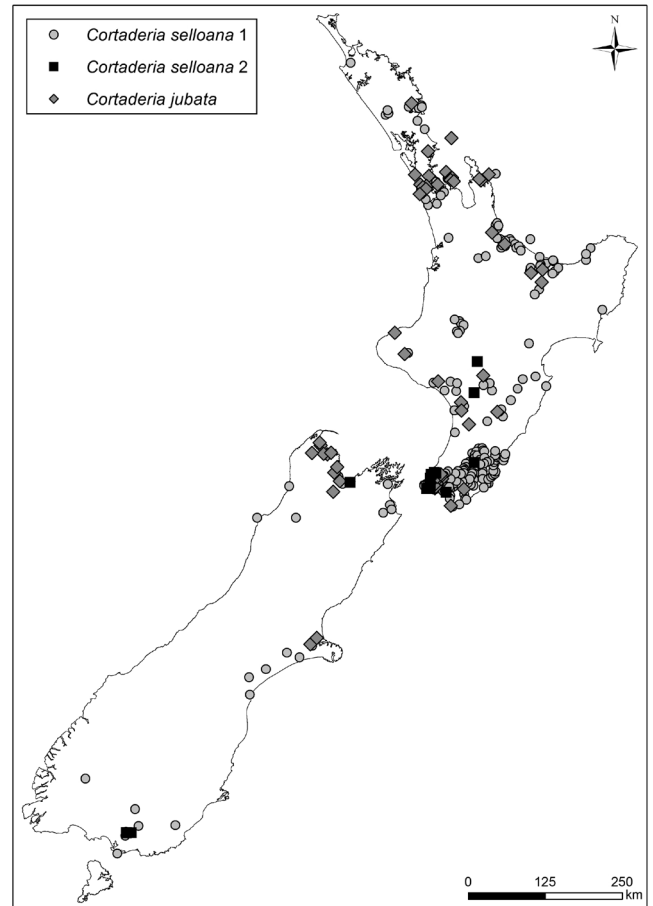


Figure 1. The distribution of the *Cortaderia* samples included in this study, labelled by species and showing the two different types of *C. selloana*.

Table 1. The number of markers per locus, by species. Numbers of alleles per individual in parentheses refer to a single individual with that number of alleles per locus. Private alleles are those only found in one or the other species included in the study. SSR marker labels refer to Ahmed et al. (2006).

SSR marker	<i>C. selloana</i>			<i>C. jubata</i>		
	Allele size range (bp)	Putative alleles / (private alleles)	Number of alleles per individual	Allele size range (bp)	Putative alleles / (private alleles)	Number of alleles per individual
Cor-7	236–272	15 (8)	1–2	244–269	7 (0)	1–2
Cor-11	166–223	12 (6)	1–4	169–223	7 (1)	1, 3 (2)
Cor-36	335–399	16 (12)	1–2 (3)	352–373	4 (0)	2–3
Cor-43	105–196	18 (15)	1–3	101–150	6 (3)	1–2
Cor-46	209–240	3 (2)	1 (3)	209–211	2 (1)	2
Cor-47	233–243	5 (2)	1–2 (3)	233–243	4 (1)	2–3
Cor-49	115–130	6 (4)	1–2	120–124	2 (0)	1–2
Cor-56-1	199–204	5 (3)	1–2 (3)	199–201	2 (0)	2
Cor-56-2	222–228	6 (3)	1–2	224–226	3 (0)	2 (1)

## Data analysis

Fragments were sized and scored using GeneMarker v.2.2.0 (SoftGenetics, USA). As per the studies of Ahmad et al. (2006) and Okada et al. (2009), scoring was modified from conventional microsatellite analysis to account for the high ploidy within *Cortaderia* species. Scores from both species were scored as binary characters and interpreted as dominant markers, due to our inability to assign dosage to the different markers, and because some material was collected from plants not flowering and therefore could not be assigned morphologically to one species or the other. The number of peaks per locus for each of the species is shown in Table 1.

Summary statistics and AMOVA (Analysis of Molecular Variance) were prepared in GenAlEx version 6.5 (Peakall & Smouse 2012) (Table 3), and a PCoA plot prepared from the complete dataset including both species, using MVSP (Multi-Variate Statistical Program) and Gower General Similarity (version 3.22, Kovach Computing Ltd). Both AMOVA were performed with 1000 iterations for each of the datasets. Significance was not recorded for the AMOVA due to these being based on divisions recovered from an assignment analysis (STRUCTURE) (Meirmans 2015). STRUCTURE v2.3.4 (Falush et al. 2003) was used to examine the optimal number of assignment classes within each of the species, using the admixture model. Burn-in was 200 000, followed by a run of 350 000 iterations, with 10 runs performed for each value of  $K$ .  $K$  values ranged from 1–5, and no prior population data were included in the model. Results are shown in Figures 1a and 1b. The optimal  $K$  value was selected following the Evanno method (Evanno et al. 2005) as implemented in STRUCTURE HARVESTER (Earl & von Holdt 2012). The two species were initially run as a single dataset, with *C. selloana* and *C. jubata* then being run individually. STRUCTURE uses Bayesian probability to assign individuals to a population (or populations in the case of admixed individuals) based on the frequencies of alleles in each of the assumed populations, with the number of populations denoted by  $K$ . Individuals that have high genetic similarity or that have exchanged genetic material recently will be clustered together, and assigned to provide the highest probability of clustering for that particular value of  $K$ .

## Results

In total, 582 individuals from New Zealand were successfully genotyped: 60 from the South Island (12 *C. jubata* and 48 *C.*

*selloana*) and 522 from the North Island (84 *C. jubata* and 438 *C. selloana*) (Fig. 1). Markers were robust with very few markers failing to produce alleles for almost all individuals. While the markers in this study were originally designed to be bi-allelic (Ahmad et al. 2006; Okada et al. 2009), we found that we commonly amplified more than two alleles per locus even in *C. selloana*. Therefore, we scored all loci as dominant, with no interpretation of dosage. While this does reduce the information on heterozygosity from our data, this reduction is of more importance to applications such as paternity analysis than population assignment. The number of alleles scored per individual for each of the species for each locus and the total number of alleles per locus and allele size range for each species are shown in Table 1.

The two species of *Cortaderia* present in New Zealand are readily identified in the dataset, with the variation in *C. jubata* being low compared to *C. selloana* (Table 2). The optimal value of  $K$  from the STRUCTURE analysis for the data was  $K = 3$ , as determined by STRUCTURE harvester.  $K = 2$  also provided a good fit, resolving *C. selloana* and *C. jubata*, with  $K = 3$  resolving a further division within *C. selloana* (see Figure S2 in Supplementary Material). Summary statistics for *C. jubata*, *C. selloana* (total), and *C. selloana* by the two STRUCTURE divisions are shown in Table 2. The geographic distributions of the two divisions of *C. selloana* are shown in Figure 1, with the more common type being widespread and the less numerous type having a restricted, yet disjunct range. Further analysis of the *C. jubata* data found that  $K = 3$  was the best fit, and assignment of individuals within this is shown in Figure S3 (see Supplementary Material).

Summary statistics for each of the species and the two divisions in *C. selloana* are shown in Table 1. *Cortaderia jubata* has lower values than *C. selloana* for most of the genetic diversity metrics compared, particularly estimated heterozygosity ( $H_e$ ) and the number of alleles per locus ( $N_a$ ) (Table 2). Statistics for the two divisions of the *C. selloana* samples were similar, especially the number of effective alleles ( $N_e$ ) and estimated heterozygosity ( $H_e$ ). The multi-locus genotypes function in GenAlEx 6.5 identified 31 multi-locus genotypes in *C. jubata*, of which 20 were singletons (11 with at least two identical individuals). By comparison, the outcrossing *C. selloana* had 435 genotypes, with 401 singletons (34 genotypes with at least two identical individuals). AMOVA between *C. selloana* and *C. jubata* partitioned 59% of variation among populations, and 41% within populations. Within *C. selloana* 38% of variation was among populations, and 62% within populations.

**Table 2.** Summary statistics for *C. selloana* and *C. jubata* from New Zealand. For *C. selloana*, statistics for the two main genetic groups found in New Zealand are also shown:  $n$ , number of samples;  $N_a$ , number of alleles;  $N_e$ , number of effective alleles;  $I$ , Shannon's information index;  $H_e$ , estimated expected heterozygosity;  $uH_e$ , unbiased estimated expected heterozygosity. Values in parentheses are Standard Errors of the Means.

	$n$	$N_a$	$N_e$	$I$	$H_e$	$uH_e$
<i>C. selloana</i>	486	1.891 (0.048)	1.152 (0.028)	0.155 (0.024)	0.095 (0.016)	0.095 (0.016)
<i>C. jubata</i>	96	0.750 (0.099)	1.099 (0.026)	0.098 (0.020)	0.061 (0.014)	0.061 (0.014)
Total	582	1.321 (0.069)	1.125 (0.019)	0.126 (0.016)	0.078 (0.011)	0.078 (0.011)
<i>C. selloana</i> only						
	$n$	$N_a$	$N_e$	$I$	$H_e$	$uH_e$
<i>C. selloana</i> type 1	460	1.804 (0.062)	1.151 (0.029)	0.148 (0.024)	0.093 (0.017)	0.093 (0.017)
<i>C. selloana</i> type 2	26	0.913 (0.103)	1.145 (0.027)	0.154 (0.023)	0.094 (0.015)	0.096 (0.016)



The three STRUCTURE divisions are shown in the PCoA plot (Fig. 2). The first axis explains 31.0% of the variation, and the second 6.9%. *Cortaderia jubata* forms a single group, with the core *C. selloana* group and the second *C. selloana* group also being clearly distinguished on the plot. Variation in *C. selloana* is much greater than that in *C. jubata*, as also seen in the summary statistics.

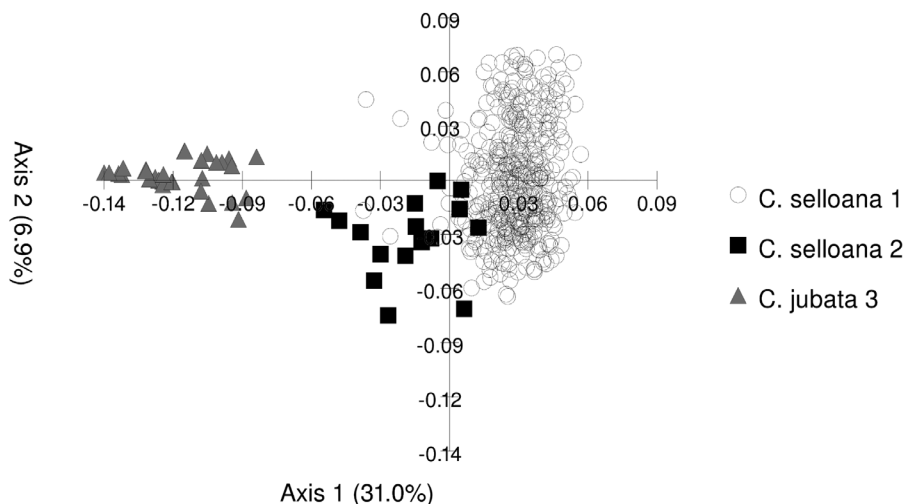
### Discussion

*Cortaderia* in New Zealand has become a significant weed due to its propensity to invade both native and non-native systems, and because its control consumes considerable resources. The aim of this study was to understand the genetic variation within *Cortaderia* species in New Zealand to better infer the pattern of invasion, with an eye towards possible biocontrol solutions. The two species of *Cortaderia* are readily distinguishable genetically, but the amount of genetic variation present in each differs markedly. This pattern is not surprising given the difference in breeding system of the two species (Connor 1974). An earlier study on the genetics of invasive *C. jubata* from around the world (Okada et al. 2009) suggested that *C. jubata* in New Zealand was almost clonal; a finding consistent with its apomictic reproductive mode. This present study included substantially more samples of *C. jubata* than that of Okada et al. (2009) to determine if this conclusion was robust. Genetic diversity in the current study is considerably higher than in Okada et al. (2009), where from 16 individuals they found a total of five multi-locus genotypes, and considerably higher diversity than in other parts of the invasive range (233 samples from California comprised 8 genotypes, while 33 samples from Hawai‘i were a single genotype). From the 96 samples assigned to *C. jubata* in the current study, we found 31 multi-locus genotypes (11 of which comprised of two or more individuals), a similar proportion to the Okada et al. (2009) study, although surprisingly high for a species that is believed to be obligately apomictic. Our samples came from a broader range of sites from across New Zealand, although this does not completely explain the high number of multi-locus genotypes present, as Okada et al. (2009) found only 11 from all of the samples in the introduced range (Table 1). The proportion of genotypes to samples observed in this study is comparable

to *C. jubata* in the native range (77 samples with 26 unique genotypes) (Okada et al. 2009). Further investigation into the reproduction of *C. jubata* may be warranted, as it is unlikely that we would see this level of genetic diversity in a purely apomictic species, especially one that is a recent invader. This result would make it seem highly unlikely that *C. jubata* has undergone any bottlenecking or reduction in genetic variation as a result of introduction. While the variation present in *C. jubata* is considerably less than observed in *C. selloana*, this is not surprising as the two species have different breeding systems.

STRUCTURE analysis indicates that there are three main groups of genotypes within the diversity of *C. jubata* in New Zealand. The high diversity in New Zealand has several possible explanations. Whereas plantings of *C. jubata* in California were for landscape or amenity purposes, *Cortaderia* in New Zealand has historically been used as a fodder species (*C. selloana*) and as an ornamental (*C. jubata*), and therefore it is likely there have been numerous introductions. It is also possible that some early records of *C. selloana* actually refer to both species, as the taxonomic treatment of these species has been problematic in New Zealand. *Cortaderia jubata* was often referred to as *C. atacamensis*, a species found in Northern Argentina, and some early reports of hybrids between *C. selloana* and the native *Austroderia* were most likely *C. jubata* (Connor 1965). The complex introduction history of *Cortaderia* in New Zealand and the numerous uses of the plant in many different habitats make it unsurprising that variation is higher than in other parts of the introduced range.

Genetic variation in *Cortaderia selloana* is notable in that there are two well differentiated genetic groups in the dataset, one of which is far more numerous in the environment than the other. These groupings are supported by Bayesian clustering methods, PCoA, and AMOVA, and would appear to be robust. These two groups of plants flower at different times of the year (Daniel Than, Richard Toft, pers. comm.), but otherwise cannot be readily identified morphologically. Non-synchronous flowering times likely represent a reproductive barrier between the two groups. The less common type of *C. selloana* is found across a range of locations in New Zealand; however, it does not seem to be as invasive, at least currently. It is unclear whether this reflects a type that is not as aggressive as most of the *C. selloana* plants across the rest of the country



**Figure 2.** PCoA plot of *C. jubata* and *C. selloana* from New Zealand based on dominant scoring of SSR data, showing the two STRUCTURE divisions of *C. selloana*.

or a type that has not been grown to the same extent (reduced propagule pressure).

Assuming that these two entities within *C. selloana* fall within the species concept for this taxon, and we currently have no evidence that this is not the case, it may be that they do represent races within *C. selloana*, with different degrees of weediness. Other studies of invasive species have shown that it is often only a subset of the genetic variation within a species that has the propensity to become weedy (Dekker 2003), and that species per se is not a good predictor of invasive potential (Baker 1965). How these two groups of genotypes vary ecologically is unclear, other than the noted difference in flowering time, and whether differences in performance explain the different frequency of these types in the environment requires further study. It is difficult to interpret this pattern in regard to the literature on the introduction of *C. selloana* into New Zealand (Grimmett 1935; Dunlop & Coup 1951). These reports often mention different strains or cultivars being used in different areas, although how well characterised these were is unknown. These earlier reports cannot explain the distribution of the two types of *C. selloana*, and it is likely that reproductive and demographic processes have been more important in shaping the variation we see today (Novak & Mack 2005).

Control of pampas grass in New Zealand is of high interest due to the expense of using herbicides, and the low efficacy of most herbicide applications on the plants (Harris & Skilton 2007). As a result there has been interest in developing biological control agents for use against *Cortaderia*. The results of this study would indicate that agents will have to be chosen carefully for best efficacy against the variation that is present in New Zealand within each of these species. *Cortaderia jubata* is known to have come from Peru and Ecuador (Okada et al. 2007), although variation within *C. jubata* is higher than found in the earlier study, so further refinement may be required to determine the correct area to search for agents. *Cortaderia selloana* is also problematic, because there are two distinctly different groups of genotypes in New Zealand, and it will be essential to determine what part of the native range relates to the more common of the two for bio-prospecting for agents. Any agents will also have to be safe against the closely related *Austroderia* species, so specificity will be critical to achieve control without adverse effects on the native flora.

Understanding the genetic variation in invasive species is important to both understand the process of invasion, and to better explore control options. *Cortaderia* is an ideal model system for these questions due to the presence of multiple species or genotypes in the introduced range. By comparing the results of the present study to work examining the variation in the native range of *Cortaderia*, we aim to prospect for biocontrol agents, and assess the risk of the different entities already present in New Zealand.

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## Supplementary Material

Additional supporting information may be found in the online version of this article:

**Table S1.** Location data and labelling for the samples included in this study.

**Figure S2.** STRUCTURE plot of the whole dataset including *Cortaderia selloana* (two divisions – red and blue) and *C. jubata* (one division – green).

**Figure S3.** STRUCTURE plot for *C. jubata* only, showing  $K = 3$  as chosen by STRUCTURE Harvester.

**Figure S4.** STRUCTURE plot for *C. selloana* only, showing  $K = 2$  as chosen by STRUCTURE Harvester.

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