Landcare Research
Manaaki Whenua

EcoGene®
DNA-based diagnostics

Getting “species” out of the box! (or putting them back in)

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Why Species?

- Species are important!
- Biodiversity
  - Management
  - Characterisation
- Biosecurity
  - Identification
  - Regulation

New Zealand Context

Native / Exotic

“New” organisms

Species concepts – Ancient Lore

- ‘The species concept is one of the oldest and most fundamental in biology. And yet it is almost universally conceded that no satisfactory definition of what constitutes a species has ever been proposed.’ Dobzhansky 1935

- “Biological species concept”
  - Interbreeding or potentially interbreeding individuals (Mayr 1942)

Species?

- Species are usually defined by “concepts”
  - Recent review found 24 different species concepts (de Queiroz 2007 Syst. Biol. 56: 879-886)
  - How they are applied by different authors can also vary

- The modern proliferation:

Biological Isolation Recognition

Ecological Evolutionary Cohension

Phylogenetic

Hennigan Monophyletic Genealogical

Diagnosible

Phenetic

Genotypic cluster

- Systematists usually know what they mean, but aren’t good at letting us know

Species?

- Does it matter?

- How often are regulations dependent on species?

How bad is it?

- Examples from the literature
  - Different treatments in different groups of things with the same issues
- Examples from NZ (Biosecurity perspective)
  - More isn’t always merrier
- Messages of hope?

Plants aren’t mammals

2n = 36
Sexual
Outcrossing

2n = 36
Asexual
Apomictic

2n=45
Asexual
Apomictic

2n = 54
Asexual
Apomictic

Species?

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Species?

Hieracium pilosella syn. *Pilosella officinarum*

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Species?

Hieracium pilosella syn. *Pilosella officinarum*

2n = 72	2n = 92	2n=104	2n = 72?
Sexual	Asexual	Asexual	Sexual
Outcrossing	Apomictic	Apomictic	??

Species?

2n = 72	2n = 92	2n=104	2n = 72?
Sexual	Asexual	Asexual	Sexual?
Outcrossing	Apomictic	Apomictic	??

Species?

Cortaderia selloana	*C. jubata**	*C. atacamensis*	*C. ???*

*Some of it – we'll get to this in a minute!

But that's just those weird polyploid things….

- ~70% of plants with polyploid histories (D. A. Levin 2002)
- Biosecurity – how many of our worst weeds are from these sorts of groups??
- How often is chromosome number checked with taxonomic work?

A simple example: “cabbages”

B. nigra

B. oleracea

B. rapa

B. carinata

B. juncea

B. napus

A simple example: cultivated Brassica

B. nigra	2n =16
B. oleracea	2n = 18
B. rapa	2n = 20
B. carinata	2n = 34
B. juncea	2n = 36
B. napus	2n = 38

A simple example: cultivated Brassica

B. nigra	2n =16, 32
B. oleracea	2n = 18, 36
B. rapa	2n = 20, 40
B. carinata	2n = 34
B. juncea	2n = 36
B. napus	2n = 38

A simple example: cultivated Brassica

B. rapa

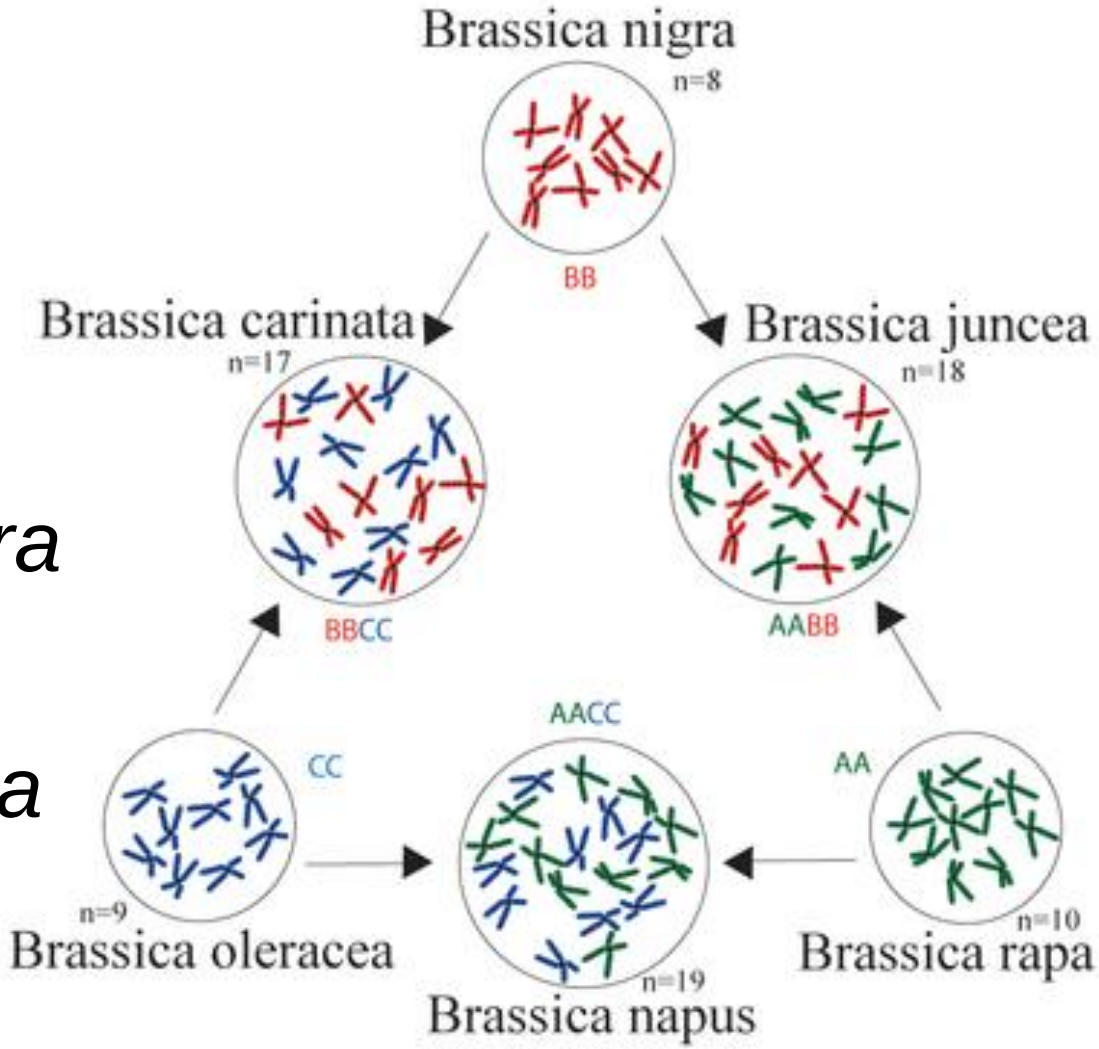
B. nigra

B. oleracea

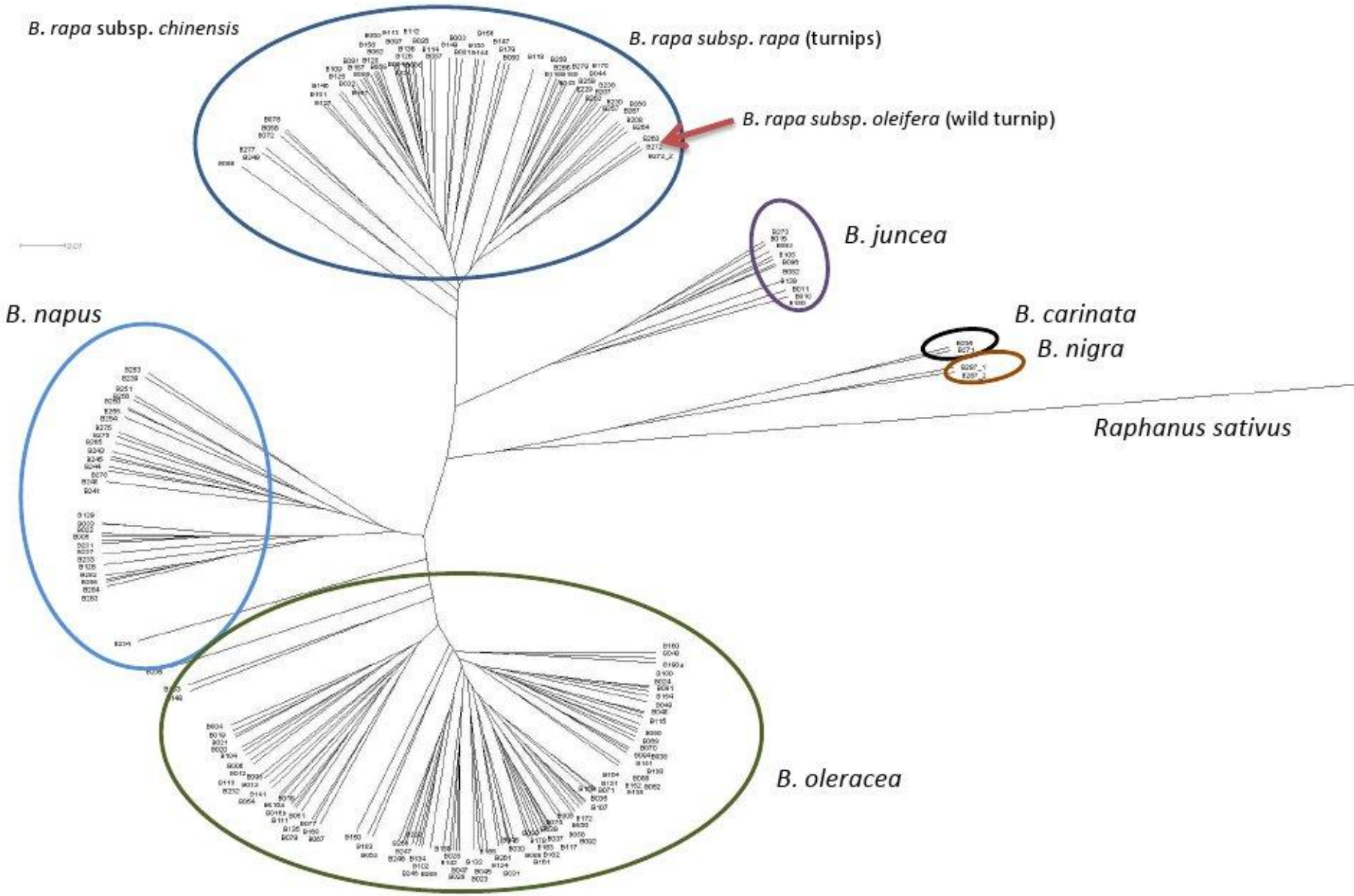
B. oleracea x nigra

B. nigra x rapa

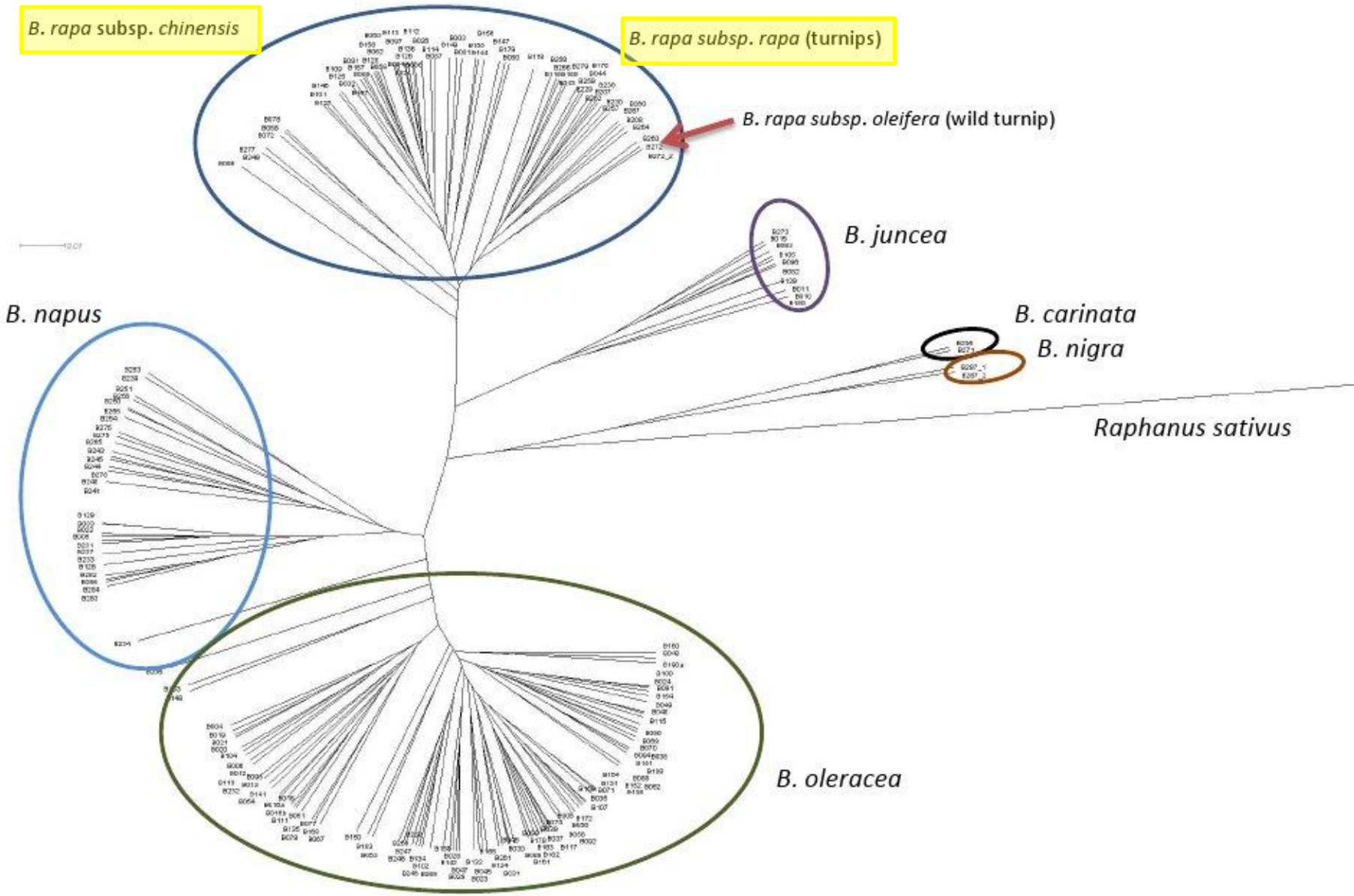
B. oleracea x rapa



Commercial *Brassica* germplasm in NZ, 2010-11



Commercial *Brassica* germplasm in NZ, 2010-11



- Kahili ginger *Hedychium gardnerianum*
- White ginger *Hedychium coronarium*
- Yellow ginger *Hedychium flavescens*

H. gardnerianum from India:

2n = 36

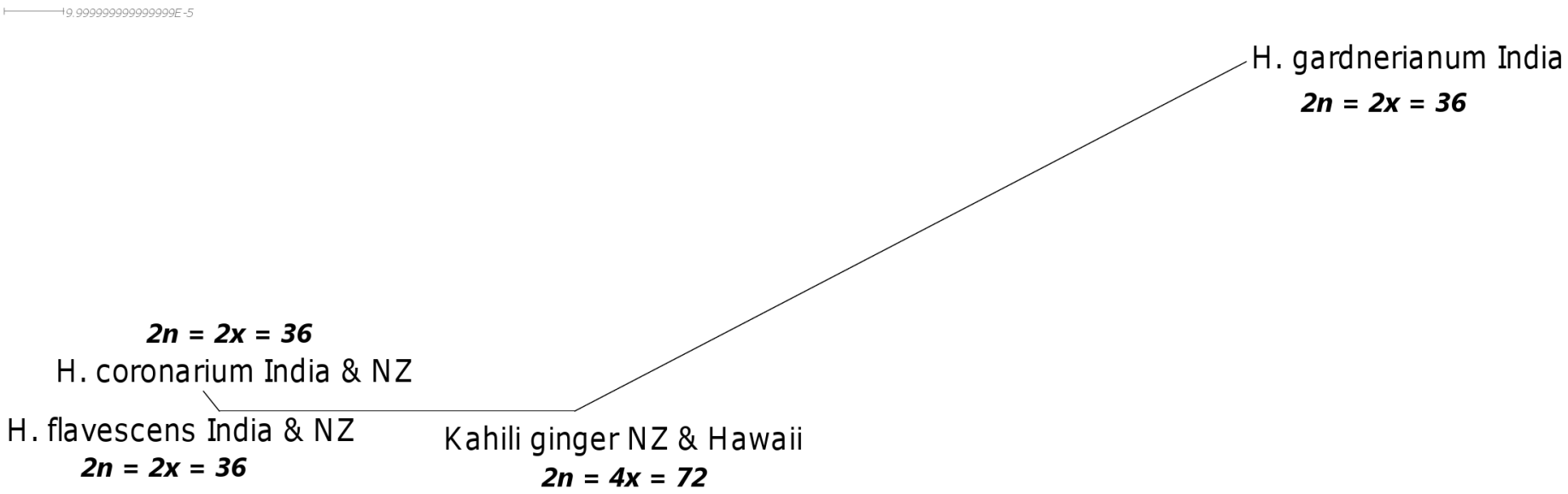
H. coronarium from India / NZ:

2n = 36

H. flavescens from India / NZ

2n = 36

“NZ Kahili” 2n = 72!



We don't have *H. gardnerianum,* we have a hybrid that looks like *H. garderianum* (matromorph?)!

Ginger grey lit describe these BIOCONTROL

2n = 72	2n = 92	2n=104	2n = 72?
Sexual	Asexual	Asexual	Sexual?
Outcrossing	Apomictic	Apomictic	??

Species?

Cortaderia selloana	*C. jubata**	*C. atacamensis*	*C. ???*

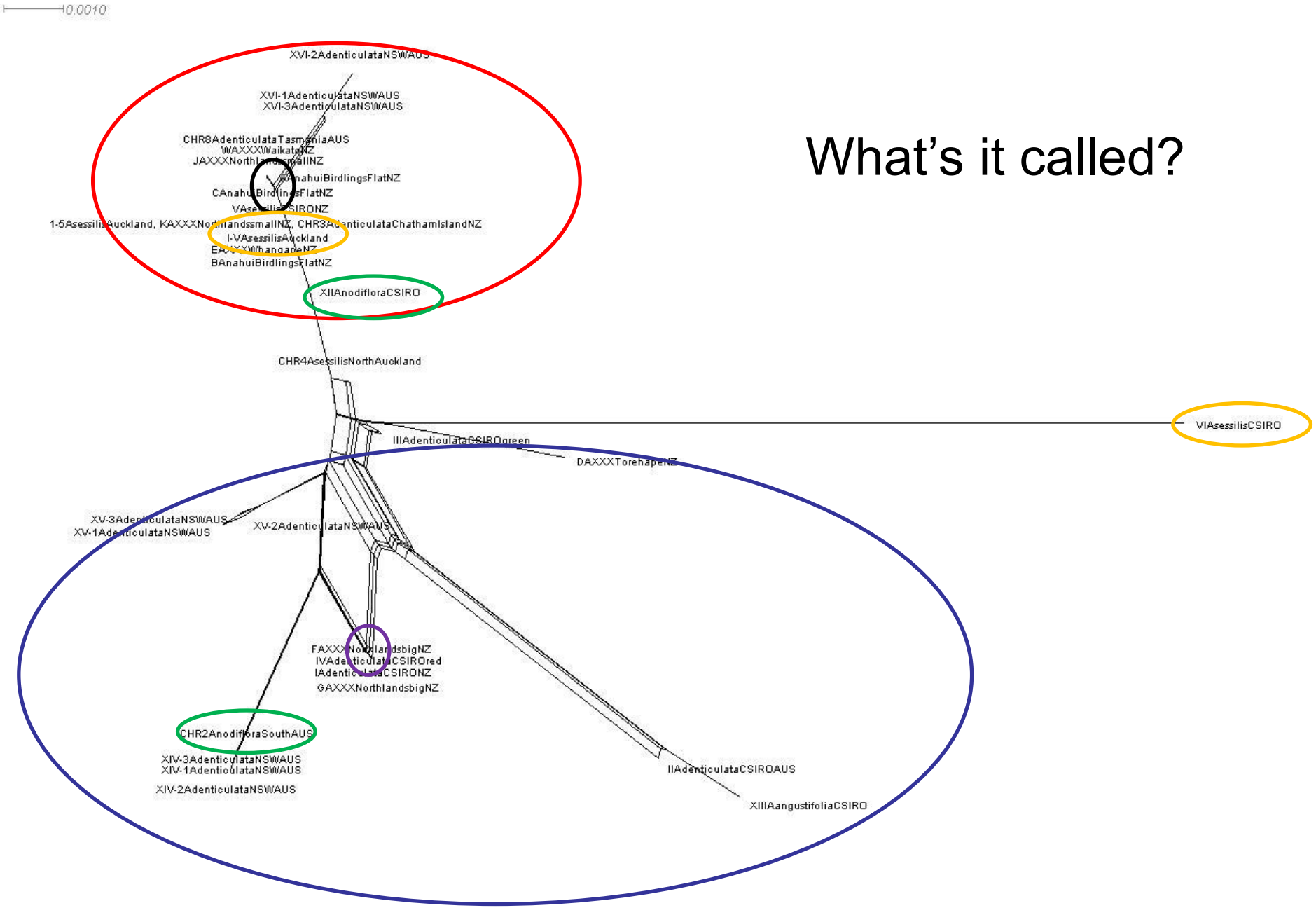
*Some of it – we'll get to this in a minute!

- New Zealand *C. selloana* and *C. jubata* from South America

- *C. jubata* is actually from Peru, Northern Ecuador (*C. quila*)
  - Single genetic clone
  - Nothing like Argentinian *C. jubata* genetically
- *C. selloana* in New Zealand isn't like *C. selloana* in Argentina*

- Do we have three pampas grasses here?

- *Alternanthera* (Alligator weed and relatives)
  - Difficult to tell apart morphologically (plasticity)
  - Uncertainty about origins of NZ material (endemic / native / introduced?)



What's it called?

Barcoding / diagnostics

- Vallisneria (Eelgrass)
  - *V. gigantea* a nationally banned species
  - Couldn't be determined accurately from morphology, and uncertainty about what some other material was.
  - Direct sequenced ITS (internal transcribed spacer of the ribosomal DNA),compared to published revision (Les et al 2008. Systematic Botany 33:49-65).

Vallisneria

- Formerly: *Vallisneria gigantea* (Eelgrass) = *V. americana*

- We found *V. australis, V. spiralis* and *V. natans*!

- Taxonomy has been confusing, and will result in changes to the Unwanted Organisms Register (*V. australis*)

Applications

- Species level diagnostics
- Intraspecific identification (eg Tradescantia, Pampas)

Limitations

- Available existing data (Genbank)
- Sample quality

What if there is no data / all have the same sequence?

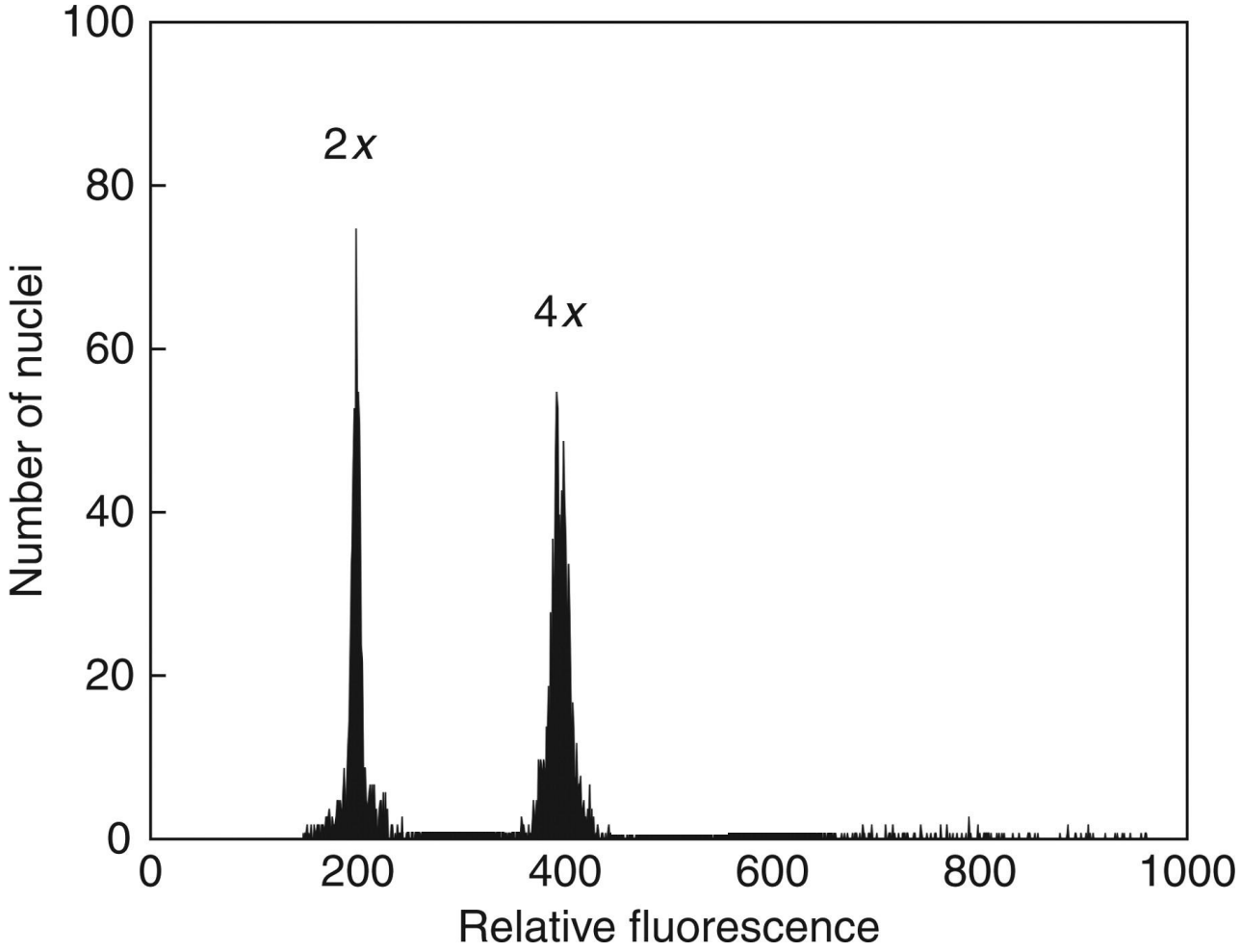
- *Sorghum halpense* is a nationally banned plant

- Morphological id of a sample from the wild came back as *S. halpense*, but wasn't ideal material / some missing characters

- All sequences for *Sorghum* spp. on Genbank were identical

Sorghum – flow cytometry

- *Sorghum halpense* is tetraploid; other species (*S. bicolor, S. xsudan*) are diploid



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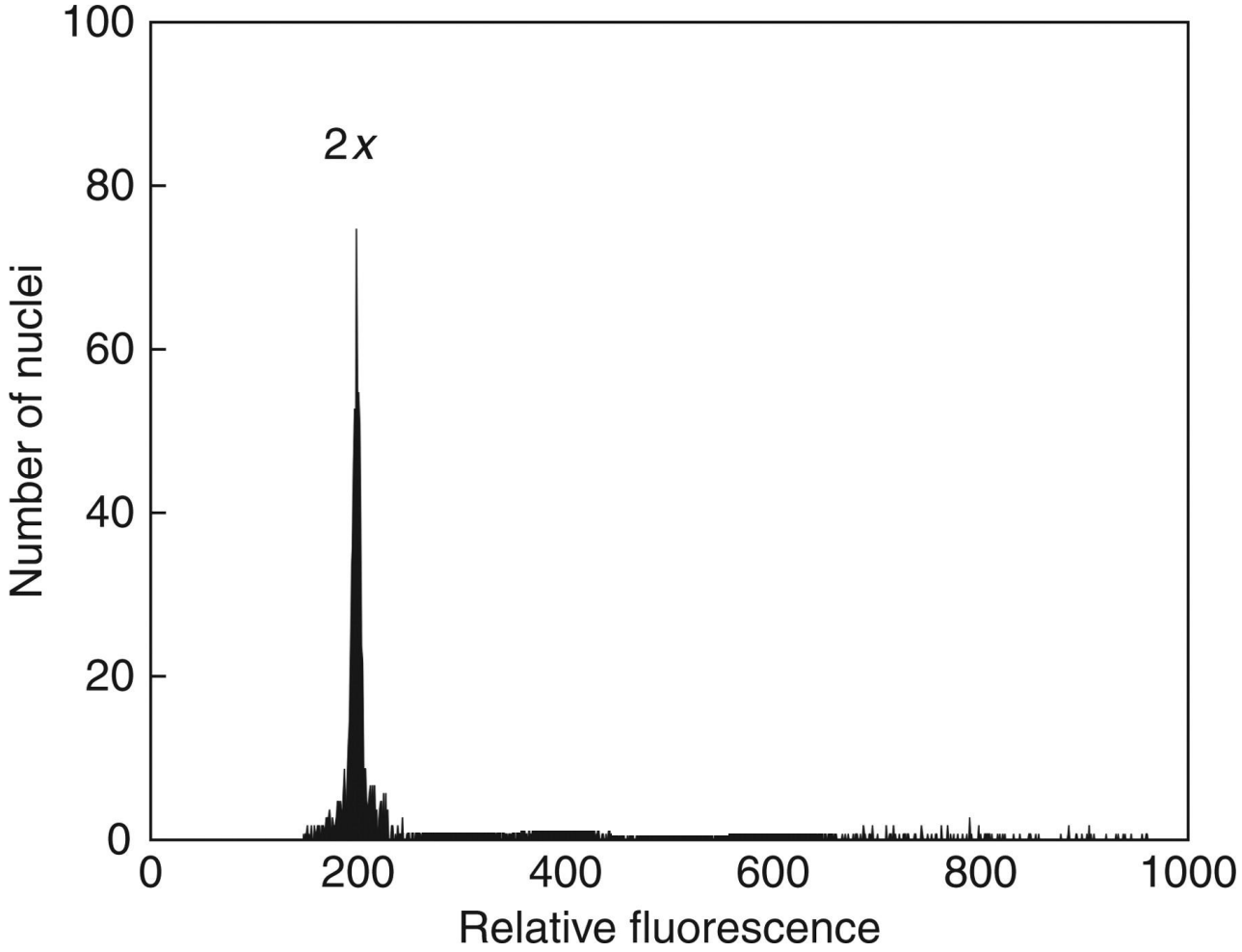
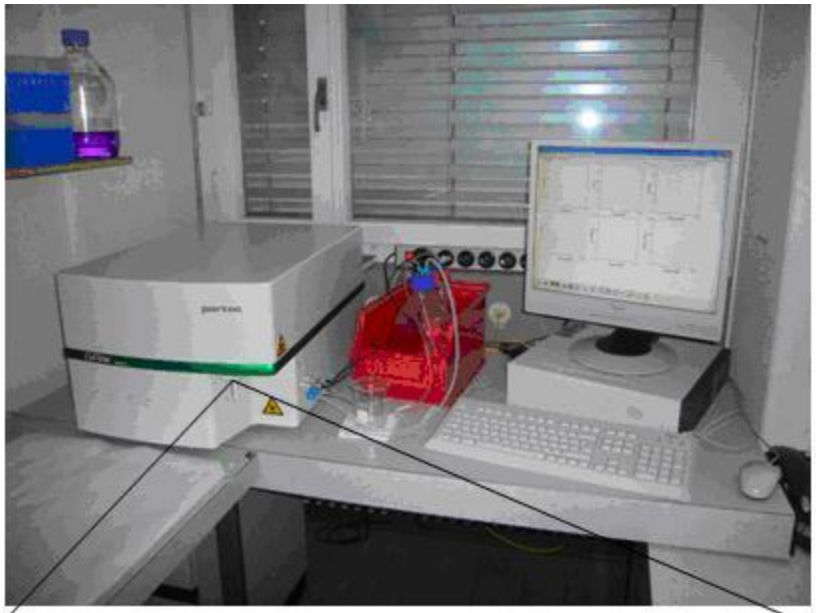
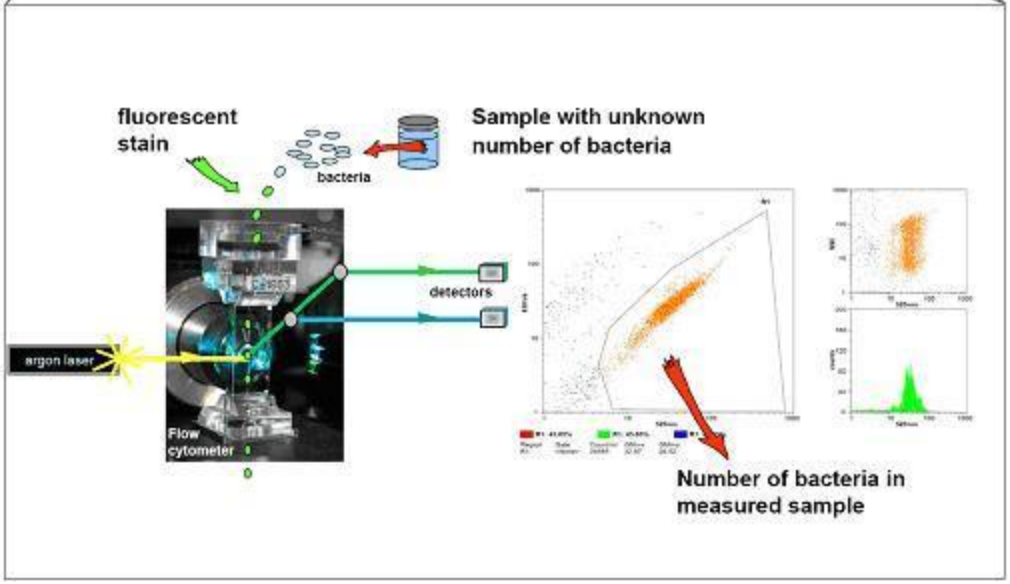
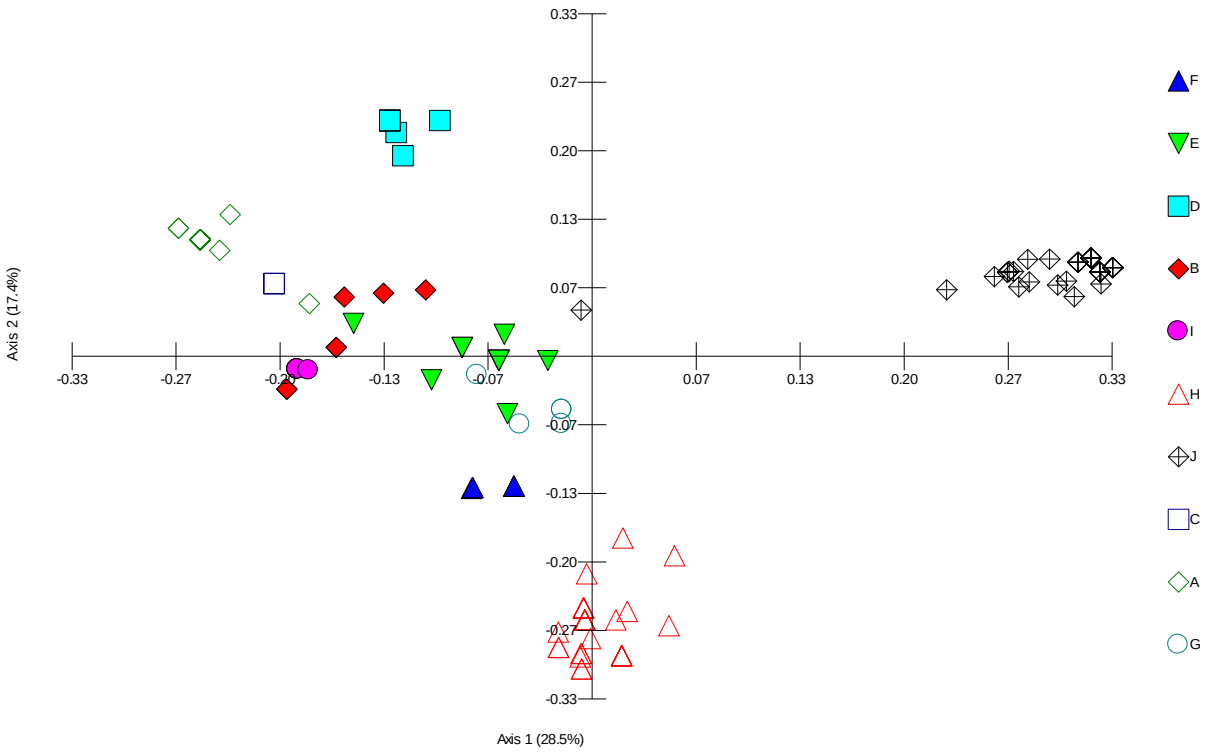


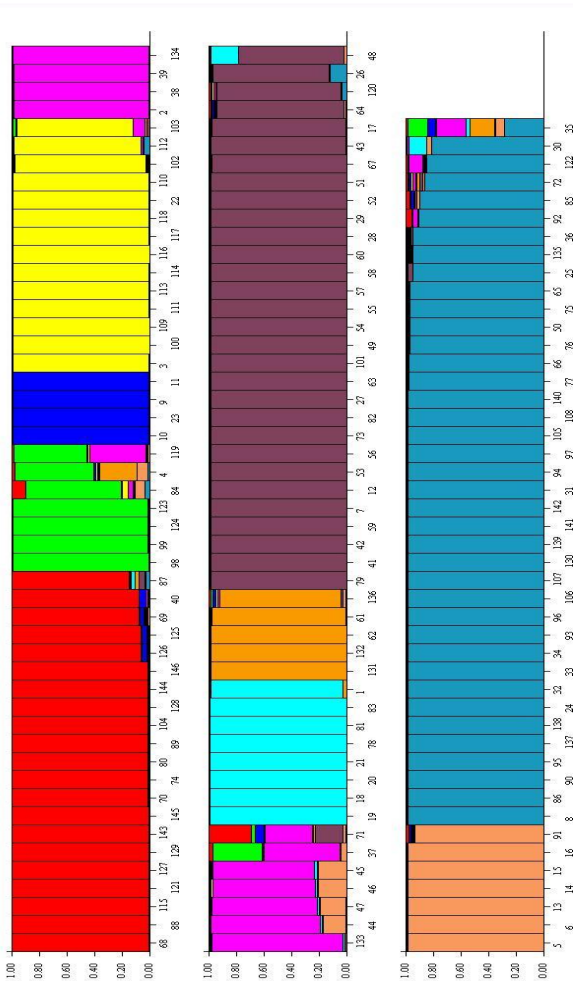



fluorescent stain
bacteria
Sample with unknown number of bacteria
detectors
argon laser
Flow cytometer
Number of bacteria in measured sample

What if we need to know more than species?

- Often the case in weed biocontrol (species complexes)





Recent examples; why / how?

- We sometimes need to know more than “species”
- Plasticity – things aren’t always as they seem
- Sometimes only fragments / processed products (diagnostics)
- We want to demonstrate this idea!

Other common problem children…

Poaceae (grasses)
Roseaceae (rose family)
Asteraceae (daisies)

Warning signs…

Clonality / Sterility
Ploidy
Apomixis!
Genus species agg.
Genus species sensu lato / stricto

Take home messages:

- We need some system to catalogue what we work with = species
- Species is important – it will point you to the literature
- It isn’t neat little boxes
- When you write species in a document, think about what this may imply

The way forward:

- Rapid assessment using modern tools
  - Genome sizing is cheap, accurate, and often informative
- Species is a starting rather than an endpoint
- Access to “new organisms” for assessment of “old organisms”
- Caution is good, information is better

Who to talk to: (shameless advertisement)

- Dr Gary Houliston, Plant Science Advisor, EcoGene
- Dr Frank Molinia, Manager, EcoGene
- Dr Ines Schonberger, Manager, Allan Herbarium, Landcare Research