

Identification and Specificity of Rhizobia in New Zealand

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NZ Native Legumes

- Kowhai (*Sophora* spp.)
- Kaka beak (*Clianthus* sp.)
- NZ Broom (*Carmichaelia* spp.)
- Scree Pea (*Montigena* sp.)



Carmichaelia



Sophora



Clianthus



Montigena



Questions - Natives

- What rhizobia are present in NZ?
 - NZ split 80 mya, became isolated
 - Co-evolution between native legumes and rhizobia since that time
- How specific are the relationships that have formed since that time?
 - Does each legume species/genus have its own rhizobia or are the relationships more general

Woody Legume Weeds in NZ

1-2 million hectares, tens of millions \$ pa.

- Gorse (*Ulex europaeus*)
- Broom (*Cytisus scoparius*)
- Wattles (*Acacia* spp.)



Gorse (*Ulex europaeus*)



Broom in Porters Pass, South Island



Acacia longifolia



Questions - Exotics

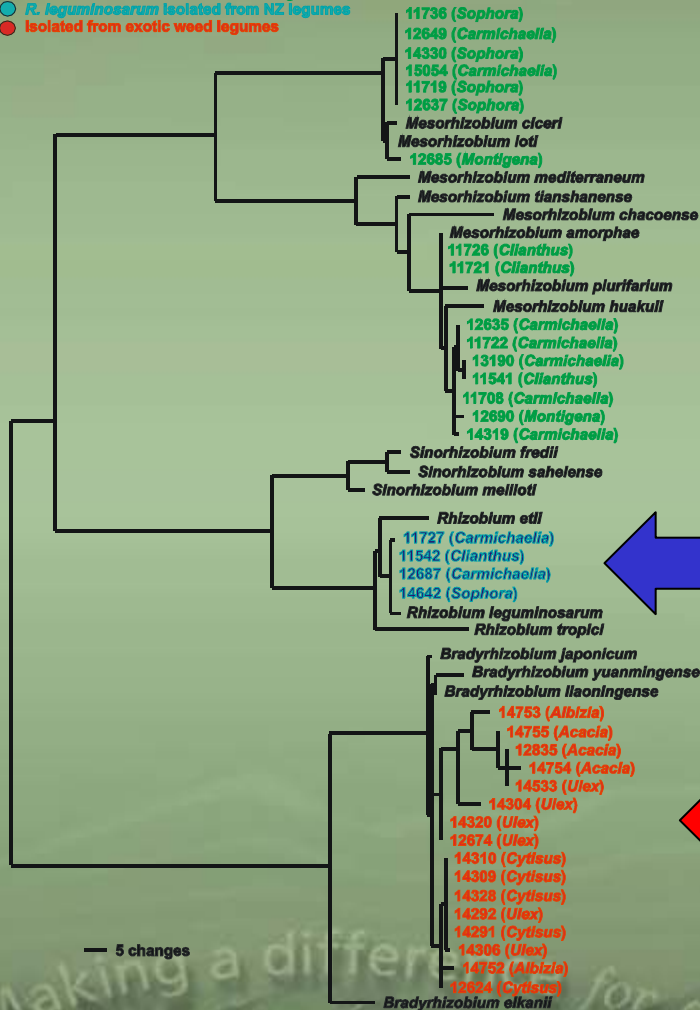
- Weed legumes were introduced
 - Present for less than 200 years
- How are the weeds able to nodulate?
- Three possibilities:
 - They have adapted to use native rhizobial populations
 - Effective rhizobia were co-introduced with the exotic legumes
 - Effective cosmopolitan rhizobial populations are present in New Zealand

Phylogenetics

- Isolated 40 strains from root nodules
- Sequenced 5 genes
 - 16S, *recA*, *atpD*, *glnII*, *nodA*
- Built phylogenetic trees
 - NJ, ML, Bayesian
 - ProtTest, Modeltest

16S Phylogeny of NZ Rhizobia

- Isolated from native New Zealand legumes
- *R. leguminosarum* isolated from NZ legumes
- Isolated from exotic weed legumes



← Native legumes - *Mesorhizobium*

← *Rhizobium leguminosarum*

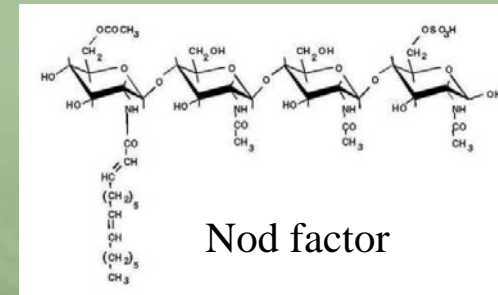
← Exotic legumes - *Bradyrhizobium*



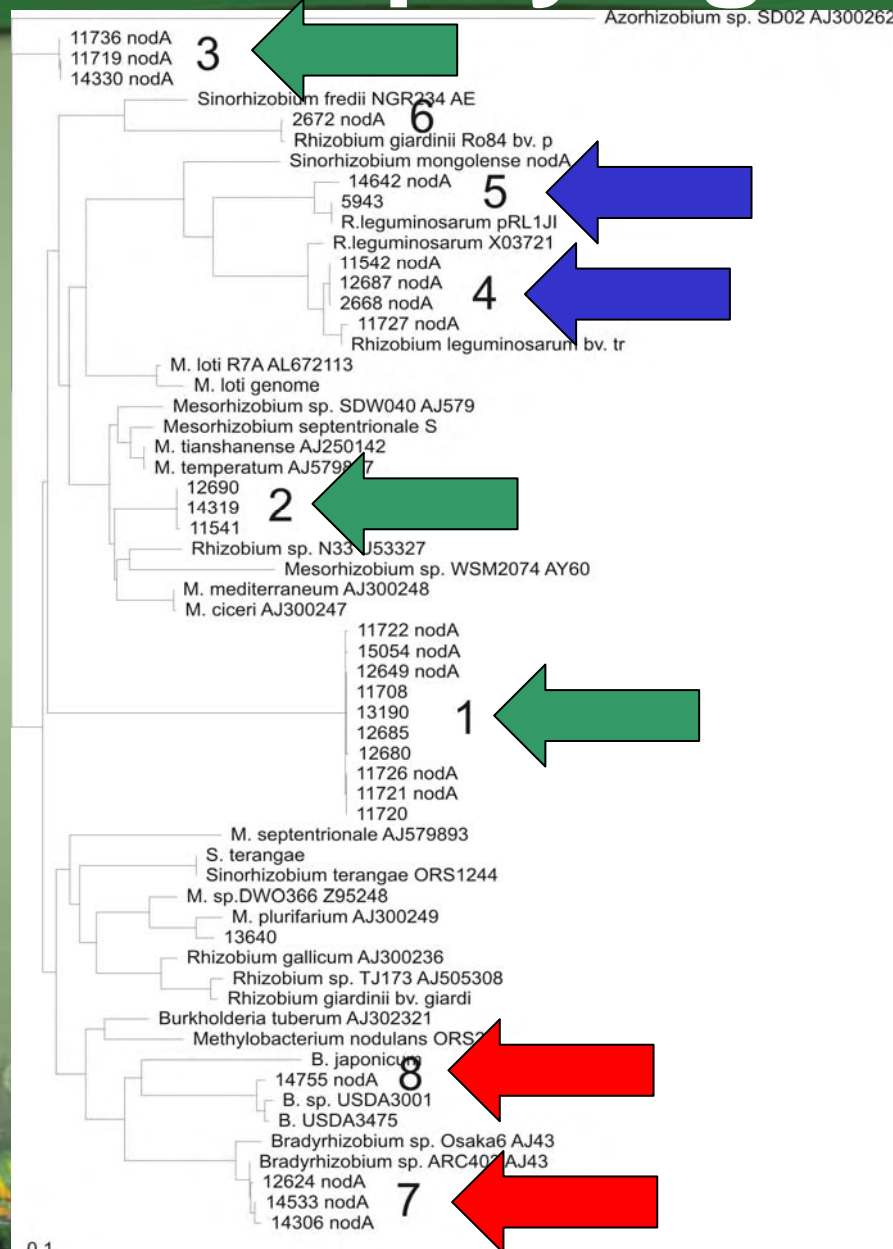
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Nodulation genes

- Nod factor is a bacterial signal molecule that initiates nodule formation
- Nod factor “core” created by three genes:
 - *nodA*, *nodB*, *nodC*
 - Some degree of relation to host range
- Sequenced *nodA*:
 - reveal evolutionary history of the gene, and
 - relation to specificity in NZ legumes



nodA phylogeny



nodA phylogeny

- Three novel *nodA* types in NZ *Mesorhizobium*
 - No correlation with house-keeping genes
 - Carried on transposable symbiosis island
 - No correlation with host legume
- *R. leguminosarum* strains have typical *nodA*
 - Haven't picked up *Mesorhizobium* symbiosis island
- *Bradyrhizobium nodA* similar to others found
 - Possibly host specific

Making a difference for a truly green New Zealand



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Research Strategy

- **Identification**

- Multiple gene sequencing, phylogenetic
- Biolog (not shown)
- Fatty acids (not shown)

- **Specificity**

- Nodulation gene
- Host-range: Inoculate plants with strains, acetylene reduction, nodule counting.



Research Objectives for Specificity

- Investigate symbiotic promiscuity of *Mesorhizobium* strains nodulating native NZ legumes.
- Investigate symbiotic promiscuity of *Bradyrhizobium* strains nodulating exotic weed legumes.
- Investigate interactions between *R. leguminosarum* and native NZ legumes.
- Verify the previously established division of *Mesorhizobium* nodulating natives, and *Bradyrhizobium* nodulation exotics.



Mesorhizobium – Native Legumes

- 10 *Mesorhizobium* strains
- 5 Native legumes
 - Two *Sophora*
 - Two *Carmichaelia*
 - *Clianthus*



- Five replicates, uninoculated controls, Full cross inoculation, vermiculite, acetylene reduction

Mesorhizobium – Native Legumes

<i>Mesorhizobium</i> strain	Genomic group	<i>nodA</i> type	nod/fix response on inoculated native legume				
			<i>Sophora</i> <i>microphylla</i>	<i>Sophora</i> <i>tetraptera</i>	<i>Carmichaelia</i> <i>australis</i>	<i>Carmichaelia</i> <i>stevensonii</i>	<i>Clianthus</i> <i>puniceus</i>
14330 (<i>Sophora</i>)	A	3	nod ⁺ fix ⁺	nod ⁺ fix ⁺	nod ⁺ fix ⁻	nod ⁺ fix ⁻	nod ⁺ fix ⁺
11719 (<i>Sophora</i>)	A	3	nod ⁺ fix ⁺	nod ⁺ fix ⁺	nod ⁺ fix ⁻	nod ⁺ fix ⁻	nod ⁺ fix ⁺
15054 (<i>Carmichaelia</i>)	A	1	nod ⁺ fix ⁺	nod ⁺ fix ⁺	nod ⁺ fix ⁺	nod ⁺ fix ⁺	nod ⁺ fix ⁺
12685 (<i>Montigena</i>)	B	1	nod ⁻ fix ⁻	nod ⁻ fix ⁻	nod ⁺ fix ⁺	nod ⁺ fix ⁺	nod ⁺ fix ⁺
11721 (<i>Clianthus</i>)	C	1	nod ⁺ fix ⁺	nod ⁺ fix ⁺	nod ⁺ fix ⁺	nod ⁺ fix ⁺	nod ⁺ fix ⁺
11726 (<i>Clianthus</i>)	C	1	nod ⁺ fix ⁺	nod ⁺ fix ⁺	nod ⁺ fix ⁺	nod ⁺ fix ⁺	nod ⁺ fix ⁺
11541 (<i>Clianthus</i>)	D	2	nod ⁺ fix ⁻	nod ⁺ fix ⁻	nod ⁺ fix ⁺	nod ⁺ fix ⁺	nod ⁺ fix ⁺
12680 (<i>Clianthus</i>)	D	1	nod ⁺ fix ⁺	nod ⁺ fix ⁺	nod ⁺ fix ⁺	nod ⁺ fix ⁺	nod ⁺ fix ⁺
12690 (<i>Montigena</i>)	D	2	nod ⁻ fix ⁻	nod ⁻ fix ⁻	nod ⁺ fix ⁺	nod ⁺ fix ⁺	nod ⁺ fix ⁺
13190 (<i>Carmichaelia</i>)	D	1	nod ⁻ fix ⁻	nod ⁻ fix ⁻	nod ⁺ fix ⁺	nod ⁺ fix ⁺	nod ⁺ fix ⁺

Mesorhizobium – Native Legumes

- Generally broad nodulation
 - All nodulated *Clianthus* effectively
- Exceptions: *Sophora*
 - 3 strains unable to form nodules, 1 ineffective
 - 2 strains isolated from *Sophora* ineffective on *Carmichaelia*
- No clear correlation to genomic or *nodA* groups
- Just how broad is the symbiosis? ...

Mesorhizobium – Exotic Legumes

- Broad nodulation in natives
 - Can they nodulate other legumes?
- Best possible targets:
- Chick Pea (*Cicer*), Milk Vetch (*Astralagus*), Lotus, other *Sophora*
- Inoculated four exotic legumes with seven *Mesorhizobium* strains

Mesorhizobium – Exotic Legumes

<i>Mesorhizobium</i> strain	Genomic group	<i>nodA</i> type	nod/fix response on inoculated legume species			
			<i>Astragalus</i> <i>membranaceus</i>	<i>Lotus</i> <i>tetragonolobus</i>	<i>Cicer</i> <i>arietinum</i>	<i>Styphnolobium</i> <i>japonicum</i>
14330 (<i>Sophora</i>)	A	3	nod ⁺ fix ⁺	nod ⁻ fix ⁻	nod ⁻ fix ⁻	nod ⁻ fix ⁻
15054 (<i>Carmichaelia</i>)	A	1	nod ⁺ fix ⁺	nod ⁻ fix ⁻	nod ⁻ fix ⁻	nod ⁻ fix ⁻
11719 (<i>Sophora</i>)	A	3	nod ⁺ fix ⁺	nod ⁻ fix ⁻	nod ⁻ fix ⁻	nod ⁻ fix ⁻
12685 (<i>Montigena</i>)	B	1	nod ⁺ fix ⁺	nod ⁻ fix ⁻	nod ⁻ fix ⁻	nod ⁻ fix ⁻
11726 (<i>Clianthus</i>)	C	1	nod ⁺ fix ⁺	nod ⁻ fix ⁻	nod ⁻ fix ⁻	nod ⁻ fix ⁻
11541 (<i>Clianthus</i>)	D	2	nod ⁺ fix ⁺	nod ⁻ fix ⁻	nod ⁻ fix ⁻	nod ⁻ fix ⁻
13190 (<i>Carmichaelia</i>)	D	1	nod ⁺ fix ⁺	nod ⁻ fix ⁻	nod ⁻ fix ⁻	nod ⁻ fix ⁻

Mesorhizobium – Exotic Legumes

- Only *Astragalus* nodulated
 - No correlation to genomic or *nodA* type
- Wagstaff et al. (1999): *Astragalus*, *Clianthus*, *Carmichaelia* are closely related.
 - *Lessertia*, *Sutherlandia*, *Swainsona*, *Colutea* [Galegeae]
 - NZ *Mesorhizobium* nodulate the Galegeae tribe and related
- Interesting that some strains nodulated exotic *Astragalus*, but not some native legumes



Rhizobium leguminosarum

- Four isolates identified as *R. leguminosarum*
 - Thought to nodulate peas, bean, clover
 - Host range extended through GM
- Hypothesis is that they are contaminants
 - Clover innoc. Widely used
- Tested the four isolates, plus one each of the three biovars: *trifolii*, *phaseoli*, *viceae*
- Inoculated 3 native legumes, and three typical hosts (pea, bean, clover)



Rhizobium leguminosarum

<i>Rhizobium leguminosarum</i> strain	<i>nodA</i> type	nod/fix response on inoculated legume species					
		<i>Sophora microphylla</i>	<i>Carmichaelia australis</i>	<i>Cliaanthus puniceus</i>	<i>Trifolium repens</i>	<i>Phaseolus vulgaris</i>	<i>Pisum sativum</i>
11542 (<i>Cliaanthus</i>)	4	nod ⁻ fix ⁻	nod ⁺ fix ⁻	nod ⁺ fix ⁻	nod ⁻ fix ⁻	nod ⁺ fix ⁺	nod ⁺ fix [±]
11727 (<i>Carmichaelia</i>)	4	nod ⁻ fix ⁻	nod ⁺ fix ⁻	nod ⁺ fix ⁻	nod ⁺ fix ⁺	nod ⁺ fix ⁺	nod ⁺ fix [±]
12687 (<i>Carmichaelia</i>)	4	nod ⁻ fix ⁻	nod ⁺ fix ⁻	nod ⁺ fix ⁻	nod ⁻ fix ⁻	nod ⁺ fix ⁺	nod ⁺ fix [±]
14642 (<i>Sophora</i>)	5	nod ⁺ fix ⁻	nod [±] fix ⁻	nod ⁺ fix ⁻	nod ⁻ fix ⁻	nod ⁺ fix ⁻	nod ⁺ fix [±]
2668 (<i>trifolii</i>)	4	nod ⁻ fix ⁻	nod ⁺ fix ⁻	nod ⁺ fix ⁻	nod ⁺ fix ⁺	nod ⁺ fix ⁻	nod ⁺ fix ⁺
2672 (<i>phaseoli</i>)	6	nod ⁻ fix ⁻	nod ⁻ fix ⁻	nod ⁻ fix ⁻	nod ⁻ fix ⁻	nod ⁺ fix ⁺	nod ⁺ fix [±]
5943 (<i>viceae</i>)	5	nod ⁻ fix ⁻	nod ⁻ fix ⁻	nod [±] fix ⁻	nod ⁺ fix ⁻	nod ⁻ fix ⁻	nod ⁺ fix ⁺

Rhizobium leguminosarum

- *R. leguminosarum* strains nodulate NZ legumes but ineffectively
- *R. leguminosarum* from natives nodulated pea, bean and clover effectively
- Probably typical of most *R. leguminosarum* strains
 - Novel host range expansion



Bradyrhizobium - Exotic Legumes

- 4 *Bradyrhizobium* strains, isolated from *Acacia*, gorse, and broom
- 3 exotic legumes:
 - *Acacia*, gorse, and broom
- Full cross inoculation



Nodulation in Broom



Bradyrhizobium - Exotic Legumes

<i>Bradyrhizobium</i> strain	Genomic group	<i>nodA</i> type	<i>nod/fix</i> response		
			<i>Cytisus</i> <i>scoparius</i>	<i>Ulex</i> <i>europaeus</i>	<i>Acacia</i> <i>longifolia</i>
12674 (<i>Ulex</i>)	I	7	nod ⁺ fix ⁺	nod ⁺ fix ⁺	nod ⁺ fix ⁻
14291 (<i>Cytisus</i>)	J	7	nod ⁺ fix ⁺	nod ⁺ fix ⁺	nod ⁺ fix ⁻
14533 (<i>Ulex</i>)	G	7	nod ⁺ fix ⁺	nod ⁺ fix ⁺	nod ⁺ fix ⁻
14755 (<i>Acacia</i>)	G	8	nod ⁺ fix ⁺	nod ⁺ fix [±]	nod ⁺ fix ⁺

Bradyrhizobium - Exotic Legumes

- Broad nodulation
 - All strains nodulated all legumes
- Gorse and Broom isolates ineffective on *Acacia*
 - Correlation to *nodA* gene



Conclusions

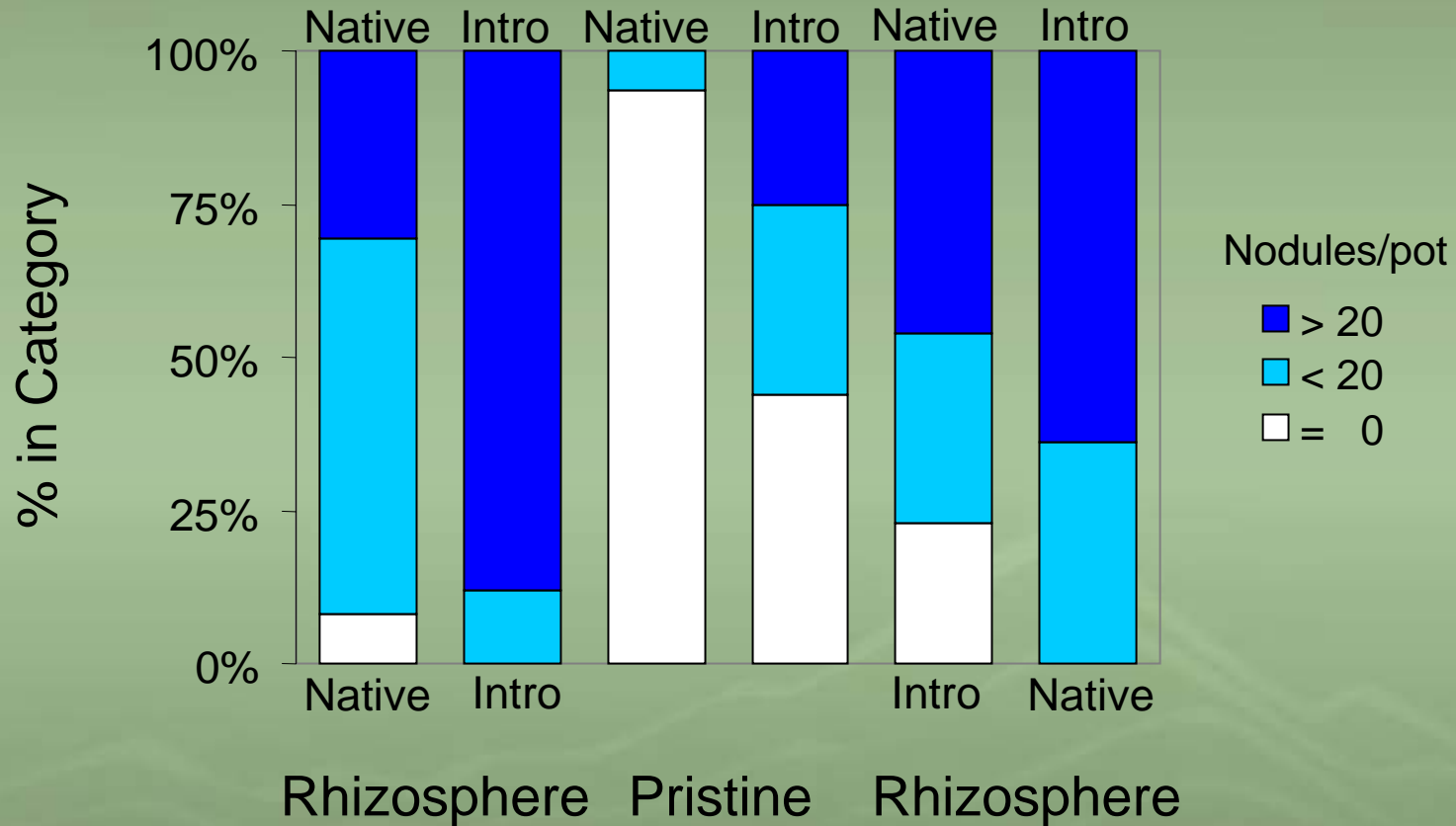
- The exotic weed legumes: Broom, Gorse, *Acacia* are effectively nodulated by diverse *Bradyrhizobium* species, and can cross nodulate.
- Confirmed that *Bradyrhizobium* strains do not nodulate the native legumes, and *Mesorhizobium* do not nodulate exotic weed legumes

Soil Investigations

- Soil samples were collected from:
 - *Carmichaelia* and *Sophora* rhizospheres
 - Broom and gorse rhizospheres
 - pristine soils (legumes not in sight)
- Soils into pots, then planted with native and introduced legumes
- Examined for nodulation after 16 weeks
- Rhizobia isolated and identified (sequencing)

Results

Bait plants



More Information

- Web: <http://www.rhizobia.co.nz>
- Email: info@rhizobia.co.nz
- Weir *et. al.* (2004) AEM 70(10): 5980-5987

The screenshot shows the homepage of the 'New Zealand Rhizobia' website. The page has a green header with the title 'New Zealand Rhizobia' and a sub-header 'home page.'. The main content area is white and contains the following text:

The Rhizobia of New Zealand

This website is about bacteria known as [rhizobia](#). These bacteria are able to form a symbiotic relationship with plants called legumes. The rhizobia live inside plant structures called root nodules, and fix nitrogen from the air into a form usable by the plants.

On these web pages you can learn about the rhizobia that are present in New Zealand, and my [PhD project](#), which is to learn more about them. Also this website contains taxonomy information about all rhizobia species, and taxonomy information about the native legume species present in New Zealand.

I keep the [rhizobia taxonomy](#) page up-to-date, with all the new species, including those from genera not normally considered to nodulate legumes such as *Devosia*. Additionally I have put up a [guide to the Modeltest program](#) that is used to find the model of DNA evolution that best describes a given sequence alignment.

My latest addition is a HTML dump of my [endnote bibliography database](#), currently this has 466 papers listed. These are some of the papers I will cite in my PhD thesis.

I recommend you use [firefox](#) to browse the internet

At the bottom of the page, there are several logos and a footer: 'W3C CSS', 'W3C XHTML 1.0', 'SITE METER 19,219', 'SOME RIGHTS RESERVED', and 'Bevan Weir'.

On the right side, there is a 'navigation' sidebar with links: Home, Site map, News, PhD project, Publications, Taxonomy, Phylogenetics, Downloads, links, Landcare Research, Auckland University, contact, contact me, about me, and Ads by Google with links to Analyze DNA Sequences, Store, BLAST and annotate millions of...



Biosecurity / Restoration

- Endangered native *Clanthus* forms effective symbiosis with all *Mesorhizobium* spp.
- But... also forms ineffective nodules with *R. leguminosarum*, could compete.
- Need to think about “contaminating” national parks with foreign rhizobia