



**COMPLEMENTATION OF SYMBIOTIC GENES IN THE  
TAXONOMICALLY DIFFERENT CO-MICROSymbionTS OF A RELIC  
LEGUME *OXYTROPIS POPOVIANA***

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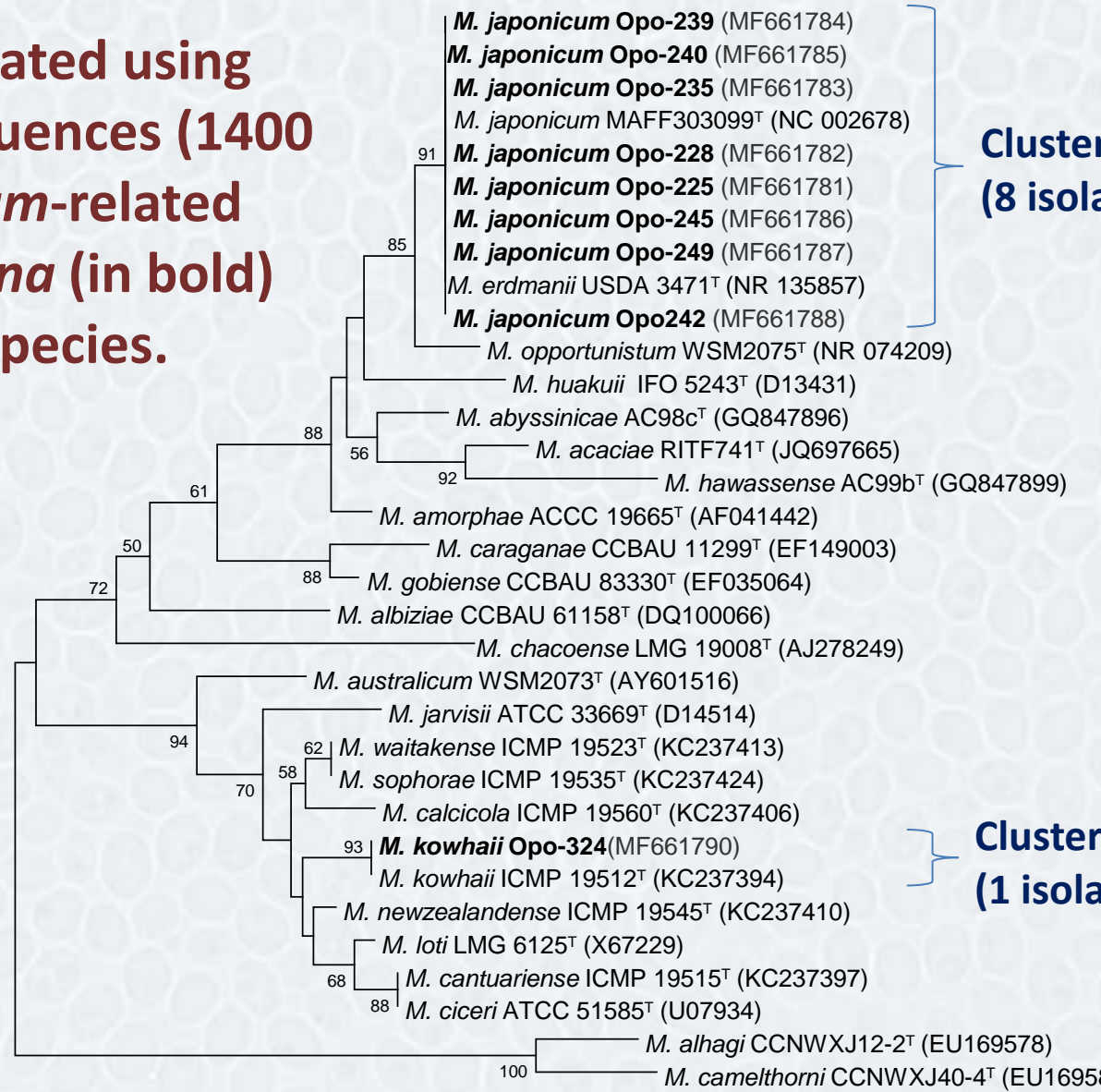
**Miocene-Pliocene relic legume  
*Oxytropis popoviana* (20 to  
3 million years ago),  
Buryatia,  
Northwestern coast of the Baikal  
Lake, Russian Federation.**

**Picture by Nadezhda Stepantsova  
(<http://www.plantarium.ru/>)**



Phylogenetic tree generated using partial 16S rRNA gene sequences (1400 nt) of the *Mesorhizobium*-related isolates from *O. popoviana* (in bold) and closely related species.

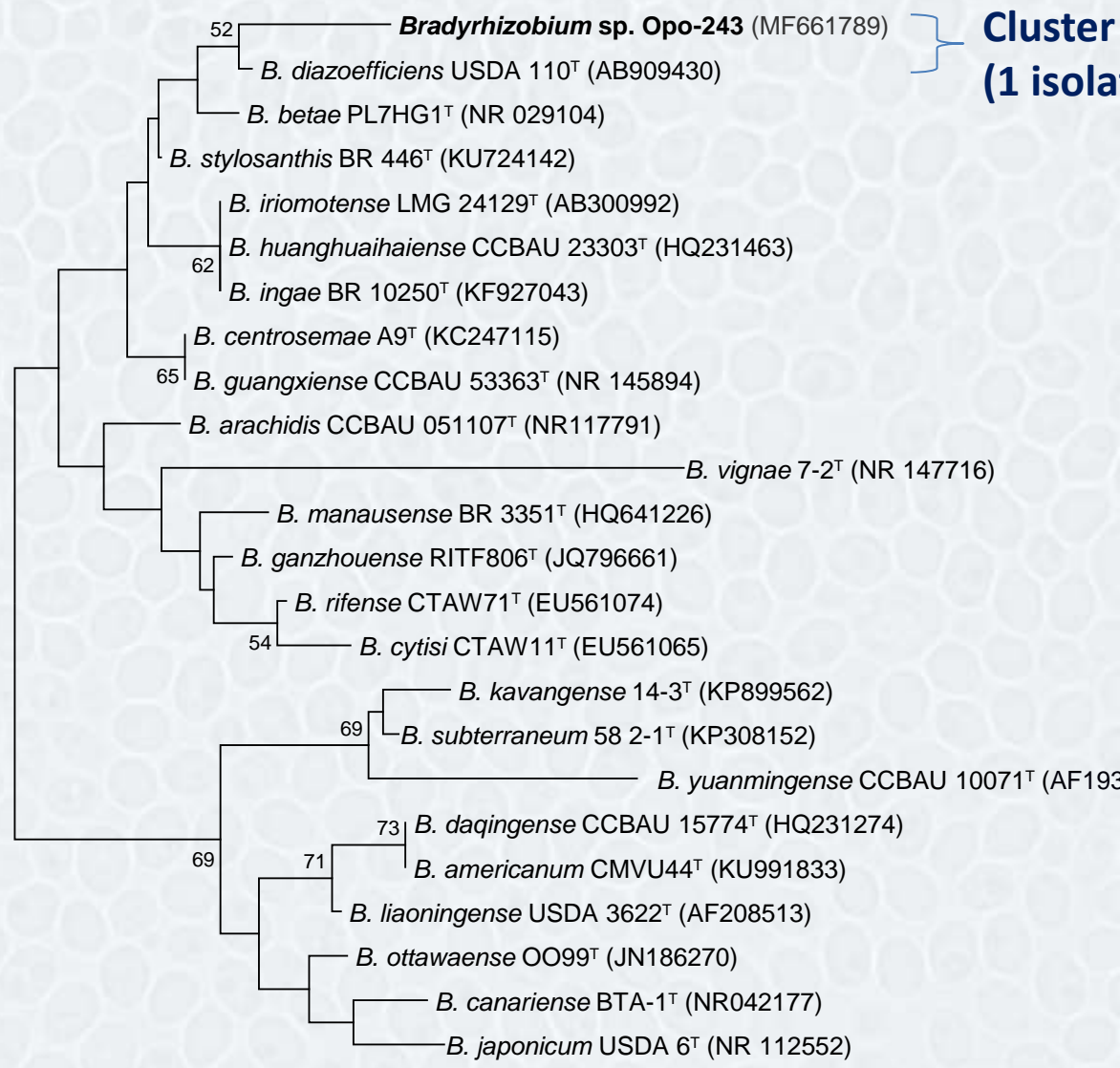
*Rrs* similarity of the isolates from Cluster I with the type strain *M. japonicum* MAFF 303099<sup>T</sup> was 99.6 -100%. Similarity of the isolate from Cluster II with the type strain *M. kowhii* ICMP 19512<sup>T</sup> was 100%.





**Phylogenetic tree generated using partial 16S rDNA sequences of the *Bradyrhizobium*-related isolate Opo-243 (in bold) and closely related species**

***Rrs* similarity of the isolate Opo-243 with the type strain *B. diazoefficiens* USDA 110<sup>T</sup> was 99.5%**



0.001



## Presence of symbiotic genes in the pare of isolates *M. Japonicum* Opo-242 and *Bradyrhizobium* sp. Opo-243

Isolate	Symbiotic genes				
	nod	nif	fix	nol	noe
Opo-242	ABCDEFGHIJFGNLMZT	HDKENABQTZXWSU	ABCXNOPQGHISJKL	-	K
Opo-243	NTG <b>PQ</b>	<b>LS</b>	NOQPGHISJKL	<b>K</b>	<b>L</b>

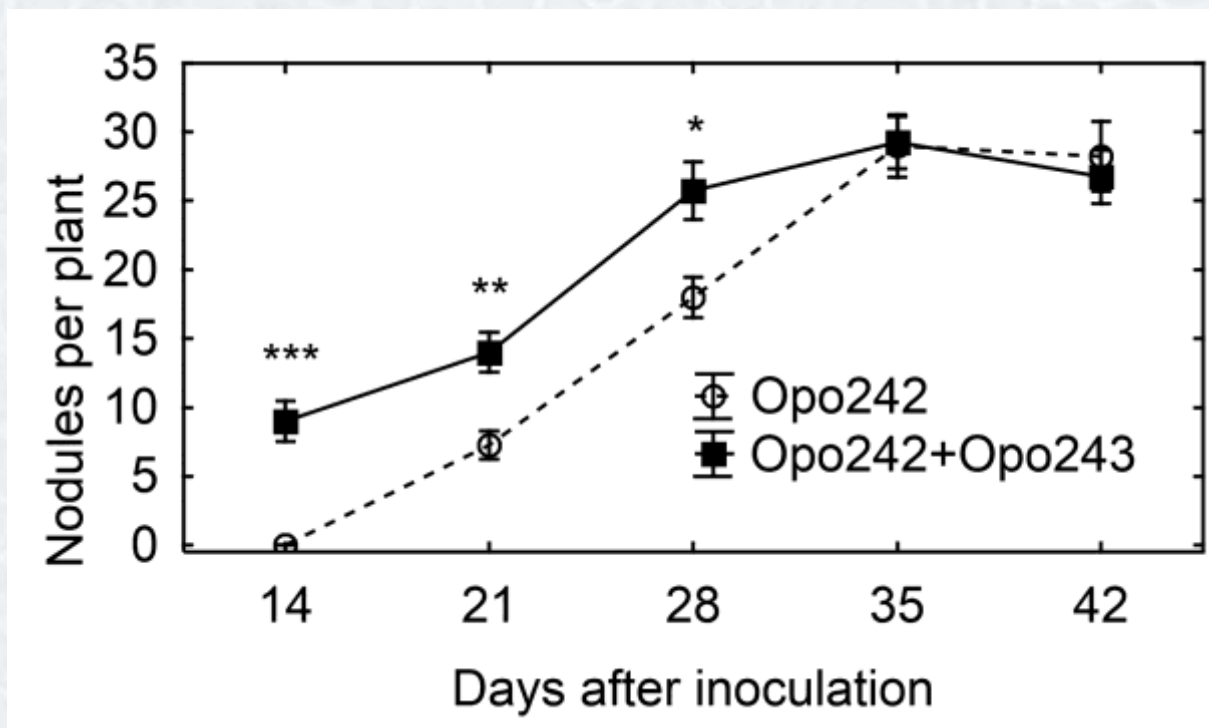
Genes present in the isolate Opo-243 and complementary to symbiotic genes of the isolate Opo-242 **in red**.

*nolK* and *noeL* genes are responsible for fucosylation of Nod-factors,  
*nodPQ* genes involved in sulfation of Nod-factors



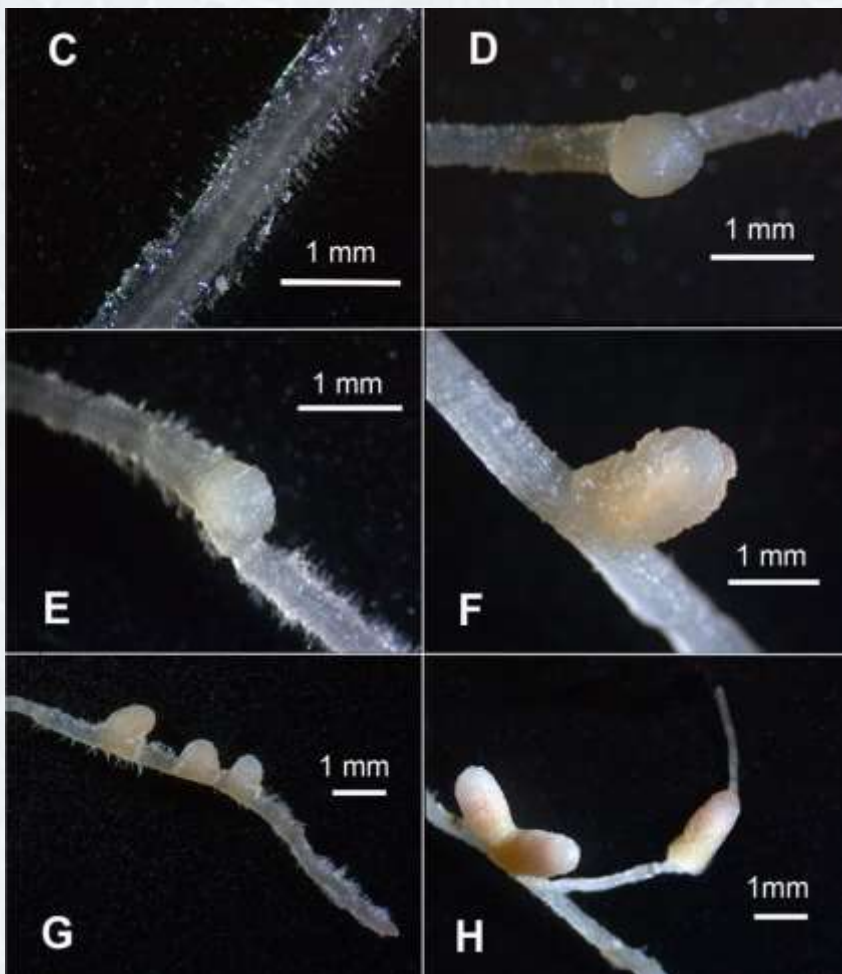
## Nodule formation on *O. popoviana* roots after mono- and co-inoculation with isolates Opo-242 and Opo-243 in the gnotobiotic plant nodulation assays

Vertical bars represent SE. Asterisks indicates probability at  $P < 0.05$  (\*),  $P < 0.01$  (\*\*) and  $P < 0.001$  (\*\*\*), respectively (Student's *t*-test). The data of two experiments with 6 replications each.





## *O. popoviana* root nodules in gnotobiotic nodulation assay after 2, 4 and 6 weeks of plant vegetation



C, E, G – inoculation with the isolate Opo-242;

D, F, H – co-inoculation with the isolates Opo-242 and Opo-243;

C, D – after 2 weeks;  
E, F – after 4 weeks;  
G, H – after 6 weeks.

The single inoculation with the strain Opo-243 did not produce nodules.



## Pairs of rhizobial co-microsymbionts isolated from nodules of the Miocene-Pliocene relic legumes (one nodule – one pair)



Plant	Isolate	Rhizobial species
<i>Vavilovia formosa</i>	Vaf-92	<i>Rhizobium leguminosarum</i>
	Vaf-93	<i>Tardiphaga robiniae</i>
<i>Oxytropis triphylla</i>	Tri-38	<i>Phyllobacterium</i> sp.
	Tri-39	<i>Bosea vestrisii</i>
	Tri-43	<i>Rhizobium leguminosarum</i>
	Tri-44	<i>Bosea vestrisii</i>
<i>Astragalus chorinensis</i>	Ach-307	<i>Bosea vaviloviae</i>
	Ach-343	<i>Mesorhizobium kowhaili</i>
<i>Glycyrrhiza uralensis</i>	Gur-139	<i>Phyllobacterium</i> sp.
	Gur-140	<i>Mesorhizobium</i> sp.





## New species of root nodule bacteria – microsymbionts of the Miocene-Pliocene relic legumes

Plant species	Geographical origin	Rhizobial species
<i>Vavilovia formosa</i>	North Ossetia, Caucasus	<i>Bosea vaviloviae</i>
<i>Oxytropis triphylla</i>	Irkutsk, Baikal Lake region	<i>Phyllobacterium zundukense</i>



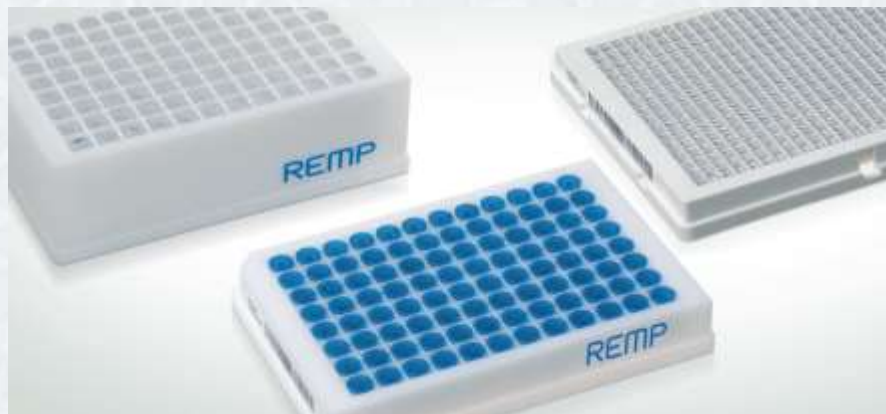


## Automated $-80^{\circ}\text{C}$ Tube Store is the reliable system for a maintenance of microbial strains



### Main store features:

- Minimal temperature fluctuation inside the storage chamber;
- 2D-Barcode identification of samples;
- Possibility of authorized storage of the commercial strains (under password);
- Online database “in real time”;
- Backup Cooling;
- Capacity 200 thousand samples



*Thanks for your attention*

