Betaproteobacterial symbioses with legumes

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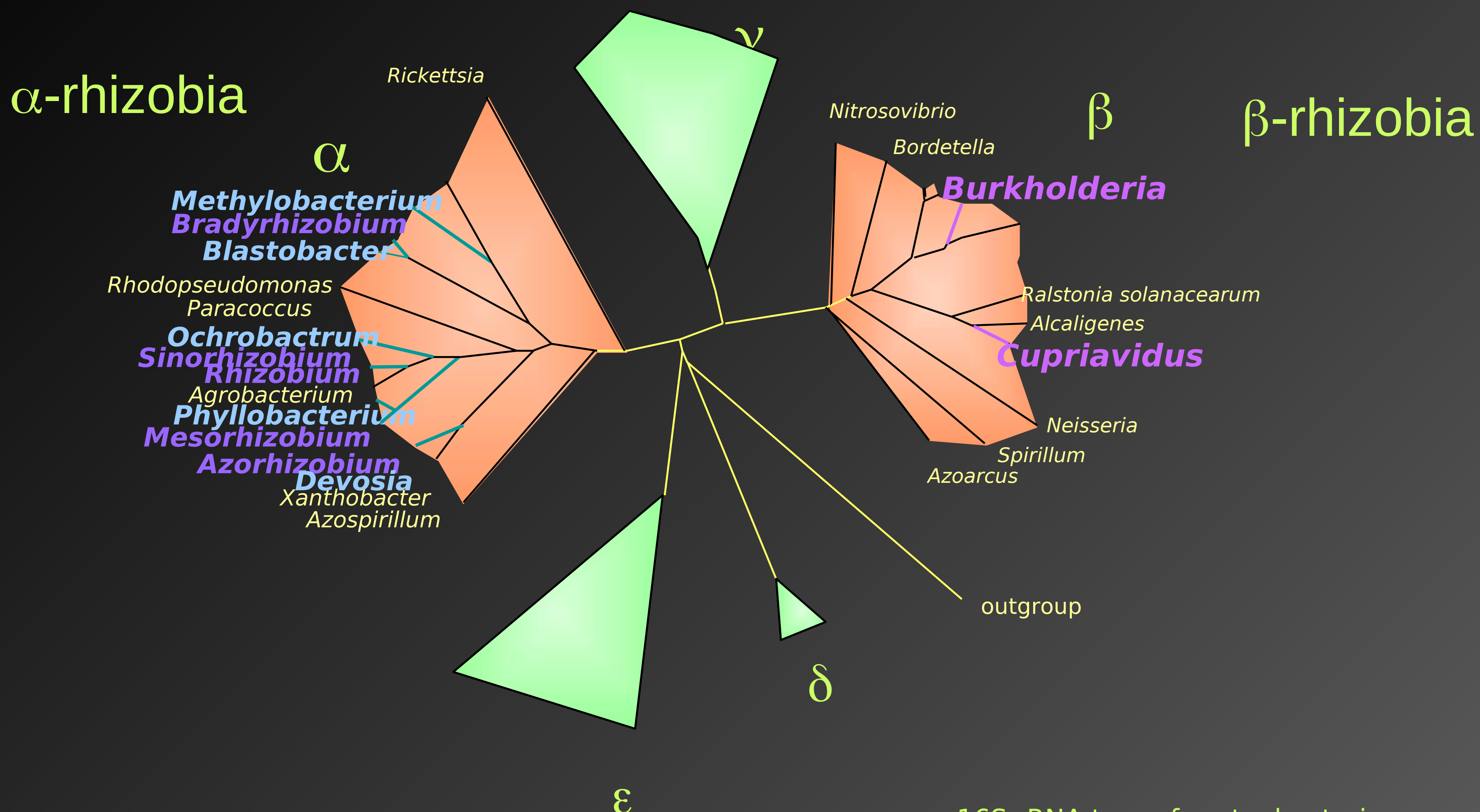
diversity of rhizobia

“rhizobia” can be a functional term to define bacteria able to fix nitrogen in symbiosis with legumes



16S rRNA tree of proteobacteria
(adapted from Moulin et al. 2001)

Beta-rhizobia: facts & questions

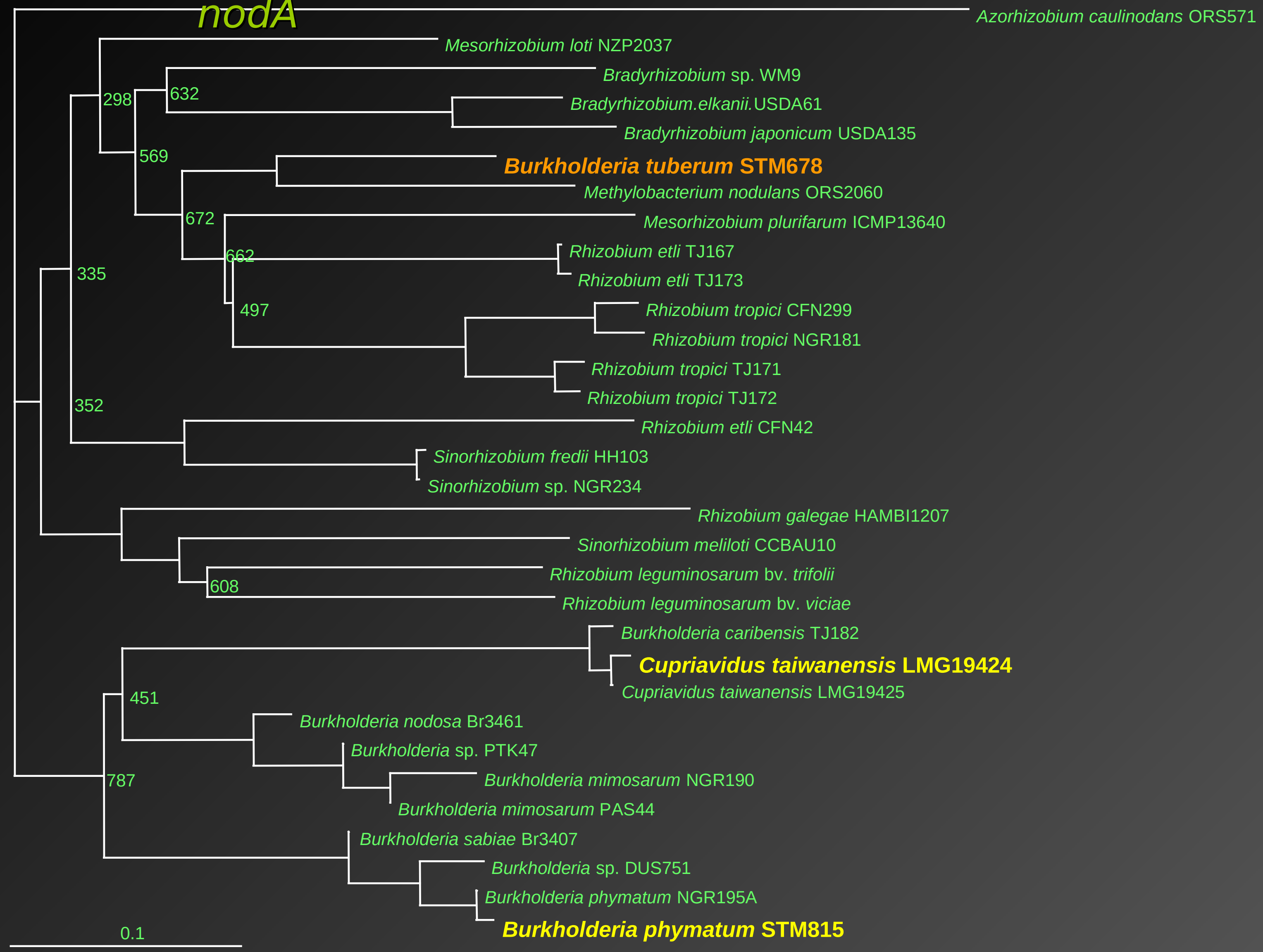
- FACTS:
- So far comprise *Cupriavidus* (*Ralstonia*) and *Burkholderia* spp.
- Not related to *Rhizobium*, but are closely related to plant-associated and endophytic Betaproteobacteria, such as other N_2-fixing *Burkholderia* spp. and to plant growth-promoting bacteria, such as *Herbaspirillum*.

- QUESTIONS:
- Are they genuine symbionts?
- From where do they originate?
- Are they widespread and important to ecology?
- Is there any taxonomic relationship between them and their legume hosts?

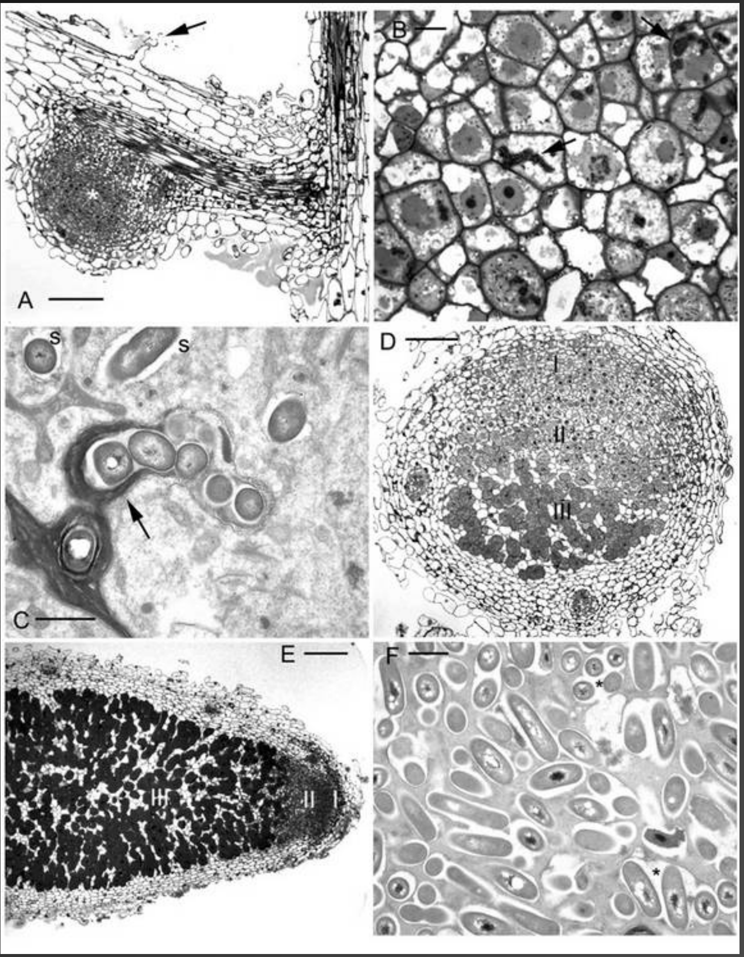
Are they genuine symbionts?

- Need to demonstrate a genetical predisposition to nodulate and fix N_2 (i.e. *nod* and *nif* genes)
- Need to demonstrate an actual ability to nodulate and fix N_2 to the benefit of their legume hosts

Beta-rhizobia from *Mimosa* spp. have *nod* and *nif* genes that cluster together e.g. *nodA*

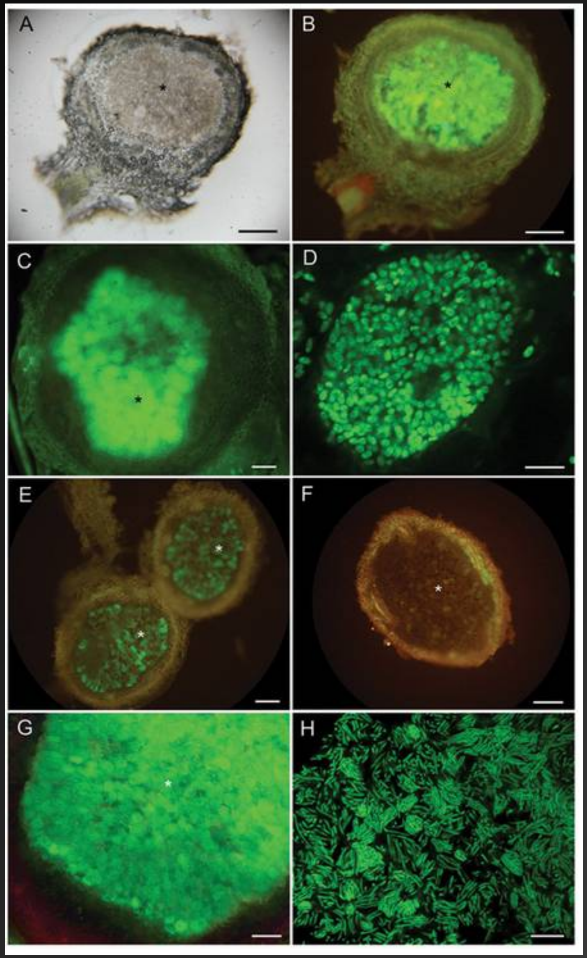


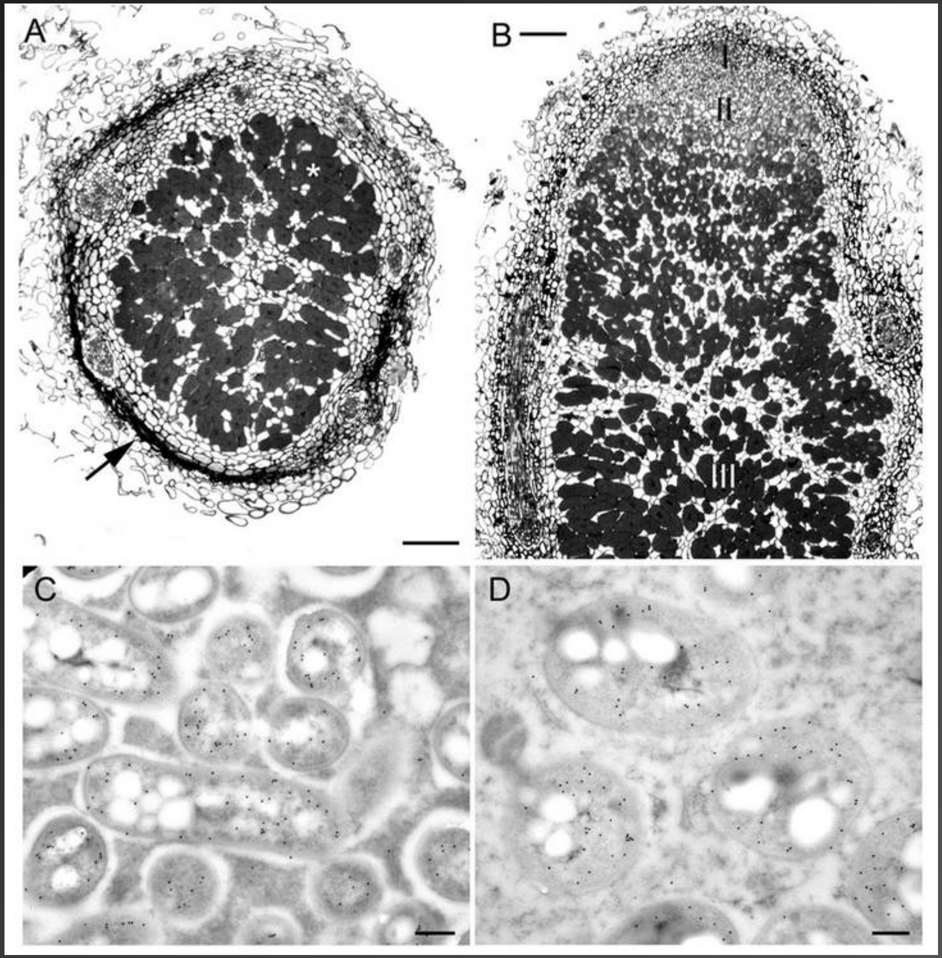
Mimosa pudica nodules formed by *C. taiwanensis* are fully functional symbiotic organs



First confirmation of symbiotic nodulation by Beta-rhizobia: Chen et al. 2003 MPMI **16:**1051-1061.

Mimosa nodules formed by *Burkholderia mimosarum* and *B. nodosa* are also fully functional





Chen et al. 2005a *AEM* **71:** 7461-7471; Chen et al. 2005b New Phytol. **168:** 661-675.

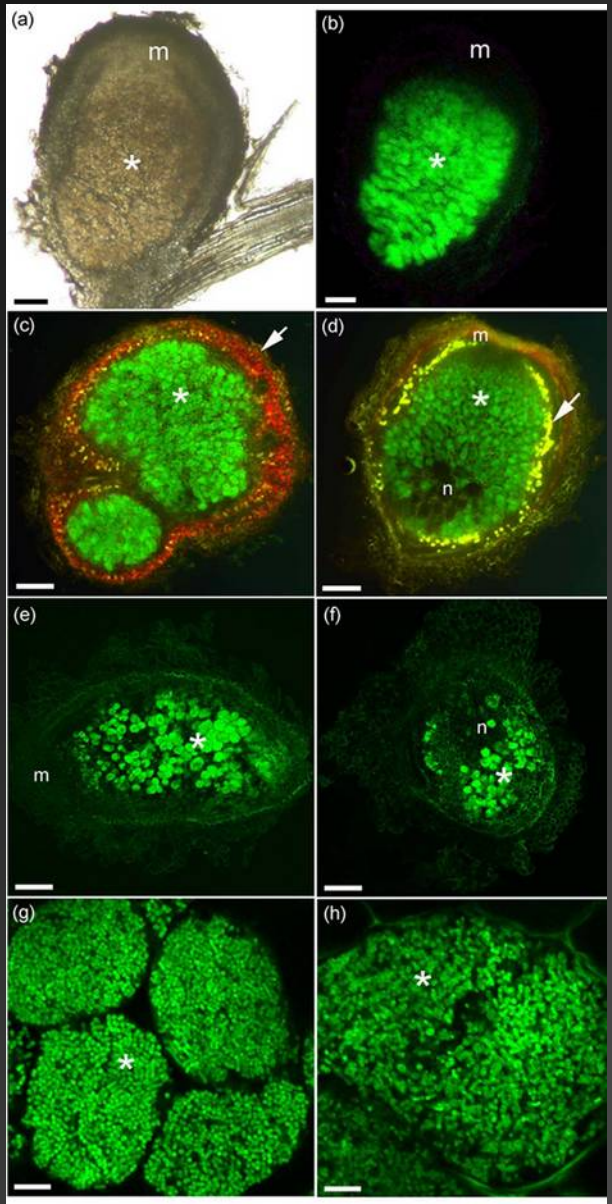
Burkholderia phymatum STM815 is also an effective symbiont of *Mimosa* spp.

M. pudica
+ Bp-GFP

M. himalayana
+ Bp-GFP

M. latispinosa
+ Bp-GFP

M. latispinosa
+ Bp-GFP



M. pudica
+ Bp-GFP

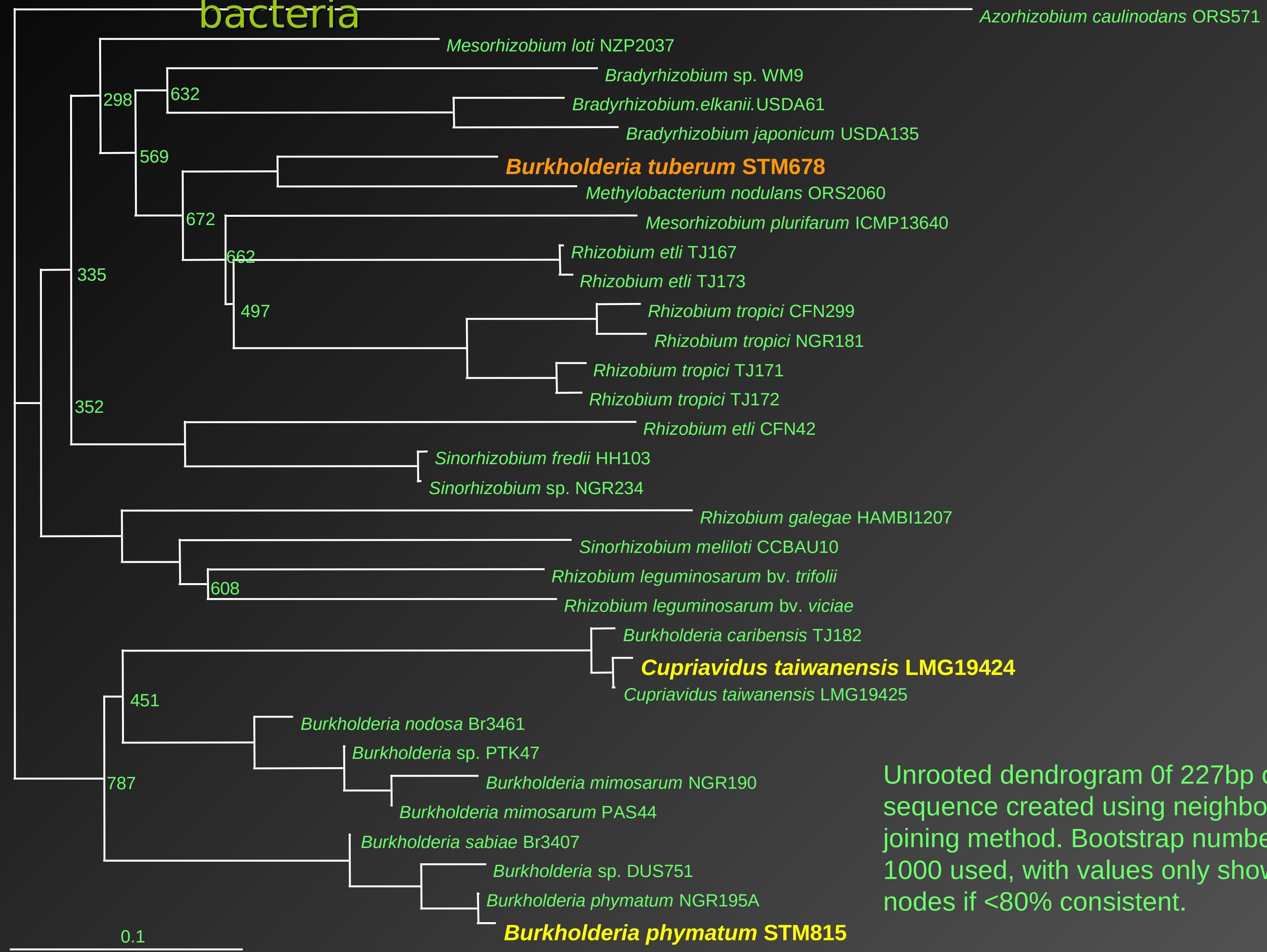
M. himalayana
+ Ct-GFP

M. latispinosa
+ Ct-GFP

M. latispinosa
+ Ct-GFP

Elliott et al. 2007a. New Phytol. **173:** 168-180.

The *nodA* gene of *B. tuberum* is distant from *Mimosa*-nodulating bacteria



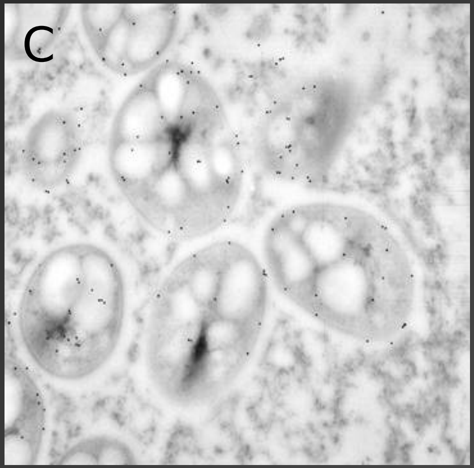
Unrooted dendrogram 0f 227bp of *nodA* sequence created using neighbor-joining method. Bootstrap number of 1000 used, with values only shown at nodes if <80% consistent.

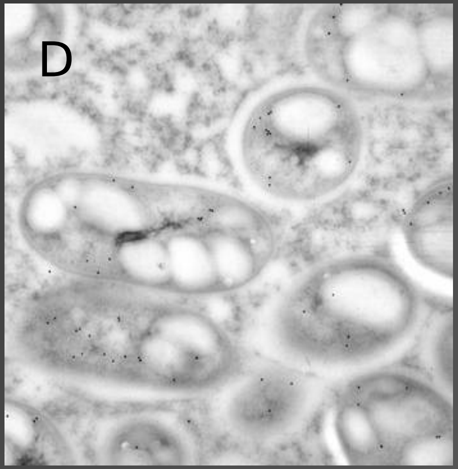
B. tuberum STM678 effectively nodulates the South African Papilionoid legume *Cyclopia*

Cyclopia genistoides
+ *B. tuberum* STM678GFP (A, B)

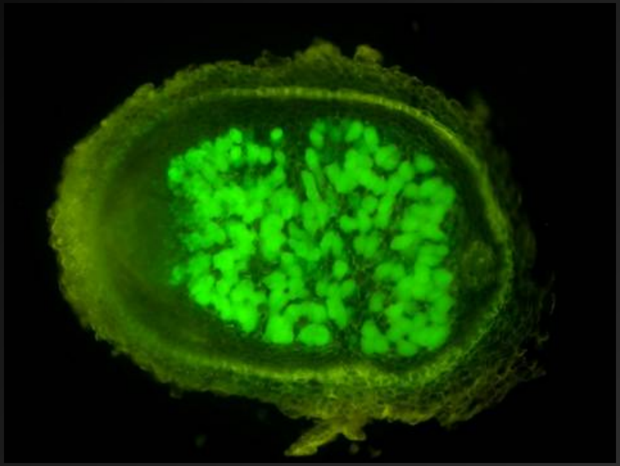
Elliott et al. 2007b. Ann. Bot. **100:** 1403-1411.

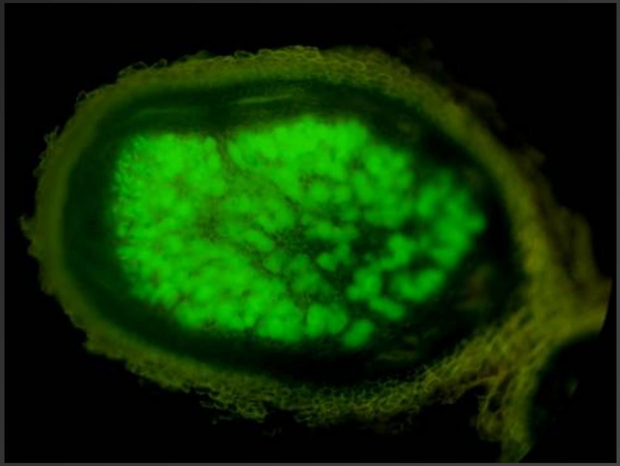
Cyclopia bacteroids labelled with antibodies against *Burkholderia* (C) and *nifH* protein (D)

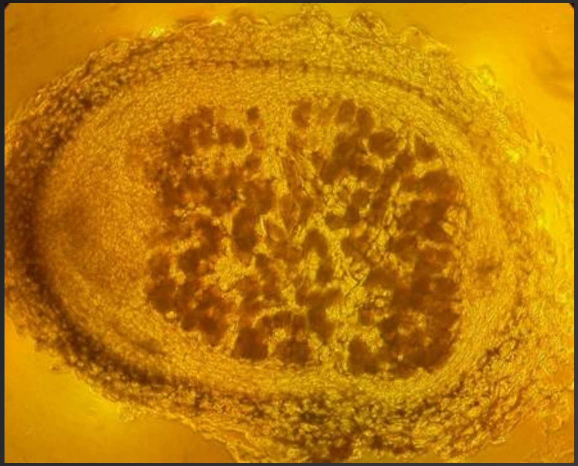


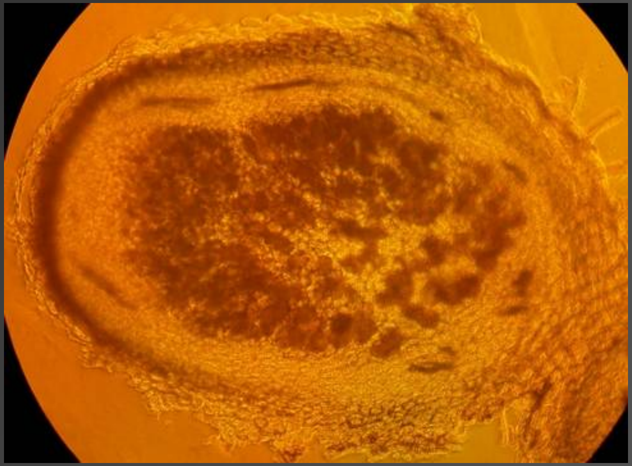


B. tuberum STM678 nodulates other legumes in the tribe Podalyreae (Papilionoideae)









Podalyria canescens

Virgilia oropoides

Does NOT nodulate *Mimosa* spp.

Beta-rhizobia are genuine symbionts:

- They possess *nod* and *nif* genes
- They can form functional nodules on their legume hosts, as demonstrated under sterile conditions with reporter gene (GFP)-tagged strains and/or specific antibodies

Where do they come from?

- In terms of geography
- In terms of evolution

Are they widespread and important to ecology?

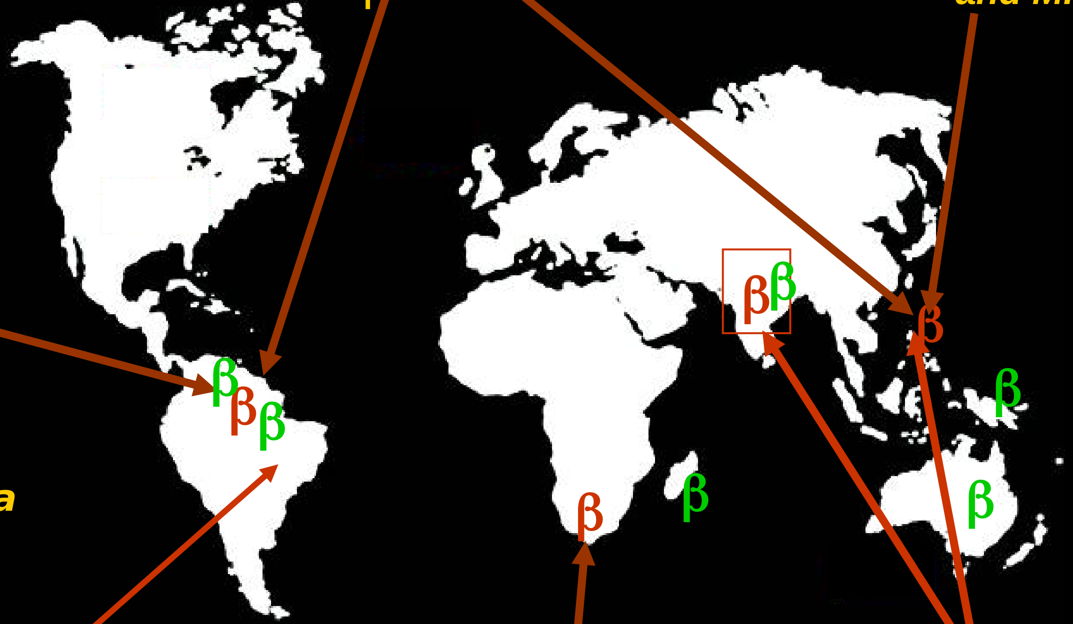
-rhizobia are widespread

13 characterised species of β-rhizobia and many other isolates of β-rhizobia

Burkholderia mimosarum
(*Mimosa pigra*)

Burkholderia caribensis
(*Mimosa pudica*
***and M. diplotricha*)**

A special affinity of *Mimosa* for β-rhizobia ?



Burkholderia phymatum
(*Mimosa spp.*)

Burkholderia nodosa
(*Mimosa bimucronata*
and *M. scabrella*)
Burkholderia sabiae
(*M. caesalpiniifolia*)
Burkholderia symbiotica
(*Mimosa cordistipula*)
Burkholderia diazotrophica
(*Mimosa spp.*)
***Cupriavidus necator* (host?)**

Burkholderia tuberum
(*Cyclopia* spp. and
other Podalyreae)
Burkholderia sprentiae
Burkholderia rhynchosiae
(*Lebeckia, Rhynchosia*)

Cupriavidus (Ralstonia)
taiwanensis
(*Mimosa pudica*
***and M. diplotricha*)**

The large legume genus *Mimosa*

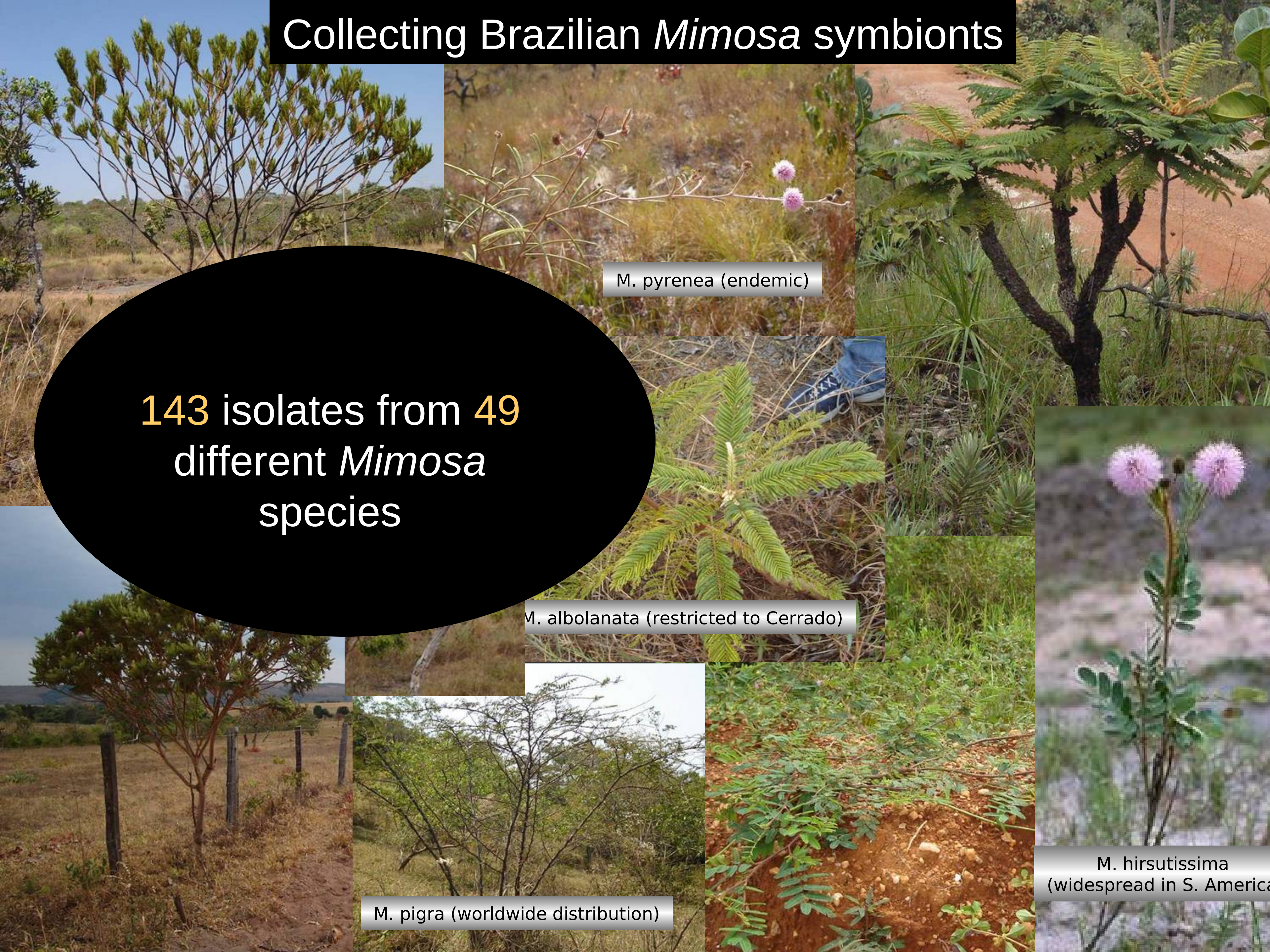
Mimosa species can be herbs but most are shrubs or trees from tropical or subtropical regions

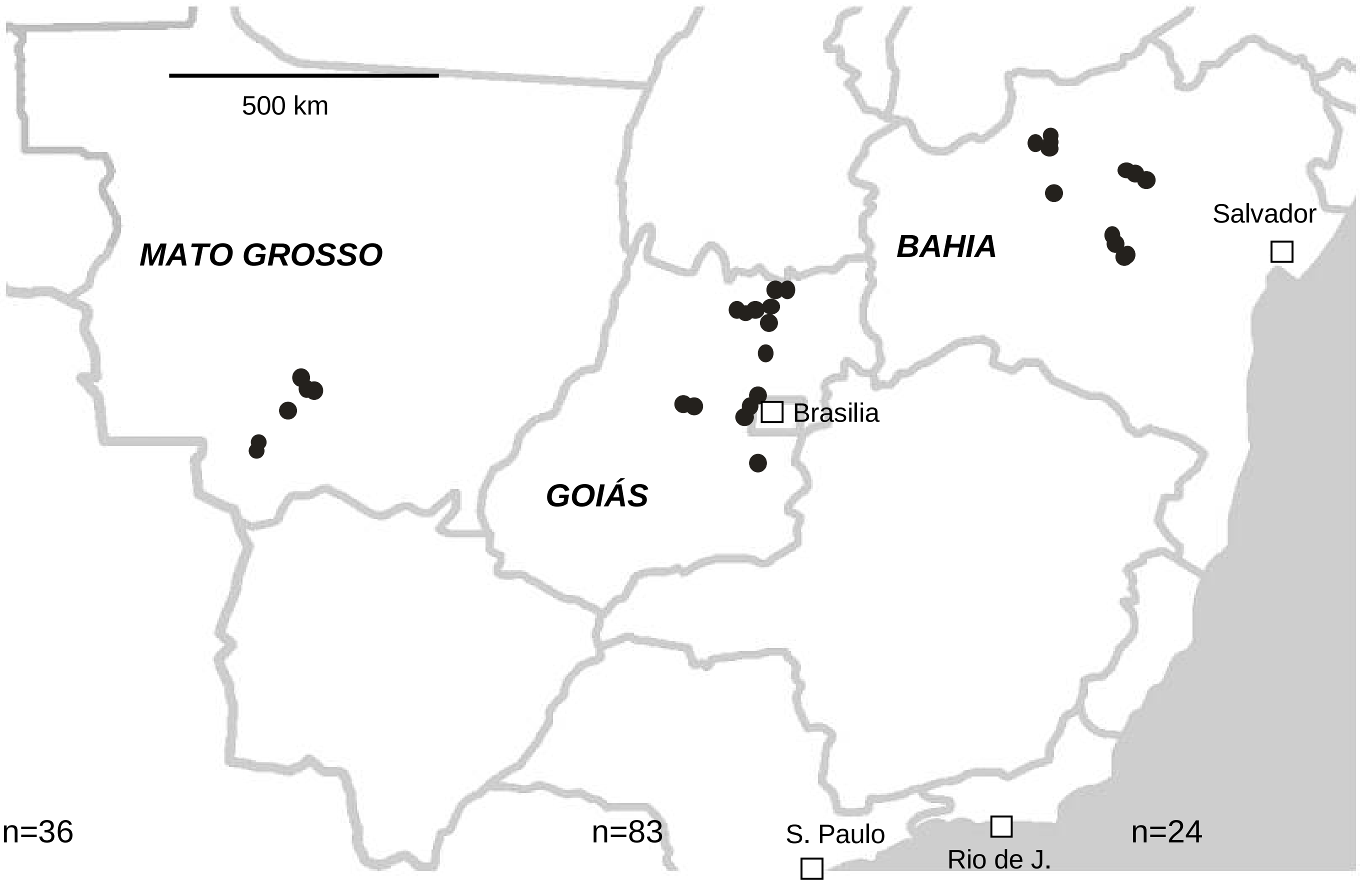
Mimosa is one of the largest genera of Mimosoid legumes, with approximately 500 species

About 450 *Mimosa* species are native to Central and South America

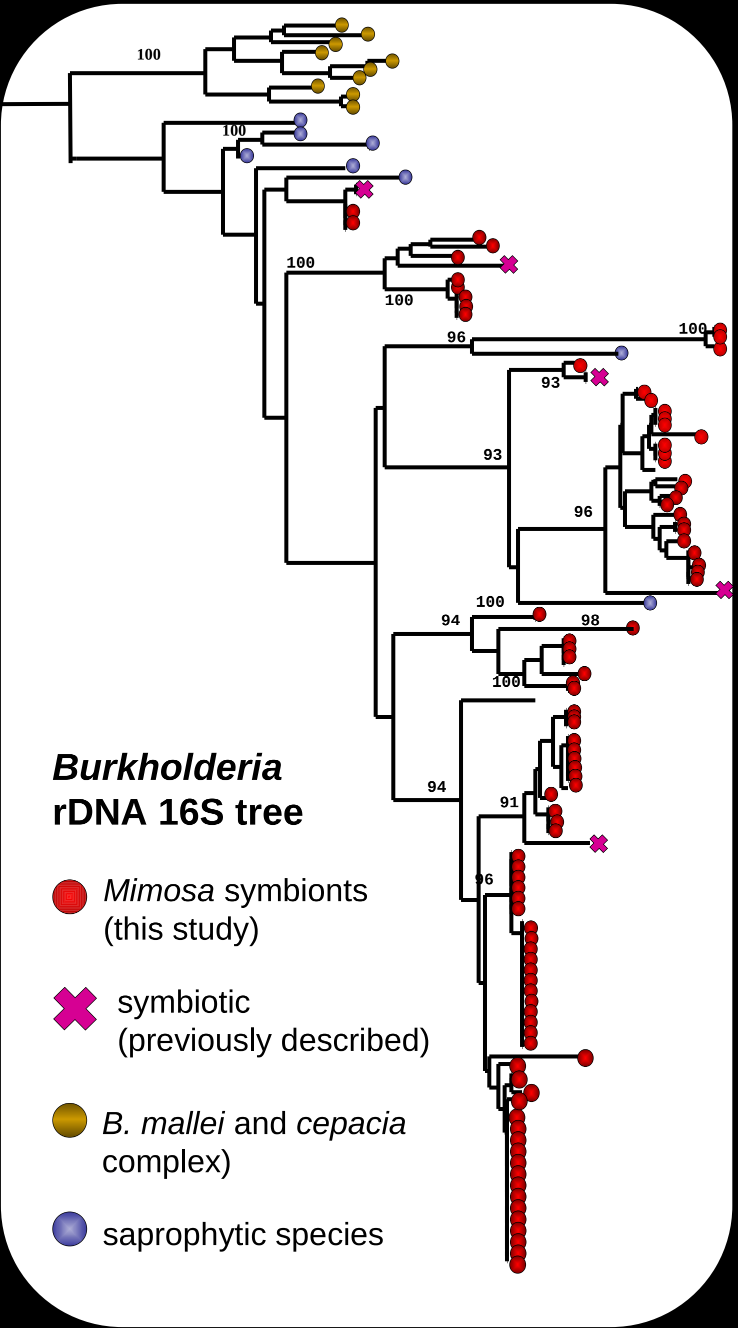
Cerrado and *Caatinga* regions of Central Brazil are the major centres of diversification

Collecting Brazilian *Mimosa* symbionts


Collecting Brazilian Mimosa symbionts
143 isolates from 49 different Mimosa species
M. pyrenea (endemic)
M. albolanata (restricted to Cerrado)
M. pigra (worldwide distribution)
M. hirsutissima (widespread in S. America


500 km
MATO GROSSO
BAHIA
Salvador
GOIÁS
Brasilia
n=36
n=83
S. Paulo
Rio de J.
n=24

What do housekeeping genes tell us?



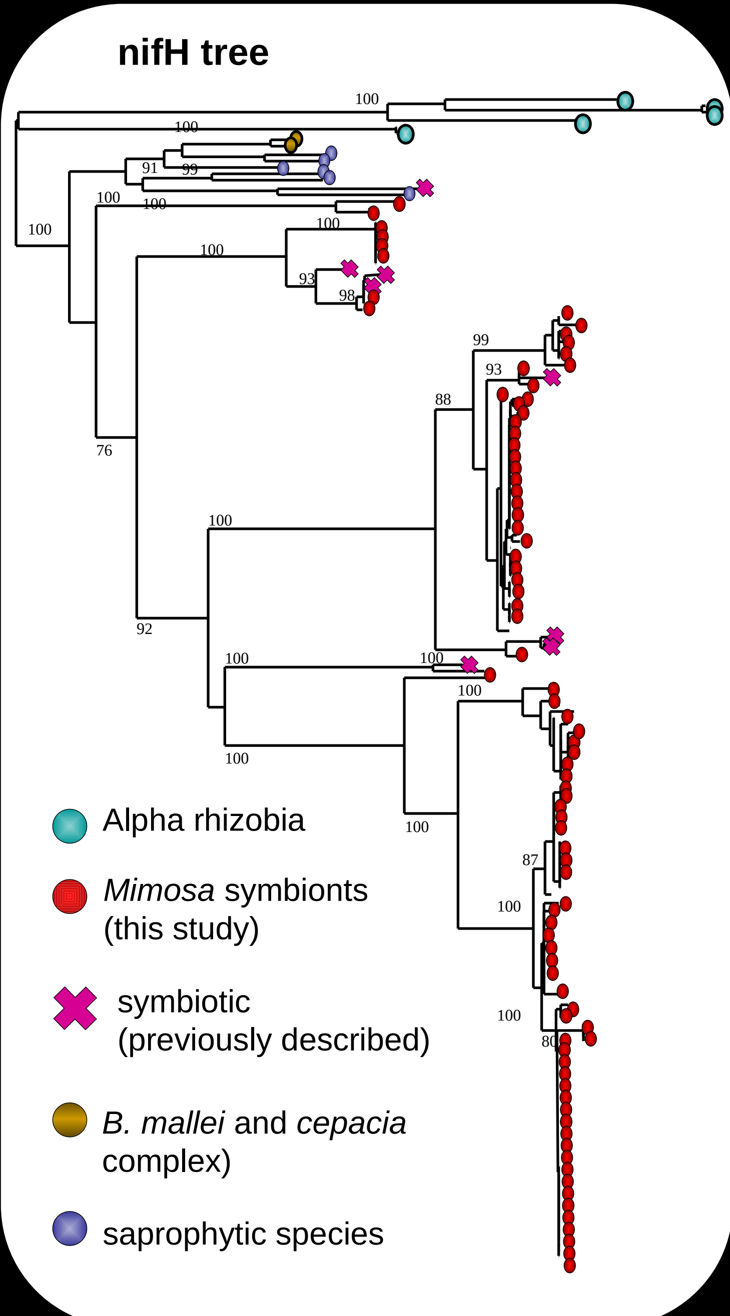
97% of *Mimosa* symbionts in Brazil belong to the *Burkholderia* genus

Burkholderia are the main symbionts of *Mimosa*

16S and *recA* tree topologies are congruent and indicate that Brazilian *Burkholderia*

- are close to already described symbiotic *Burkholderia*
- may constitute several new species
- are closer to saprophytic and generally diazotrophic *Burkholderia* rather than to pathogenic *Burkholderia*

What do nitrogen fixation genes tell us?



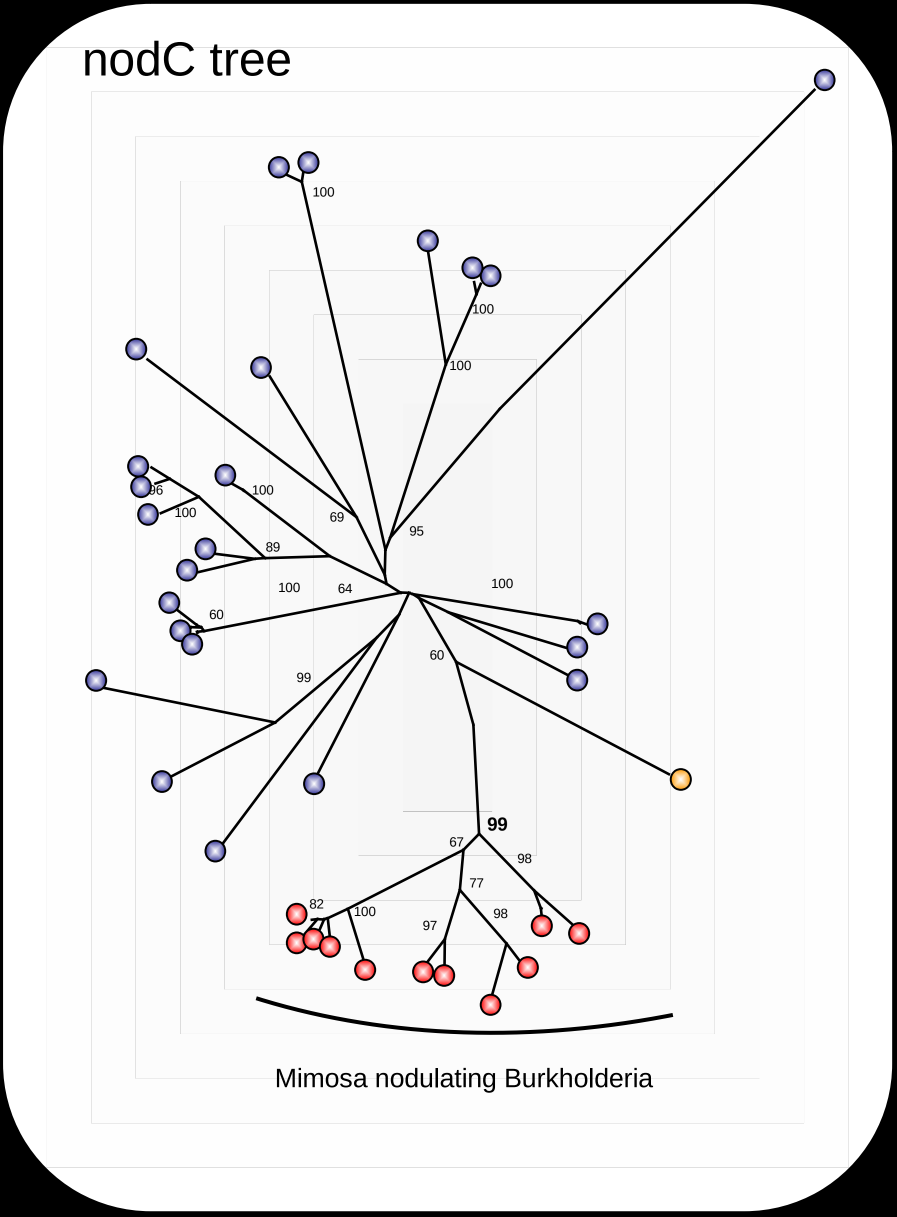
The diazotrophic character is present in many non-symbiotic *Burkholderia*

Symbiotic *Burkholderia nifH* genes are related to those of free-living burkholderias

Suggests that symbiotic *Burkholderia* didn't acquire their nitrogen fixing capabilities from α-rhizobia, but added nodulation to pre-existing N-fixing capabilities

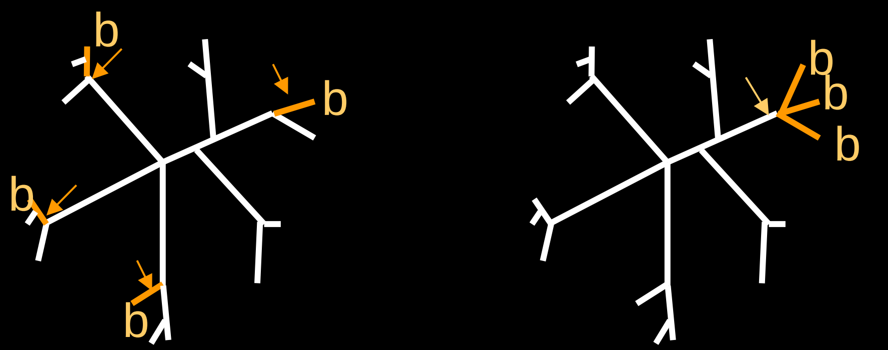
In contrast to most α-rhizobia, some of them are known to be free living diazotrophs

What do nodulation genes tell us?



- *nodC* genes of *Burkholderia* associated with *Mimosa* form a monophyletic group

Suggests a unique acquisition of symbiotic genes



Cerrado & Caatinga spp. grow in low pH soils (>3.0) and prefer acid-tolerant *Burkholderia*

Nodules from 67 *Mimosa* spp. gave a positive signal with an antibody against *Burkholderia*, and no signal with an antibody against *Cupriavidus*

M. ursina inoculated with *Burkholderia* (left) or *Cupriavidus* (right)

Summary – The Betas from Brazil

- *Burkholderia* species are the natural symbionts of native *Mimosa* species in Brazil.
- Symbionts are found in 7 distinct species complexes.
- Symbiosis-related genes have the same phylogeny as core genes – HGT is rare.
- Ancient symbionts (>50 million years)
- Species differ in environmental preference, not host specificity.
- Endemic Cerrado/Caatinga spp. cannot form effective symbioses with other bacteria.

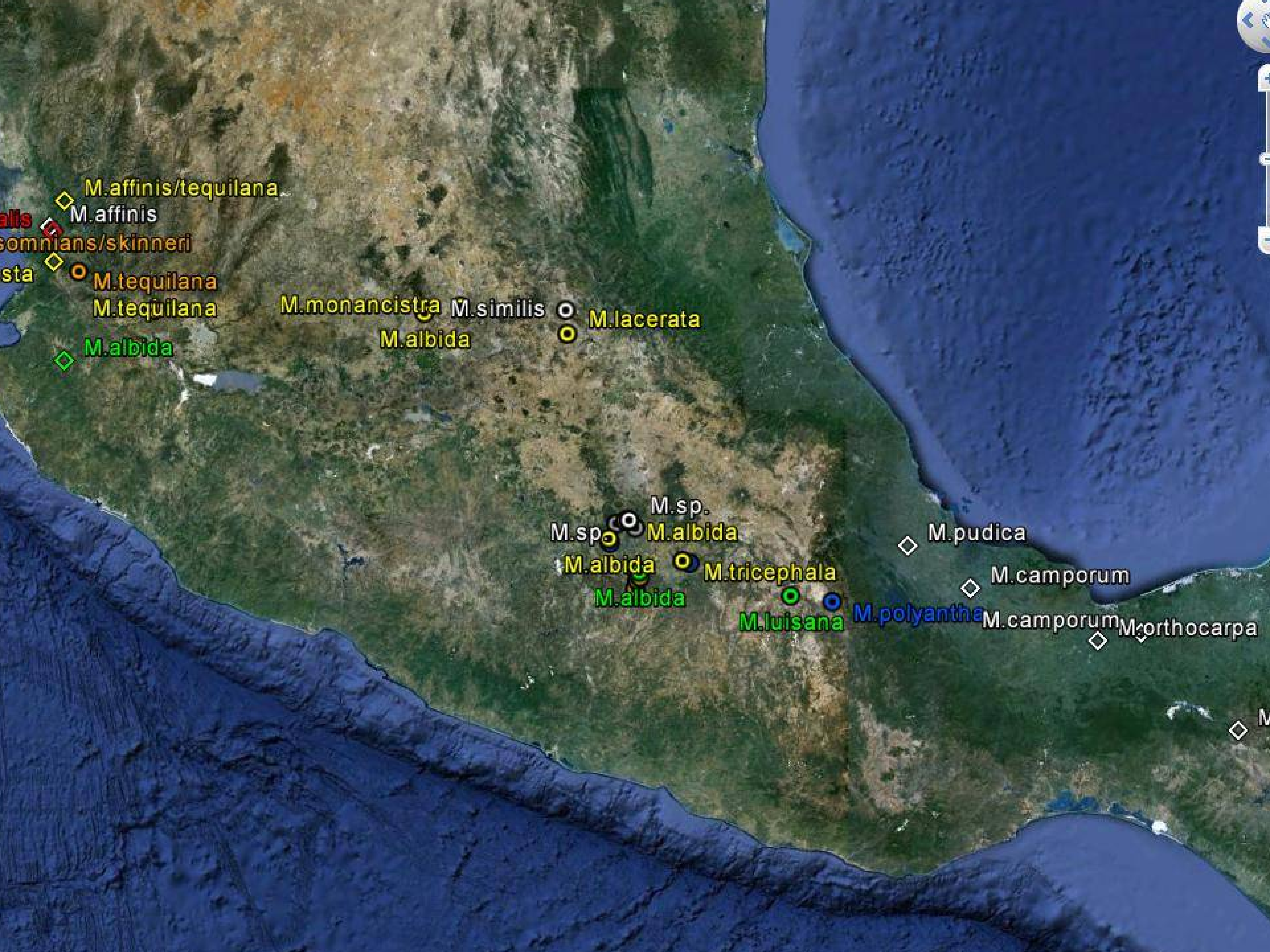
Mimosa spp. are nodulated exclusively by Beta-rhizobia

Or are they?

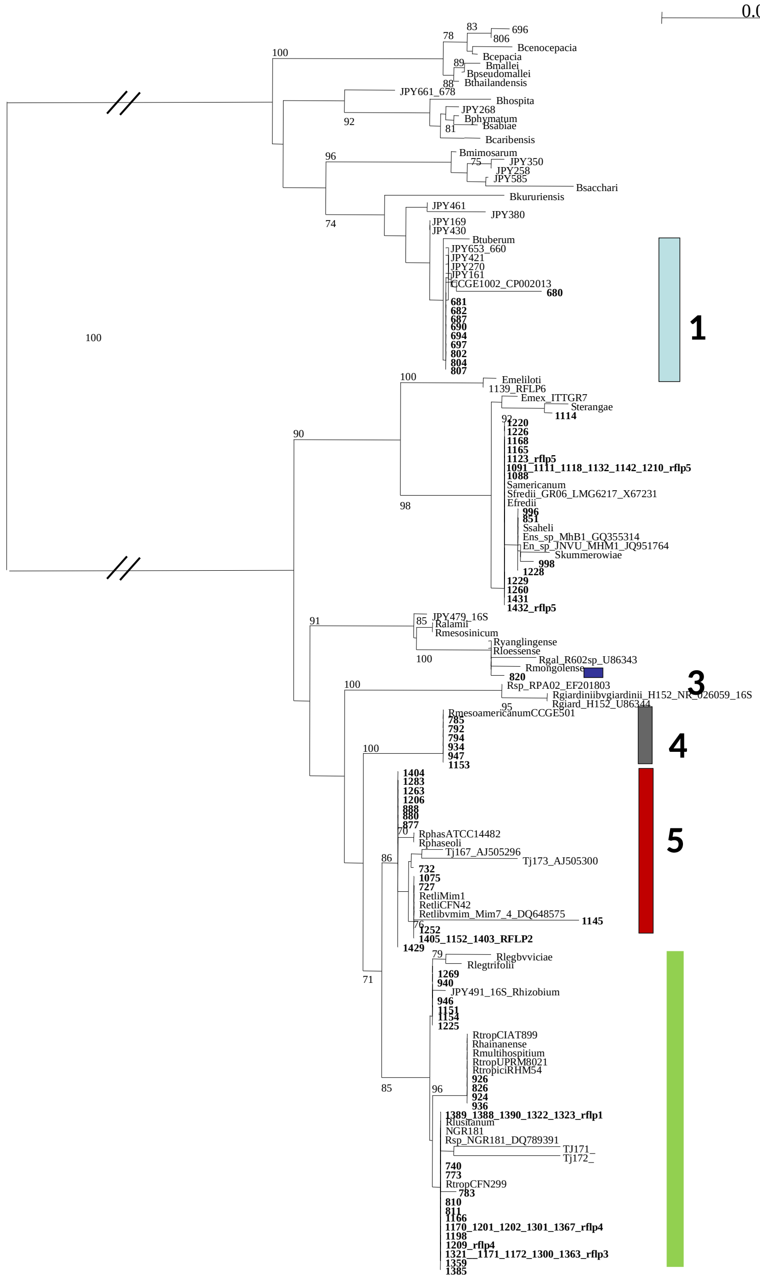
Outside Brazil…..

Mexico is the most important centre of *Mimosa* diversity in the Northern hemisphere (>100 spp.), so what are the symbionts of Mexican *Mimosa* spp.?

>1000 isolates from 30 *Mimosa* spp.


M.affinis/tequilana
alis
M.affinis
somnians/skinneri
sta
M.tequilana
M.tequilana
M.monancistra
M.similis
M.lacerata
M.albida
M.albida
M.sp.
M.sp
M.albida
M.albida
M.tricephala
M.albida
M.pudica
M.camporum
M.luisana
M.polyantha
M.camporum
M.orthocarpa

16S rRNA



Burkholderia

Ensifer (Sinorhizobium)

Rhizobium mesoamericanum

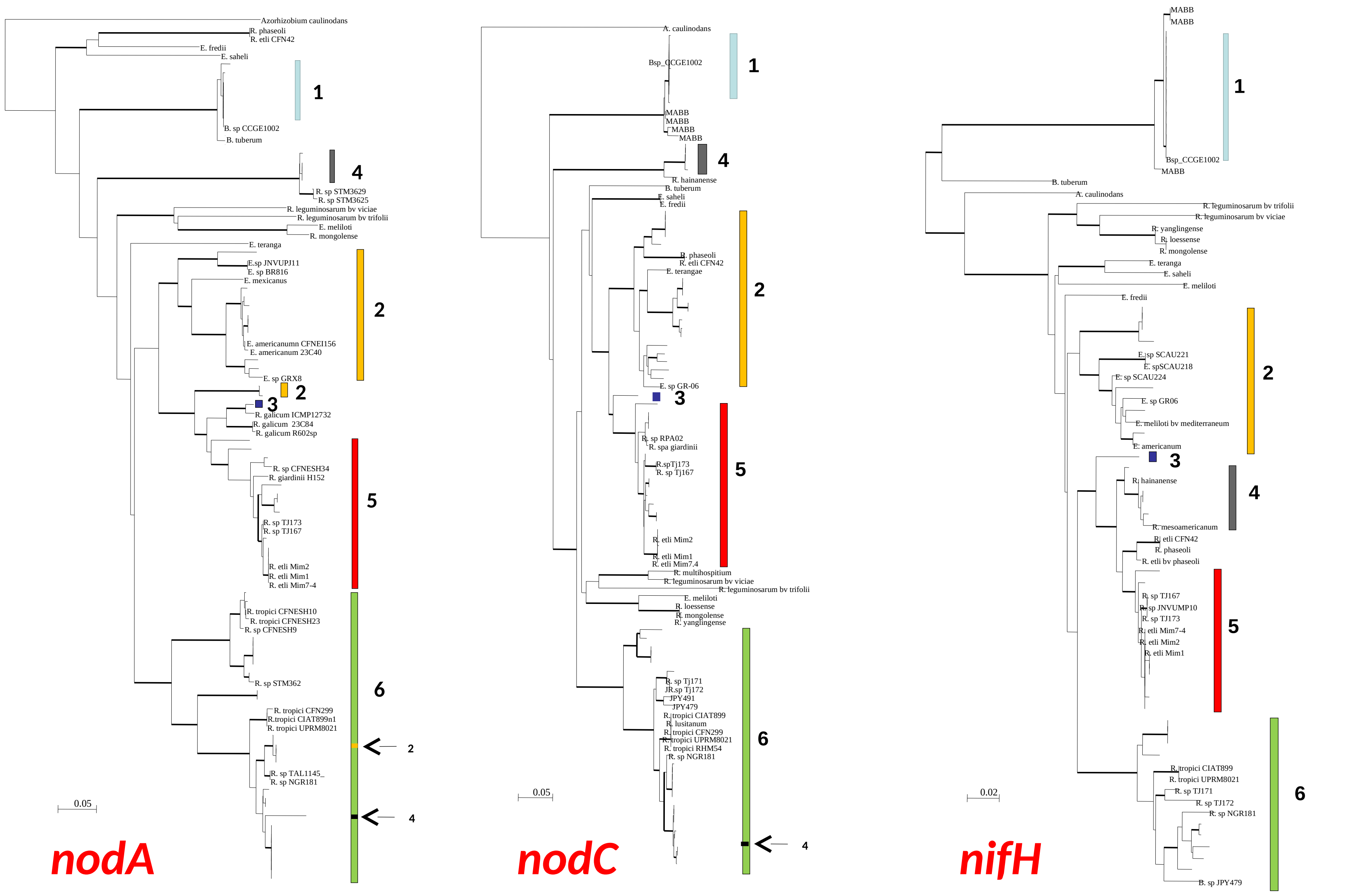
Rhizobium etli

Rhizobium tropici

16S

Bontemps et al. New Phytol (in press)

Symbiosis-related genes have the same phylogeny as the house-keeping genes



Mexican *Mimosa* symbionts

- 25 *Mimosa* spp. have been sampled from central Mexico as far south as Tehuacan (Puebla), and as far west as Tequila (Jalisco).
- The majority of the symbionts are either *Rhizobium* or *Sinorhizobium*; these are closely related to species already present in central Mexico, particularly in common bean (*Phaseolus vulgaris*).
- Mexican endemic *Mimosa* spp. cannot nodulate effectively with Beta-rhizobia, but will nodulate with native *Rhizobium* strains.
- “Widespread” Mexican species can nodulate with *Burkholderia*
- **Soils are neutral - alkaline.**

Other centres of *Mimosa* diversity

- Madagascar and East Africa: 30 spp.
- India: 4 – 6 spp.
- Uruguay: 12 spp.

“Old World” *Mimosa* spp. are not nodulated by Beta-rhizobia

M. hamata = Sinorhizobium

Does NOT nodulate effectively with Beta-rhizobia

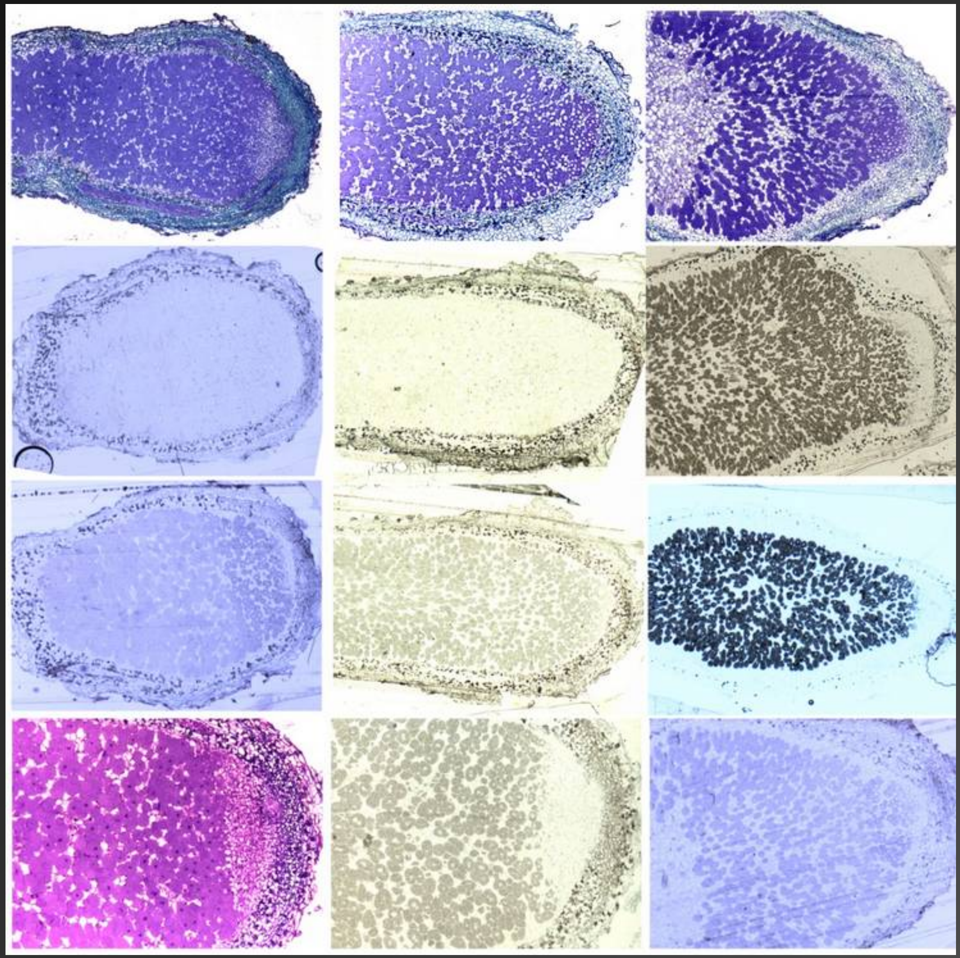
Gehlot et al. (2013) Ann Bot **112:** 179-196

M. himalayana = Sinorhizobium

ndia *Mimosa* symbionts are (Old World) *Sinorhizobium* spp., but we know
nost nothing about Madagascan endemics

Southern *Mimosa* spp. are nodulated by *Cupriavidus*?

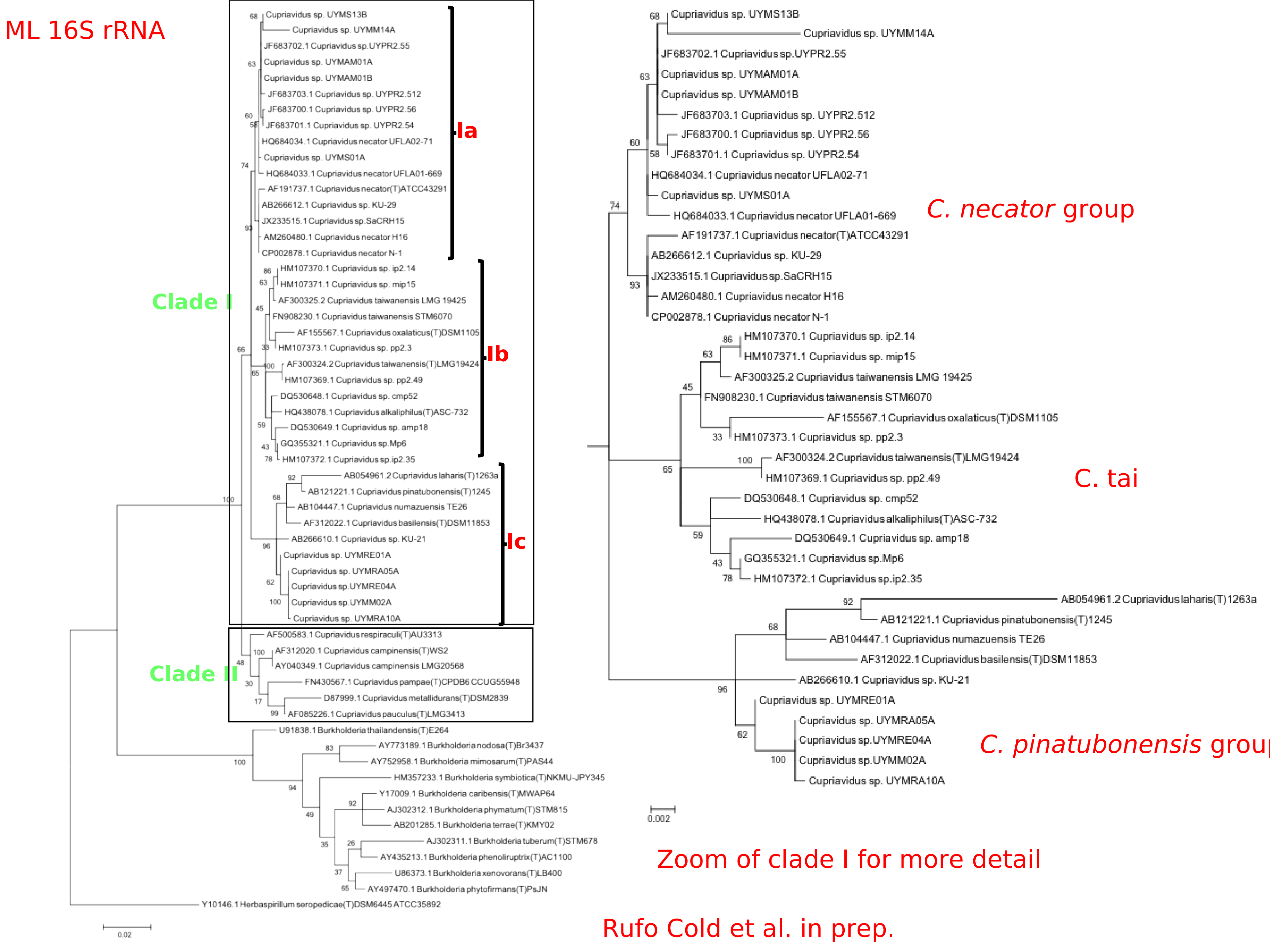
M. magentea *M. ramulosa* *M. schledeneii*



Burkholderia

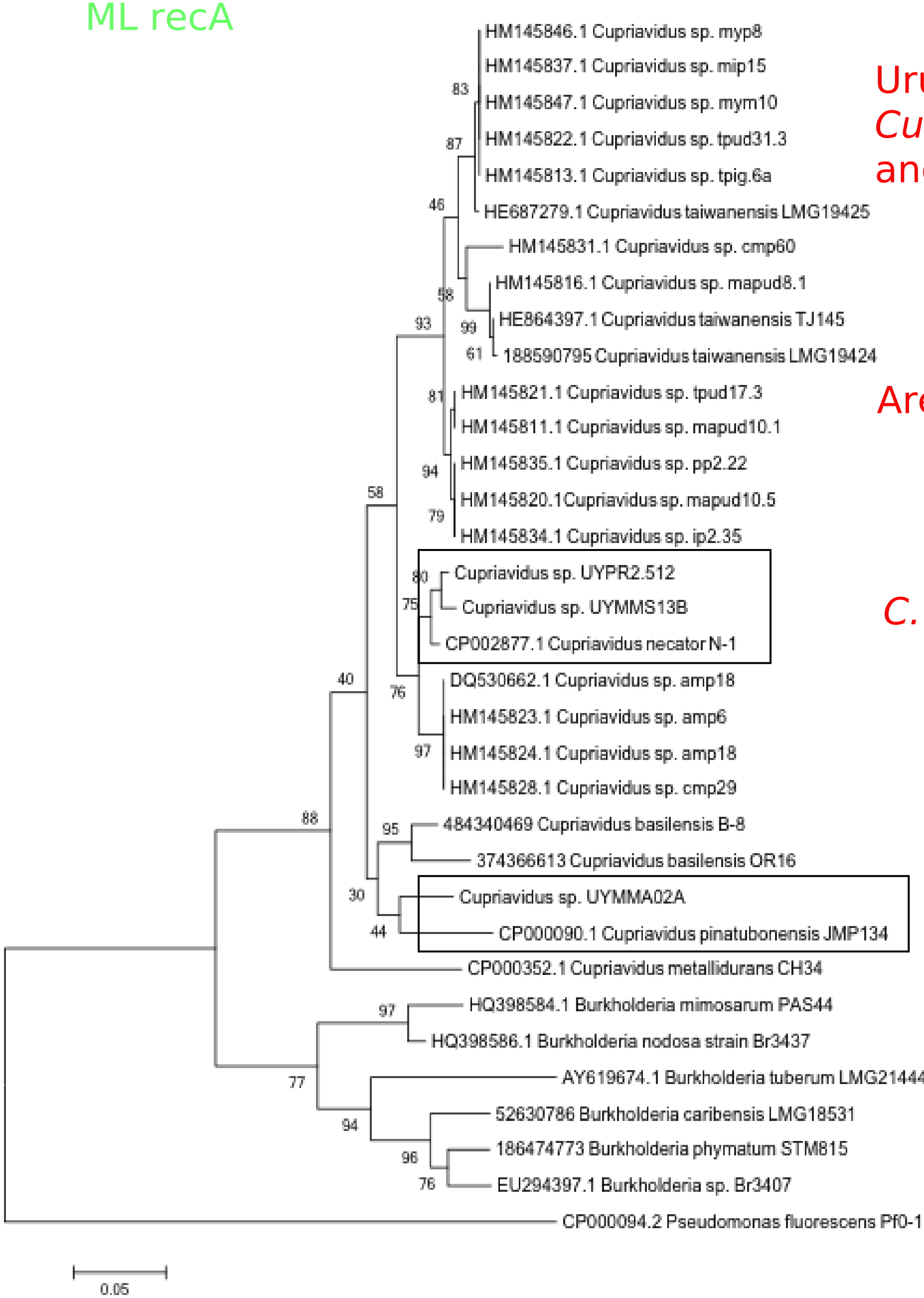
Cupriavidus

But NOT *C. taiwanensis*


ML 16S rRNA
Clade I
Clade II
Ia
Ib
Ic
Cupriavidus sp. UYMS13B
Cupriavidus sp. UYMM14A
JF683702.1 Cupriavidus sp.UYPR2.55
Cupriavidus sp. UYMAM01A
Cupriavidus sp. UYMAM01B
JF683703.1 Cupriavidus sp. UYPR2.512
JF683700.1 Cupriavidus sp. UYPR2.56
JF683701.1 Cupriavidus sp. UYPR2.54
HQ684034.1 Cupriavidus necator UFLA02-71
Cupriavidus sp. UYMS01A
HQ684033.1 Cupriavidus necator UFLA01-669
AF191737.1 Cupriavidus necator(T)ATCC43291
AB266612.1 Cupriavidus sp. KU-29
JX233515.1 Cupriavidus sp.SaCRH15
AM260480.1 Cupriavidus necator H16
CP002878.1 Cupriavidus necator N-1
HM107370.1 Cupriavidus sp. ip2.14
HM107371.1 Cupriavidus sp. mip15
AF300325.2 Cupriavidus taiwanensis LMG 19425
FN908230.1 Cupriavidus taiwanensis STM6070
AF155567.1 Cupriavidus oxalaticus(T)DSM1105
HM107373.1 Cupriavidus sp. pp2.3
AF300324.2 Cupriavidus taiwanensis(T)LMG19424
HM107369.1 Cupriavidus sp. pp2.49
DQ530648.1 Cupriavidus sp. cmp52
HQ438078.1 Cupriavidus alkaliphilus(T)ASC-732
DQ530649.1 Cupriavidus sp. amp18
GQ355321.1 Cupriavidus sp.Mp6
HM107372.1 Cupriavidus sp.ip2.35
AB054961.2 Cupriavidus laharis(T)1263a
AB121221.1 Cupriavidus pinatubonensis(T)1245
AB104447.1 Cupriavidus numazuensis TE26
AF312022.1 Cupriavidus basilensis(T)DSM11853
AB266610.1 Cupriavidus sp. KU-21
Cupriavidus sp. UYMRE01A
Cupriavidus sp. UYMRA05A
Cupriavidus sp.UYMRE04A
Cupriavidus sp.UYMM02A
Cupriavidus sp. UYMRA10A
AF500583.1 Cupriavidus respiraculi(T)AU3313
AF312020.1 Cupriavidus campinensis(T)WS2
AY040349.1 Cupriavidus campinensis LMG20568
FN430567.1 Cupriavidus pampae(T)CPDB6 CCUG55948
D87999.1 Cupriavidus metallidurans(T)DSM2839
AF085226.1 Cupriavidus pauculus(T)LMG3413
U91838.1 Burkholderia thailandensis(T)E264
AY773189.1 Burkholderia nodosa(T)Br3437
AY752958.1 Burkholderia mimosarum(T)PAS44
HM357233.1 Burkholderia symbiotica(T)NKMU-JPY345
Y17009.1 Burkholderia caribensis(T)MWAP64
AJ302312.1 Burkholderia phymatum(T)STM815
AB201285.1 Burkholderia terrae(T)KMY02
AJ302311.1 Burkholderia tuberum(T)STM678
AY435213.1 Burkholderia phenoliruptrix(T)AC1100
U86373.1 Burkholderia xenovorans(T)LB400
AY497470.1 Burkholderia phytofirmans(T)PsJN
Y10146.1 Herbaspirillum seropedicae(T)DSM6445 ATCC35892
0.02
C. necator group
C. tai
C. pinatubonensis group
0.002
Zoom of clade I for more detail
Rufo Cold et al. in prep.

ML recA

Uruguayan *Mimosa* spp. are nodulated by novel *Cupriavidus* symbionts close to *C. necator* and *C. pinatubonensis*



Are the heavy metals in the soils (mining areas) a facto

C. necator

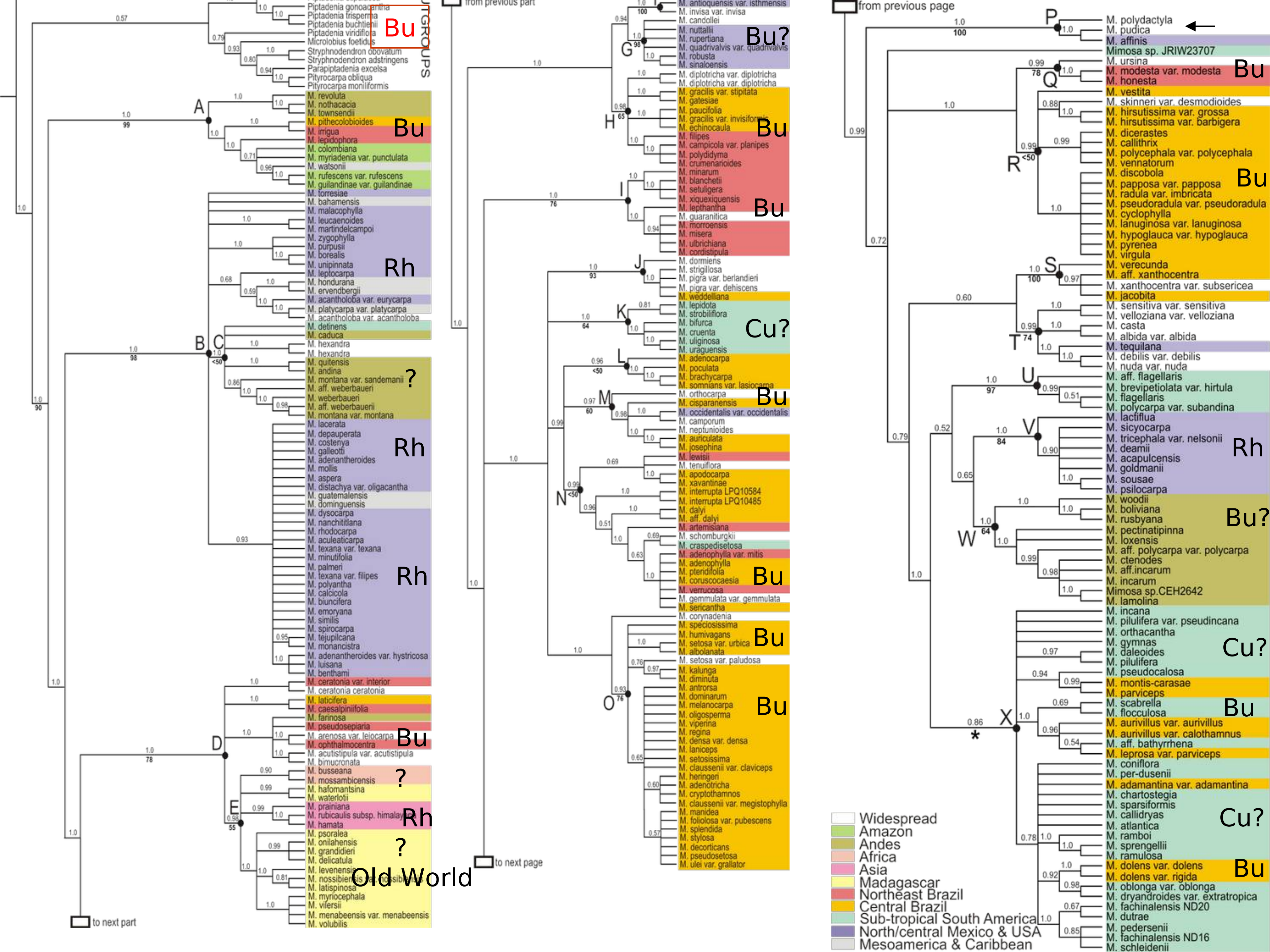
C. pinatubonensis

Rufo Cold et al. in prep.

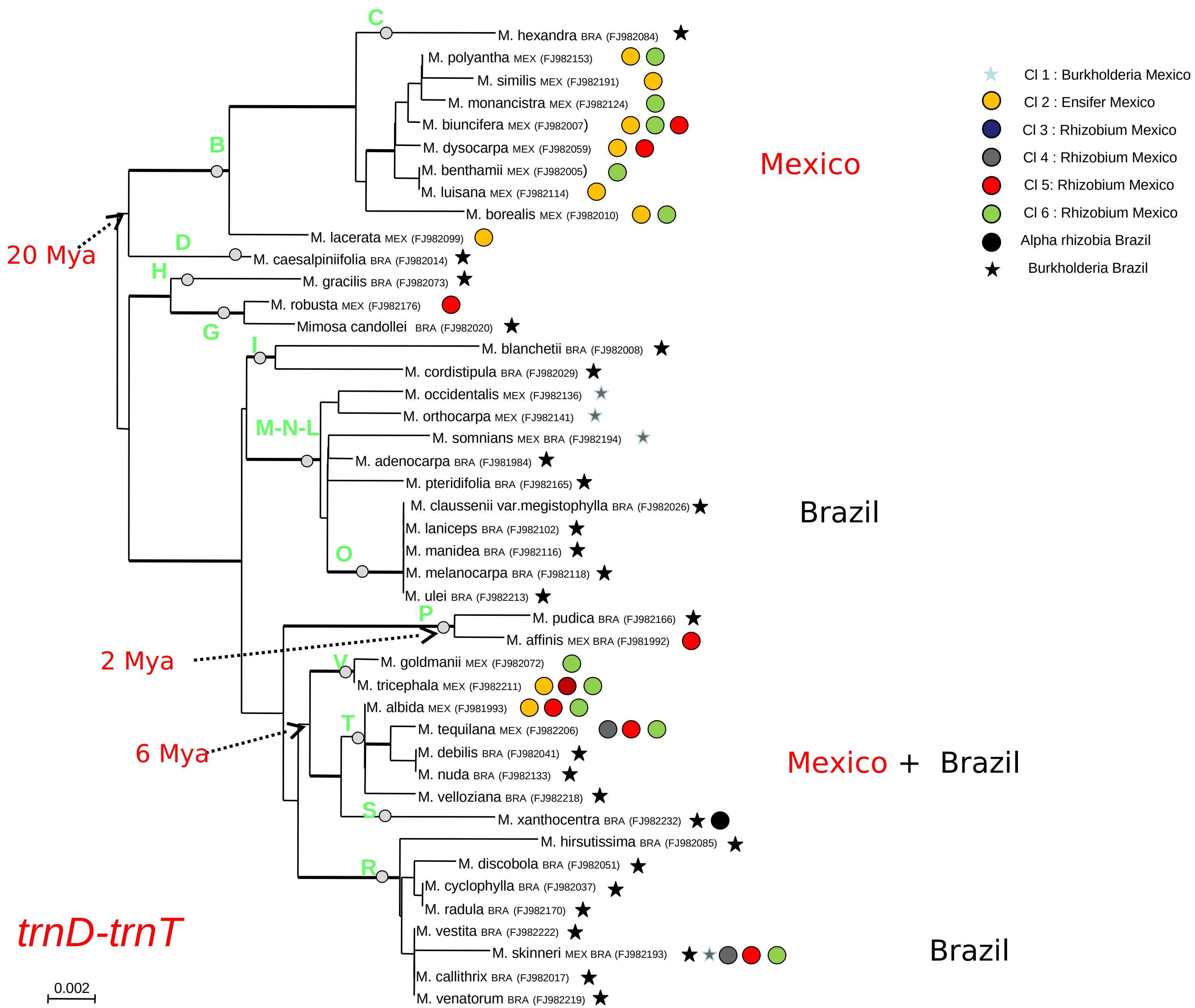
Summary of endemic *Mimosa* symbionts by biome

- Cerrado (Brazil) = *Burkholderia*
- Caatinga (Brazil) = *Burkholderia*
- Mata Atlantica (Brazil) = *Burkholderia*
- Central highlands (Mexico) = *Rhizobium/Sinorhizobium*
- Uruguay (heavy metal soils) = *Cupriavidus*
- Madagascar/E. Africa = ?
- India = *Sinorhizobium*

- Does *Mimosa* phylogeny/taxonomy have a role in this distribution or is it soil/environment that drives it?



Piptadenia gonoacantha
Piptadenia trisperma
Piptadenia buchtienii
Piptadenia viridiflora
Microlobius foetidus
Stryphnodendron obovatum
Stryphnodendron adstringens
Parapiptadenia excelsa
Pityrocarpa obliqua
Pityrocarpa moniliformis
OUTGROUPS
Bu
A
M. revoluta
M. nothacacia
M. townsendii
M. pithecolobioides
M. irrigua
M. lepidophora
M. colombiana
M. myriadenia var. punctulata
M. watsonii
M. rufescens var. rufescens
M. guilandinae var. guilandinae
Bu
M. torresiae
M. bahamensis
M. malacophylla
M. leucaenoides
M. martindelcampoi
M. zygophylla
M. purpusii
M. borealis
M. unipinnata
M. leptocarpa
M. hondurana
M. ervendbergii
M. acantholoba var. eurycarpa
M. platycarpa var. platycarpa
M. acantholoba var. acantholoba
Rh
B
C
M. detinens
M. caduca
M. hexandra
M. hexandra
M. quitensis
M. andina
M. montana var. sandemanii
M. aff. weberbaueri
M. weberbaueri
M. aff. weberbaueri
M. montana var. montana
?
M. lacerata
M. depauperata
M. costenya
M. galeottii
M. adenantheroides
M. mollis
M. aspera
M. distachya var. oligacantha
M. guatemalensis
M. dominguensis
M. dysocarpa
M. nanchititlana
M. rhodocarpa
M. aculeaticarpa
M. texana var. texana
M. minutifolia
M. palmeri
M. texana var. filipes
M. polyantha
M. calcicola
M. biuncifera
M. emoryana
M. similis
M. spirocarpa
M. tejupilcana
M. monancistra
M. adenantheroides var. hystricosa
M. luisana
M. benthami
Rh
Rh
D
M. ceratonia var. interior
M. ceratonia ceratonia
M. laticifera
M. caesalpiniifolia
M. farinosa
M. pseudosepiaria
M. arenosa var. leiocarpa
M. ophthalmocentra
M. acutistipula var. acutistipula
M. bimucronata
Bu
E
M. busseana
M. mossambicensis
M. hafomantsina
M. waterlotii
M. prainiana
M. rubicaulis subsp. himalayana
M. hamata
M. psoralea
M. onilahensis
M. grandidieri
M. delicatula
M. levenensis
M. nossibiensis var. nossibiensis
M. latispinosa
M. myriocephala
M. viersii
M. menabeensis var. menabeensis
M. volubilis
?
Rh
?
Old World
to next part
from previous part
F
G
M. antioquensis var. isthmensis
M. invisa var. invisa
M. candollei
M. nuttallii
M. rupertiana
M. quadrivalvis var. quadrivalvis
M. robusta
M. sinaloensis
Bu?
H
M. diplotricha var. diplotricha
M. diplotricha var. diplotricha
M. gracilis var. stipitata
M. gatesiae
M. paucifolia
M. gracilis var. invisiformis
M. echinocaula
M. filipes
M. campicola var. planipes
M. polydidyma
M. crumenarioides
M. minarum
M. blanchetii
M. setuligera
M. xiquexiquensis
M. lepthantha
Bu
I
M. guaranitica
M. morroensis
M. misera
M. ulbrichiana
M. cordistipula
Bu
J
M. dormiens
M. strigillosa
M. pigra var. berlandieri
M. pigra var. dehiscens
M. weddelliana
K
M. lepidota
M. strobiliflora
M. bifurca
M. cruenta
M. uliginosa
M. uraguensis
Cu?
L
M. adenocarpa
M. poculata
M. brachycarpa
M. somnians var. lasiocarpa
M
M. orthocarpa
M. cisparanensis
M. occidentalis var. occidentalis
M. camporum
M. neptunioides
M. auriculata
M. josephina
M. lewisii
Bu
N
M. tenuiflora
M. apodocarpa
M. xavantinae
M. interrupta LPQ10584
M. interrupta LPQ10485
M. dalyi
M. aff. dalyi
M. artemisiana
M. schomburgkii
M. craspedisetosa
M. adenophylla var. mitis
M. adenophylla
M. pteridifolia
M. coruscocaesia
M. verrucosa
M. gemmulata var. gemmulata
M. sericantha
Bu
M. corynadenia
M. speciosissima
M. humivagans
M. setosa var. urbica
M. albolanata
M. setosa var. paludosa
Bu
O
M. kalunga
M. diminuta
M. antrorsa
M. dominarum
M. melanocarpa
M. oligosperma
M. viperina
M. regina
M. densa var. densa
M. laniceps
M. setosissima
M. claussenii var. claviceps
M. heringeri
M. adenotricha
M. cryptothamnos
M. claussenii var. megistophylla
M. manidea
M. foliolosa var. pubescens
M. splendida
M. stylosa
M. decorticans
M. pseudosetosa
M. ulei var. grallator
Bu
to next page
from previous page
P
M. polydactyla
M. pudica
M. affinis
Mimosa sp. JRIW23707
Q
M. ursina
M. modesta var. modesta
M. honesta
M. vestita
Bu
R
M. skinneri var. desmodioides
M. hirsutissima var. grossa
M. hirsutissima var. barbigera
M. dicerastes
M. callithrix
M. polycephala var. polycephala
M. vennatorum
M. discobola
M. papposa var. papposa
M. radula var. imbricata
M. pseudoradula var. pseudoradula
M. cyclophylla
M. lanuginosa var. lanuginosa
M. hypoglauca var. hypoglauca
M. pyrenea
M. virgula
M. verecunda
M. aff. xanthocentra
Bu
S
M. xanthocentra var. subsericea
M. jacobita
T
M. sensitiva var. sensitiva
M. velloziana var. velloziana
M. casta
M. albida var. albida
M. tequilana
M. debilis var. debilis
M. nuda var. nuda
U
M. aff. flagellaris
M. brevipetiolata var. hirtula
M. flagellaris
M. polycarpa var. subandina
V
M. lactiflua
M. sicyocarpa
M. tricephala var. nelsonii
M. deamii
M. acapulcensis
M. goldmanii
M. sousae
M. psilocarpa
Rh
W
M. woodii
M. boliviana
M. rusbyana
M. pectinatipinna
M. loxensis
M. aff. polycarpa var. polycarpa
M. ctenodes
M. aff.incarum
M. incarum
Mimosa sp.CEH2642
M. lamolina
Bu?
X
M. incana
M. pilulifera var. pseudincana
M. orthacantha
M. gymnas
M. daleoides
M. pilulifera
M. pseudocalosa
M. montis-carasae
M. parviceps
M. scabrella
M. flocculosa
M. aurivillus var. aurivillus
M. aurivillus var. calothamnus
M. aff. bathyrrhena
M. leprosa var. parviceps
Cu?
Bu
M. coniflora
M. per-dusenii
M. adamantina var. adamantina
M. chartostegia
M. sparsiformis
M. callidryas
M. atlantica
M. ramboi
M. sprengellii
M. ramulosa
M. dolens var. dolens
M. dolens var. rigida
M. oblonga var. oblonga
M. dryandroides var. extratropica
M. fachinalensis ND20
M. dutrae
M. pedersenii
M. fachinalensis ND16
M. schleidenii
Cu?
Bu
Widespread
Amazon
Andes
Africa
Asia
Madagascar
Northeast Brazil
Central Brazil
Sub-tropical South America
North/central Mexico & USA
Mesoamerica & Caribbean



C
M. hexandra BRA (FJ982084)
M. polyantha MEX (FJ982153)
M. similis MEX (FJ982191)
M. monancistra MEX (FJ982124)
M. biuncifera MEX (FJ982007)
M. dysocarpa MEX (FJ982059)
M. benthamii MEX (FJ982005)
M. luisana MEX (FJ982114)
M. borealis MEX (FJ982010)
B
M. lacerata MEX (FJ982099)
D
M. caesalpiniifolia BRA (FJ982014)
20 Mya
H
M. gracilis BRA (FJ982073)
M. robusta MEX (FJ982176)
G
Mimosa candollei BRA (FJ982020)
I
M. blanchetii BRA (FJ982008)
M. cordistipula BRA (FJ982029)
M. occidentalis MEX (FJ982136)
M. orthocarpa MEX (FJ982141)
M-N-L
M. somnians MEX BRA (FJ982194)
M. adenocarpa BRA (FJ981984)
M. pteridifolia BRA (FJ982165)
M. claussenii var.megistophylla BRA (FJ982026)
M. laniceps BRA (FJ982102)
M. manidea BRA (FJ982116)
O
M. melanocarpa BRA (FJ982118)
M. ulei BRA (FJ982213)
P
M. pudica BRA (FJ982166)
2 Mya
M. affinis MEX BRA (FJ981992)
V
M. goldmanii MEX (FJ982072)
M. tricephala MEX (FJ982211)
M. albida MEX (FJ981993)
T
M. tequilana MEX (FJ982206)
6 Mya
M. debilis BRA (FJ982041)
M. nuda BRA (FJ982133)
M. velloziana BRA (FJ982218)
S
M. xanthocentra BRA (FJ982232)
M. hirsutissima BRA (FJ982085)
R
M. discobola BRA (FJ982051)
M. cyclophylla BRA (FJ982037)
M. radula BRA (FJ982170)
M. vestita BRA (FJ982222)
M. skinneri MEX BRA (FJ982193)
M. callithrix BRA (FJ982017)
M. venatorum BRA (FJ982219)
Mexico
Brazil
Mexico + Brazil
Brazil
Cl 1 : Burkholderia Mexico
Cl 2 : Ensifer Mexico
Cl 3 : Rhizobium Mexico
Cl 4 : Rhizobium Mexico
Cl 5: Rhizobium Mexico
Cl 6 : Rhizobium Mexico
Alpha rhizobia Brazil
Burkholderia Brazil
trnD-trnT
0.002

Nodulation of *Mimosa*: conclusions

- *Mimosa* has a propensity for endemism (particularly at high altitudes), and this has resulted in large clades that have strict preferences for particular symbiont genotypes: *Burkholderia* in the case of Brazil and *Rhizobium*/*Ensifer* in the case of Mexico.
- **Soil characteristics, particularly pH, appear to be the main determining factors in the selection of symbionts.**
- Geographical separation has resulted in these older clades no longer being able to nodulate with other symbiont types.
- BUT, other (much younger) clades are “split” between Brazil and Mexico; these have retained their ancestral ability to nodulate with *Burkholderia*.
- The “switch” from a basal *Burkholderia* preference to the “local” microsymbionts can occur remarkably quickly (<2 my).
- **CONCLUSION 1: Beta-rhizobia are very widespread.**
- **CONCLUSION 2: *Mimosa* is very plastic in terms of adapting to nodulate with a wide range of symbionts, and this has assisted it’s spread into diverse environments.**

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