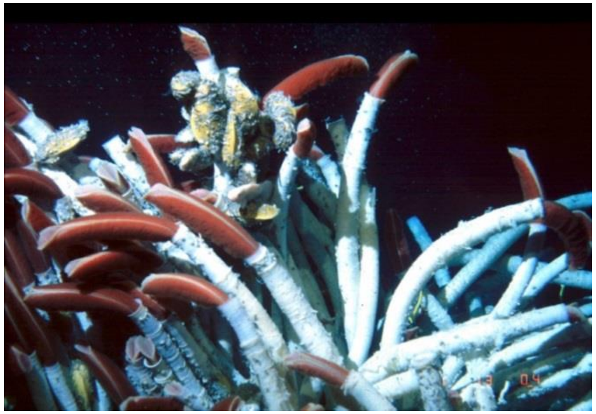
***Buchnera*' s flatmates** : repeated replacement of nutritional symbionts in aphids revealed by a phylogenomic approach

GENOSCOPE
Centre National de Séquençage

FRANCE GÉNOMIQUE

Symbioses with bacteria have favored the colonization of nutrient poor environments



Deep sea tube worms use bacteria for chemosynthesis

AA Nikoh, Hosokawa, Moriyama, Oshima, Hattori & Fukatsu. 2014.

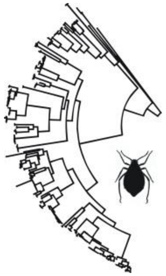
Blood-feeding arthropods host bacteria that synthesize Vitamins

Sap-feeding insects host bacteria that synthetize AAs and vitamins

Mutualistic bacteria synthesize nutrients that are missing in their host diet

Bacteria that live within their host = endosymbionts

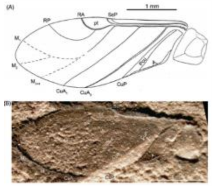
Aphids (Hemiptera: Aphididae)

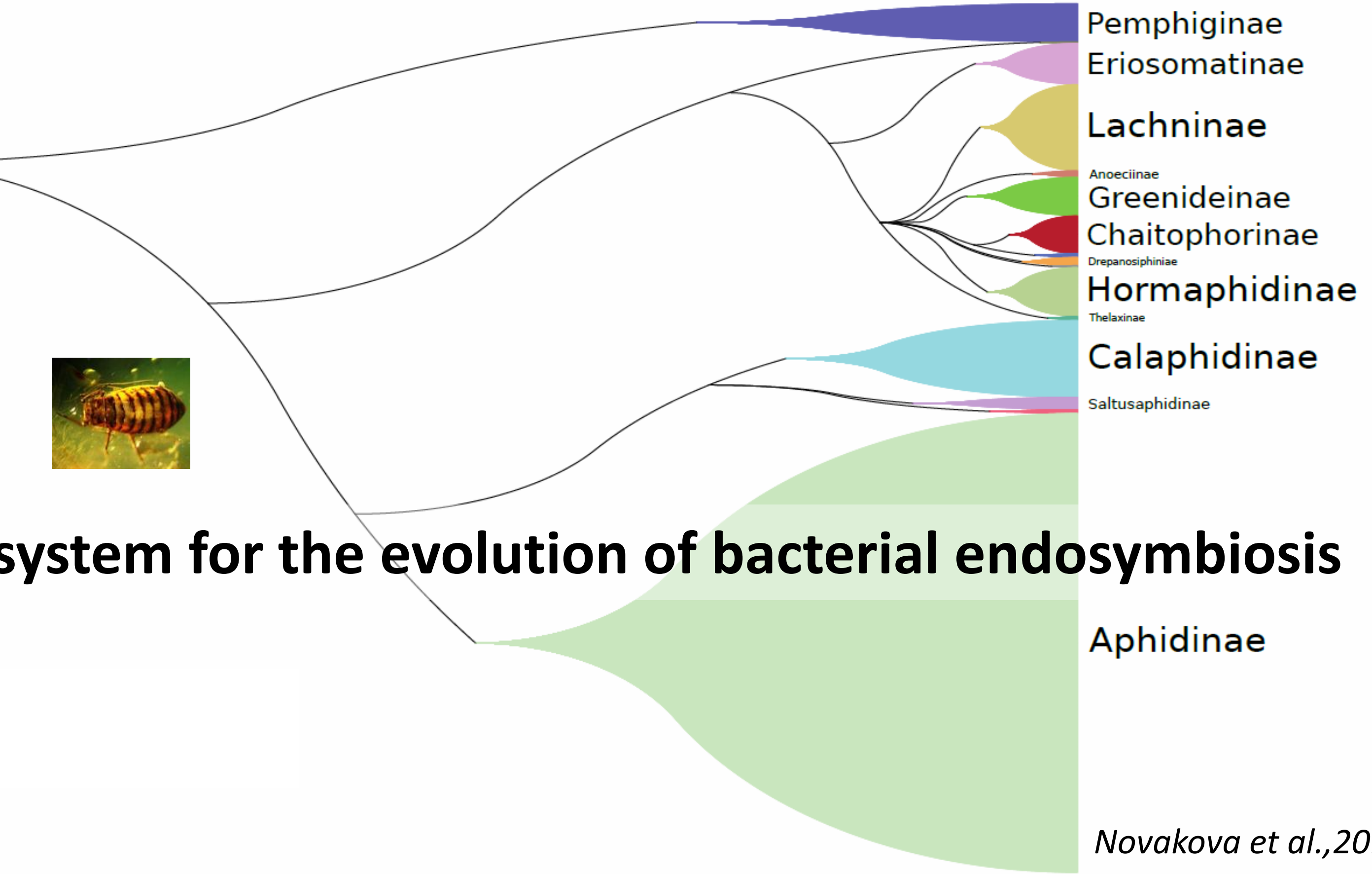


Major pests!

But also

200-150 M yr

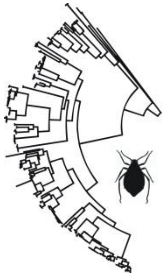




Study system for the evolution of bacterial endosymbiosis

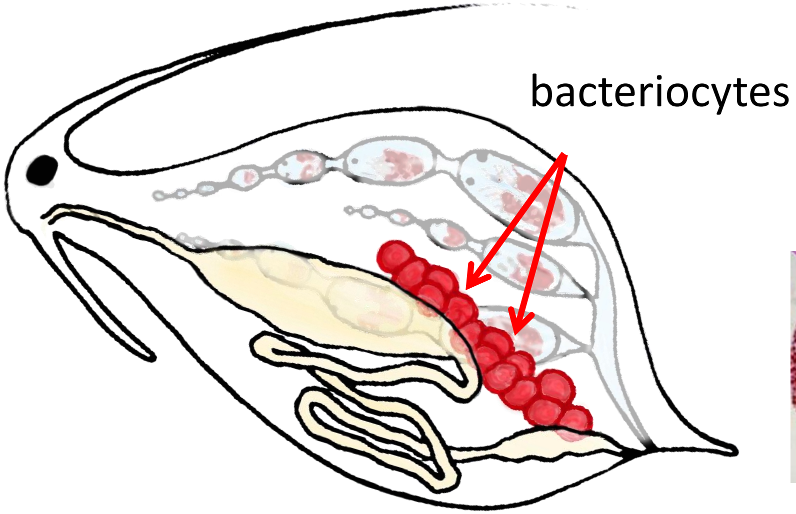
Novakova et al.,2013

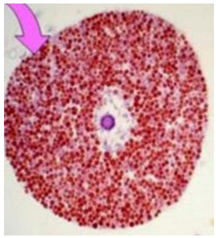
Bacterial endosymbiosis in aphids



Aphids primary symbiont: *Buchnera aphidicola (Buchner 1965)*

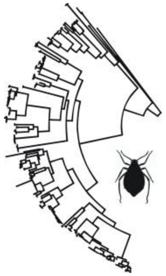
Synthetizes AA and vitamins for its host (i.e. nutrients lacking in the phloem)





About 80-100 bacteriocytes/individual in the pea aphid adult stage: 1.2×10^7 bacteria (Simonet *et al.* 2016)

Bacterial endosymbiosis in aphids



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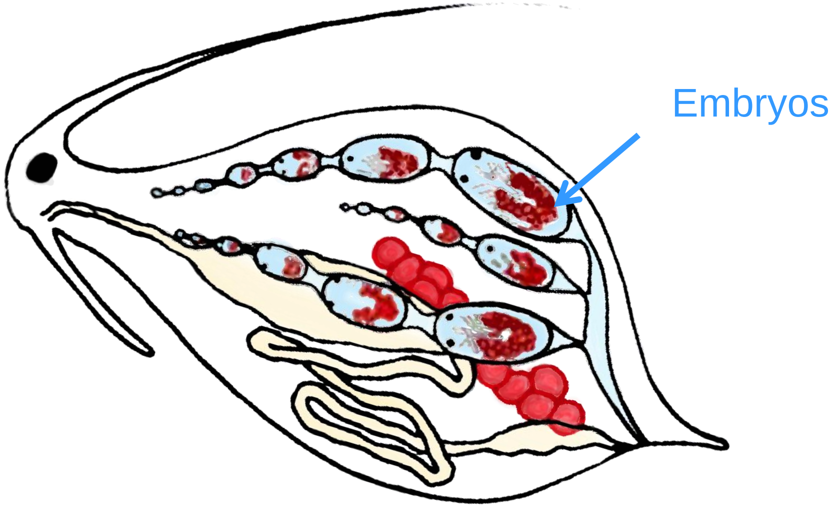
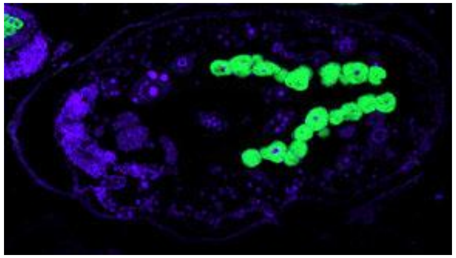
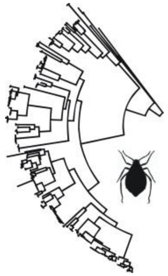
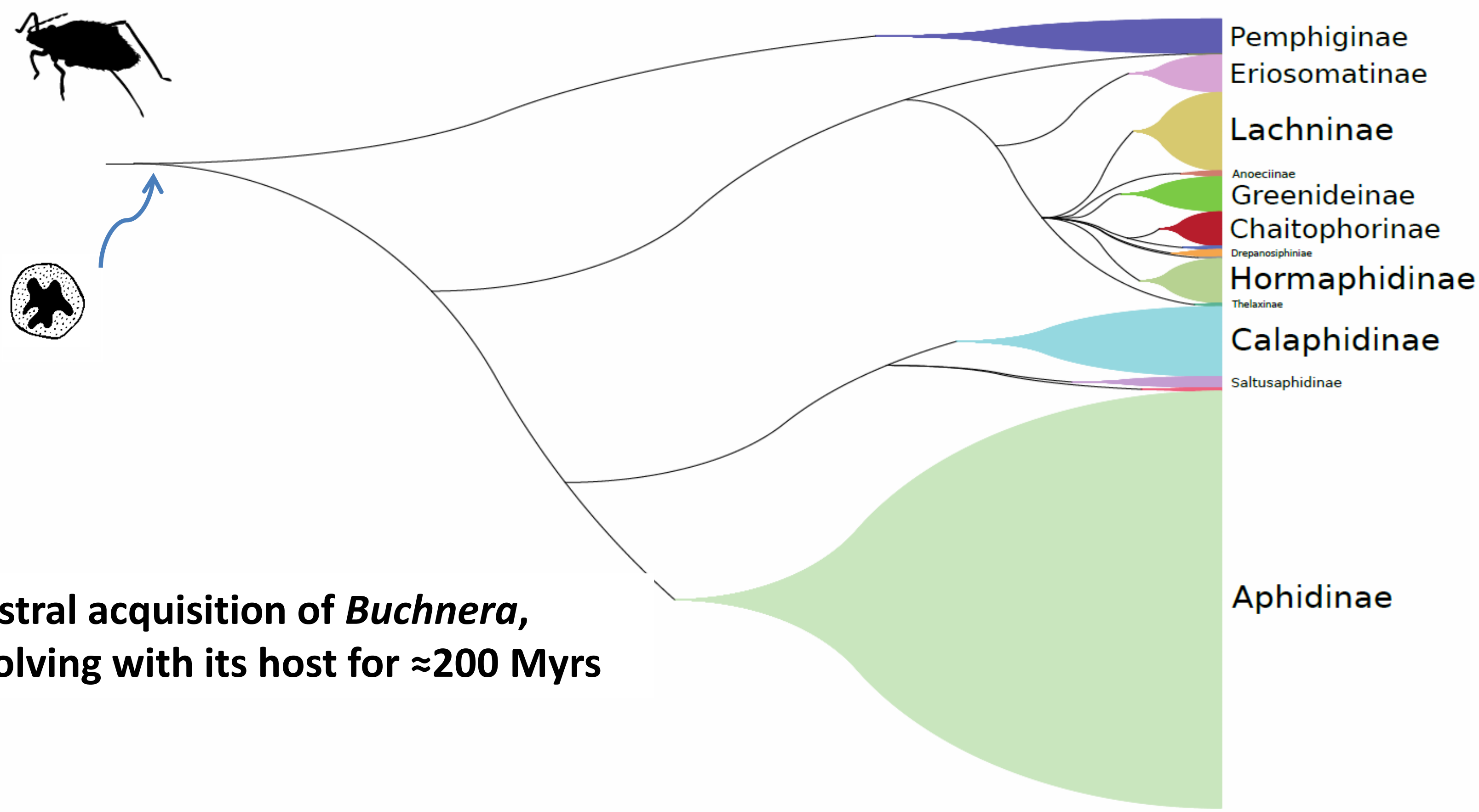




Vertical transmission of *Buchnera*: a few bacteria are transmitted from the maternal bacteriocytes to the forming embryos

Bacterial endosymbiosis in aphids





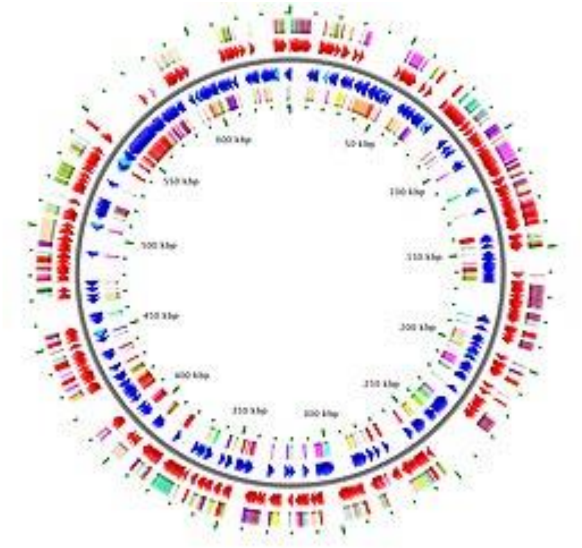
Ancestral acquisition of *Buchnera*, coevolving with its host for ≈200 Myrs

Evidence for cospeciation (*i.e. parallell phylogenies*) with aphids at several taxonomic levels

Bacterial endosymbiosis in aphids

Buchnera **genomes are SMALL**

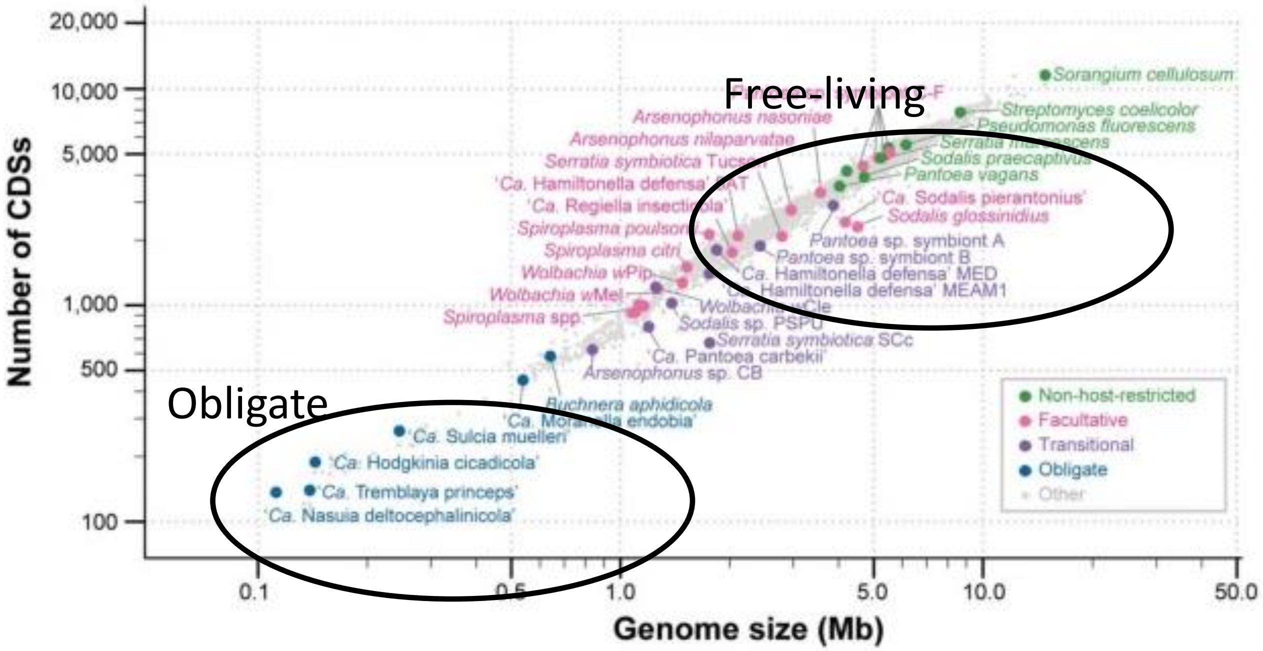
The first bacterial endosymbiont genome to be sequenced , the pea aphid's: 0,6Mb (*Shigenobu et al. 2000*) (small in comparison with free living bacteria)



Have retained genes that serve the hosts (synthesis of AA, vitamins), deletion of genes involved in cell enveloppe biogenesis, transporters etc…

Bacterial endosymbiosis in aphids

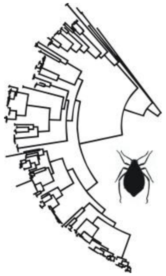
Endosymbiotic bacteria have a very small genomesin comparison with free-living relatives



Lo *et al.* (2016). *FEMS Microbiol Rev* **40**(6):855-874

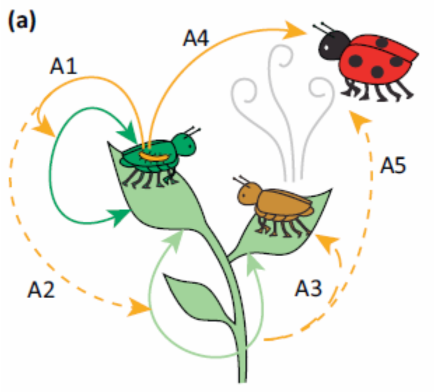
<u>Genomic erosion caused by </u>: **loss of genes useless to intracellular life-style , strong genetic drift due to recurrent bottlenecks (fixation of deleterious mutations, pseudogeneization, deletion)**

Bacterial endosymbiosis in aphids



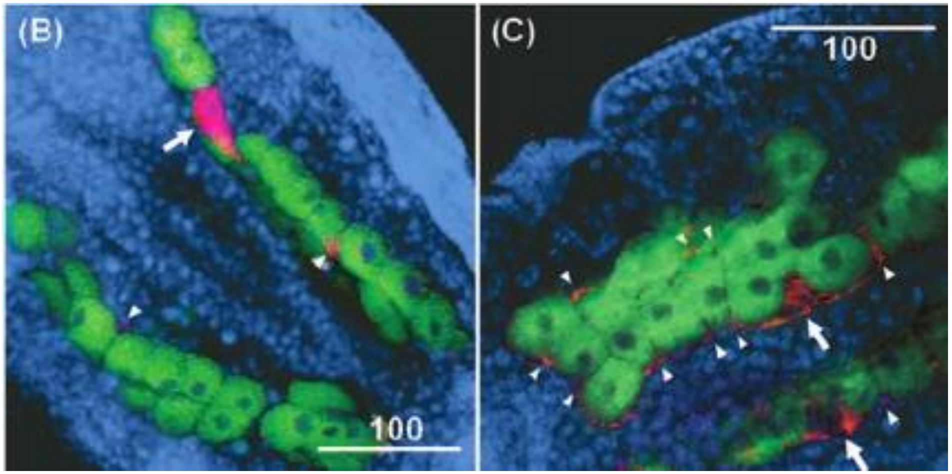
Facultative symbionts:

Serratia, Hamiltonella, Regiella, Rickettsia, Wolbachia, Arsenophonus…..



- Not fixed in species
- Advantageous in certain environnemental conditions: mediate adaptations to biotic and abiotic factors

Oliver *et al.* 2010 Ann. Rev. Ent
Frago *et al. 2012,TREE,* Frago *et al.* 2017, Nat. Com

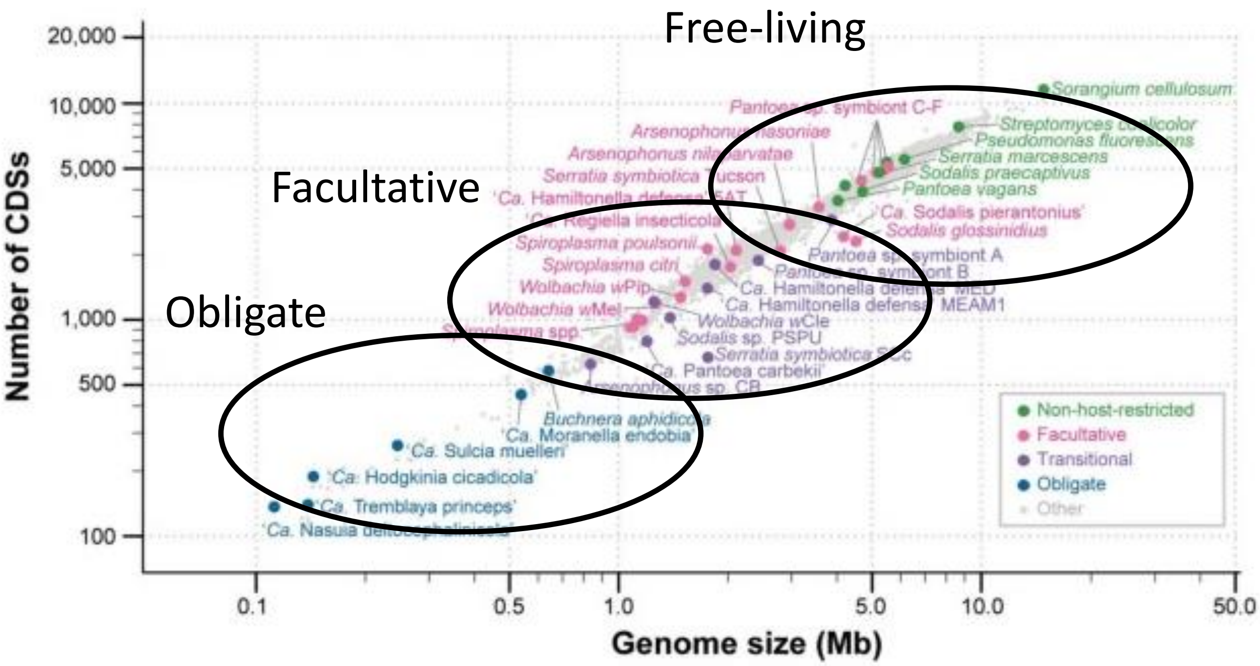


Various localizations, sometimes in their own bacteriocytes.

Usually vertically transmitted but horizontal transfers possible

Bacterial endosymbiosis in aphids

Facultative bacteria



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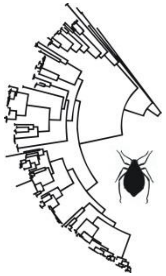
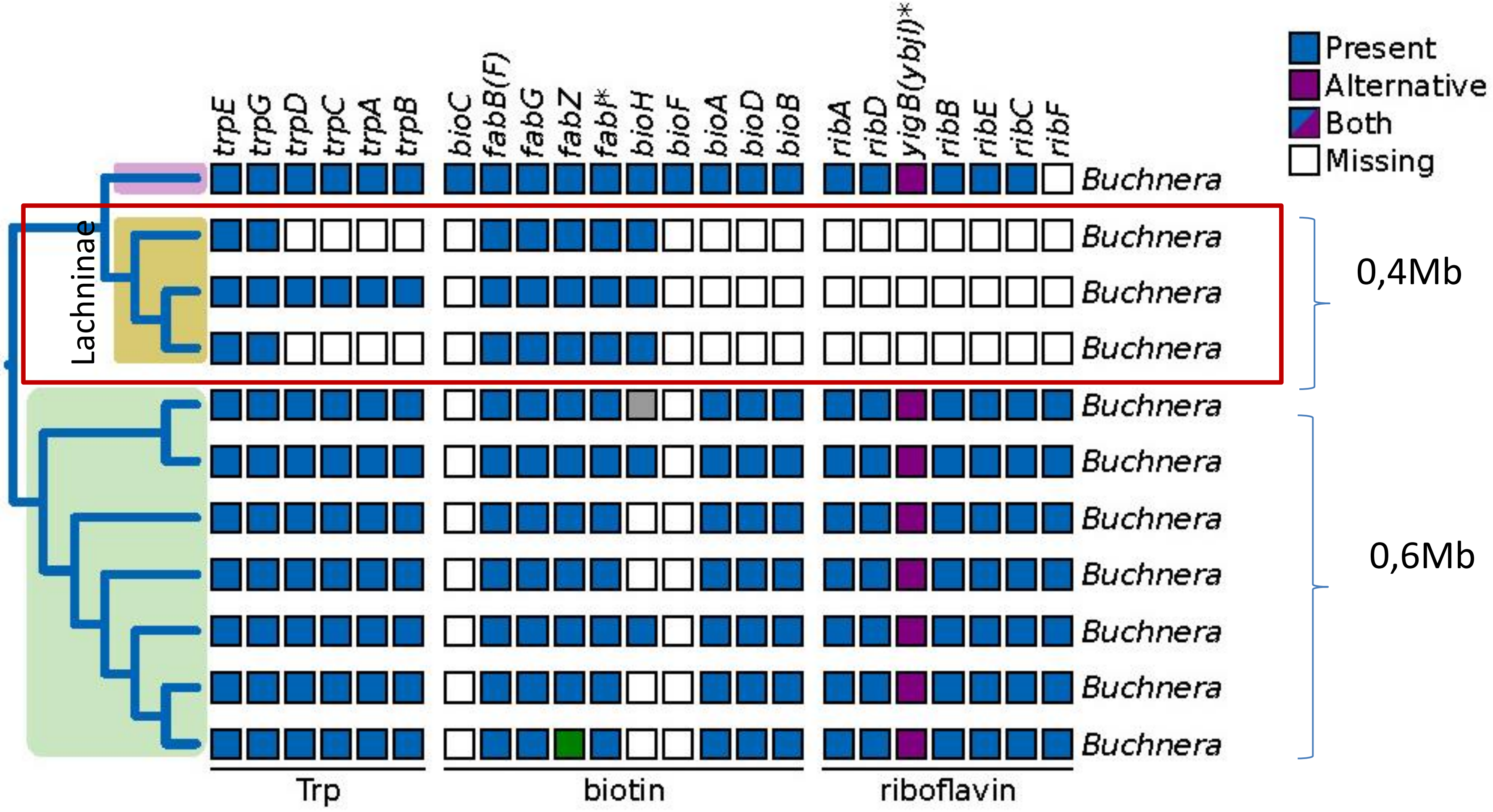
Bacterial endosymbiosis in aphids



In *Lachninae* , *Buchnera is* only 0,4Mb and has lost some key metabolic functions

Who does it if not *Buchnera*?

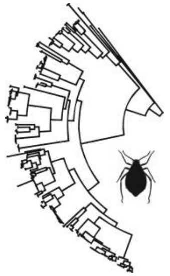


🟦 Present
🟪 Alternative
Both
☐ Missing

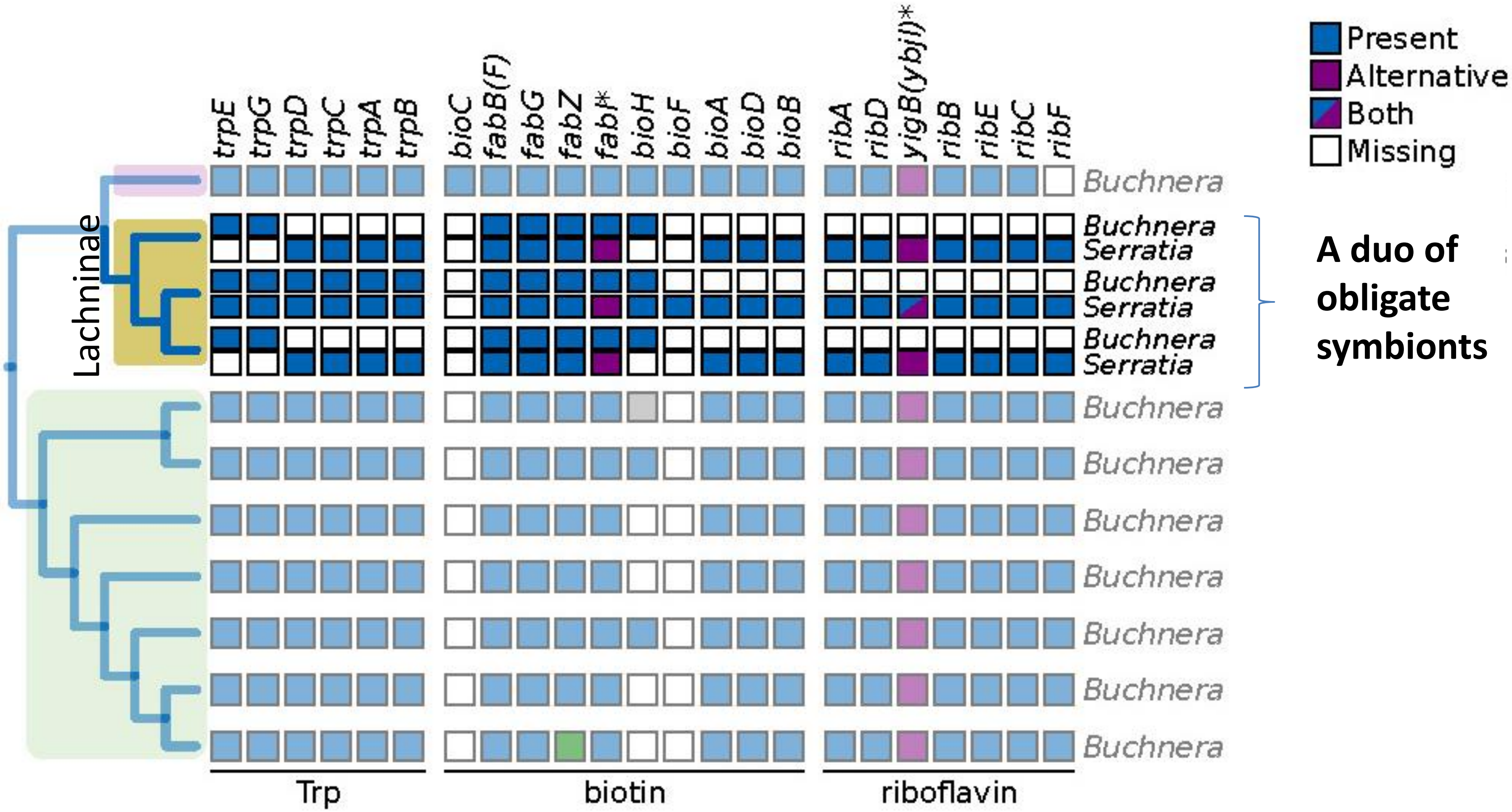
	trpE	*trpG*	*trpD*	*trpC*	*trpA*	*trpB*	*bioC*	*fabB(F)*	*fabG*	*fabZ*	*fabI**	*bioH*	*bioF*	*bioA*	*bioD*	*bioB*	*ribA*	*ribD*	*yigB(ybjI)**	*ribB*	*ribE*	*ribC*	*ribF*
Buchnera	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟪	🟦	🟦	🟦	☐
Buchnera	🟦	🟦	☐	☐	☐	☐	☐	🟦	🟦	🟦	🟦	🟦	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Buchnera	🟦	🟦	🟦	🟦	🟦	🟦	☐	🟦	🟦	🟦	🟦	🟦	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Buchnera	🟦	🟦	☐	☐	☐	☐	☐	🟦	🟦	🟦	🟦	🟦	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Buchnera	🟦	🟦	🟦	🟦	🟦	🟦	☐	🟦	🟦	🟦	🟦	▨	☐	🟦	🟦	🟦	🟦	🟦	🟪	🟦	🟦	🟦	🟦
Buchnera	🟦	🟦	🟦	🟦	🟦	🟦	☐	🟦	🟦	🟦	🟦	🟦	☐	🟦	🟦	🟦	🟦	🟦	🟪	🟦	🟦	🟦	🟦
Buchnera	🟦	🟦	🟦	🟦	🟦	🟦	☐	🟦	🟦	🟦	🟦	☐	☐	🟦	🟦	🟦	🟦	🟦	🟪	🟦	🟦	🟦	🟦
Buchnera	🟦	🟦	🟦	🟦	🟦	🟦	☐	🟦	🟦	🟦	🟦	☐	☐	🟦	🟦	🟦	🟦	🟦	🟪	🟦	🟦	🟦	🟦
Buchnera	🟦	🟦	🟦	🟦	🟦	🟦	☐	🟦	🟦	🟦	🟦	🟦	☐	🟦	🟦	🟦	🟦	🟦	🟪	🟦	🟦	🟦	🟦
Buchnera	🟦	🟦	🟦	🟦	🟦	🟦	☐	🟦	🟦	🟦	🟦	☐	☐	🟦	🟦	🟦	🟦	🟦	🟪	🟦	🟦	🟦	🟦
Buchnera	🟦	🟦	🟦	🟦	🟦	🟦	☐	🟦	🟦	🟩	🟦	☐	☐	🟦	🟦	🟦	🟦	🟦	🟪	🟦	🟦	🟦	🟦

(Perez brocal et al, 2006, Lamelas et al. 2011, Manzano-Marin et al. 2014, 2016)

Bacterial endosymbiosis in aphids



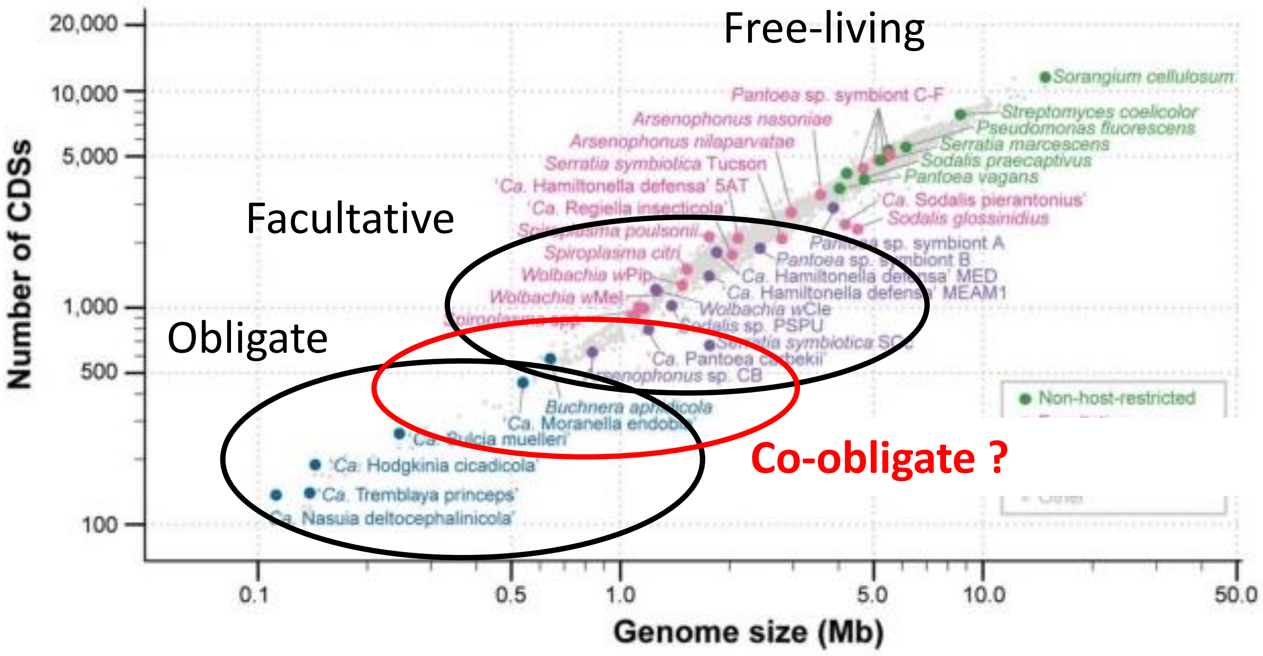
A previously facultative symbiont, ***Serratia symbiotica***, is complementing *Buchnera*



A duo of obligate symbionts

(*Perez brocal et al, 2006, Lamelas et al. 2011, Manzano-Marin et al. 2012, 2016*)

Bacterial endosymbiosis in aphids



Free-living

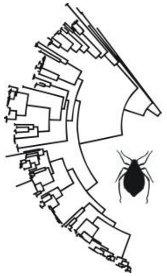
Facultative

Obligate

Co-obligate ?

Lo *et al.* (2016). *FEMS Microbiol Rev* **40**(6):855-874

Bacterial endosymbiosis in Lachninae



Questions:

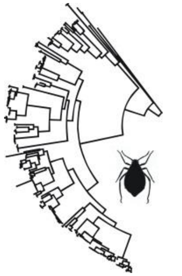
-History of the associations between *Lachninae* and their new co-obligate symbionts: who, when??

-Role of bacterial associations in aphids evolutionary transitions: do new symbionts carry new metabolic functions associated with aphids ecological niches?

-**Rates and patterns of molecular evolution in bacterial endosymbionts:**
Horizontal gene transfer between symbionts?
Pace of genome “decay” in symbionts ?

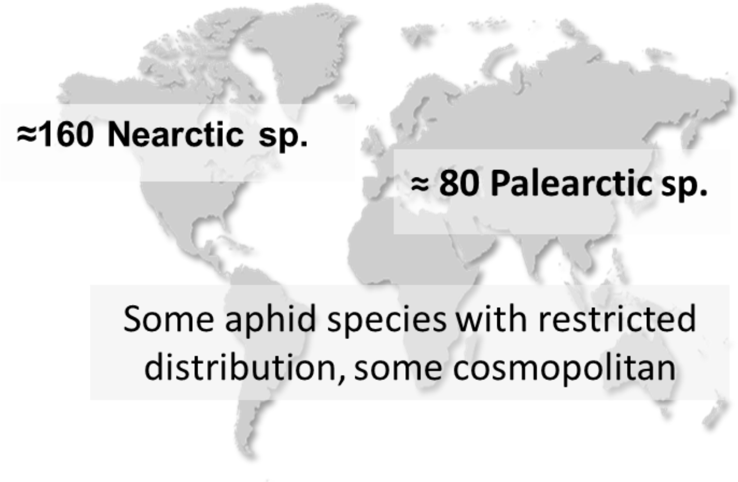
What evolutionary processes drive the evolution of nutritional bacterial symbionts and determine their succession ?

Bacterial endosymbiosis in Lachninae



≈240 spp. of *Cinara*

Have diversified 40-50mya

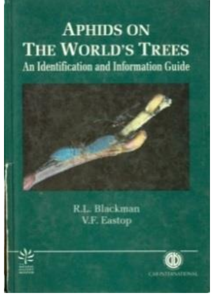


≈160 Nearctic sp.

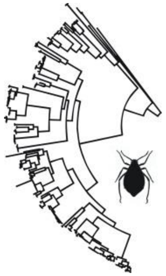
≈ 80 Palearctic sp.

Some aphid species with restricted distribution, some cosmopolitan

Evolution of endosymbiosis throughout the ecological diversification of *Cinara*?


APHIDS ON
THE WORLD'S TREES
An Identification and Information Guide
R.L. Blackman
V.F. Eastop

Building a robust & comprehensive phylogenetic framework for *Cinara*



≈ 400 specimens, ≈ 100 morphological species

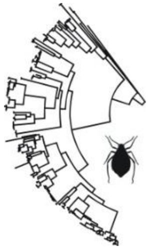
Aphid phylogenetic reconstruction

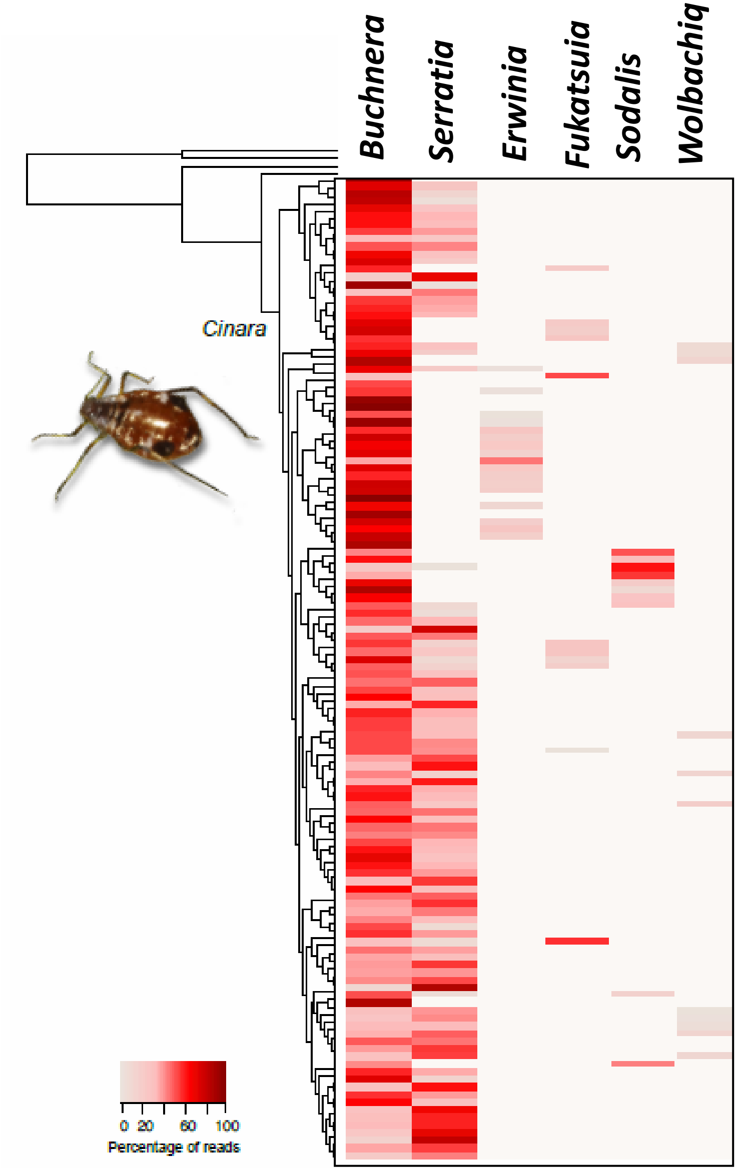
-5500bp (mitoch DNA, nuclear DNA, *Buchner*a DNA)

Endosymbiont characterisation

16S rRNA gene (V4) (MiSeq, 2*251bp**)**

Endosymbiont diversity in *Cinara*





16S rRNA gene deep sequencing (Illumina MiSeq, 2*251bp)

Heatmap showing endosymbionts that are « fixed » within species (i.e. *present in all specimens of a species*)

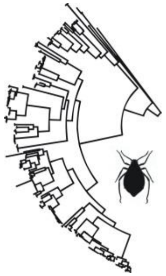
420 specimens / about 100 *Cinara* species

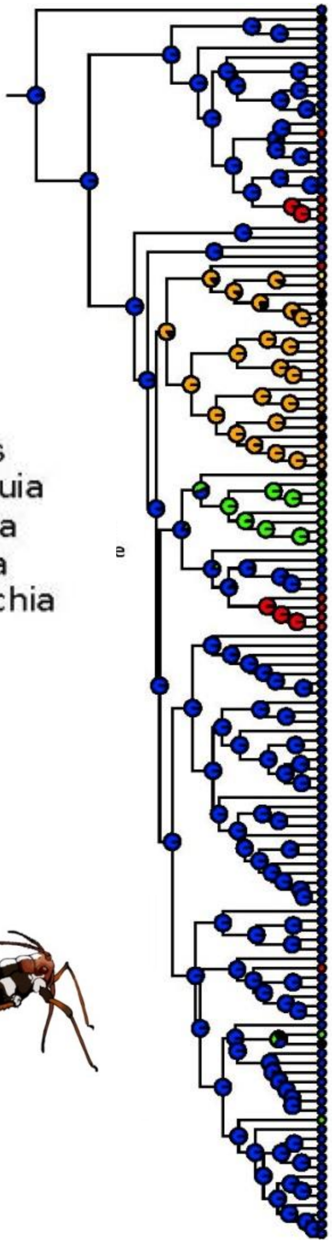
***Buchnera* has a flatmate:**

***Serratia, Erwinia , Fukatsuia* or *Sodalis* sometimes two. ..**

Meseguer et al. 2017. Mol Ecol

Endosymbiont diversity in *Cinara*





-Several fixed , probably « co-obligate », symbiont lineages detected throughout the phylogeny
-Strong phylogenetic signal in associations

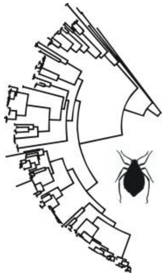
There was probably an association with *Serratia symbiotica* in the common ancestor of *Cinara*

When *Serratia* is « lost », it is replaced by another symbiont (belonging to diverse bacterial lineages)

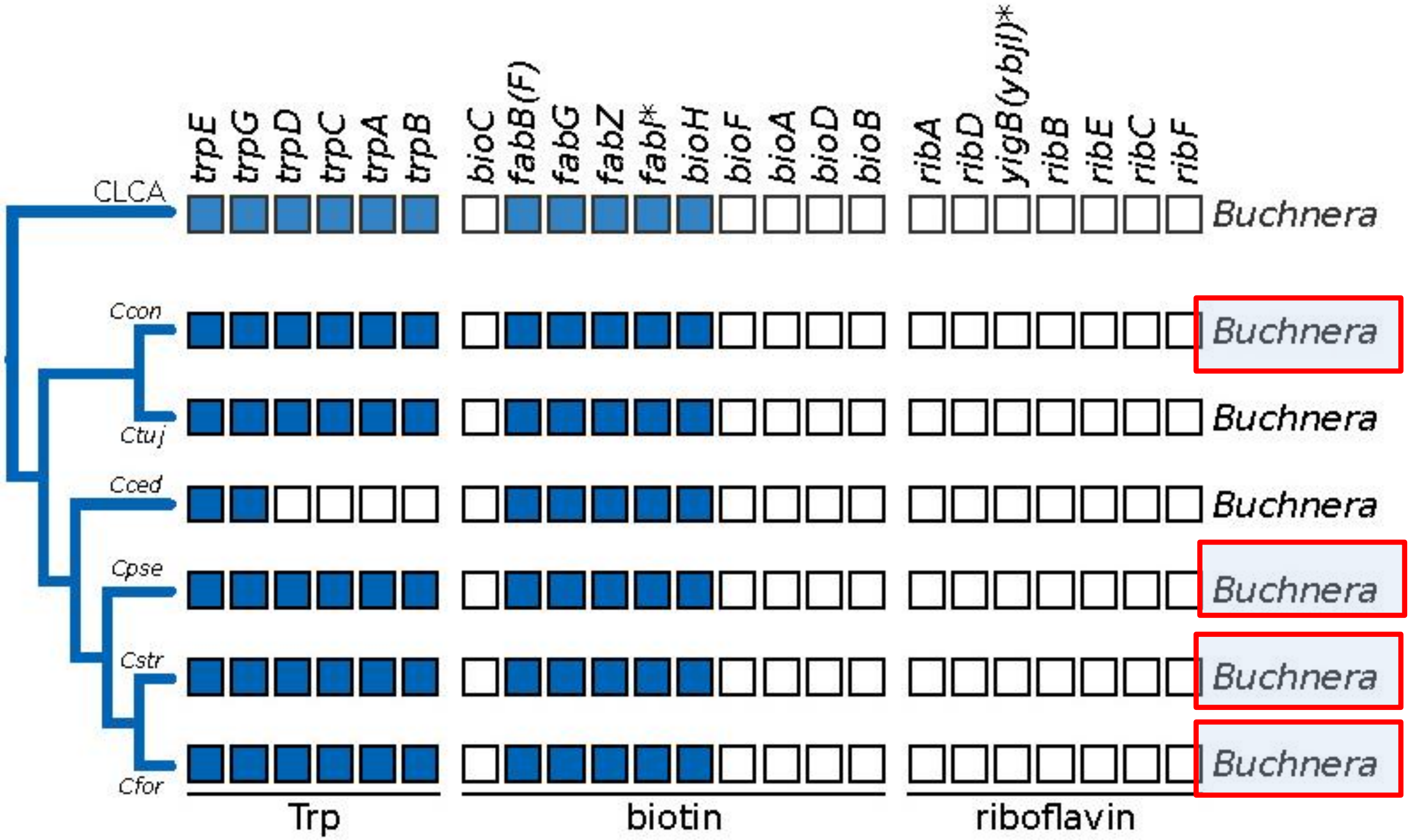
Metabolic complementarity of *Buchnera* and these new endosymbionts?
Metagenome of the endosymbiotic community of about 70 aphid species (sequencing the DNA of filtrates excluding eukaryotic cells)

Meseguer et al. 2017. Mol Ecol

Nutritional complementation of co-symbionts in *Cinara*



Assembly and full annotation of 4 new *Buchnera* in *Cinara*:
losses of the same key functions in all *Buchnera*

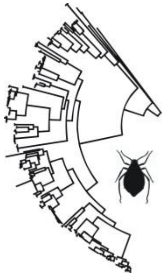


	trpE	*trpG*	*trpD*	*trpC*	*trpA*	*trpB*	*bioC*	*fabB(F)*	*fabG*	*fabZ*	*fabI**	*bioH*	*bioF*	*bioA*	*bioD*	*bioB*	*ribA*	*ribD*	*yigB(ybjI)**	*ribB*	*ribE*	*ribC*	*ribF*	
CLCA	■	■	■	■	■	■	□	■	■	■	■	■	□	□	□	□	□	□	□	□	□	□	□	*Buchnera*
Ccon	■	■	■	■	■	■	□	■	■	■	■	■	□	□	□	□	□	□	□	□	□	□	□	*Buchnera*
Ctuj	■	■	■	■	■	■	□	■	■	■	■	■	□	□	□	□	□	□	□	□	□	□	□	*Buchnera*
Cced	■	■	□	□	□	□	□	■	■	■	■	■	□	□	□	□	□	□	□	□	□	□	□	*Buchnera*
Cpse	■	■	■	■	■	■	□	■	■	■	■	■	□	□	□	□	□	□	□	□	□	□	□	*Buchnera*
Cstr	■	■	■	■	■	■	□	■	■	■	■	■	□	□	□	□	□	□	□	□	□	□	□	*Buchnera*
Cfor	■	■	■	■	■	■	□	■	■	■	■	■	□	□	□	□	□	□	□	□	□	□	□	*Buchnera*

Ancient loss of biotin and riboflavin genes followed by genome « stasis »

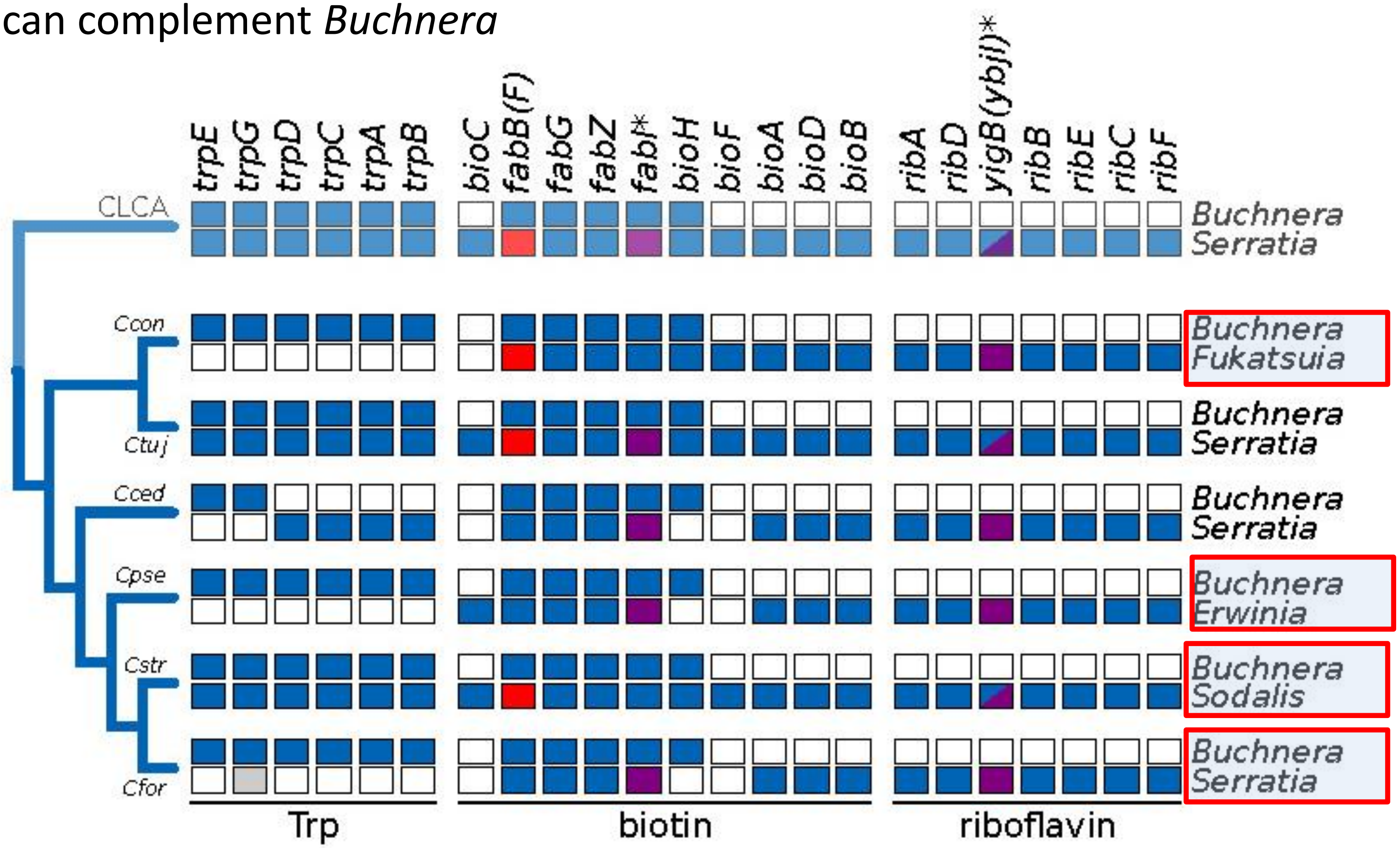
Meseguer et al. 2017. Mol Ecol

Nutritional complementation of co-symbionts in *Cinara*



Assemblies confirm the presence of the co-symbiont detected through 16S

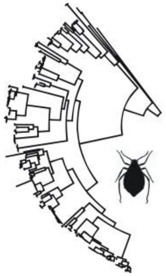
Searches for biotin and riboflavin genes confirm that all co-symbiotic lineages can complement *Buchnera*



Di-symbiotic system in which one of the partners is repeatedly replaced

Meseguer et al. 2017. Mol Ecol

Ecological factors associated with the replacement of co-symbionts?



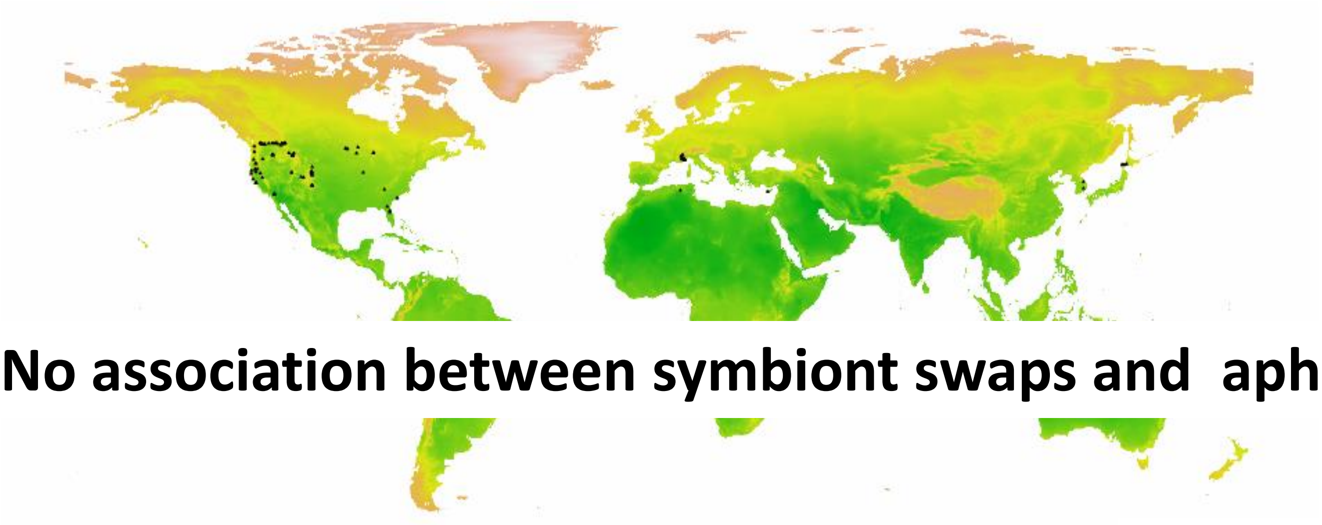
Is the identify of the symbiont lineage dependant on host plant genus?

Logistic phylogenetic regression

Not significant, changes in symbiotic partner are not associated with shifts to new host-plant genera (nor feeding site, nor feeding diet etc..)

Is the presence/absence of a symbiont lineage dependant on climatic factors?

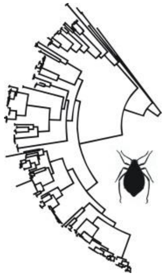
(*we retrieved climatic data for all 420 samples, from WorldClim*)



The climatic enveloppes of specimens hosting different symbionts are similar

No association between symbiont swaps and aphid's niche shifts

Meseguer et al. 2017. Mol Ecol

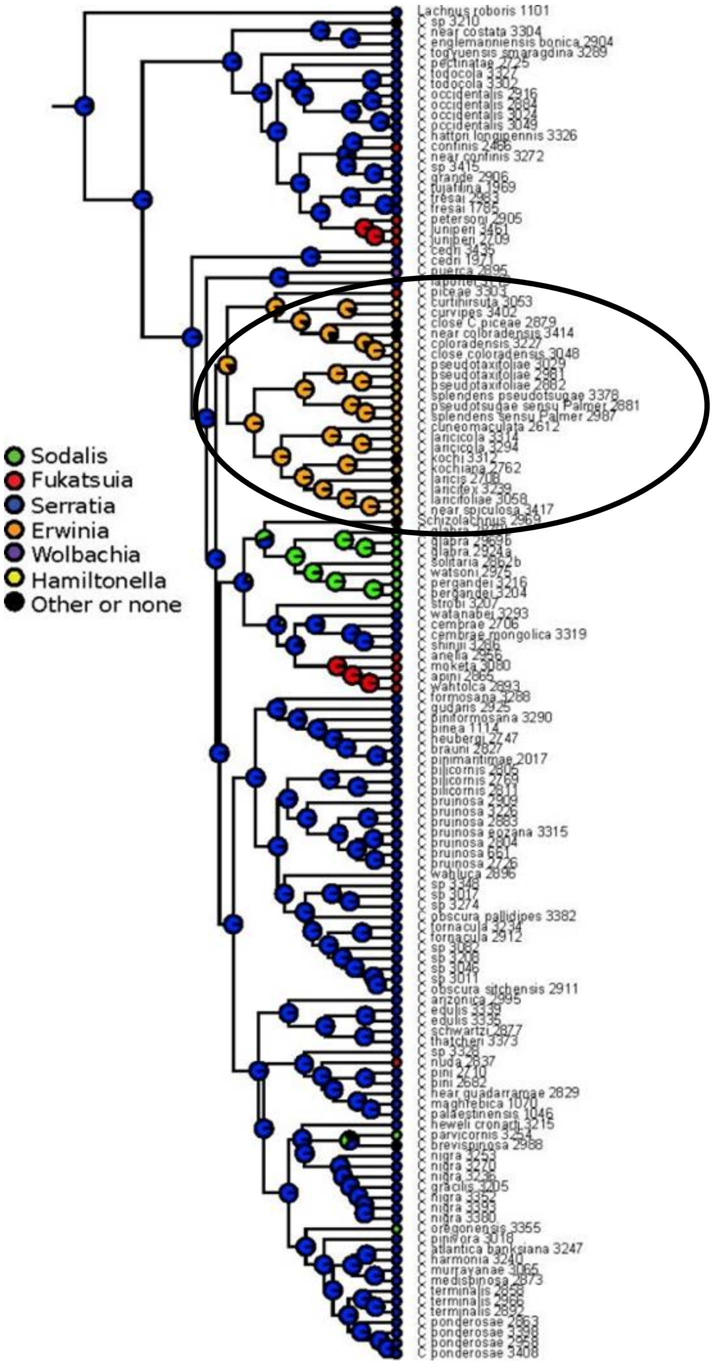


To better understand the evolutionary history of the associations:

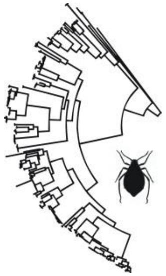
-Reconstruct the phylogenies of each symbiotic lineage and investigate co-diversification scenarios

-Investigate metabolic functions in symbiont genomes

The story of *Erwinia* in *Cinara* aphids

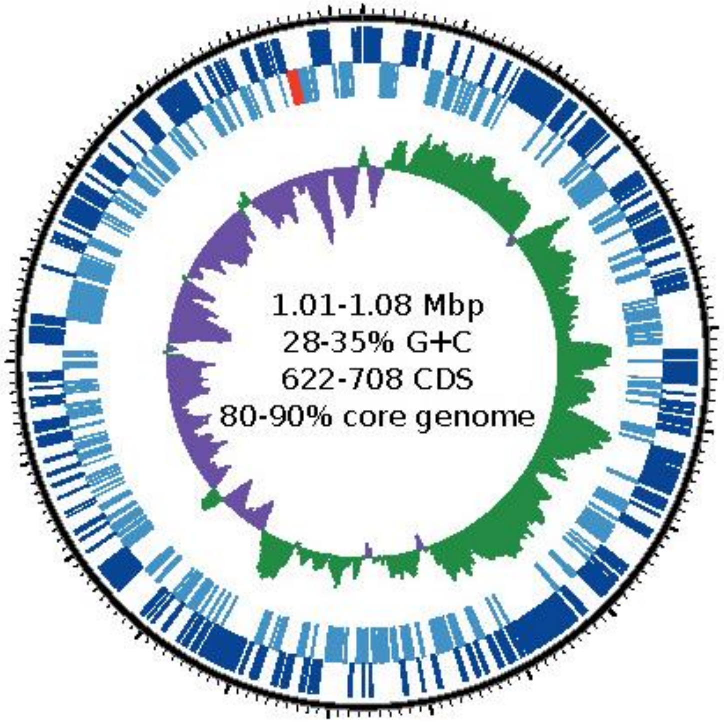


The story of *Erwinia* in *Cinara* aphids



Buchnera/*Erwinia* genomes sequenced, assembled and annotated in 9 aphid species

Erwinia has a small genome, AT rich



Manzano-Marin et al. 2019.ISME

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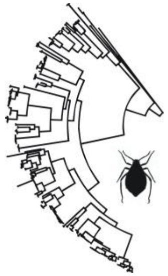
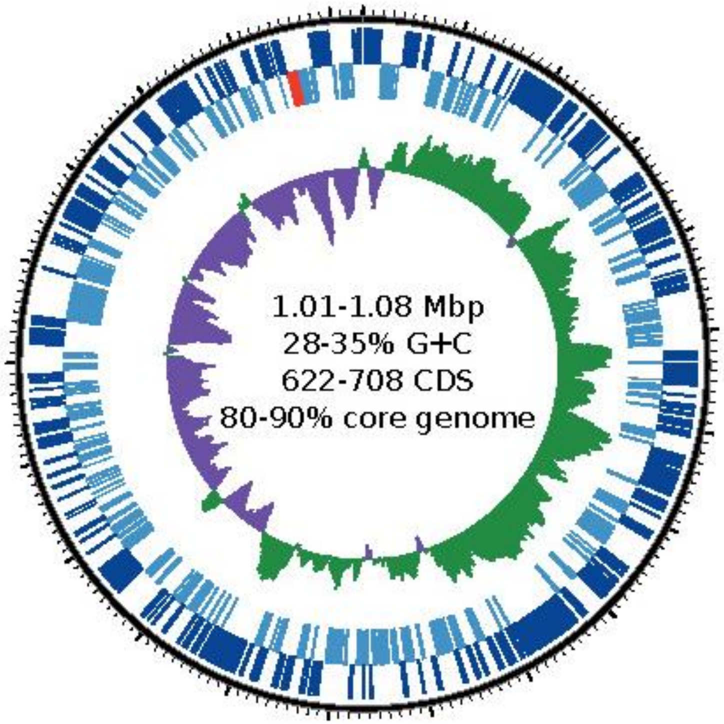
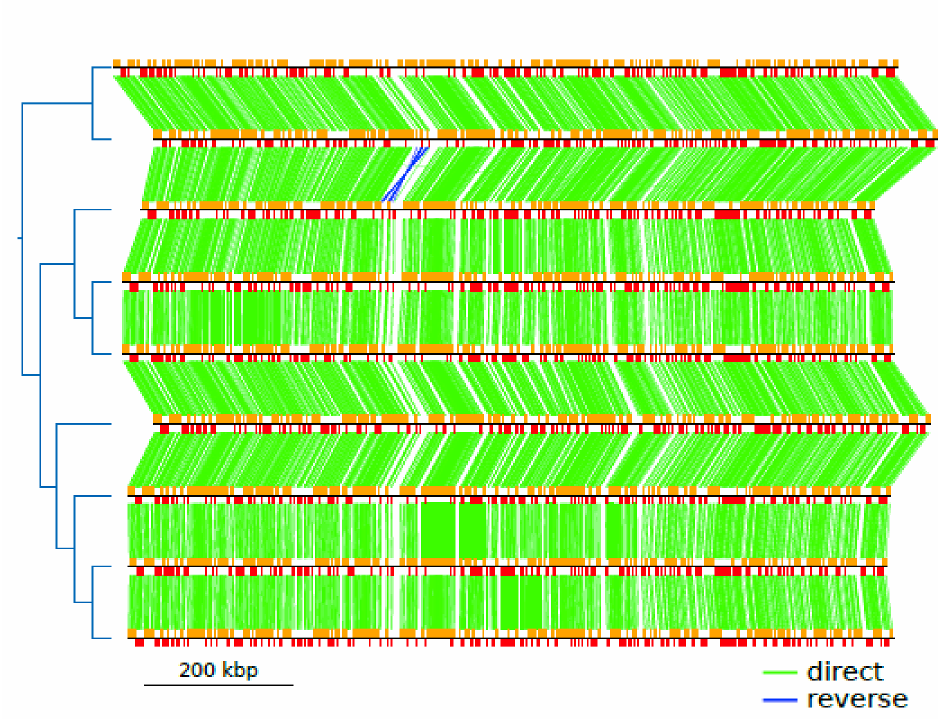


The 9 *Erwinia* genomes are syntenic





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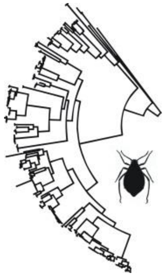
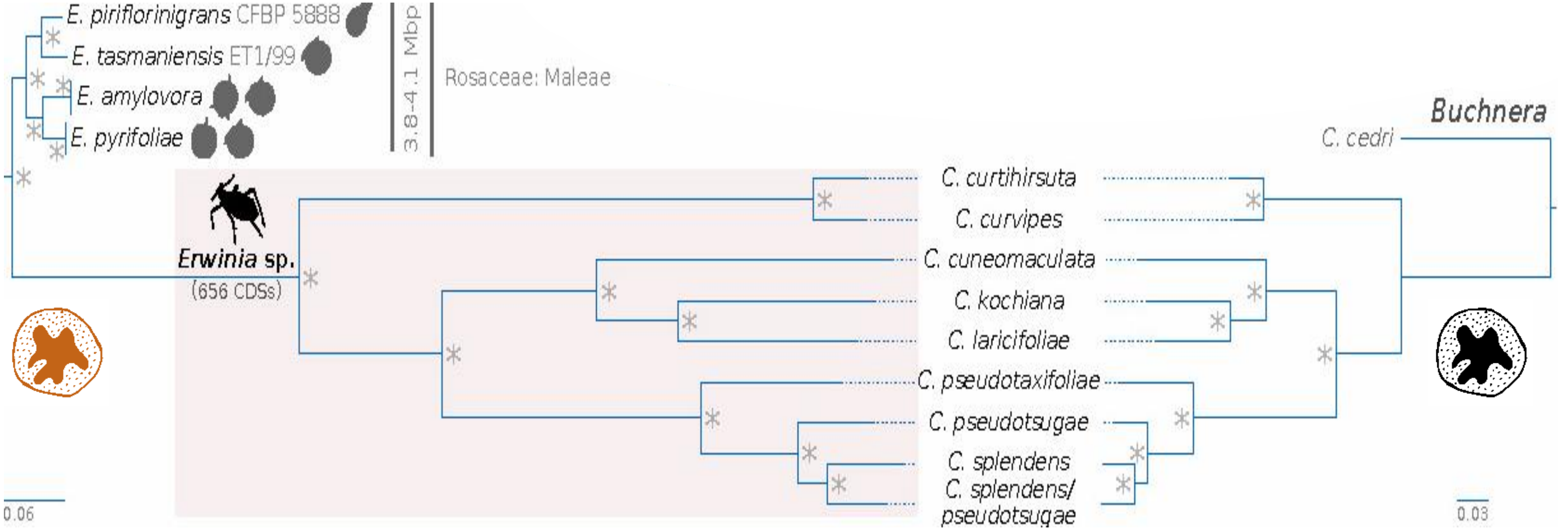


Erwinia **has cospeciated with its host since its acquisition, closest relatives are bacterial phytopathogens**



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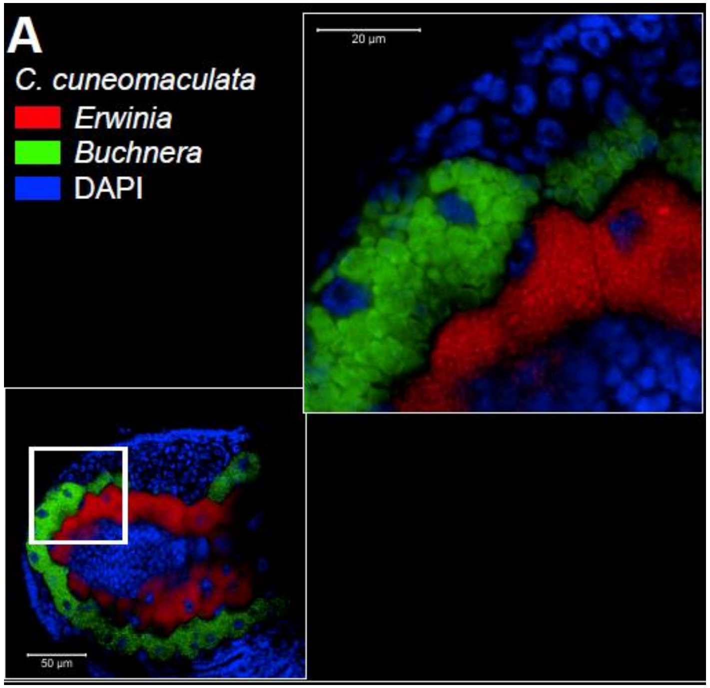
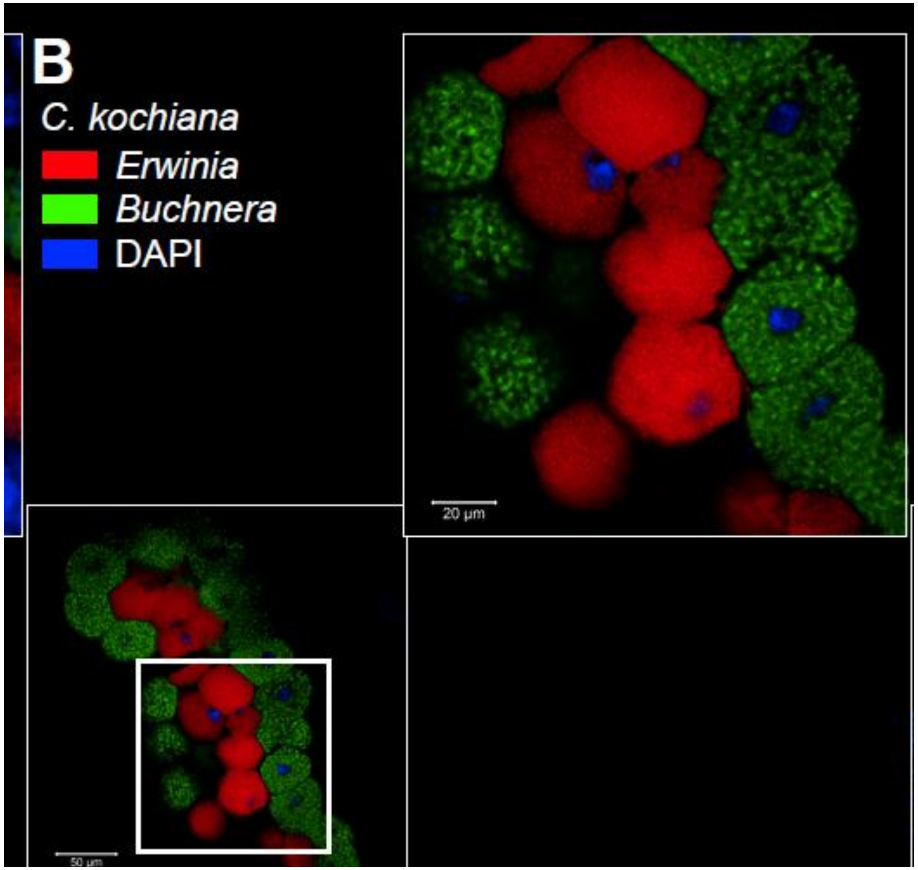
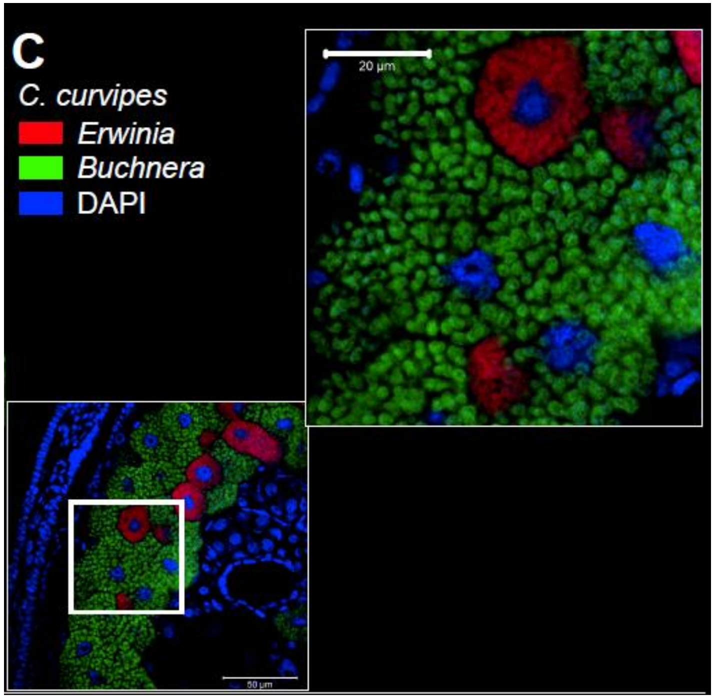


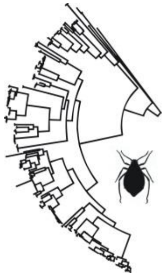




Erwinia found in specialized cells (bacteriocytes), probably vertically transmitted

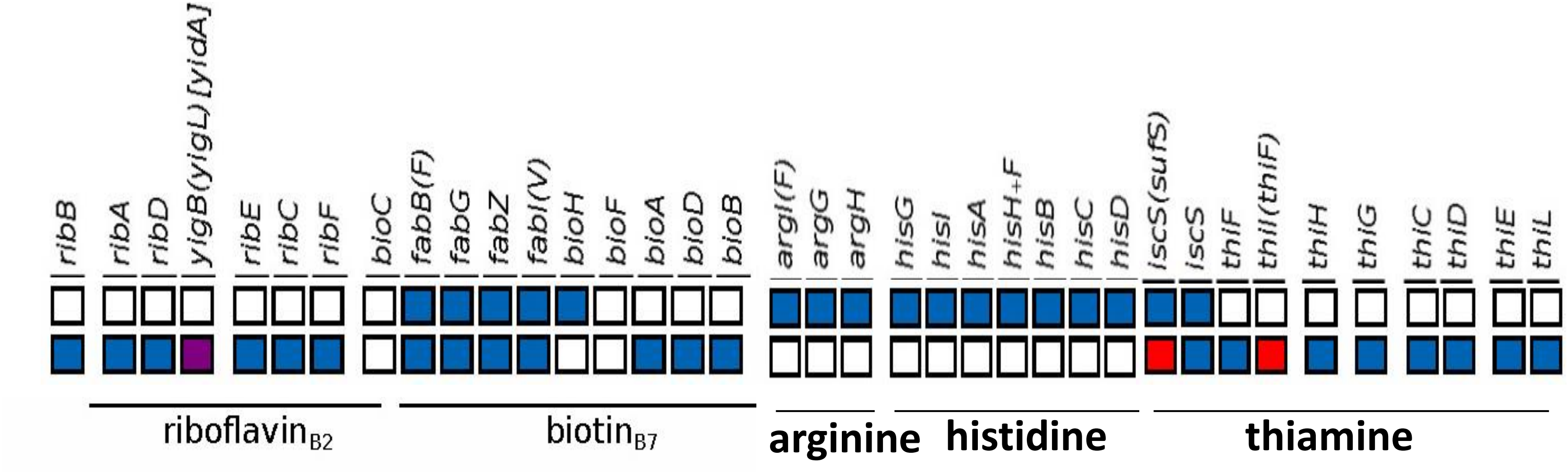
Manzano-marin et al. 2019.ISME

The story of *Erwinia* in *Cinara* aphids



Full annotation of *Erwinia* and Buchnera:

Erwinia involved in biotin, riboflavine, biosynthesis, can complement Buchnera for these functions but also has **thiamin biosynthesis capacity (never present in *Buchnera* or Serratia)**



ribB	*ribA*	*ribD*	*yigB(yigL) [yidA]*	*ribE*	*ribC*	*ribF*	*bioC*	*fabB(F)*	*fabG*	*fabZ*	*fabI(V)*	*bioH*	*bioF*	*bioA*	*bioD*	*bioB*	*argI(F)*	*argG*	*argH*	*hisG*	*hisI*	*hisA*	*hisH+F*	*hisB*	*hisC*	*hisD*	*iscS(sufS)*	*iscS*	*thiF*	*thiI(thiF)*	*thiH*	*thiG*	*thiC*	*thiD*	*thiE*	*thiL*
☐	☐	☐	☐	☐	☐	☐	☐	🟦	🟦	🟦	🟦	🟦	☐	☐	☐	☐	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	☐	☐	☐	☐	☐	☐	☐	☐
🟦	🟦	🟦	🟪	🟦	🟦	🟦	☐	🟦	🟦	🟦	🟦	☐	☐	🟦	🟦	🟦	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	🟥	🟦	🟦	🟥	🟦	🟦	🟦	🟦	🟦	🟦

riboflavin$_{B2}$

biotin$_{B7}$

arginine **histidine** **thiamine**

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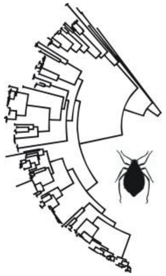
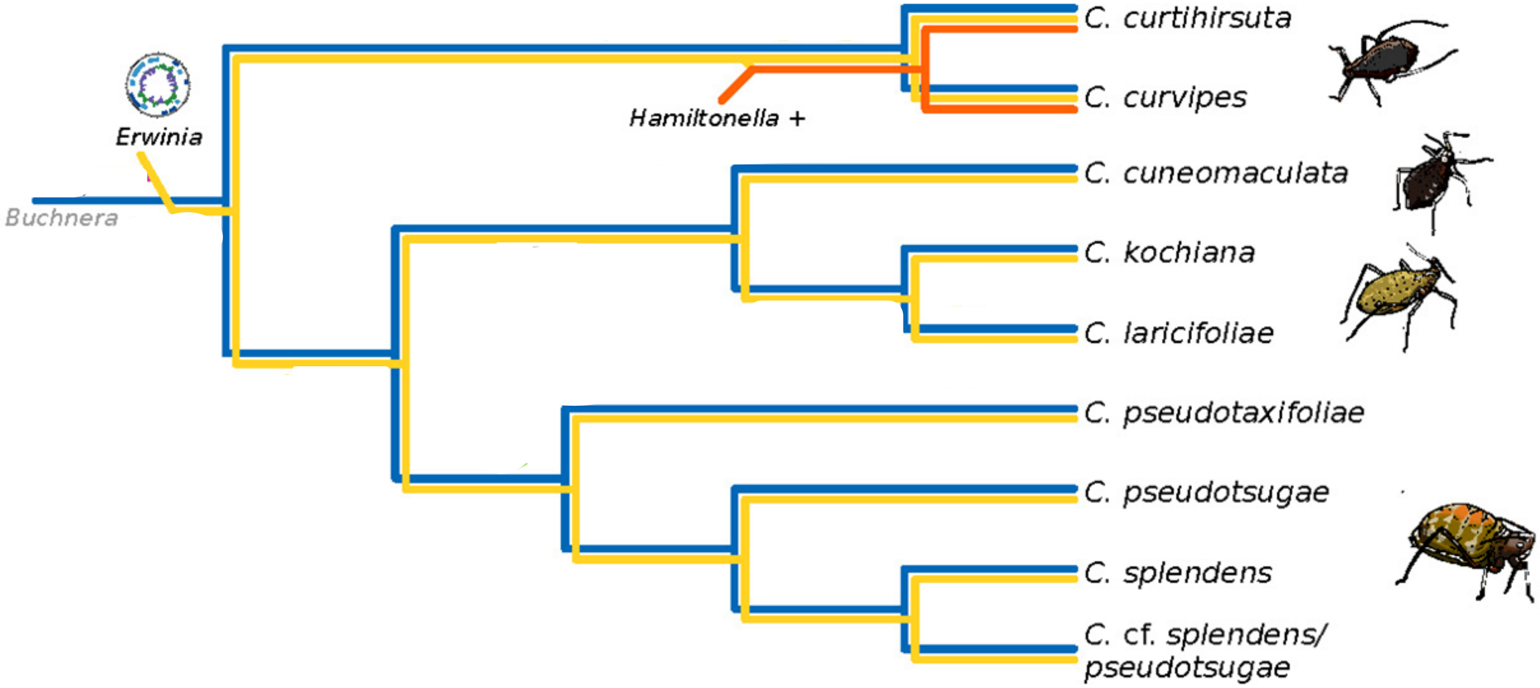
The story of *Erwinia* in *Cinara* aphids



Sequencing data revealed, a third small symbiont, *Hamiltonella* (1,4 Mbp) in two species



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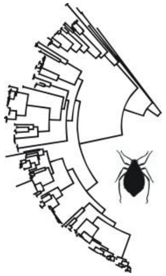
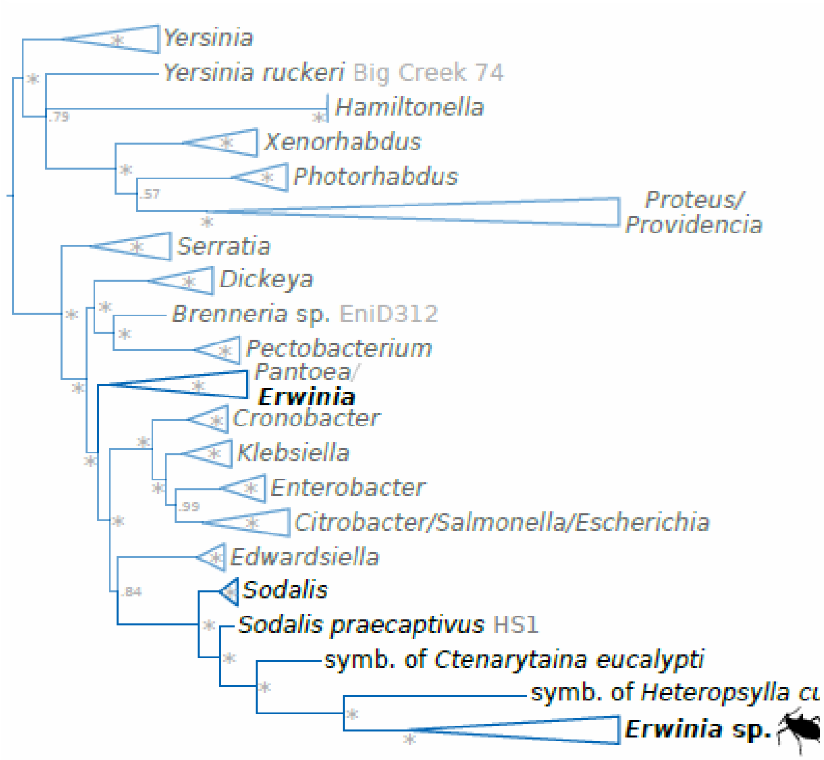
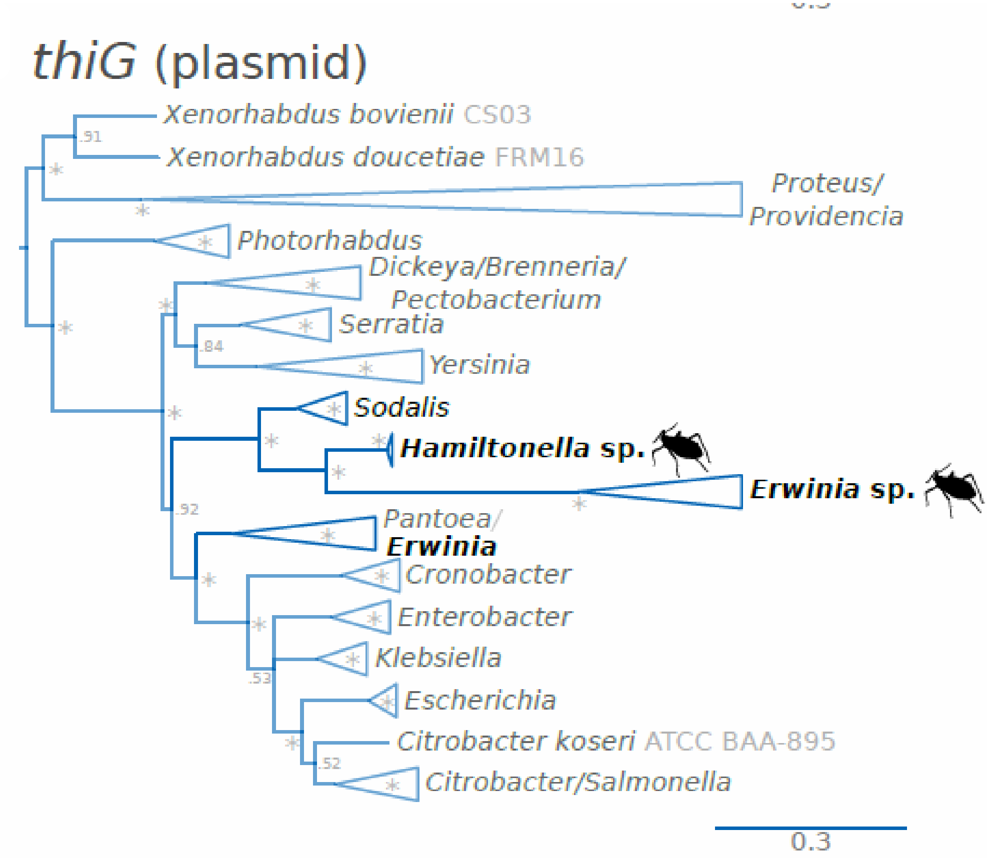
The story of *Erwinia* in *Cinara* aphids



Biotin and thiamine biosynthetic genes horizontally acquired from a *Sodalis*-related bacteria in Erwinia and then Hamiltonella

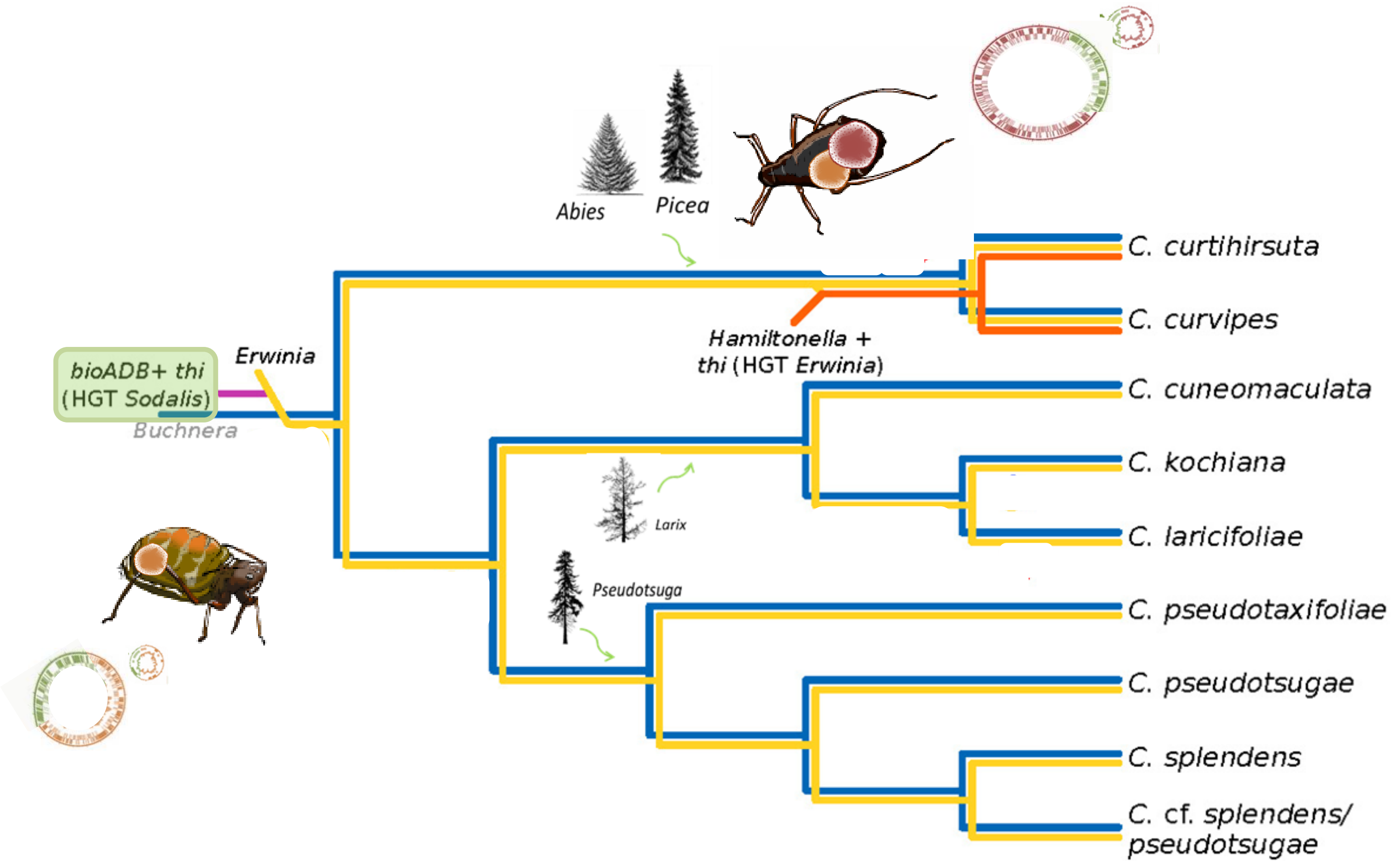
BioA (plasmid)





Manzano-Marin et al. 2019.ISME

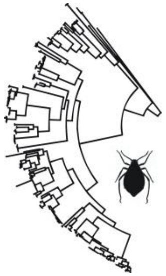
The story of *Erwinia* in *Cinara* aphids

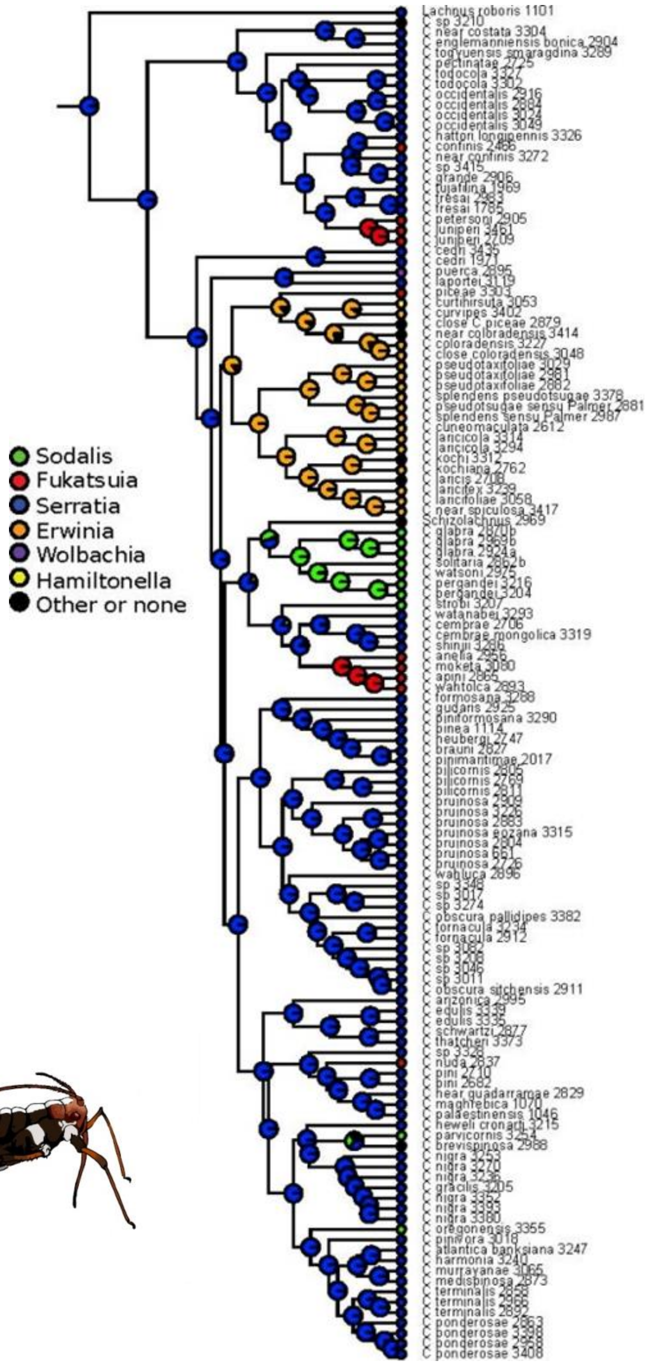


Horizontal gene transfers between co-resident symbionts play a role in symbiont replacements

Erwinia and then *Hamiltonella* have herited **Thiamine genes** –not present in *Buchnera*.

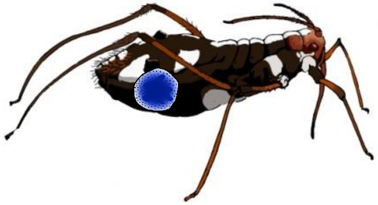
Does it play a role in adaptation to *Pseudotsuga/Larix*?



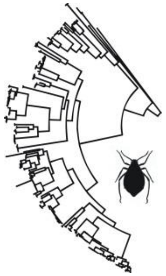


The story of *Serratia*

The most parcimonious scenario from presence / absence of *Serratia* data suggests that *Serratia* was ancestral and has been repeatedly replaced



The story of *Serratia*



Cinara’s phylogeny (*whole mitochondria*)

Cospeciating *Serratia* lineage

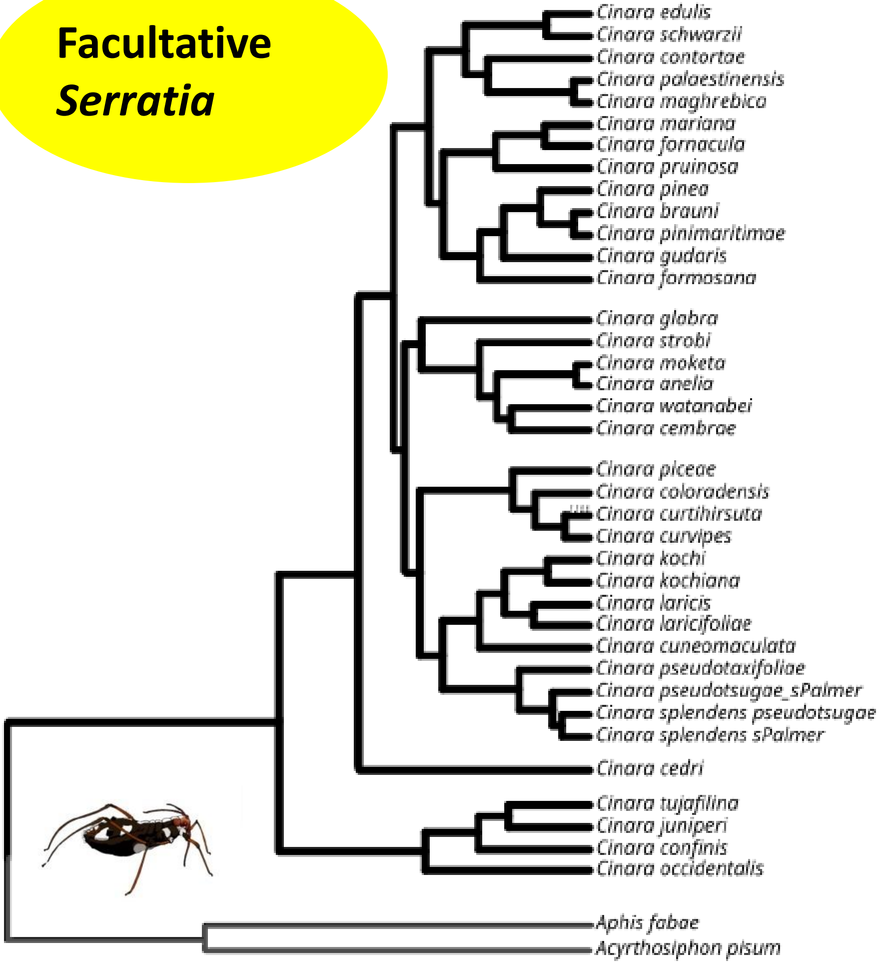
Serratia’s Phylogeny (180 CDS)

Facultative *Serratia*

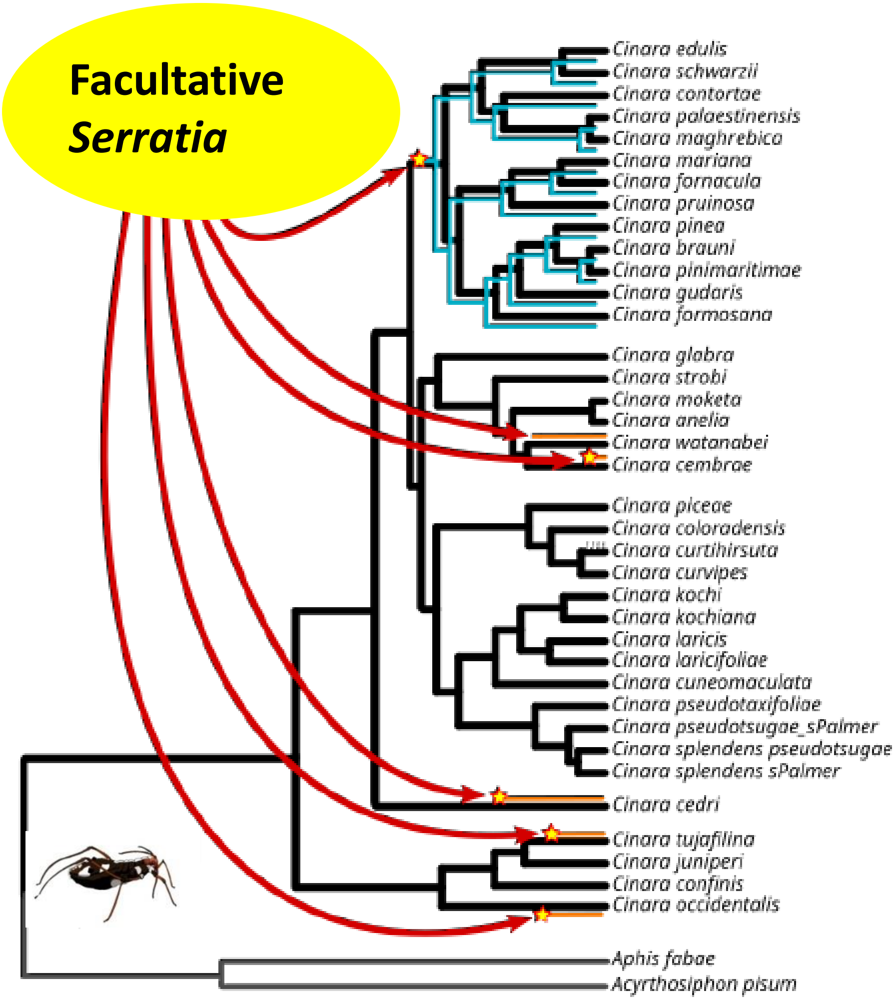
A pattern that suggests a more complicated story …

The story of *Serratia*

Facultative *Serratia*



The story of *Serratia*

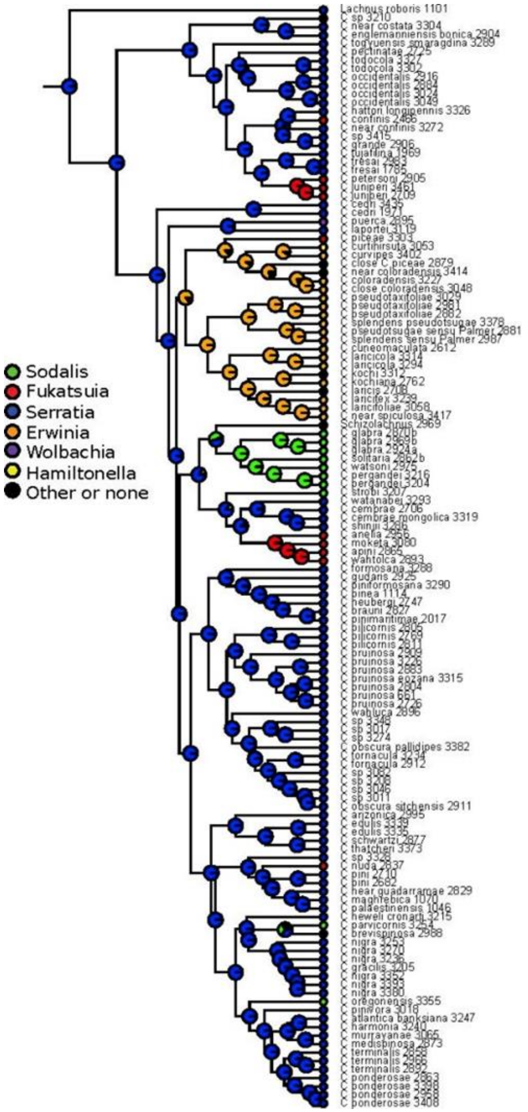


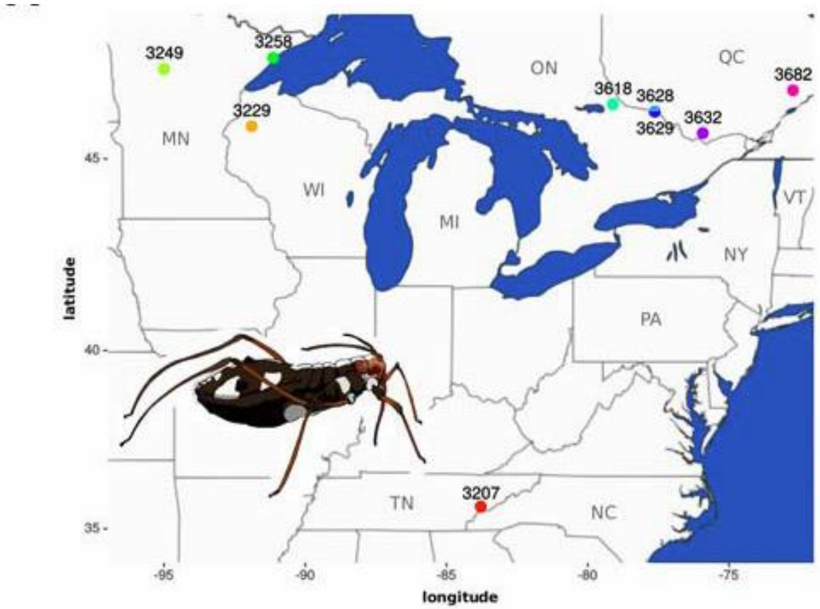
Reconciliation analyses (i.e. reconciling *Serratia* phylogeny with aphid host phylogeny) suggest multiple acquisitions of *Serratia in Cinara*!

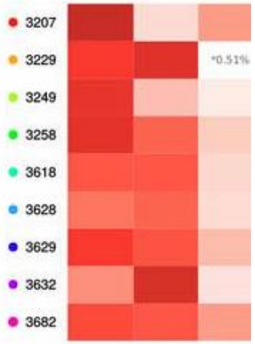
« Serratia association » might not be the ancestral state in *Cinara*, there might be losses followed by reacquisition

The story of *Serratia*

The case of *C. strobi:* three obligate symbionts?





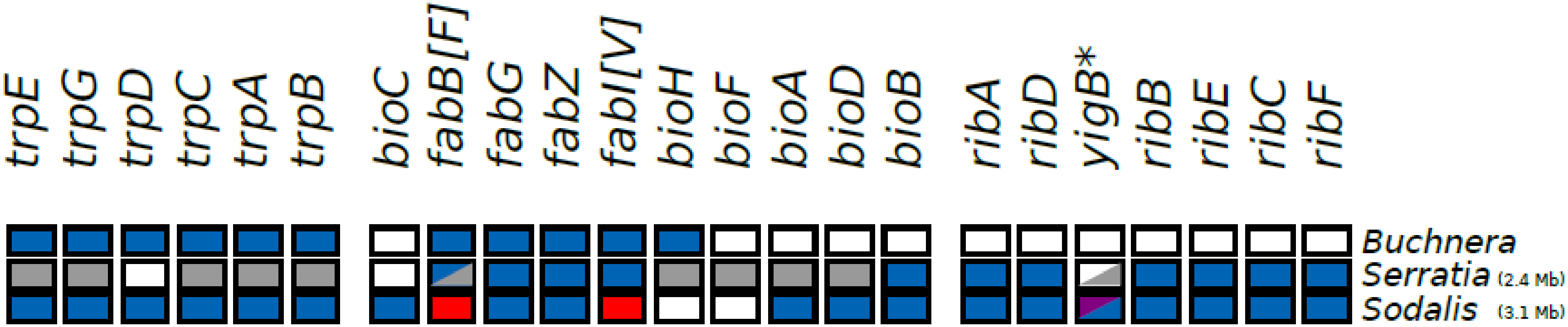


C. strobi has three fixed endosymbionts:
Buchnera, Serratia, Sodalis

Manzano-Marin *et al. GBE* 2018

The case of *Cinara strobi*

Who does what, in this system?



70% of the genome of Serratia is pseudogenized?

Serratia: a free-loader?

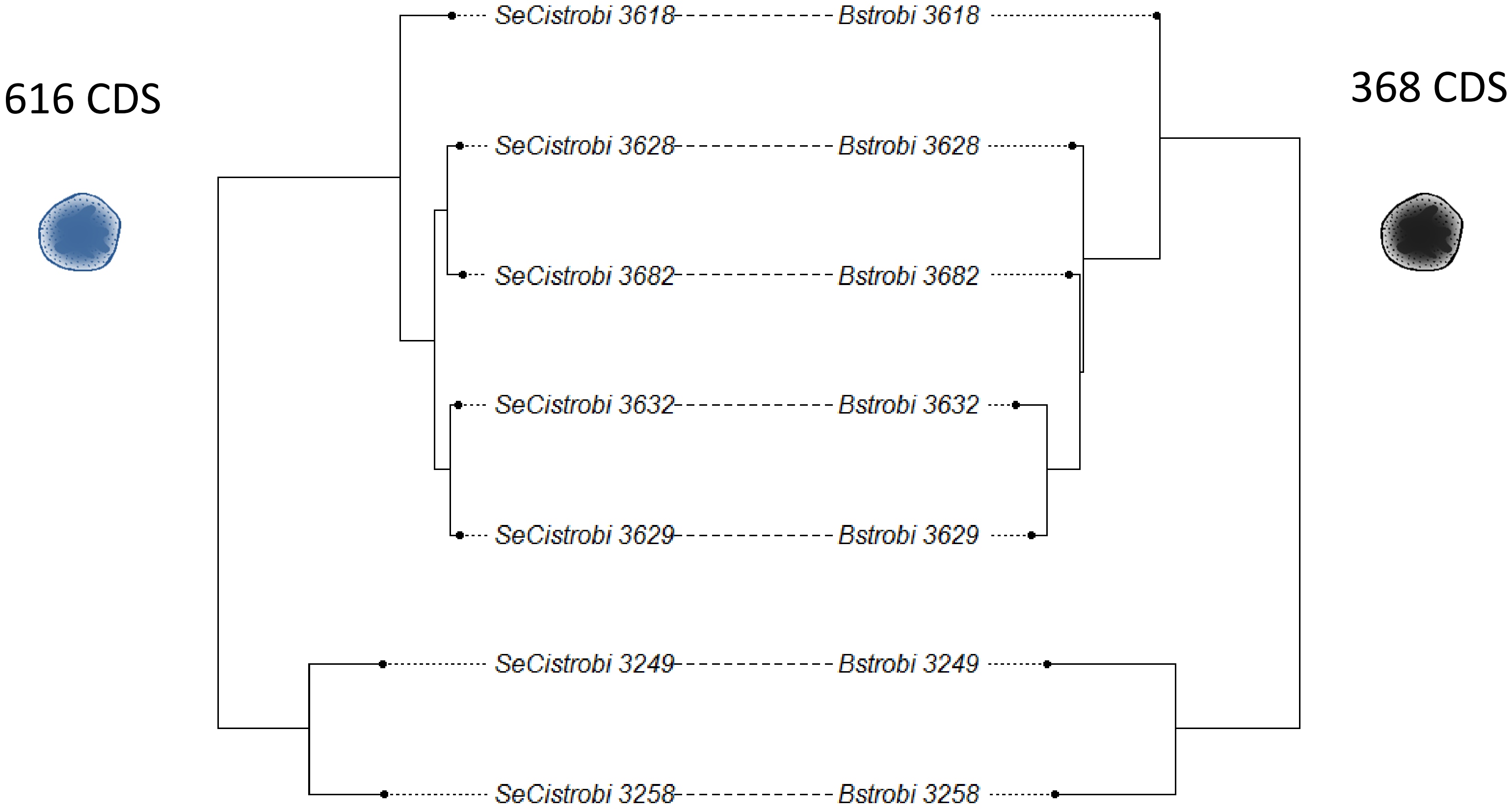
Scenario:

Sodalis is replacing *Serratia* alonside *Buchnera* as an essential endosymbiont, relaxed selective pressures on *Serratia* led to pseudogeneization?

Manzano-Marin *et al.* *GBE* 2018

The case of *C. strobi* and its very unusual *Serratia*

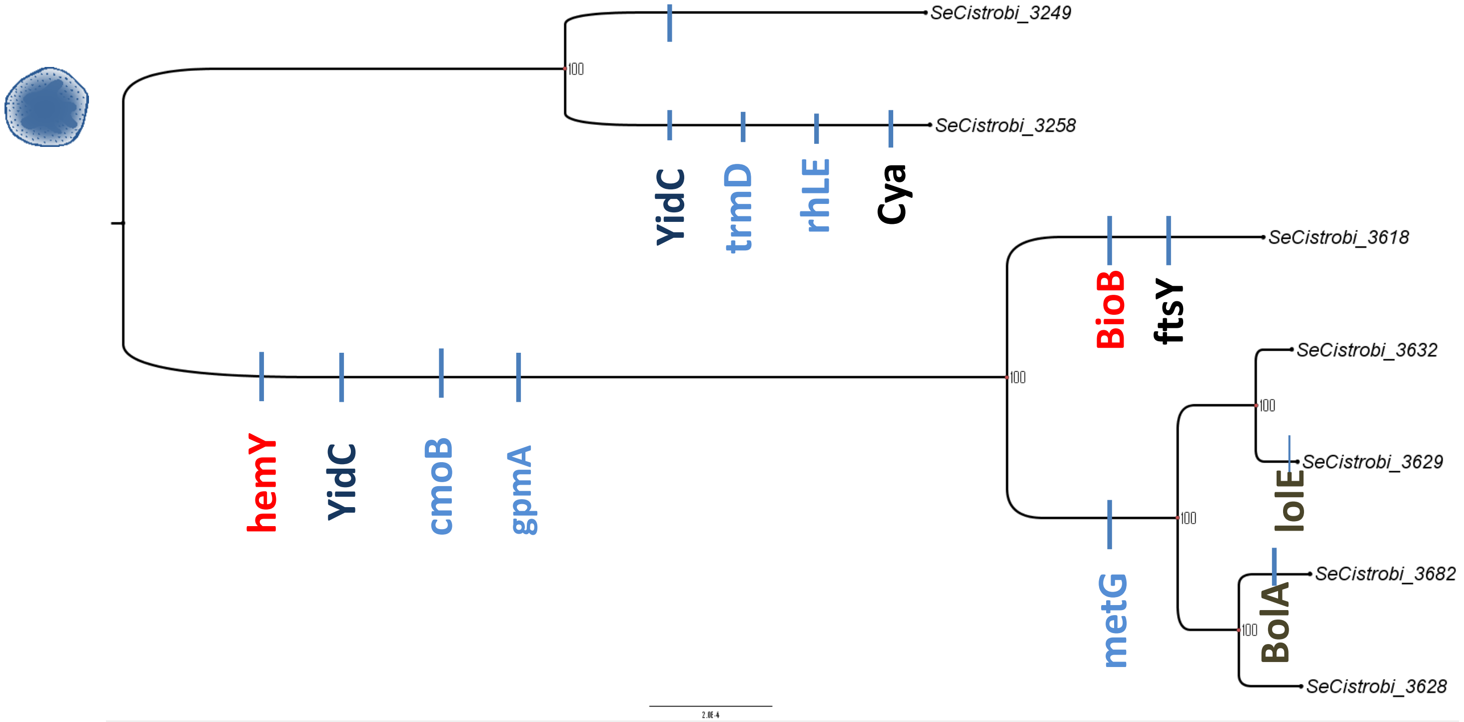
Assembly of *Buchnera* and *Serratia* chromosomes from 6 C. strobi samples
→ 6 new pairs of genomes



Confirms that the « weird » *Serratia* is vertically inherited and is probably a former obligate symbiont that is hanging there…

The case of *C. strobi* and its very unusual *Serratia*

- Losses of genes in *Serratia* (*metab*, *cell process*, ***DNA replication, recomb. & repair)***



Indels in homopolymers, in 11 out of 12 cases , causing frameshifts and premature stop codons: DNA replication slippage

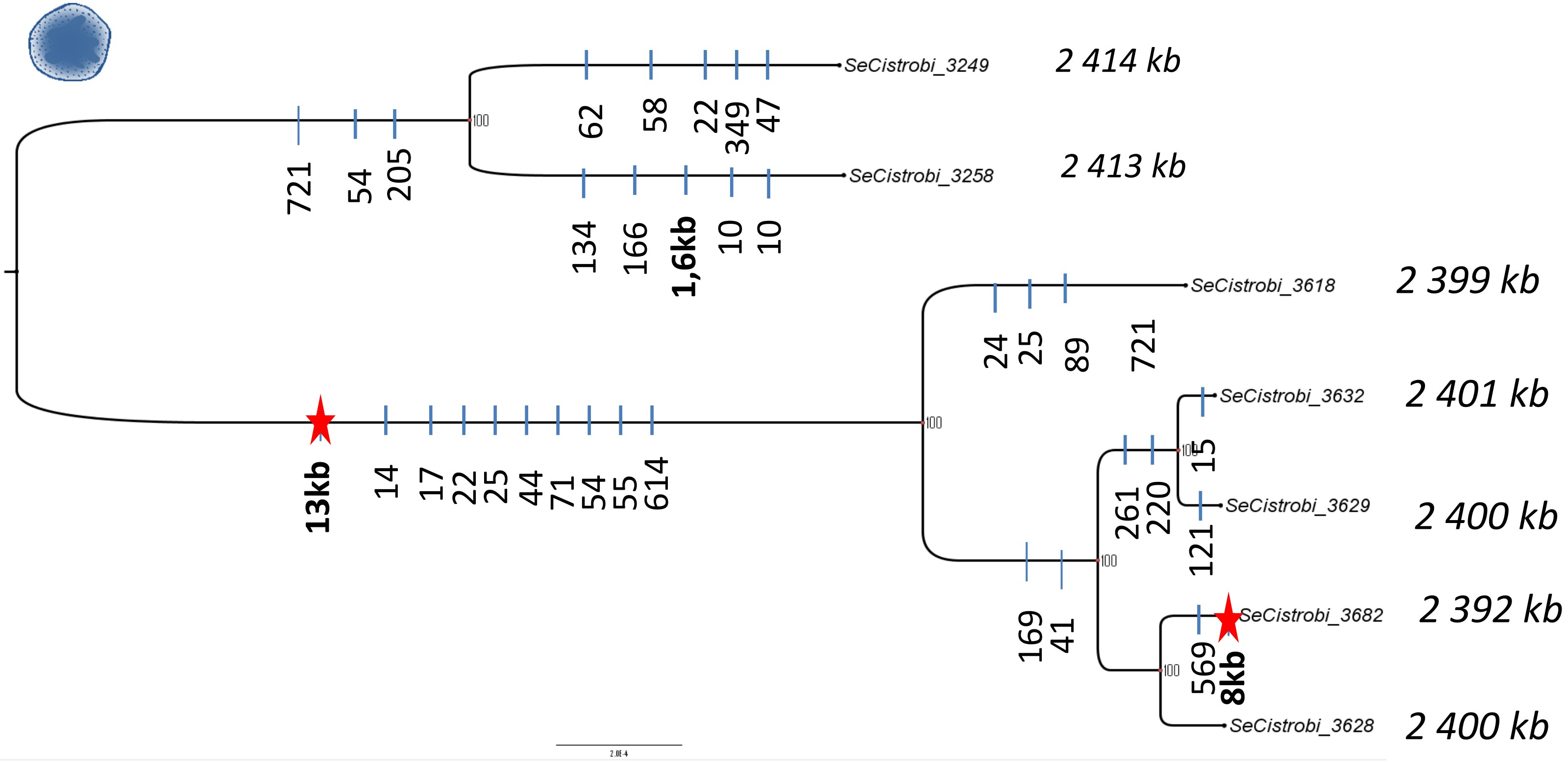
CTCATAAAAGCG
CTCAT- AAAGCG

as in *Moran et al.* 2009

The case of *C. strobi* and its very unusual *Serratia*

History of 34 « large » deletions (i.e.> 10bp) in *Serratia*

(*assuming no large insertions can occur*)



Genome reduction occurs through ‘big’ deletions in non CDS, deletion up to 13 kb, small deletions occur in homopolymers throughout non coding regions

The case of *C. strobi* and its very unusual *Serratia*

Snapshots of a shrinking partner

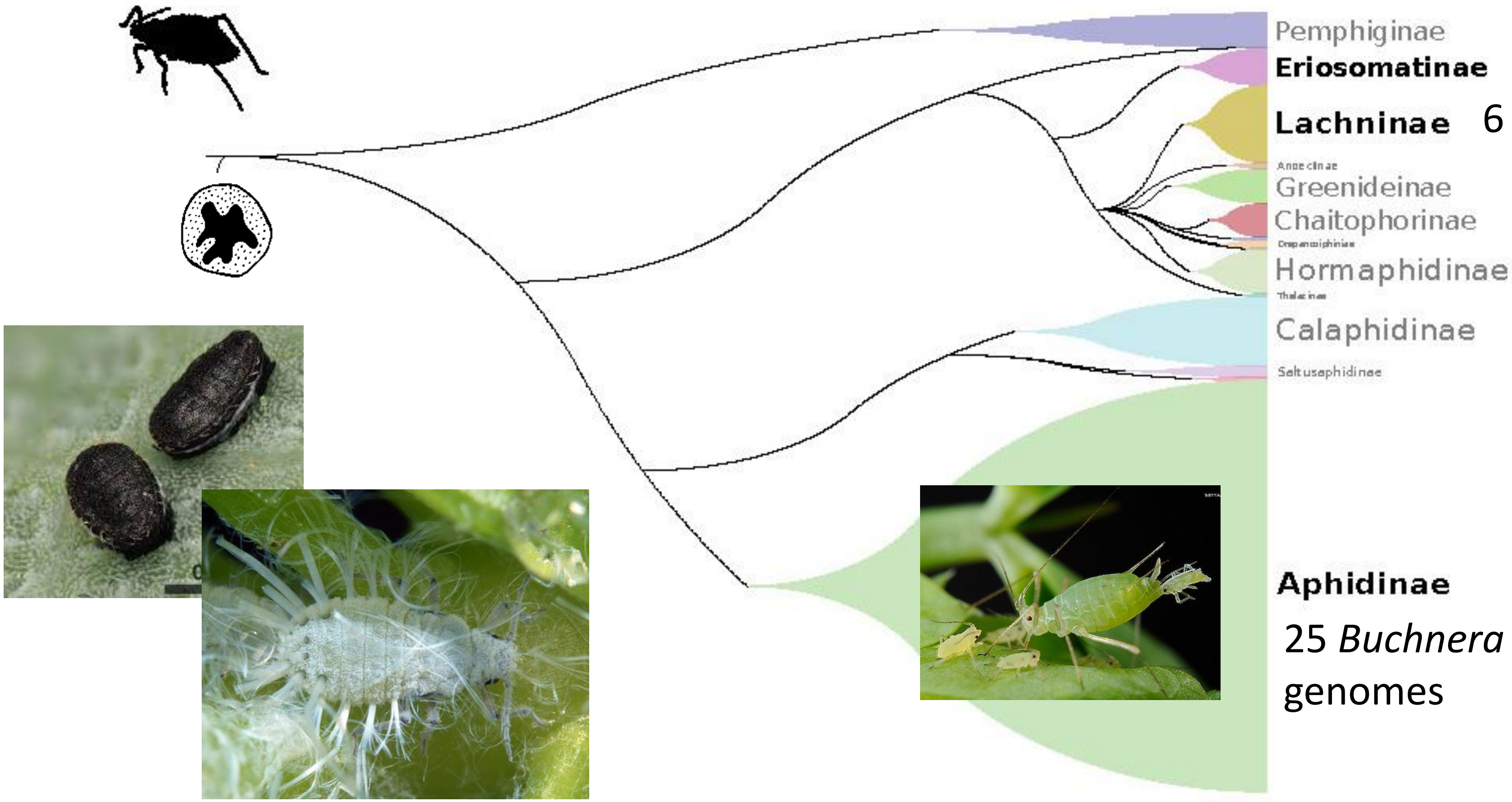
- Critical role of homopolymers in small deletions in both coding and non-coding regions

- Junk DNA then disappears through « large » stochastic deletion in pseudogenes

Very similar to what has been observed in the only intraspecific investigation on Buchnera (Moran et al. 2009)

Outside of the pea aphid and now *Cinara* spp., we know little about *Buchnera*

Altogether, 37 Buchnera genomes, 23 newly sequenced by Chong & Moran 2019, MBE

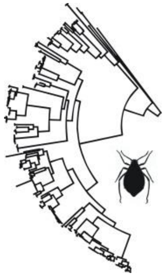


3 sp

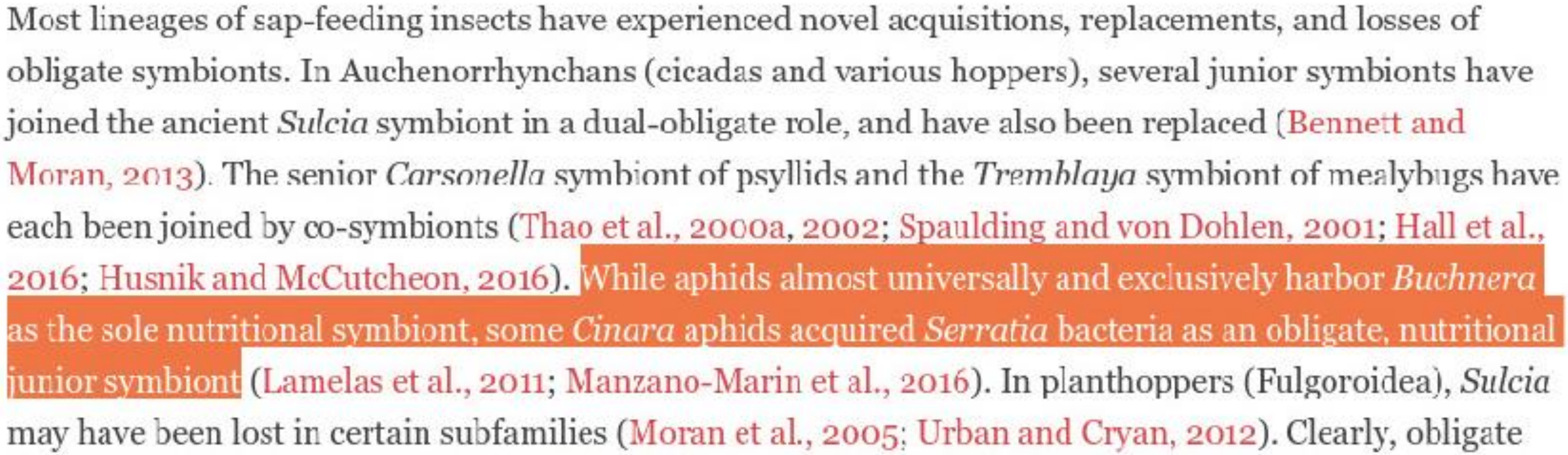
6 sp

25 *Buchnera* genomes

Our knowledge comes from 6 subfamilies, with a strong focus on Aphidinae



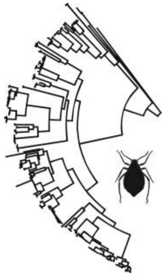
But it is generally alone…right? *Cinara* (Lachninae) are an exception?



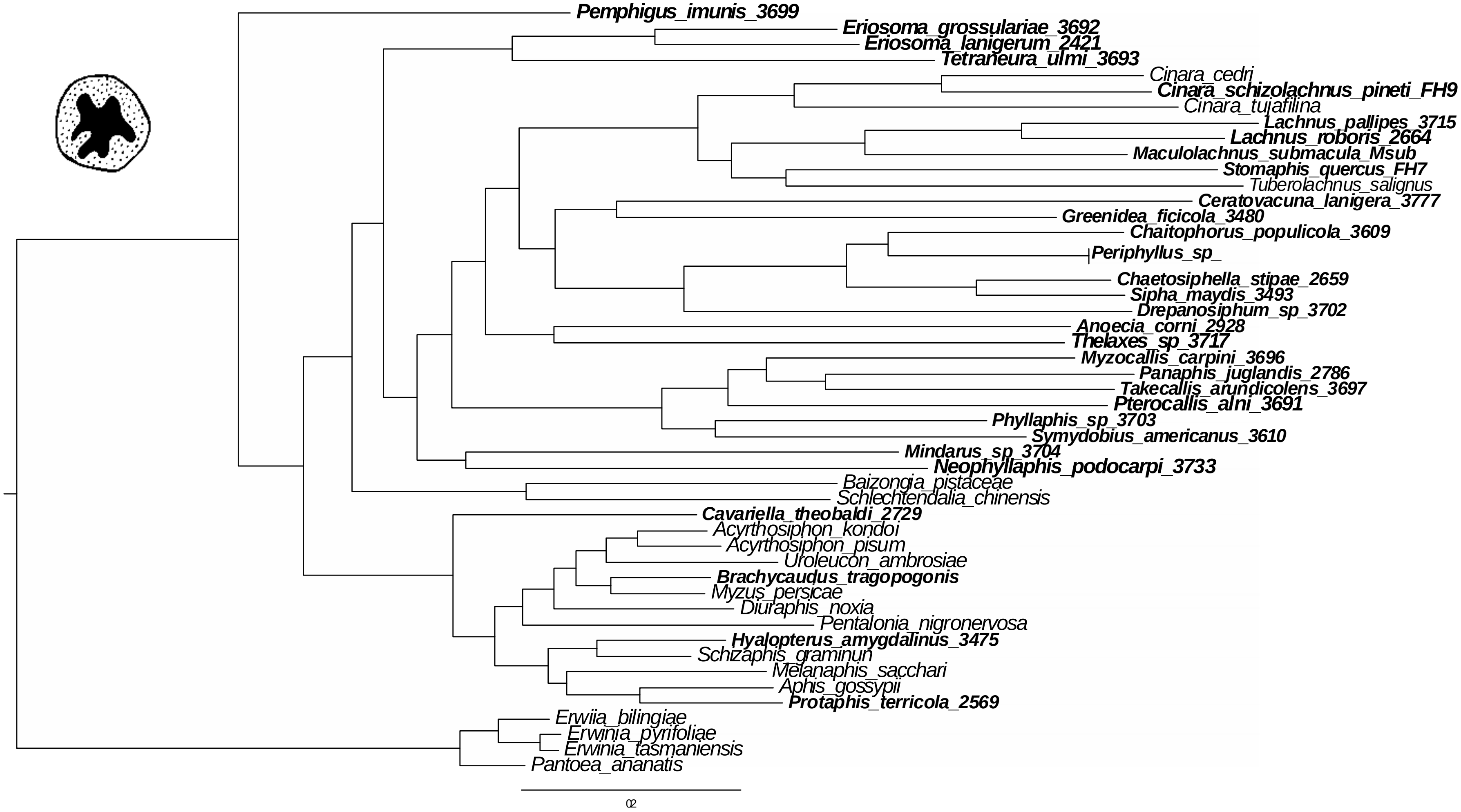
Most lineages of sap-feeding insects have experienced novel acquisitions, replacements, and losses of obligate symbionts. In Auchenorrhynchans (cicadas and various hoppers), several junior symbionts have joined the ancient *Sulcia* symbiont in a dual-obligate role, and have also been replaced (Bennett and Moran, 2013). The senior *Carsonella* symbiont of psyllids and the *Tremblaya* symbiont of mealybugs have each been joined by co-symbionts (Thao et al., 2000a, 2002; Spaulding and von Dohlen, 2001; Hall et al., 2016; Husnik and McCutcheon, 2016). While aphids almost universally and exclusively harbor *Buchnera* as the sole nutritional symbiont, some *Cinara* aphids acquired *Serratia* bacteria as an obligate, nutritional junior symbiont (Lamelas et al., 2011; Manzano-Marin et al., 2016). In planthoppers (Fulgoroidea), *Sulcia* may have been lost in certain subfamilies (Moran et al., 2005; Urban and Cryan, 2012). Clearly, obligate

Excerpt from vonDohlen *et al.* (2017). *Front Microbiol* **8**:1037

Exploring *Buchnera* across aphids

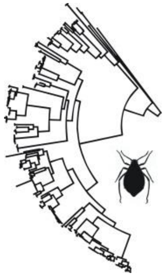


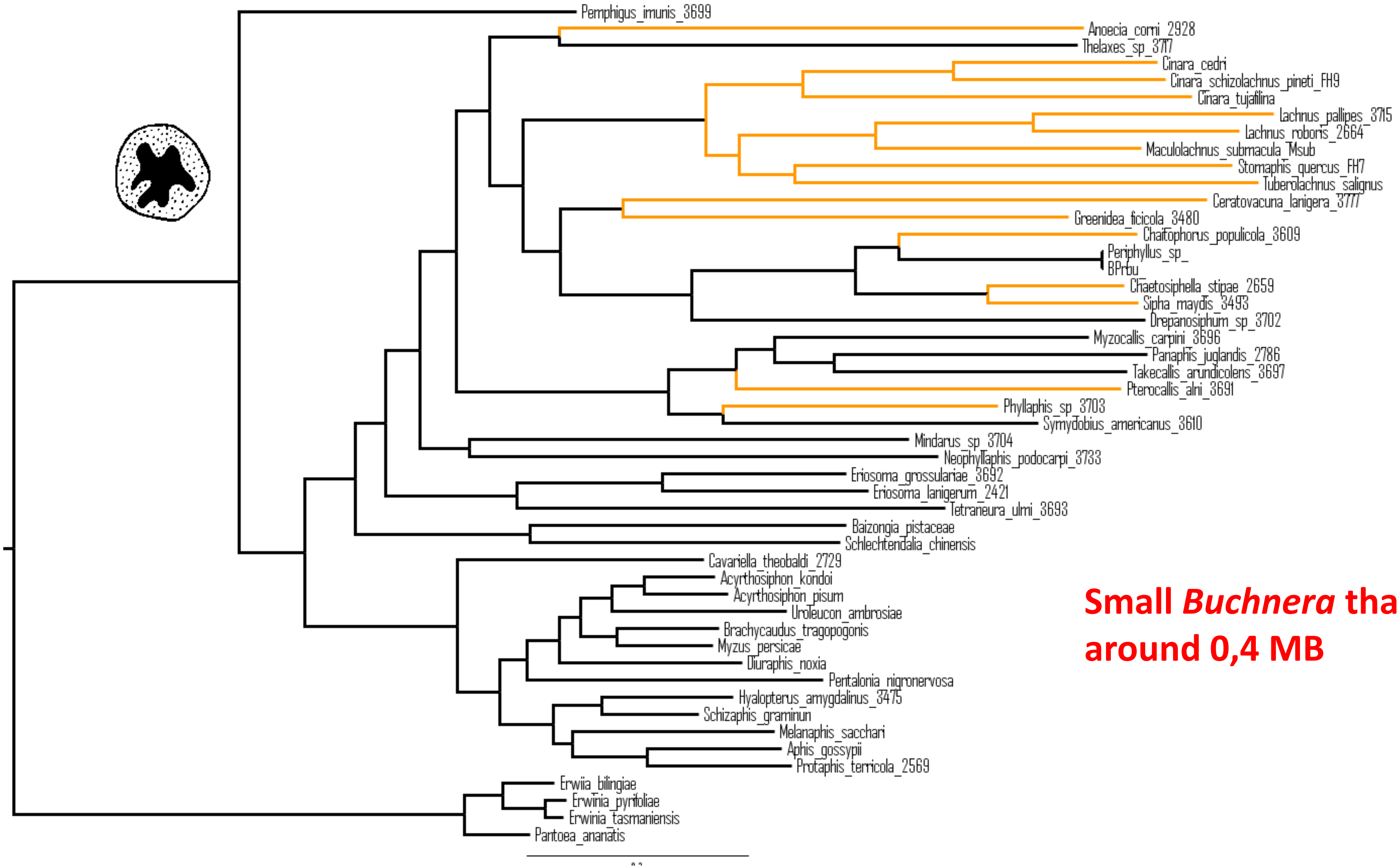
Buchnera's phylogeny (+ 27 species from 14 subfamilies)



Manzano-Marin *et al.* in prep

Exploring *Buchnera* across aphids



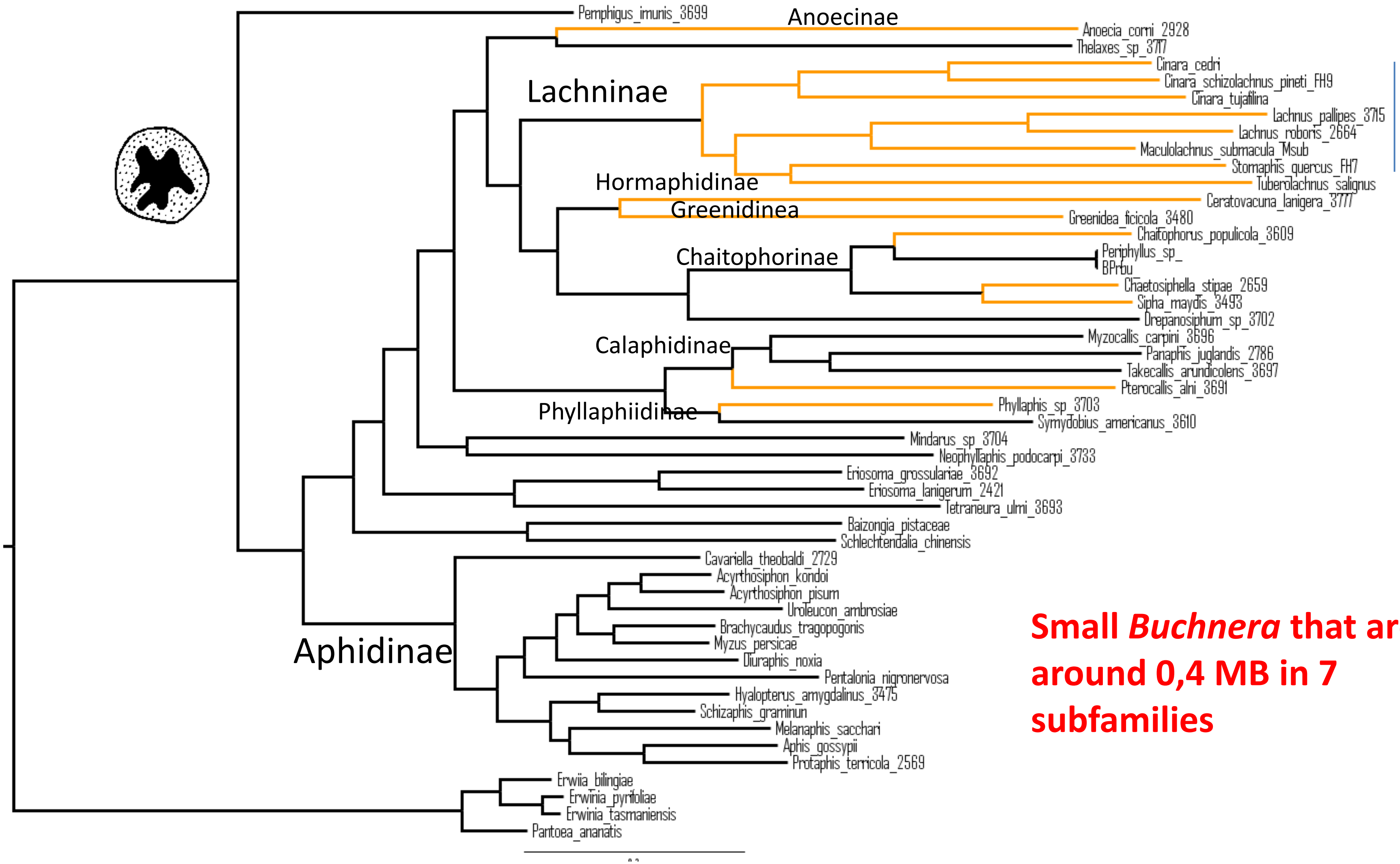


Small *Buchnera* that are around 0,4 MB

Several cases of genome reduction

Manzano-Marin *et al.* in prep

Exploring *Buchnera* across aphids

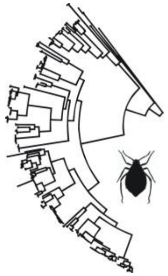


Small *Buchnera* that are around 0,4 MB in 7 subfamilies

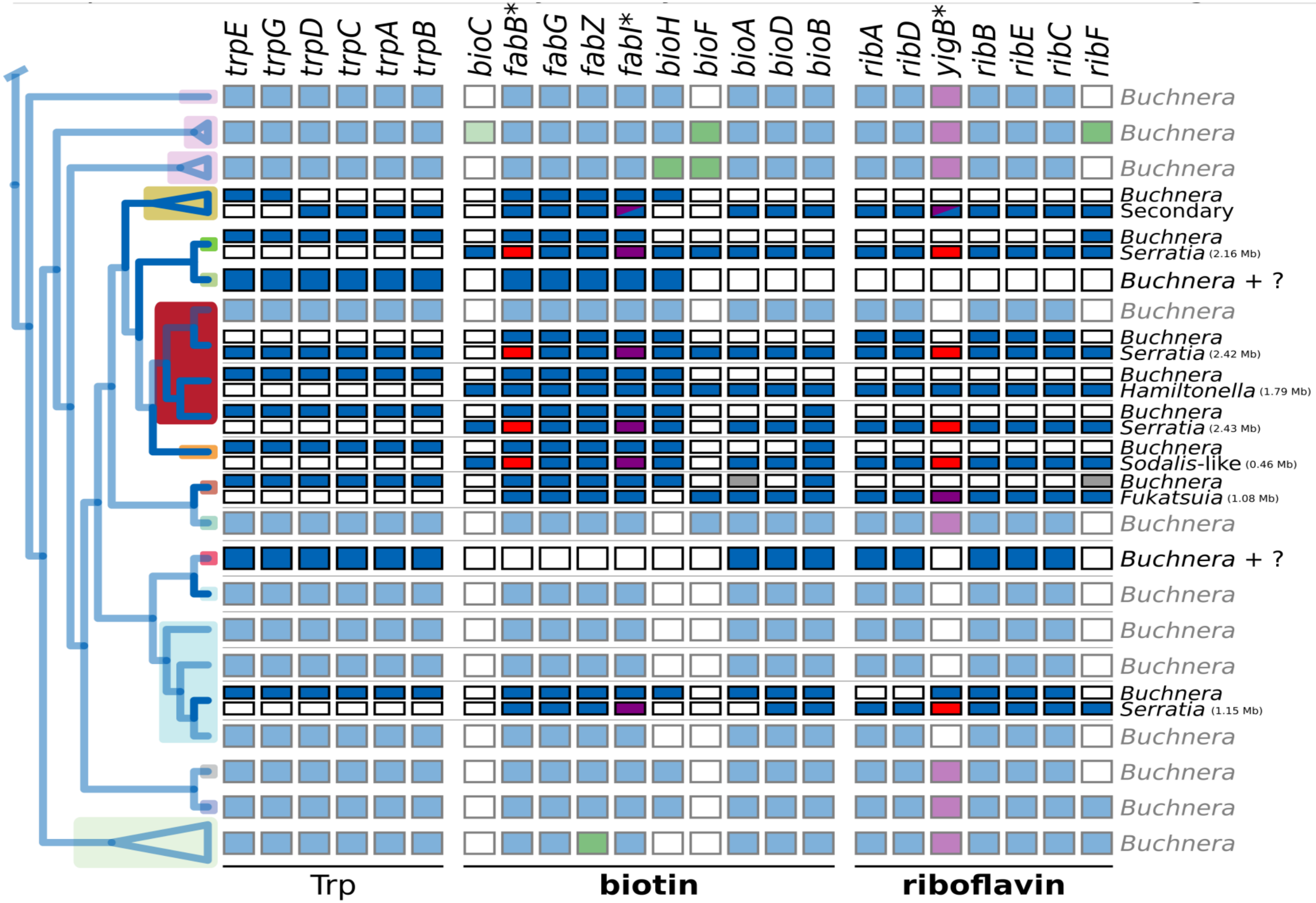
Several cases of genome reduction

Manzano-Marin *et al.* in prep

Exploring *Buchnera* across aphids

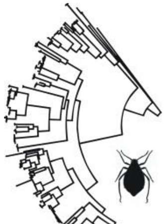


Auxotrophy in aphids from 7 subfamilies !! Some variations within and between subfamilies; not the same genes lost, recruitement of *Serratia, Hamiltonella, Wolbachia, Bacteroides*

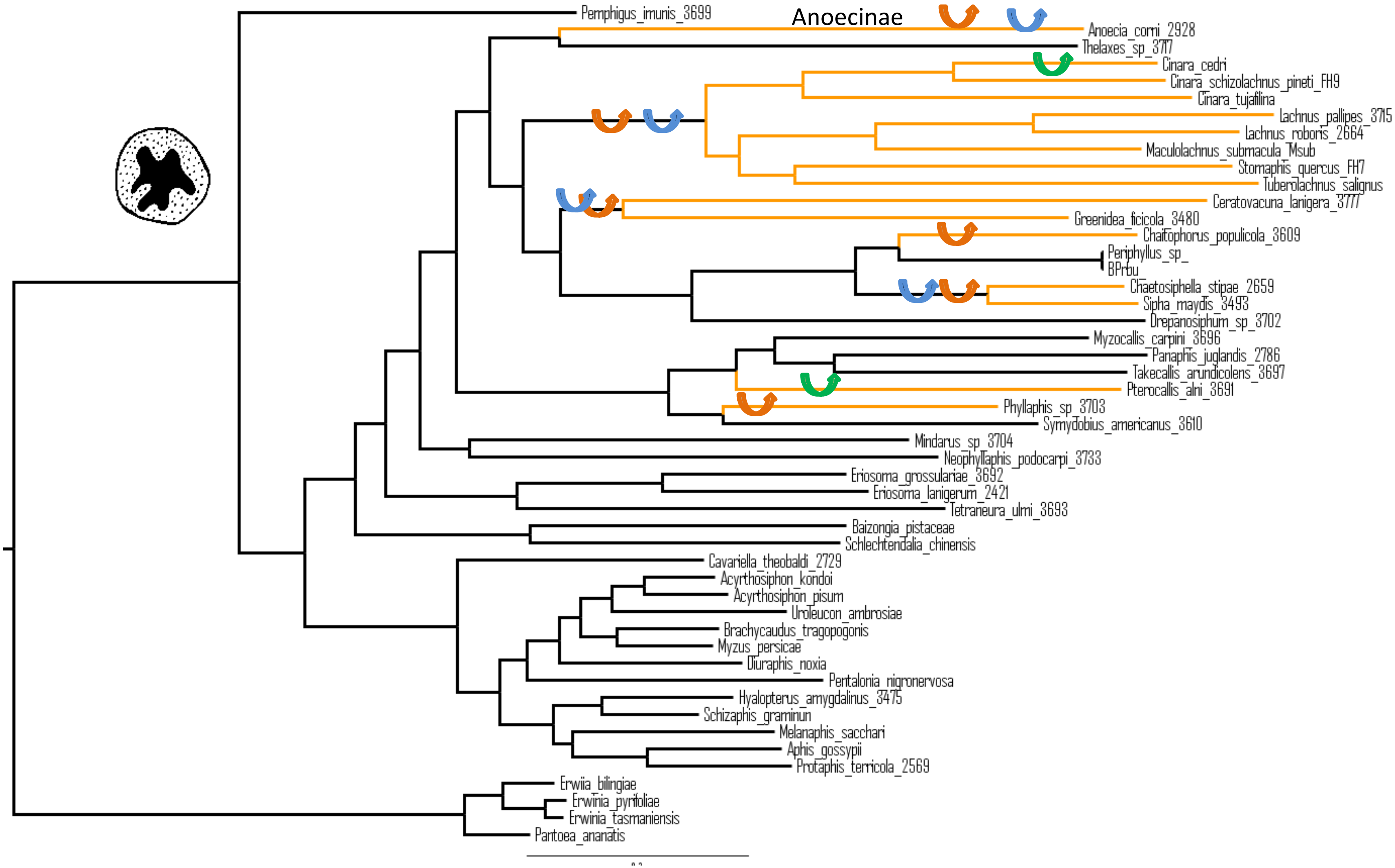


Manzano-Marín *et a*

Exploring *Buchnera* across aphids

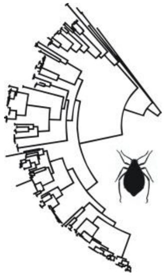


Multiple losses of **trp biosynthesis** genes, **biotin** and **riboflavin** genes



Manzano-Marin *et al.* in prep

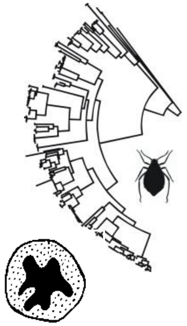
Conclusions



Diversity and history of the associations between *aphids* and their co-obligate symbionts:
Di-symbiotic systems occur repeatedly in aphids
The partners may diversify through cospeciation until the co-symbiont is replaced, there is recurrent replacement of the "new-comer"

Role of bacterial associations in aphids evolutionary transitions:
No association between niches and symbiont ID
New co-symbionts carry essential metabolic functions that compensate *Buchnera*'s deficiencies, but they can also carry **specific functions.**
Are these new functions associated with aphids ecological niche ?

Rates and patterns of evolution in bacterial endosymbionts:
Chimeric endosymbionts: HGT could favour symbiont replacement
Genomic shrinkage of symbionts occurs through slippage of DNA replication in homopolymers and large deletions in pseudogenes

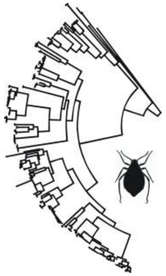


A fragile mono-symbiotic association between *Buchnera* and its host.
The establishement of a new symbiont could result from **« competition » between symbionts** that carry similar functions and/or **selection on the host** for a new function

Ongoing work :

- **Refine evolutionary history of associations in *Cinara***
- **Estimate rates of endosymbiont genome evolution using the calibrated phylogeny of the aphids**
- **Investigate symbiont localization and cell dynamics within aphids to better understand transmission / losses**
- **Comparative genomics of endosymbionts throughout Aphididae: investigate metabolic functions in co-symbionts**

My obligate symbionts



Alejandro Manzano-Marin (post-doc)

Armelle Cœur d'acier (aphid systematics)

Anne-Laure Clamens (Molecular biology/ collect)

GENOSCOPE
Centre National de Séquençage

Valerie Barbe
Céline Orvain
Corinne Cruaud

Andrea Sanchez-Meseguer (post-doc phylogeny)

M. Galan (Molecular biology)

Marianne Annonier (Master student)

Udem, Jesse Shapiro'Lab

Colin Favret, IRBV, Aphids

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