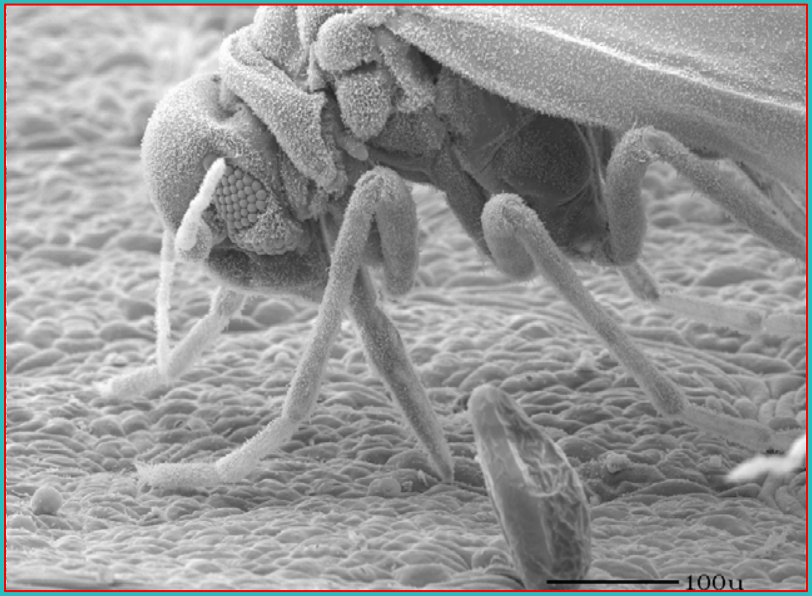
Origin of the Q biotype and global view of the genetics of the whitefly *Bemisia tabaci*

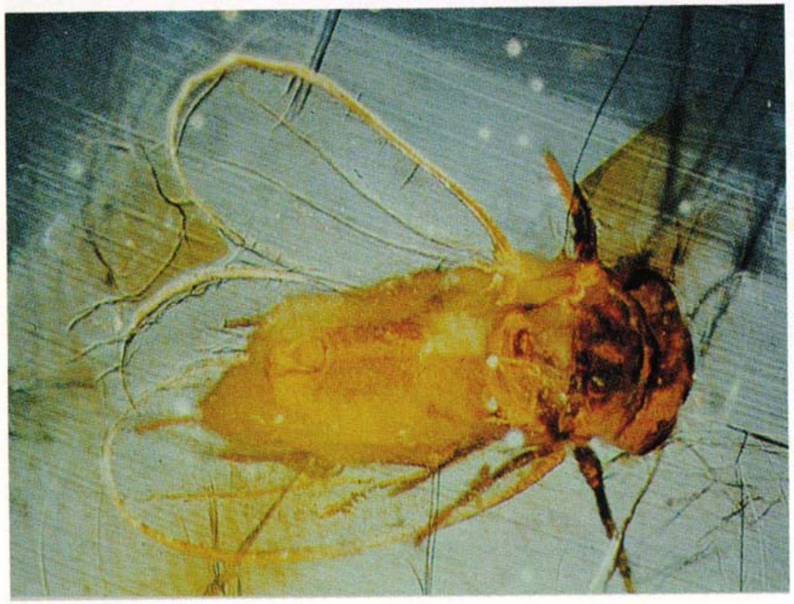
J.K. Brown
Department of Plant Sciences
The University of Arizona, Tucson



Bemisia tabaci Gennadius [Aleyrodidae]

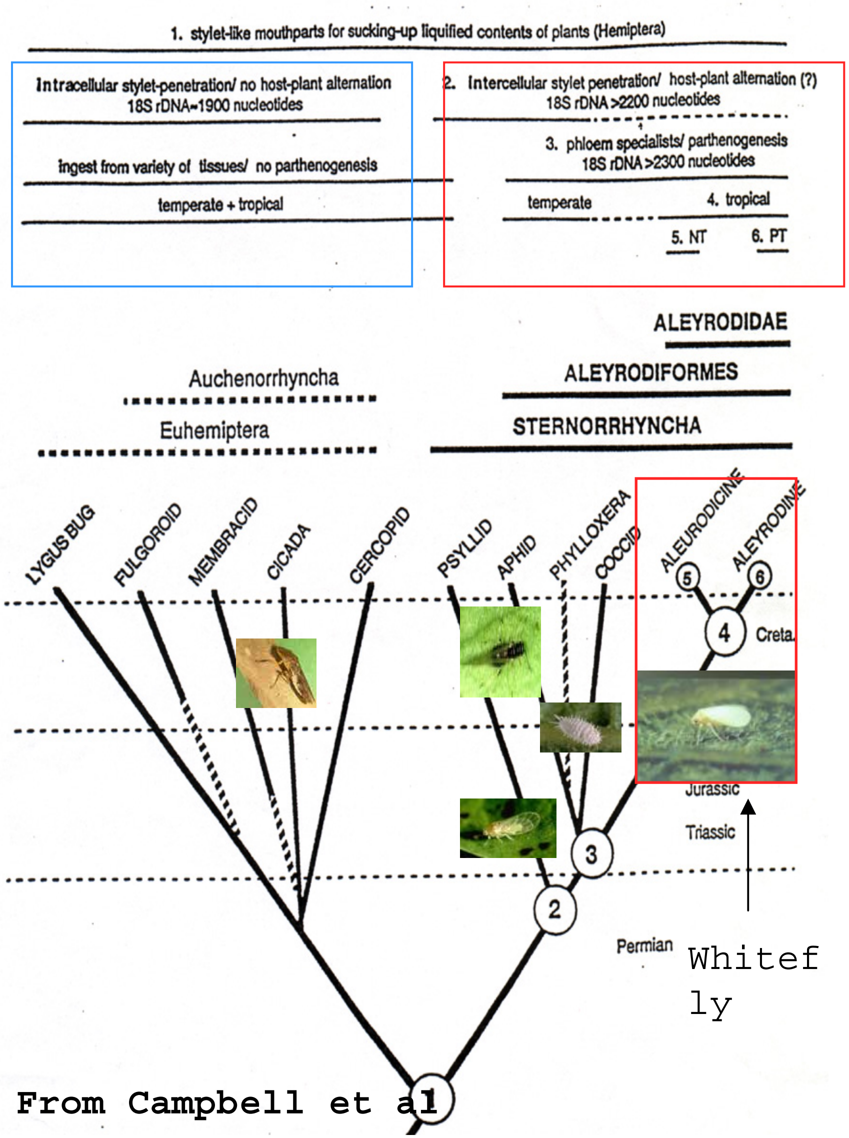
- Homopteran-tropical origin
- Phloem specialist

  intercellular stylet pene
- Haplodiploidy (2n female/1n m



Primitive whitefly *Bernaea neocomica* Schlee in Lebanese amber: 120 to130 MY BP

[Stuttgart Natural History Museum Collection]

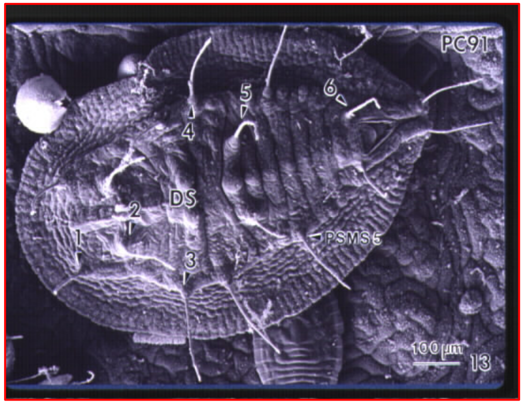


Whitefly

From Campbell et al

Adult

Pupal stage /4th Instar



Bemisia tabaci (Genn.)

- Worldwide, most important whitefly vector of plant viruses: irrigated cropping systems subtropics/tropics (local & invasive).
- Only whitefly vector of the genus: *Begomovirus.*
- Cryptic species = rich in biological variants-lacks distinguishing morphological (4th instar) characteristics.
- Synonymized from numerous species and variants by Russell, 1957 (morphological plasticity=host responsiveness [Mound; Basu and Mohanty, 1970's))
- Evidence for phenotypic (Bird, others 1950's) & genotypic variants.
- Species group/complex (A group of closely related species, usually w/ partially overlapping ranges; discontinuous gene flow) or group of species (no gene flow).

Concept of biologically distinct populations or 'races'

Now know - different behaviors influence outbreaks, virus spread, & diversification

Bird (1957) recognized Jatropha race was 'host-restricted'; Low fecundity

Sida race was polyphagous; moderate to high fecundity

Proposed 'race concept'

Jatropha

Costa and Russell (1975) *B. tabaci* - Brazil did not colonize cassava (center of origin)

Cassava-associated populations - Africa (Burban et al., 1991) (transmitted ACMV, others)

= added to Bird's' race' hypothesis

Dr. Julio Bird

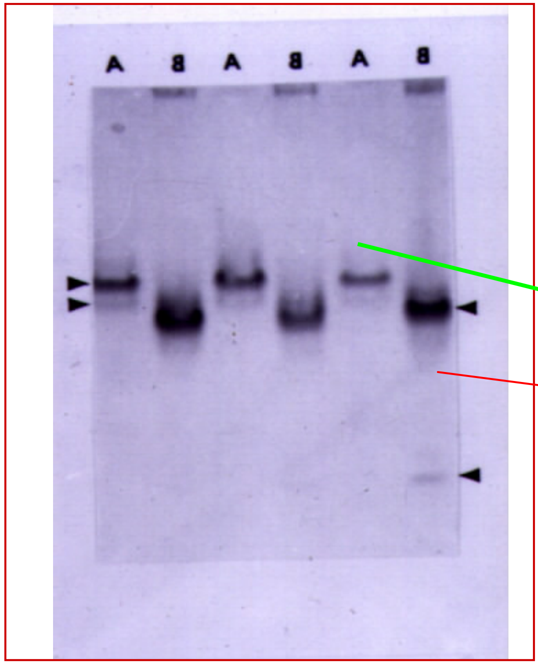
AZ-A and B biotypes recognized after introduction of B biotype 1987-88 (Costa & Brown, 1990)

Sida

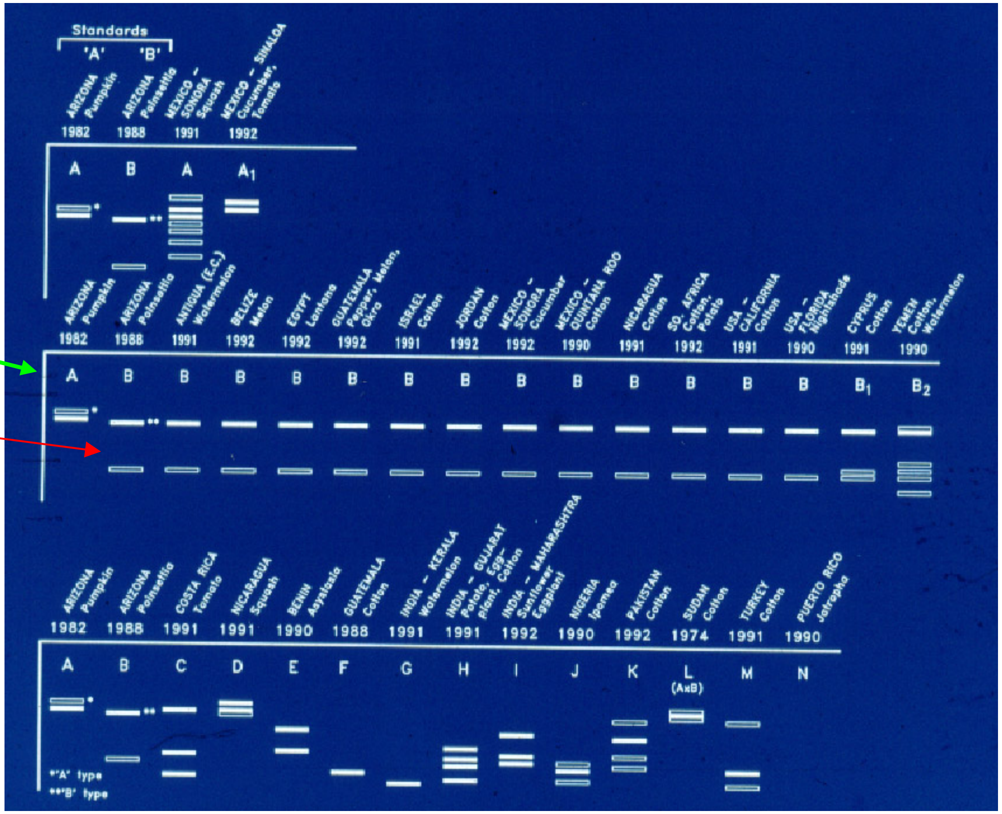
First evidence for genetic polymorphisms

(Burban et al., 1992; Costa and Brown, 1991-USA; Wool et al., 1989-Israel-Colombia)

- B biotype introduced to US & Caribbean, Central & So America & worldwide;
- Polyphagous, high fecundity, insecticide resistant; could transmit NW viruses
- Esterase patterns revealed genetic polymorphism b/t local ‘A type’ and invasive ‘B’ type + SSL symptoms in *Cucurbita* spp.
- Then … genetically diverse worldwide populations



Protein polymorphisms



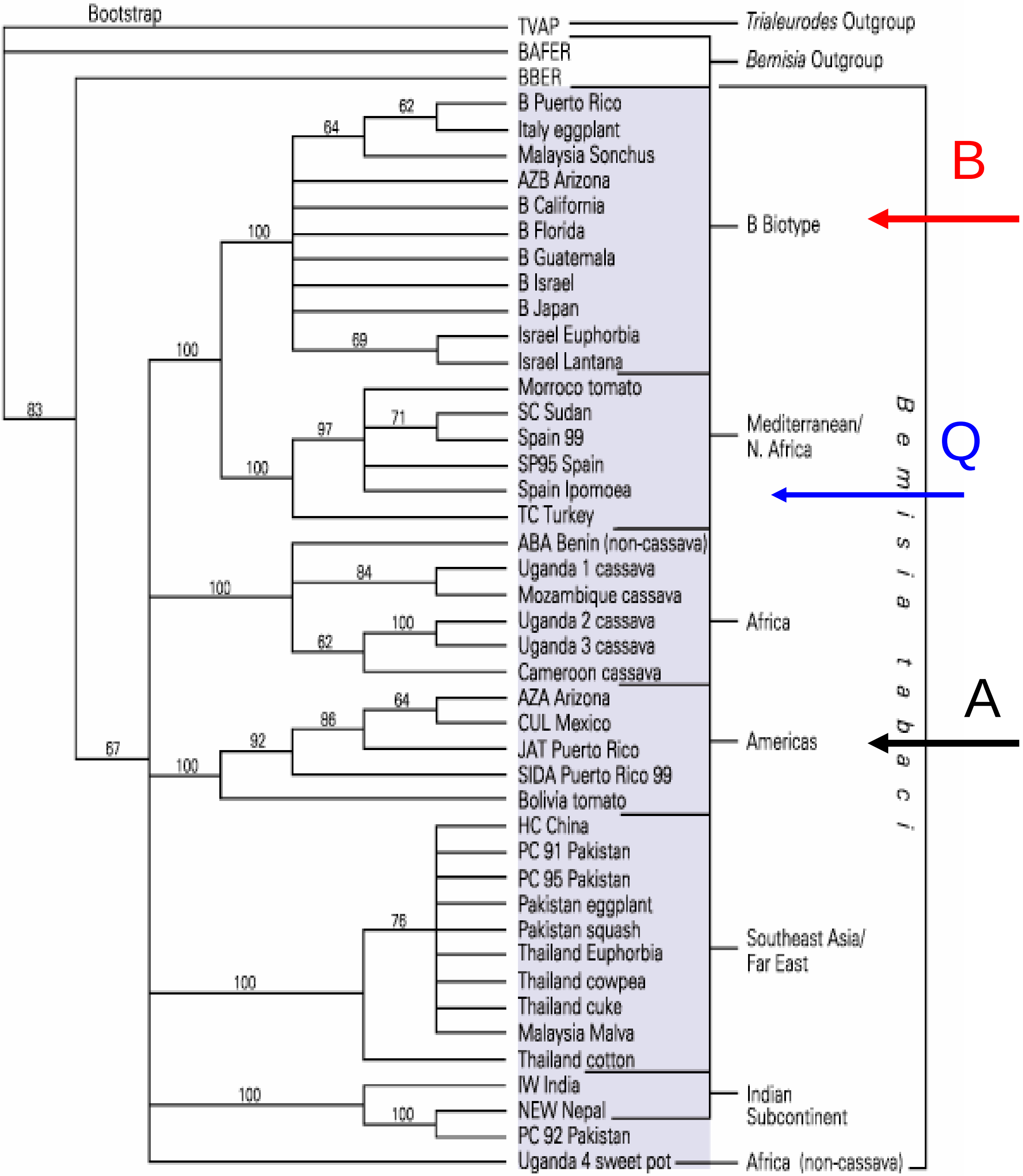
MT COI
sequence analysis

-- Seven+ phylogeographical groups

-- B biotype-Old World; A-New World

Hypotheses

***B. tabaci* is a species group .. or**
a number of separate species?
(Gene flow?)

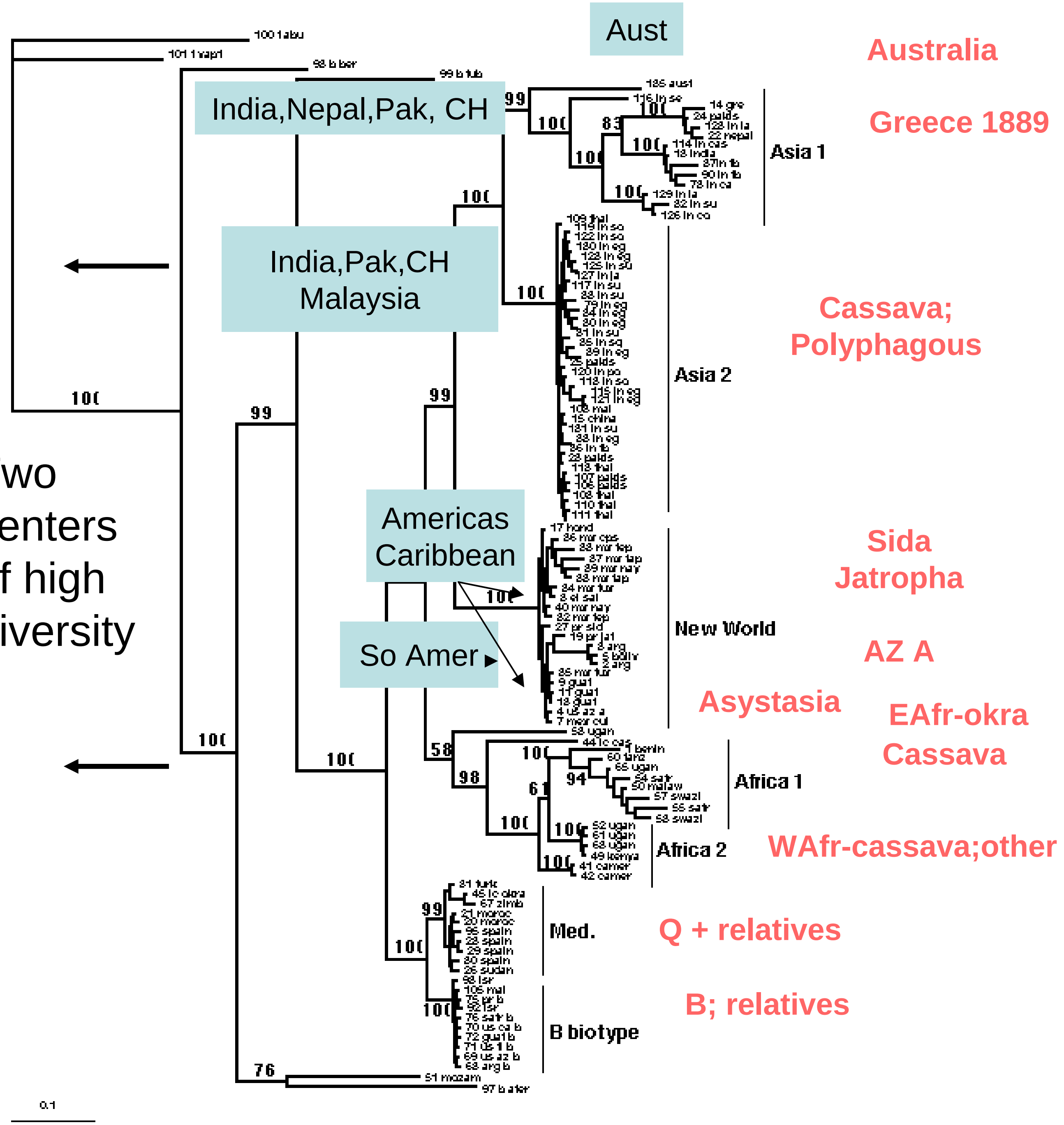


More populations worldwide

Haplotypes of the *B. tabaci* numerous

Distributed phylogeo-graphically,
(except extant introduced species)

Two centers of high diversity



Aust

India,Nepal,Pak, CH

India,Pak,CH
Malaysia

Americas
Caribbean

So Amer

Australia

Greece 1889

Cassava;
Polyphagous

Sida
Jatropha

AZ A

Asystasia

EAfr-okra
Cassava

WAfr-cassava;other

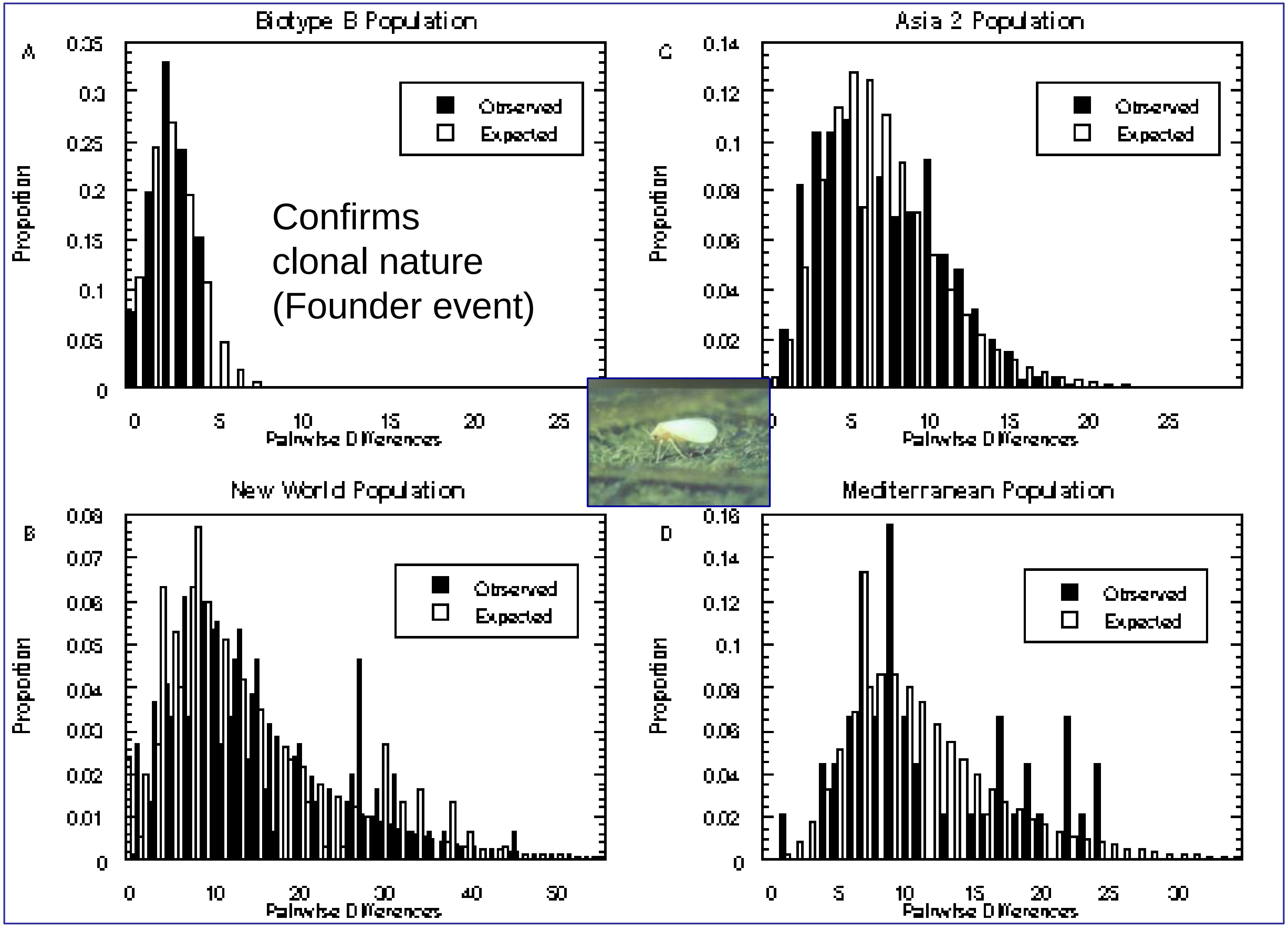
Q + relatives

B; relatives

Between clade comparisons

Clade	**% nt identity**	**% nt divergence**
New World **n = 8**	91.4 - 99.0	1.0 - 8.6
Indian Subcontinent/ Far East **n = 7**	82.8 - 98.6	1.4 - 17.2
N. Africa- Middle East- Mediterranean Region **n = 13**	81.9 - 97.2	2.8 - 18.1
Sub-Saharan Africa (w/o ABA, IC cassava) **n = 42**	88.9 - 99.7	0.3 - 11.1
Sub-Saharan Africa **N = 44**	80.3 - 99.7	0.3 - 19.7
Outgroups		
T. vaporariorum* x All *B. tabaci	71.3 - 76.5	23.5 - 28.7
B. afer* x All *B. tabaci	71.6	28.4

Population growth curves (Rogers,1997) **based on pair wise differences suggest different population histories**



Confirms
clonal nature
(Founder event)

(Brown & French,in preparation)

Greece 1889 holotype (upsurgent local or invasive population?)

Analysis of mtCOI sequences for the Greece population indicated it clustered with a Asia I (specifically, India)

Suggests that it originated in India? from where it was introduced to Greece as an 'invasive species'. [Note: the Italian T biotype has affinity with Indian clade)

Historical Notes

Origin of the B biotype (N. Africa/Medit/MidEast clade)

The B biotype traceable to the Sahel region of E Africa: Sudan, Ethiopia, Eretria, Uganda (arid, seasonal rainfall/food;dispersal phenotype). Transported to Israel 1970's (SSL, 1975); resistance to pyrethroids Transported on ornamentals to Europe, Americas/Caribbean, and worldwide.Named as a separate species (*B. argentifolii*); but it constitutes one variant!

Origin of the Q biotype (2005 introduced into US, Guat, MEX, Japan,China) Mediterranean clade; Resistance to neonicotinoids widely used to control the 'B'; Q regained predominance in Spain - resistance to nn/ what will be outcome in US? [not adapted to arid zones?]

A number of biotypes (~20) are now recognized based on behavorial & genetic differences

Host, geographical, environmental specialization

- -Geographic range: limited geographical/host range, widely distributed
- -Host range: monophagous, narrow, highly polyphagous

Vector Competency

- -Variation in transmission competency suggests virus-vector co-adaptation (concordant phylogeography viral CP/mtCOI)

Other Biotic Characters

- -Dispersal behavior: short and long distance
- -Insecticide resistance - highly variable and plastic
- -Fecundity: ~50 to 300+ offspring

Molecular studies

- -Highly divergent >24% mt COI (mt16S, ITS1-similar groups)
- -Phylogeographical distribution
- -Identical primary symbiont (homopteran paradigm-single infection/ species)

Unanswered Questions

What will the next invasive biotype be and why? Can we predict this?

What is the underlying mechanism driving biotype formation, invasiveness, adaptability, fitness?

When does displacement occur versus sympatric populations?

How important are insecticides to selection?

How important is hybridization to invasiveness, fitness?

Are hybrids more fit than parents?

Is *B. tabaci* a group of species or a species group?

If so, between clades or within clades?

What are the ‘species’ barriers?

Summary of Reciprocal Cross Experments

AZ-A X CUL	Females, bidirectional (% like AZ-A)	
AZ-A X RiverA	Females, bidirectional (“ “)	
AZ-A x JAT	Female, bidirectional (lower % than A)	Caballero et al., 2004
AZ-A x AZ-B	No females, bidirectional	Bedford et al., 1994
	No females, bidirectional	Perring et al., 1993
	Females, unidirectional (B-m x A-f)	Costa et al., 1993
	Females, unidirectional (B-m x A-f)	Caballero et al., 2004
B x AUS EAN	No females, bidirectional	
B x AUS WAN	Few females, bidirectional	
EAN X WAN	Females, bidirectional (lower % vs. homol)	De Barro & Hart, 2000
B x JAT	Females, unidirectional	Caballero et al., 2004
B x Q-Spain	Low-level females, bidirectional	Adan et al., 1999
B x Q-Israel	No females, bidirectional	Horowitz et al., in prep
B x SUD	Rare female, unidirectional	Byrne et al., 1995
INDCassxUGCass	No females, bidirectional	Maruthi & Colvin, 2001
UGEastxUGWest	Females, bidirectional	Maruthi & Colvin, 2001

SUMMARY

Evidence for impeded gene flow is incomplete

Little standardization of experimental conditions, including host plant

Single vs. 5 pairs vs. 10 pairs (pheromones?)

Difficult experiments to conduct (biologically, regulatory)

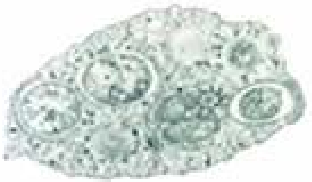
Unavailability of representative colonies

A, B, Jat are compatible (Africa-Sahel; New World); others produce females at low frequencies, or not at all unless same host and/or same extant origin.

Some exhibit unidirectional incompatibility (CI hypothesis?)

Species complex or group is probably still the best term to describe this cryptic whitefly species.

Hypothesis
Role of CI-inducing bacteria?



I. *Wolbachia* is widespread in arthropods (Werren 1997) (single and double infection)

<u>Reproductive isolation</u> -Transovarial (vertical) transmission
Feminization of males
Parthenogenesis
Male killing
Sex ration distortion
Rapid spread of bacteria through population
Short term fitness - increases competitiveness
Haplodiploid insects: females from fertilized eggs/males from unfertilized-so female killing

<u>Mechanism</u>(s)- Unknown but evidence for post-fertilization mode of action.
Bacterial modification of sperm in testes; cell division is inhibited-mortality.
Bacterial ‘rescue’ in the fertilized egg (same strain) is known.

II. Cytophaga-Flavobacterium-Bacteroides (CFB) Group identified (TEM, PCR)

Ixodes scapularis Kurtti *et al.* (1996) - no function known

Brevipalpus phoenicis Weeks *et al*. (2001) -no function

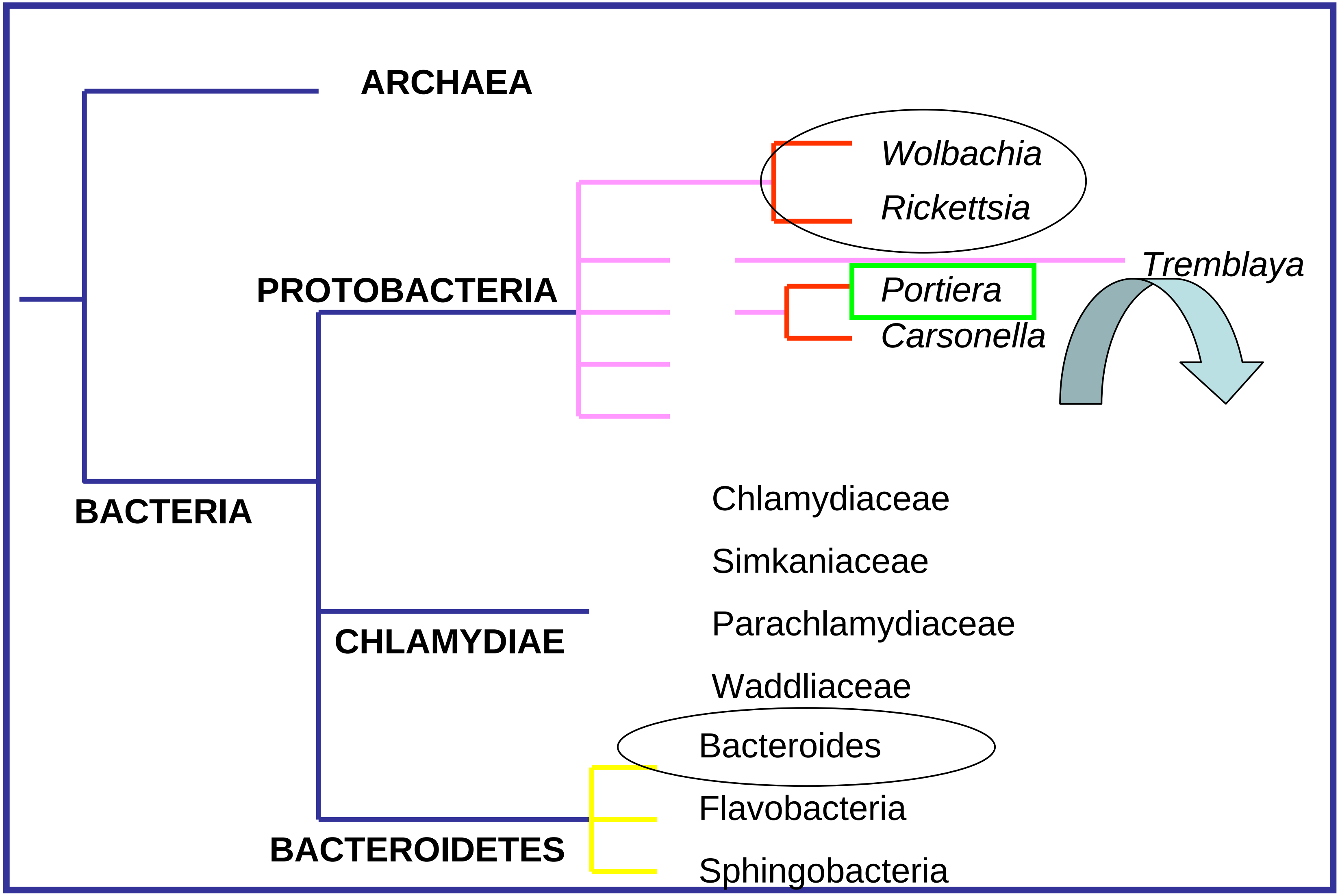
B. tabaci Weeks (2002) -no function

Encarsia bacterium (EB) Zchori-Fein *et al.* (2003)

Encarsia w/ EB: parthenogenesis Hunter *et al* (2004)

Candidatus Cardinium spp.(2005) Bacteriodetes Phylum

CLASSIFICATION



ARCHAEA

PROTOBACTERIA

Wolbachia

Rickettsia

Tremblaya

Portiera

Carsonella

BACTERIA

CHLAMYDIAE

Chlamydiaceae

Simkaniaceae

Parachlamydiaceae

Waddliaceae

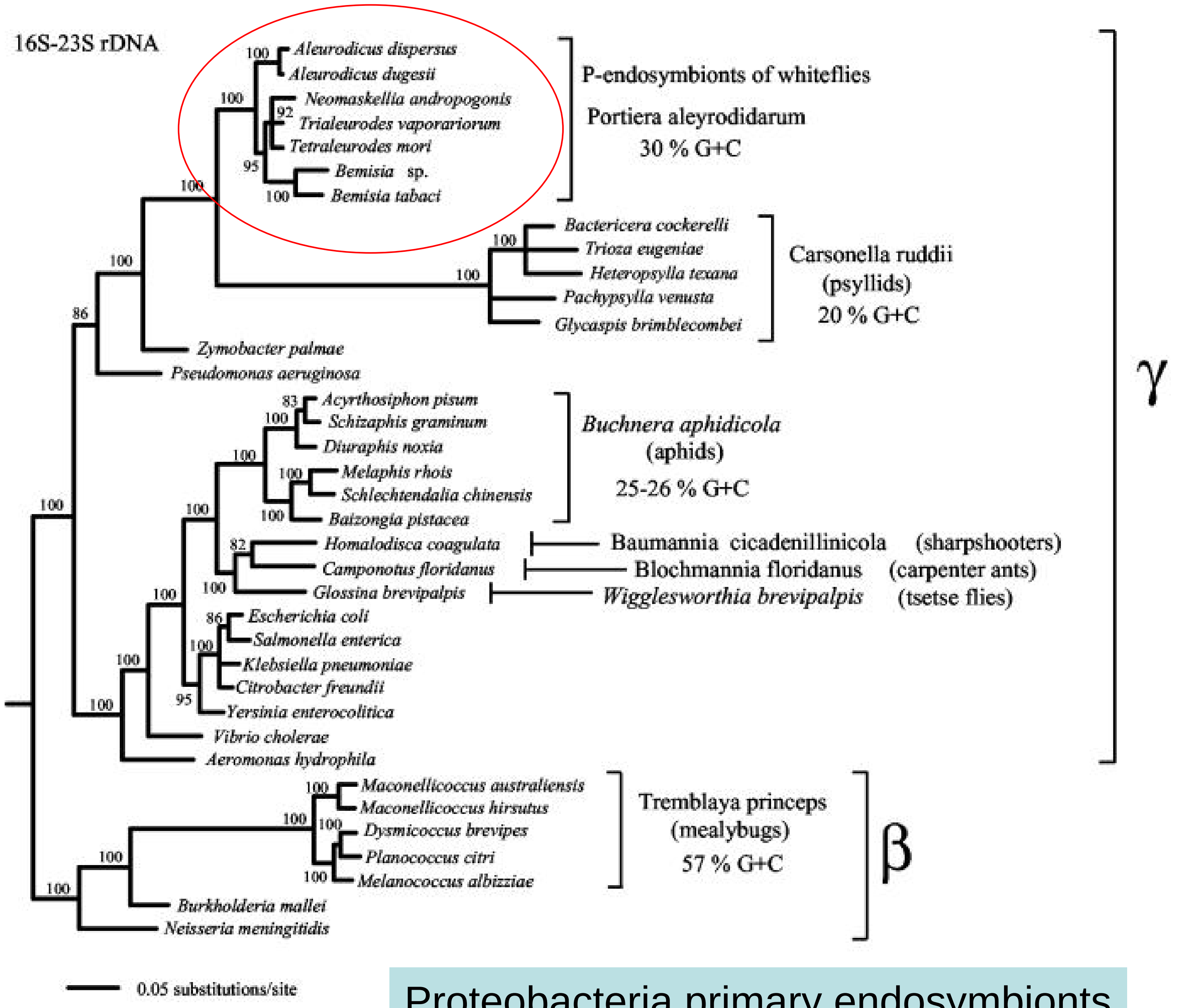
BACTEROIDETES

Bacteroides

Flavobacteria

Sphingobacteria

(Garrity & Holt 2001)

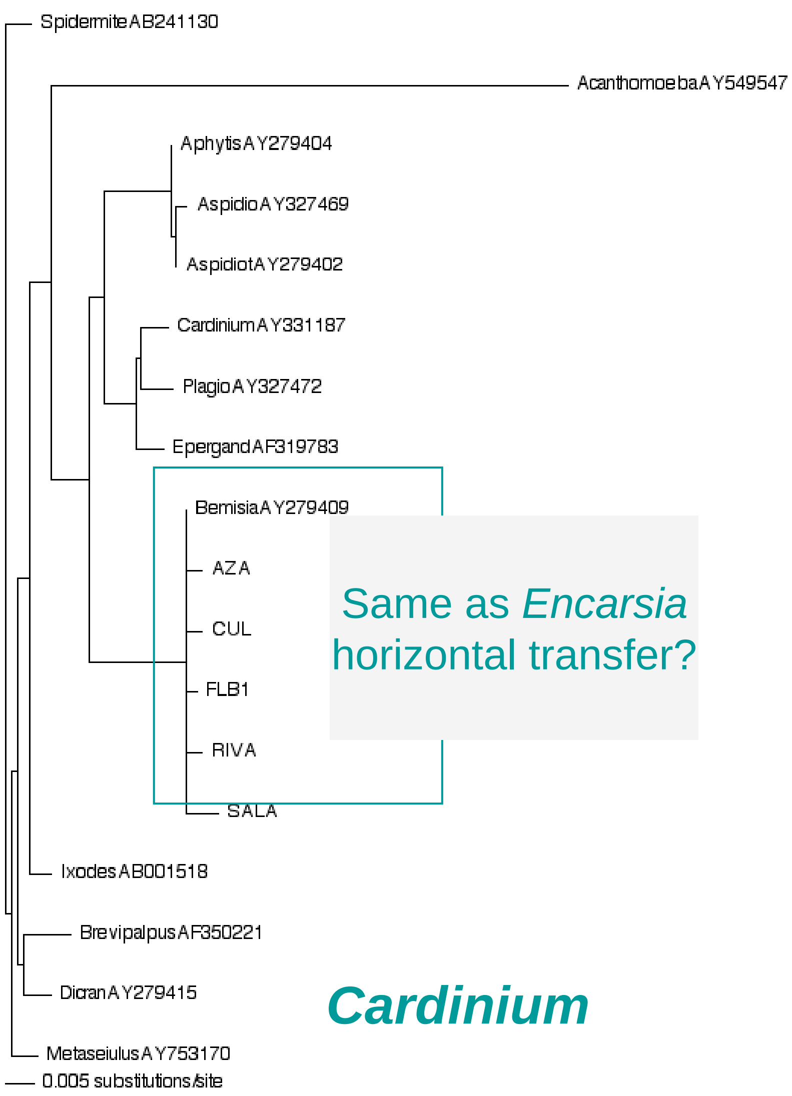


Proteobacteria primary endosymbionts

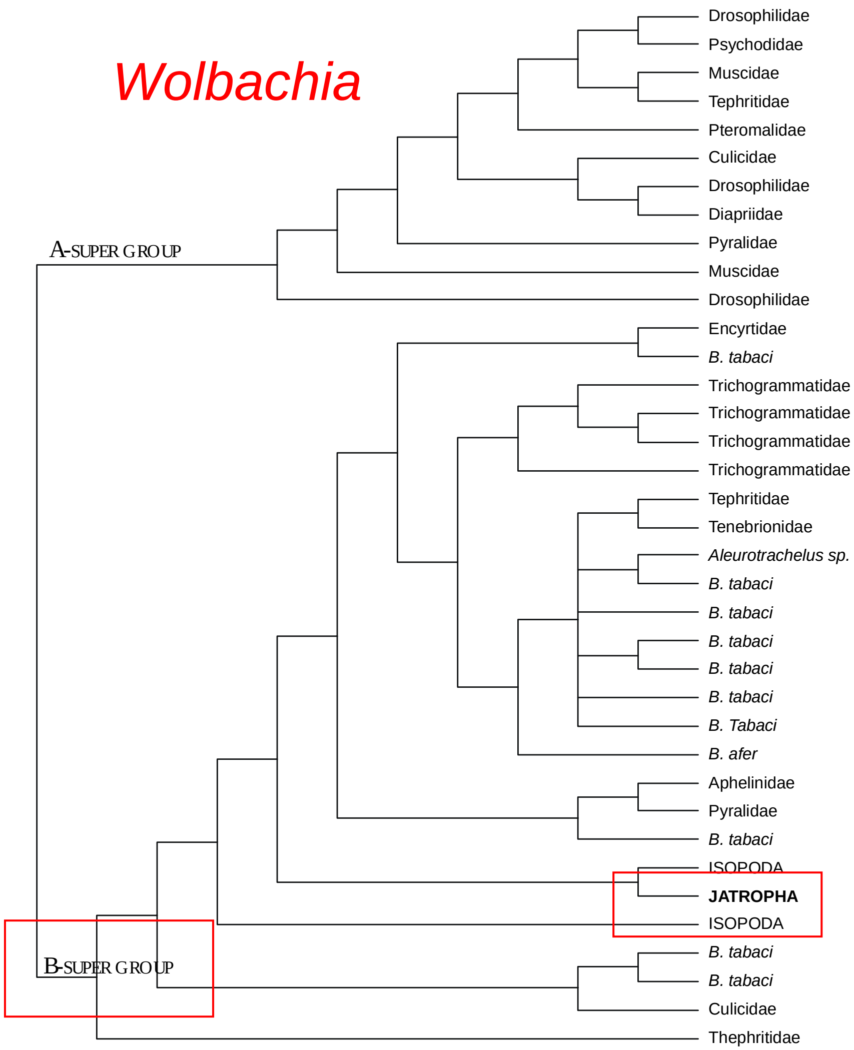
Reproductive patterns caused by CI bacteria between uninfected and infected populations

MALE	FEMALE	
	UNINFECTED	INFECTED
UNINFECTED	UNINFECTED PROGENY	INFECTED PROGENY
INFECTED	NO PROGENY	INFECTED PROGENY

16S rDNA bacteria identified in AZ-A and JAT; not in AZ-B



Same as *Encarsia* horizontal transfer?



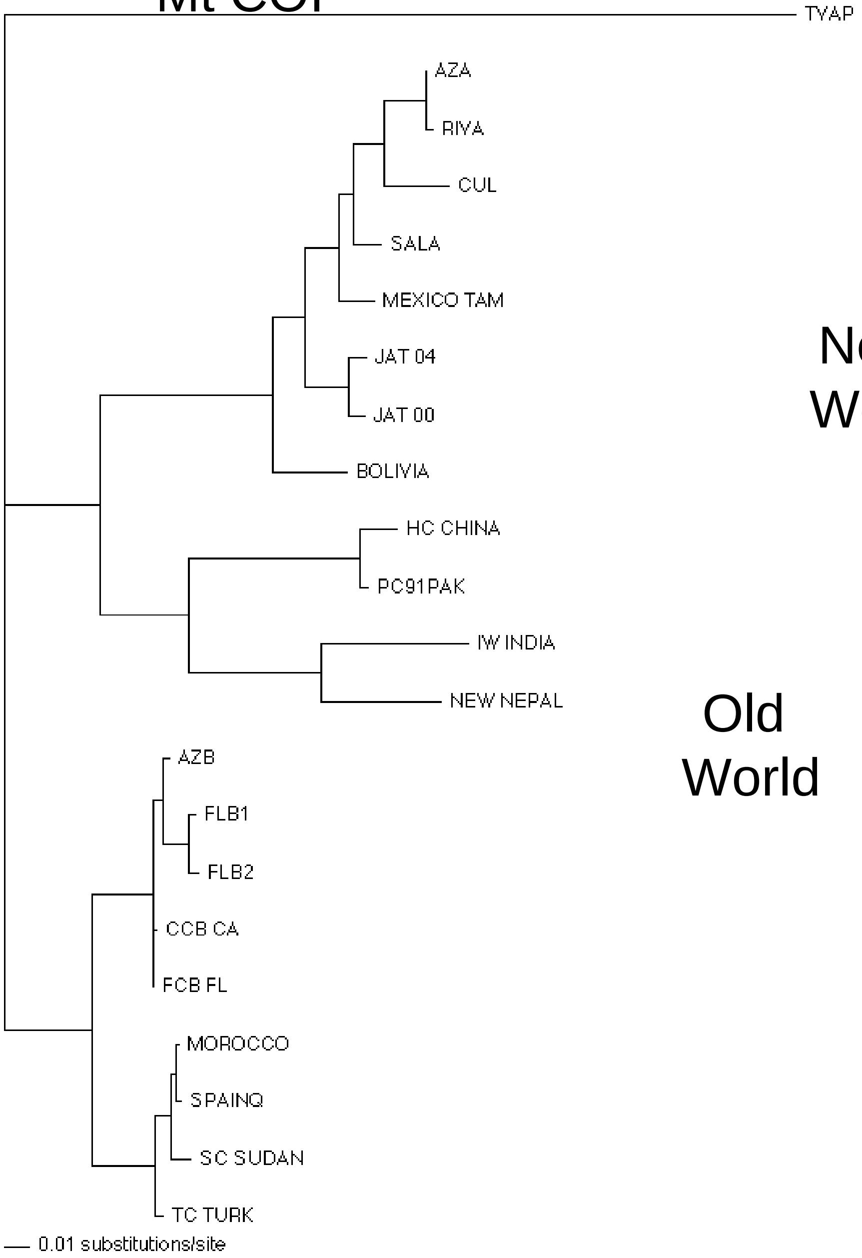
Jat harbors unique Wolb- monophagous?

OFFSPRING PRODUCED IN CROSSES IN GROUPS OF 20 FEMALES AND 20 MALES

GENETIC CROSS	OFFSPRING			
	FEMALE	MALE	TOTAL	RATIO
AzB Female x AzA Male	0	1213	1213	0: 1213
AzB Female x Jat Male	0	158	158	0: 158
AzA Female x AzB Male	84	886	970	1: 10.5
AzA Female x Jat Male	335	457	792	1: 1.4
Jat Female x AzB Male	64	189	253	1: 2.9
Jat Female x AzA Male	51	279	330	1: 5.5
AzB Female x AzB Male	447	565	1012	1: 1.3
AzA Female x AzA Male	99	163	262	1: 1.6
Jat Female x Jat Male	88	128	216	1: 1.5

Pedigree Analysis

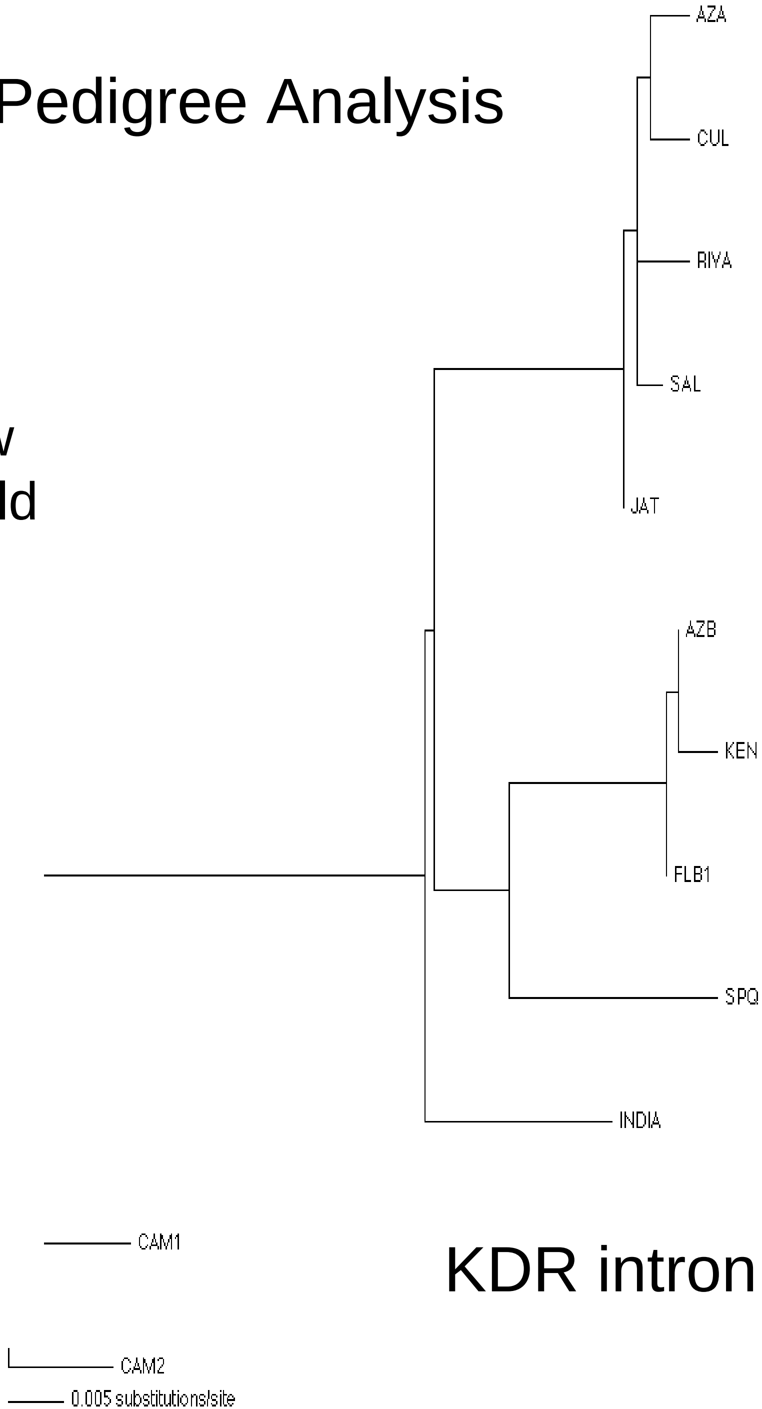
Mt COI



New
World

Old
World

KDR intron



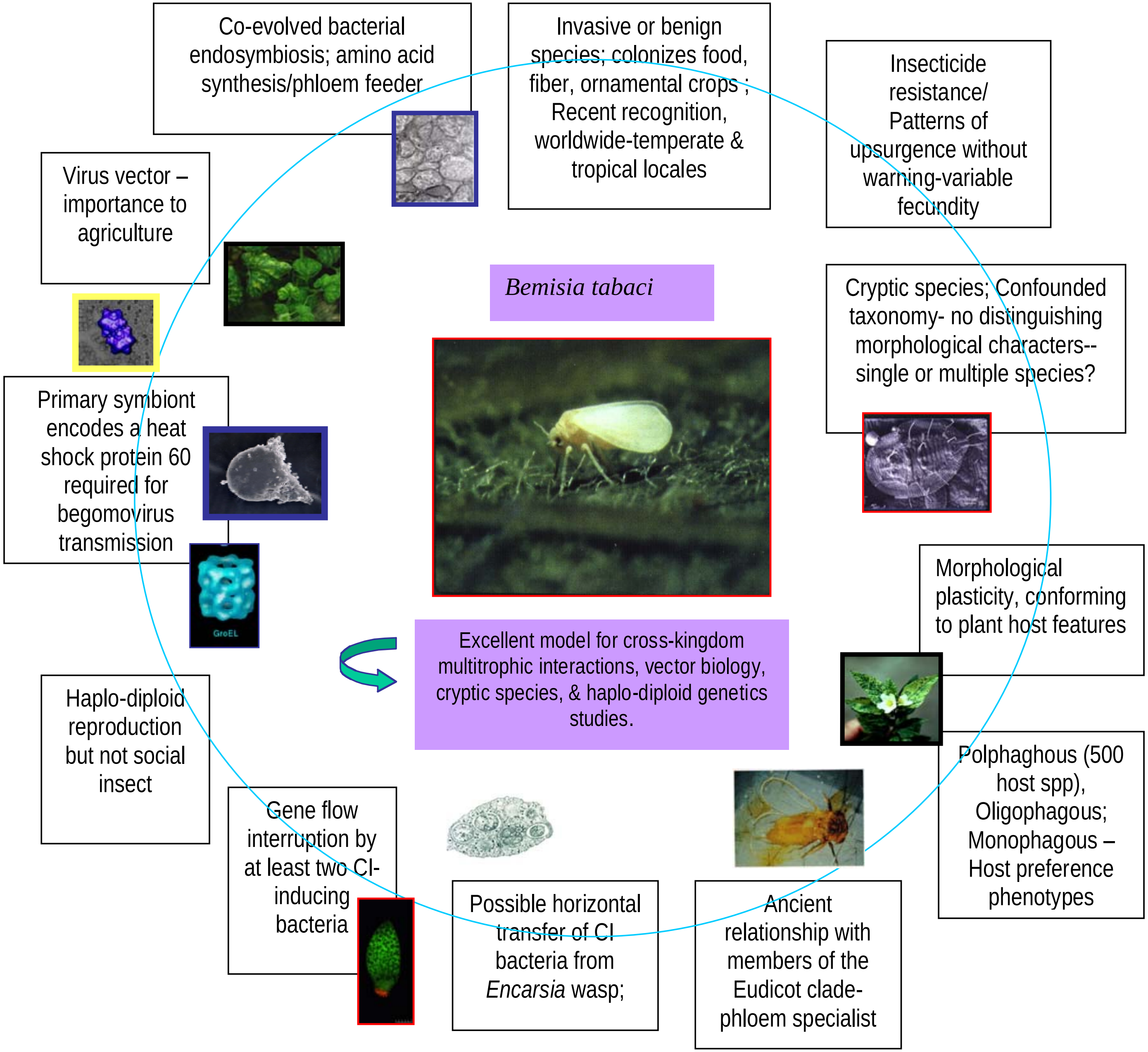
Summary

- Association of *Wolbachia* with Jat but not AZB
    - Jat x AZB-poin88 crosses=unidirectional gene flow
- Association of *Cardinium* with AZ-A
    - AZA x AZB-poin88 crosses = unidirectional gene flow
- Jat x AZA yielded bidirectional female offspring production
- Divergent (different phyla) CI-inducers should kill females;
    - Sex ratio bias indicative of rescue phenomenon
- Unprecedented - in *Wolbachia*, rescue is by related strains

- Gene flow between geographical- & host preference-isolated biotypes -- monophag vs polyphag & NW vs OW; provides support for species group or complex --not separate species.
- Interactions involving CI-bacteria = uni/bidirectional gene flow
- Results have important implications to the underlying basis for ‘invasive’ and other phenotypes, for which this species is notable.

Future Work

Implications of CI to species concept/speciation?
Implications to *B.tabaci* host range polymorphisms?
Host specialization-imposed isolation?
Implications to natural enemy- *B.tabaci* dynamics
Sex ratio and population dynamics
Cryptic species status
Status of important vector populations/biotypes
related to CI bacteria associations?
Understanding CI-mechanism - mobilize genes in populations?
How do biotypes form and why are they successful or not?
What constitutes invasiveness?
Underscores need for additional biological studies; link genomics
and functional genomics approaches



Bemisia tabaci

Excellent model for cross-kingdom multitrophic interactions, vector biology, cryptic species, & haplo-diploid genetics studies.

Co-evolved bacterial endosymbiosis; amino acid synthesis/phloem feeder

Invasive or benign species; colonizes food, fiber, ornamental crops ; Recent recognition, worldwide-temperate & tropical locales

Insecticide resistance/ Patterns of upsurgence without warning-variable fecundity

Cryptic species; Confounded taxonomy- no distinguishing morphological characters--single or multiple species?

Morphological plasticity, conforming to plant host features

Polphaghous (500 host spp), Oligophagous; Monophagous – Host preference phenotypes

Ancient relationship with members of the Eudicot clade- phloem specialist

Possible horizontal transfer of CI bacteria from *Encarsia* wasp;

Gene flow interruption by at least two CI-inducing bacteria

Haplo-diploid reproduction but not social insect

Primary symbiont encodes a heat shock protein 60 required for begomovirus transmission

Virus vector – importance to agriculture

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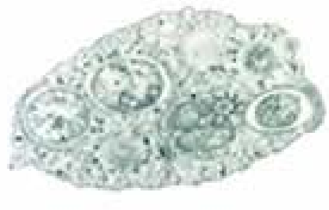
AZ LAB Colonies



Biotype	Geographic Origin	Host Plant	Year	Host Range	CI-inducing bacteria
AZA NW	Brawlee, CA	Cotton	1987	Moderate	*Cardinium*
Culiacan A NW	Culiacan,Mex	Cucumber	1993	Moderate	*Cardinium*
Salinas A NW	Salinas, CA	Lettuce	1977	Moderate	*Cardinium*
Jatropha NW*	Puerto Rico	*Jatropha*	1990	Monophagous	*Wolbachia*
AZB OW**	Tucson, AZ	Poinsettia	1988	Broad	Uninfected
FLB1 OW**	Apopka,FL	Tomato	1996	Broad	*Cardinium*
FLB2 OW**	Gainesville, FL	Tomato	1996	Broad	Uninfected

*2001-*Chlamydia* ; *2005 *Rickettsia bellii*

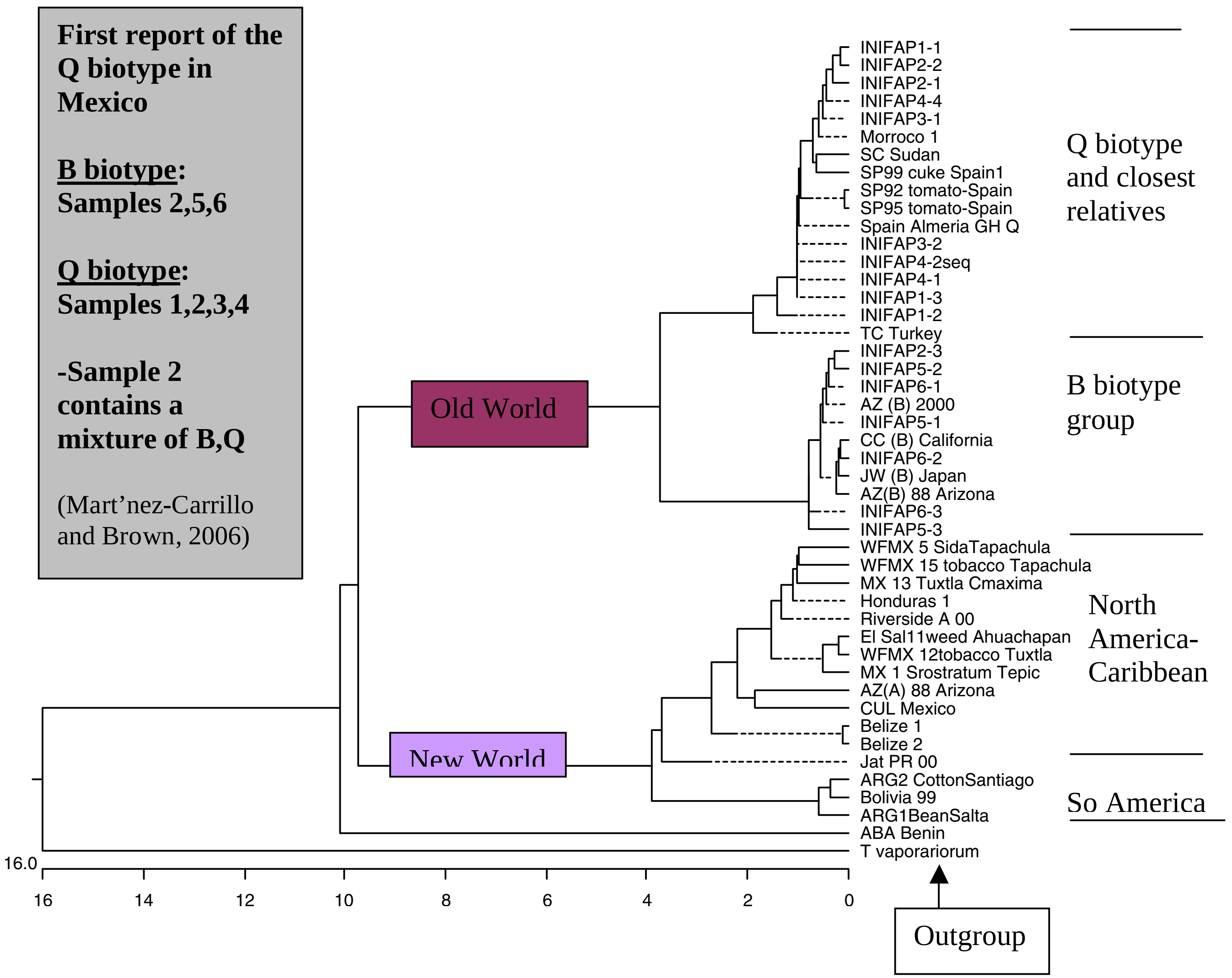
First report of the Q biotype in Mexico

B biotype:
Samples 2,5,6

Q biotype:
Samples 1,2,3,4

-Sample 2 contains a mixture of B,Q

(Mart’nez-Carrillo and Brown, 2006)



Mitochondria cytochrome oxidase I tree (750 bp) for *Bemisia tabaci* samples [INIFAP1-6] collected in Sonora, Mexico during December 2005 (Clustal W, DNASTAR, Lasergene, Madison WI). Reference sequences were taken from the Arizona whitefly mtCOI database. The outgroup is the greenhouse whitefly *Trialeurodes vaporariorum*.