

Population genetics and transcriptomics of Manila clam (*Ruditapes philippinarum*) and carpet-shell clam (*R. decussatus*): implications for aquaculture

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Ruditapes philippinarum



Production

World: 3,9 Million T

Europe: 30,000 T

R. decussatus

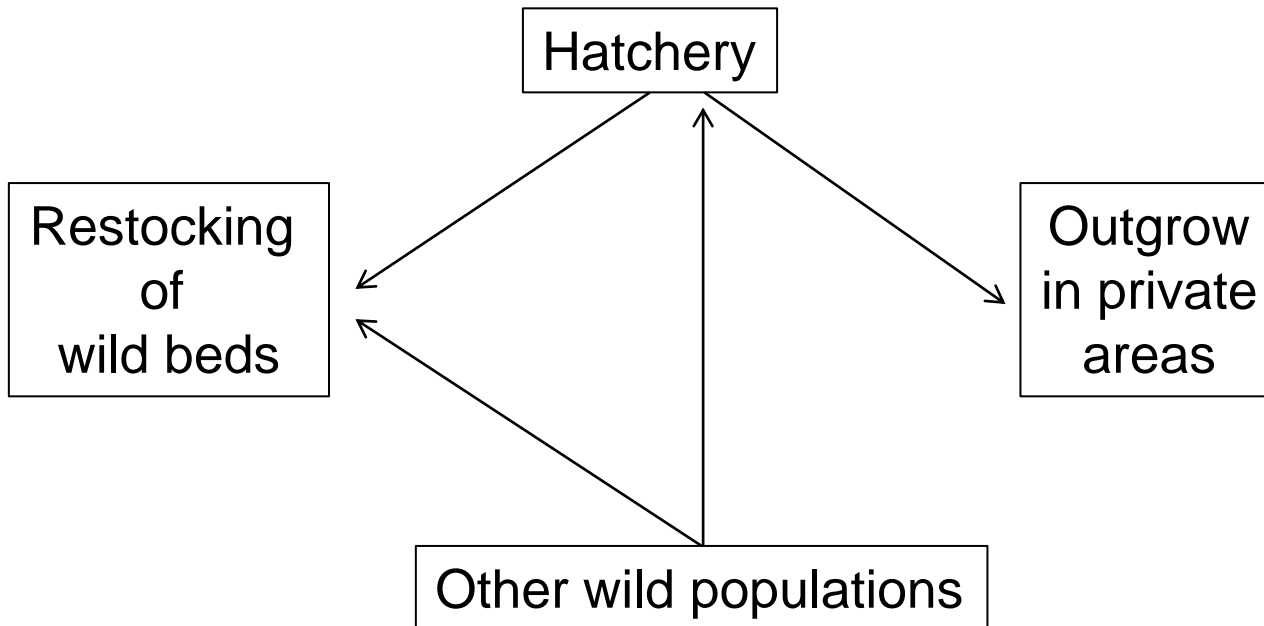


Production:

Portugal, Italy, Spain, France

5,200 T

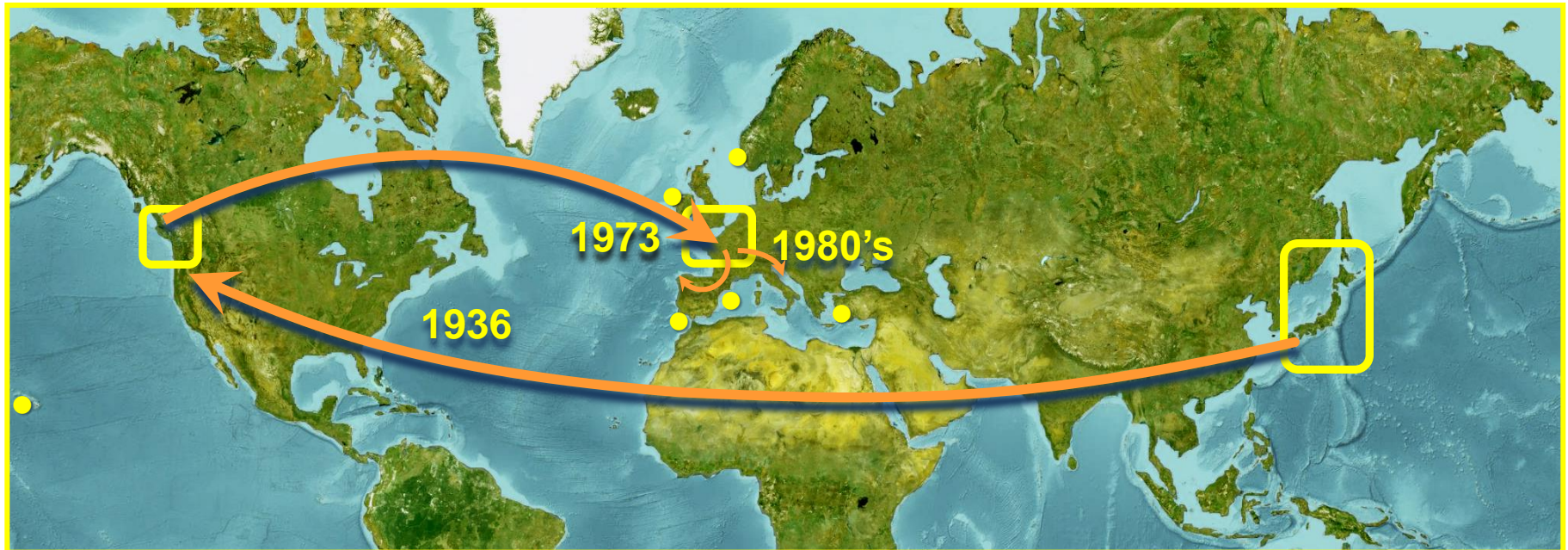




Population Genetic Studies in Clams

- Introduction of animals from other regions and countries has taken place
- Supplementary hatchery seed can affect genetic variability of populations at local scales
- We carried out population genetic studies of the 2 clam species with genetic markers (mitochondrial and nuclear)

Manila Clam Introduction in Europe



Sources: Quayle 1964; Flassch and Leborgne 1983; Ponurovsky and Yakovlev 1992; ; Breber 2002; Campos and Cachola 2006; Herbert et al 2012



Coxi

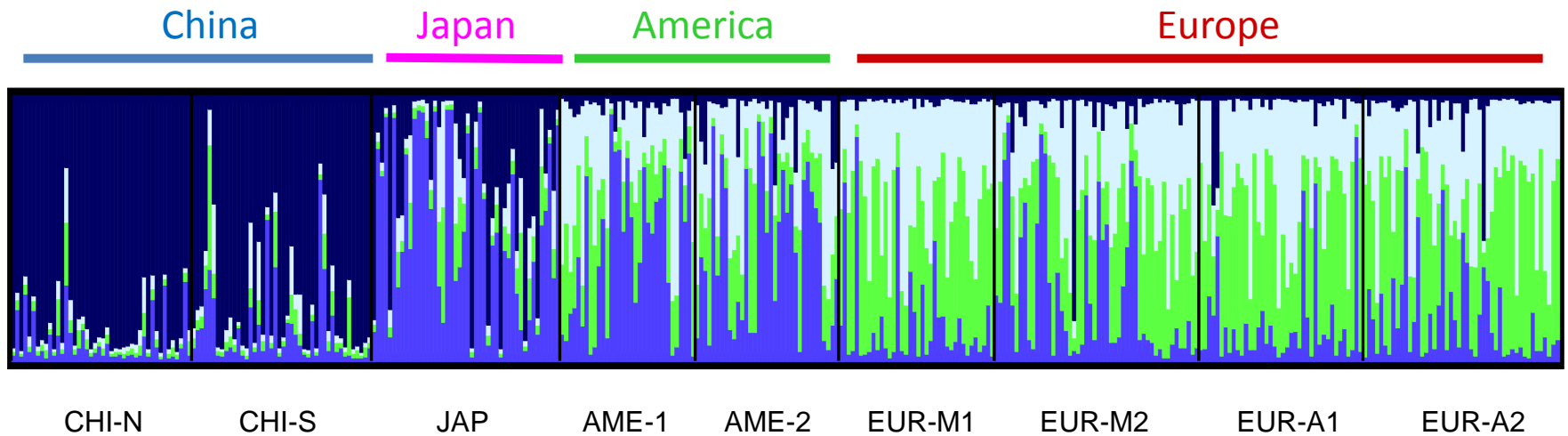
- Clade A
- Clade B
- Clade C

Sekine et al 2007
 Mao et al 2011
 Cordero et al, unpublished

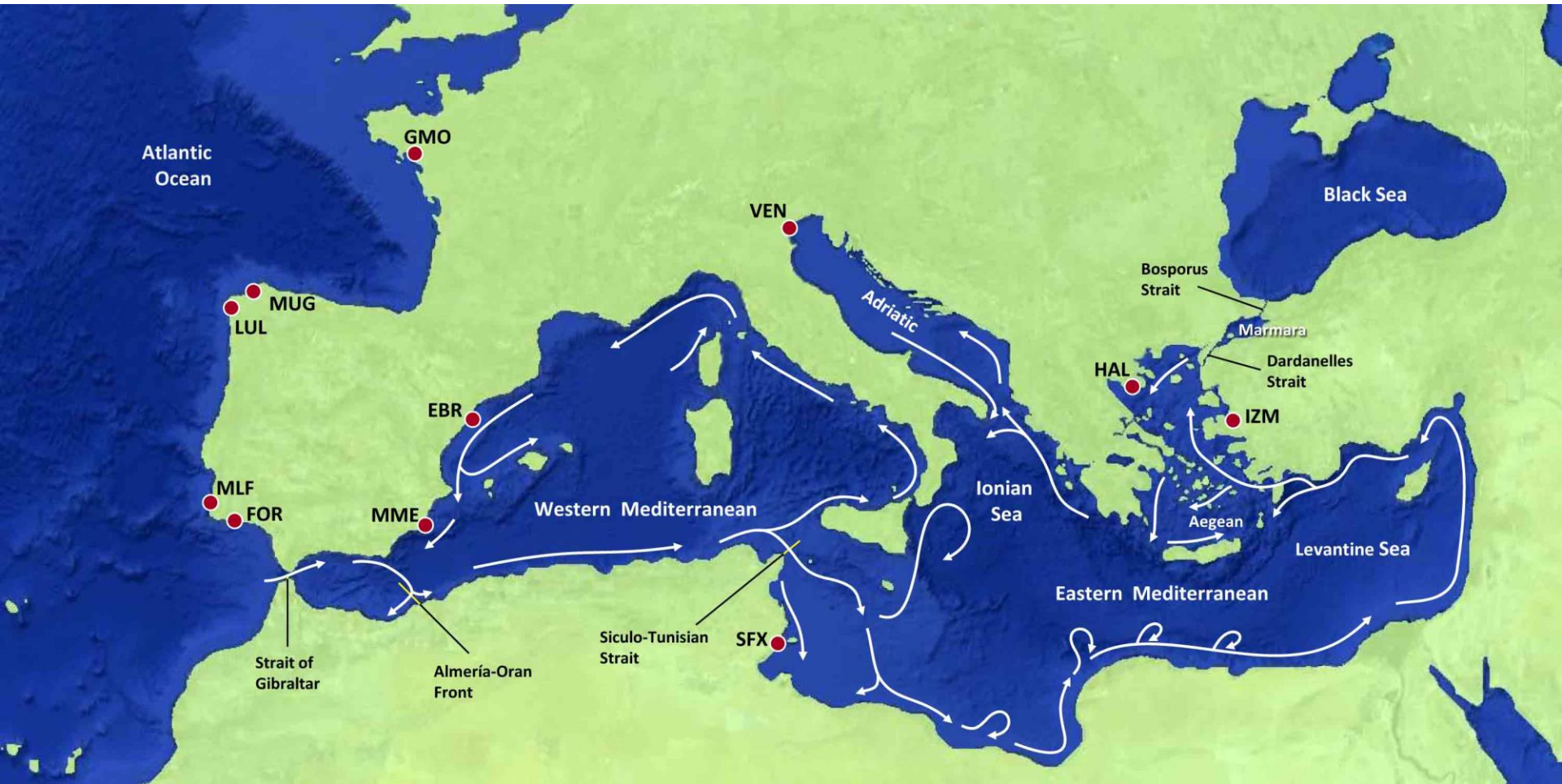
■ 555 bp
 ■ 335 samples

Manila clam – 7 Microsatellites

Bayesian STRUCTURE Analysis – 378 samples



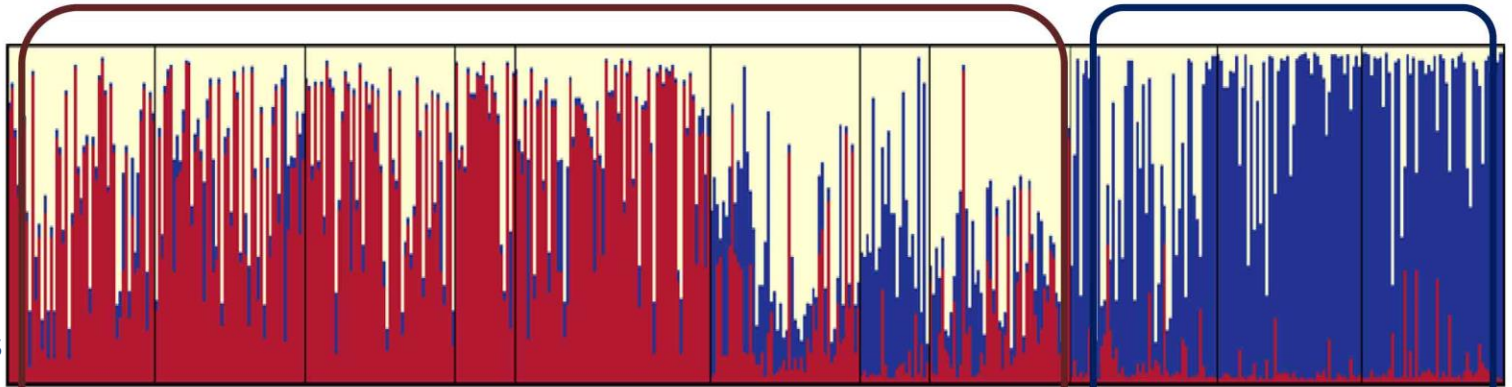
Cordero et al, unpublished



Carpet-shell clam

Introns (6)

450 bp, 311 samples



GMO

MUG

LUL

MLF

FOR

MME

EBR

SFX

VEN

HAL

IZM

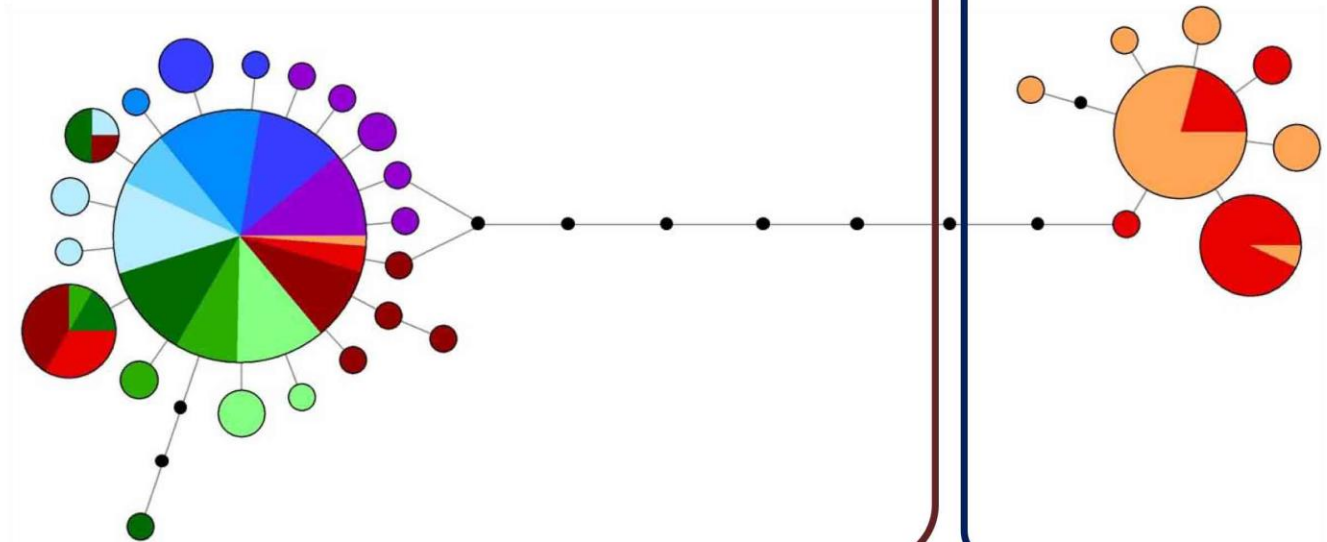
ATL

WMED

AEGAD

mtDNA (COI)

450 bp, 311 samples



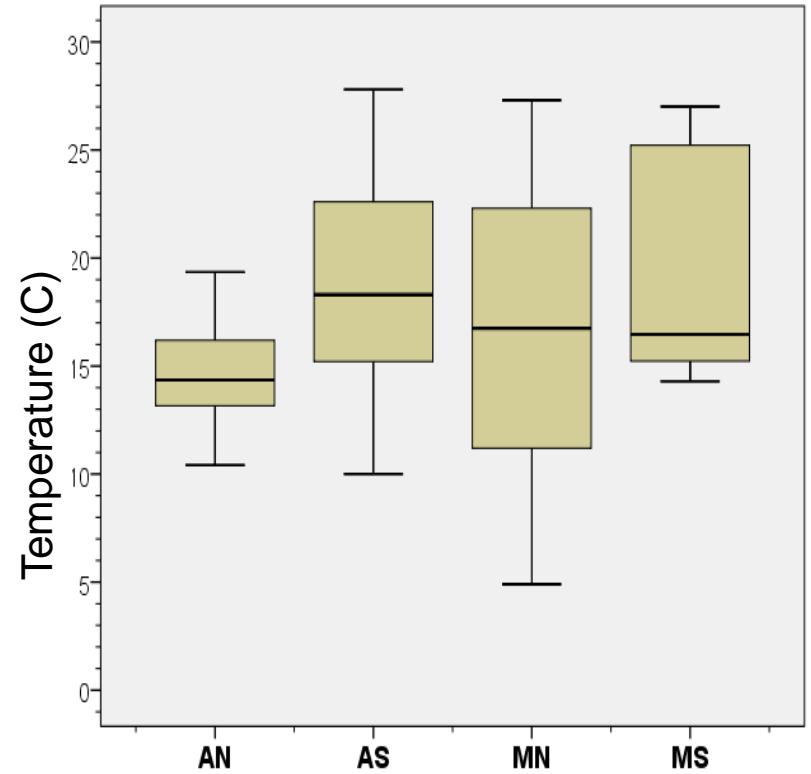
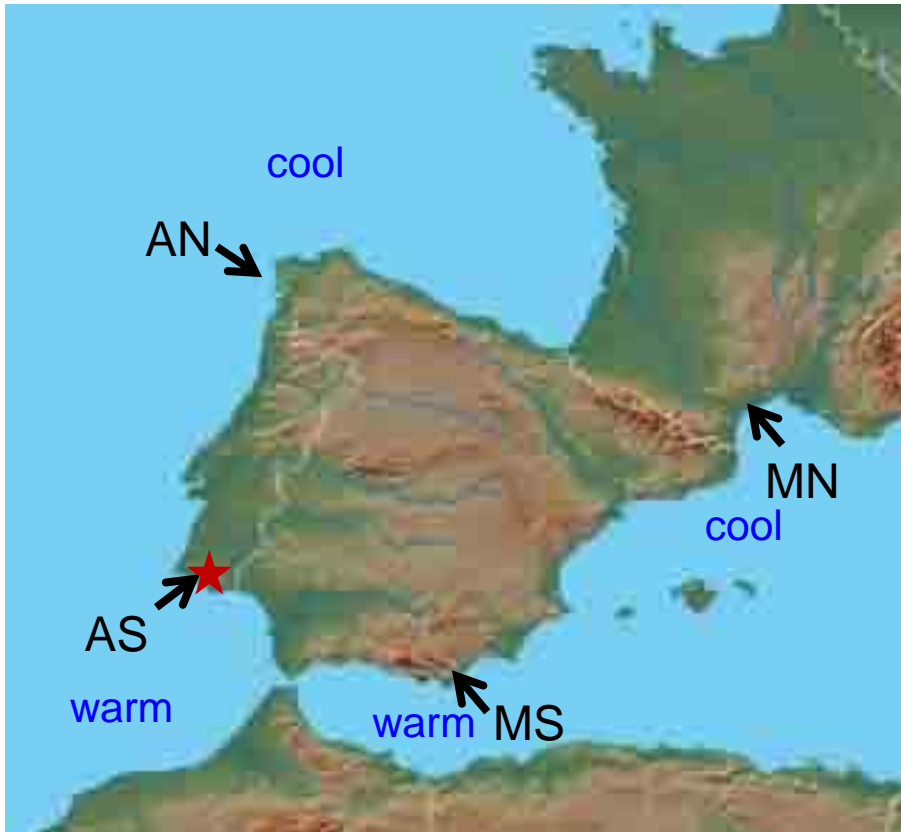
Relevance of Genetic Subdivision for Aquaculture

- Do phylogeographic lineages discovered with molecular markers exhibit differences in growth, disease susceptibility, response to stress....?
- No specific study has addressed these questions so far
- The differences between races or subspecies that were documented before molecular markers were available suggest that cryptic lineages could show important differences in traits relevant for aquaculture
- However, experiments are labour intensive and take long time.
- An alternative is available now: functional characterization through studies of differential gene expression.

Gene Expression Study of Phylogeographic Lineages in Clams

- Grooved carpet-shell clam: 2 Atlantic and 2 Mediterranean populations
- 3 months in a common environment - March-June
- RNA and DNA extracted from gills
- 32 individual clams - Genotyped for 6 nuclear markers (Cordero et al 2014, MPE) – Introns-RFLP
- Microarray – 14,003 probes
- After normalization and filtering –10,928 probes

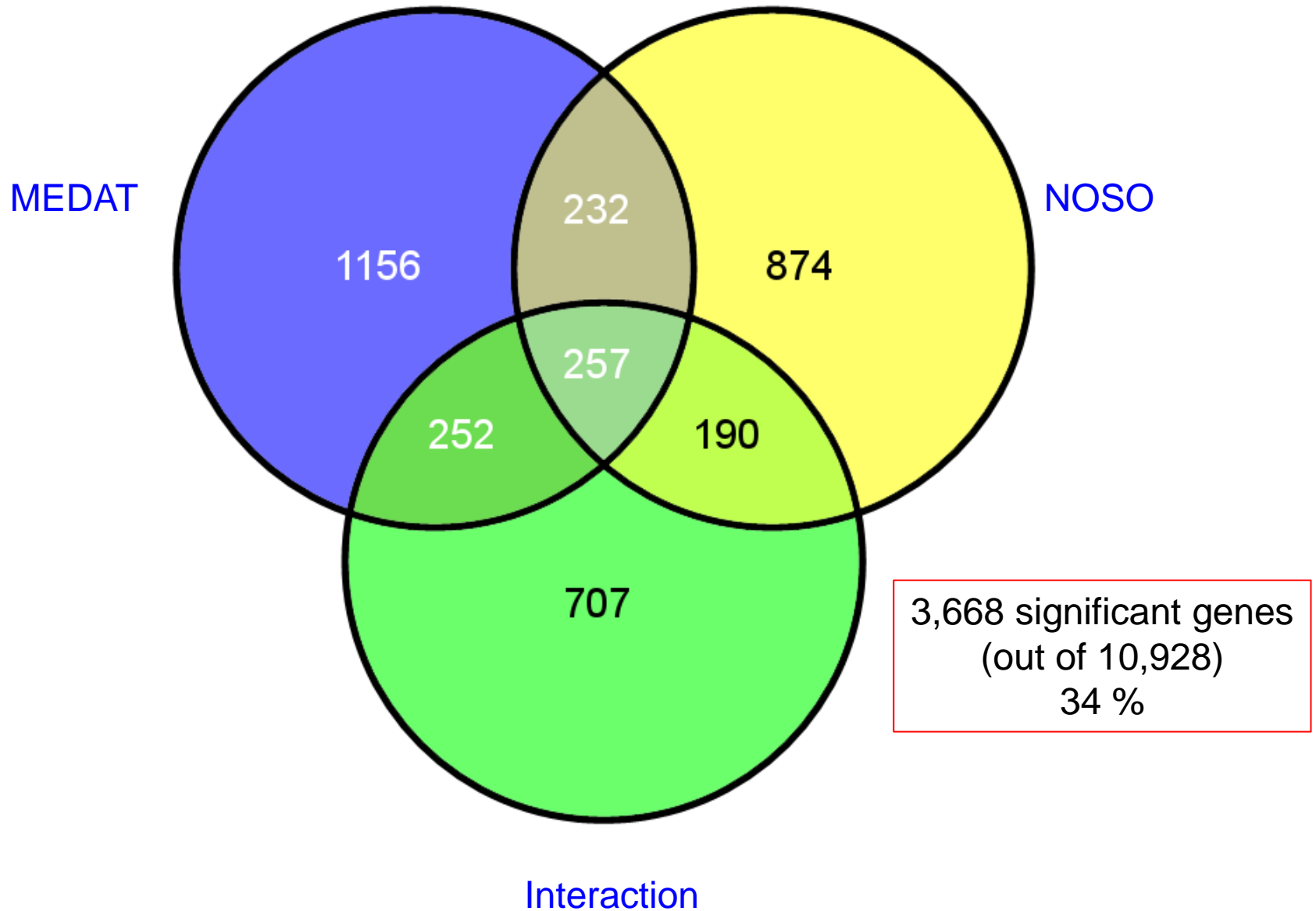
Samples and Environments



Statistical Analysis

- One-way ANOVA
 - 2,214 significant genes ($p < 0.05$)
 - 1,021 significant genes (FDR 5%)
- Two-way ANOVA (ATMED + NOSO + 2-way interaction)
- Principal components
- Multiple Linear Regression – individual genetic makeup introduced (PII)
 - PII + ATMED + NOSO + 2-way interactions + 3-way interaction
 - Significance obtained through permutation tests (R package *ImPerm*)

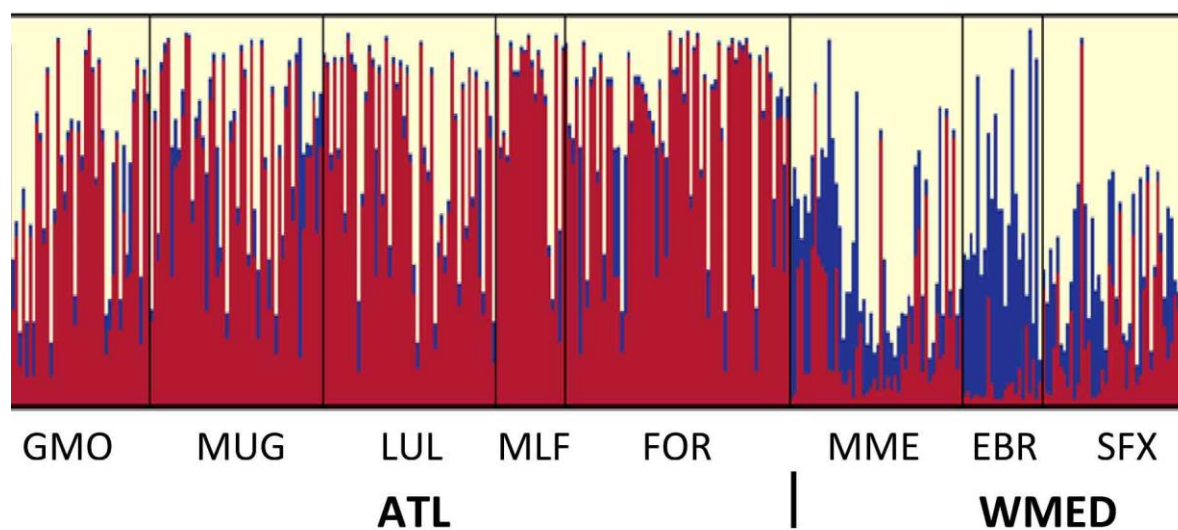
2-way ANOVA



Multiple Linear Regression

- Individual genetic makeup introduced (PII)
- PII + ATMED + NOSO + 2-way interactions + 3-way interaction
- Significance obtained through permutation tests (R package *ImPerm*)

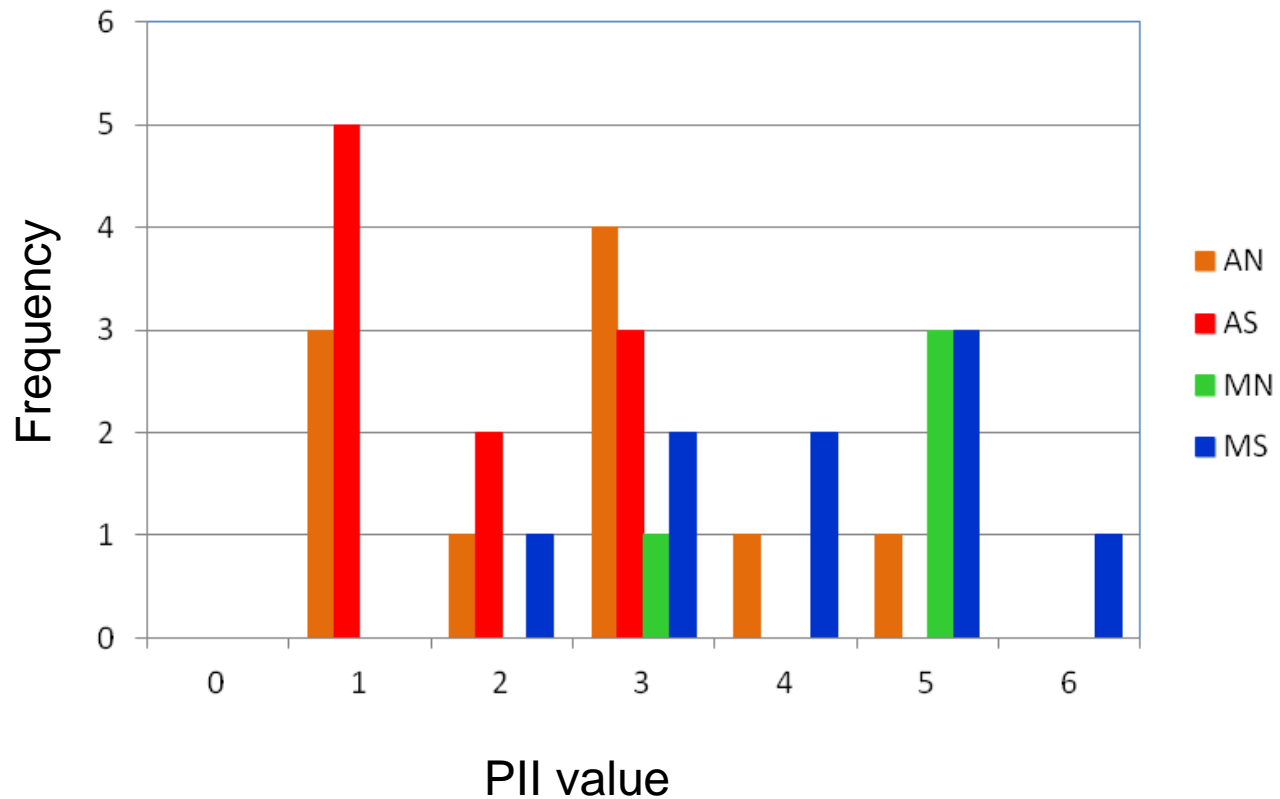
Bayesian analysis of population structure – 6 Intron RFLP



The observation of clams carrying typical Mediterranean genotypes in the Atlantic, and viceversa, is an indication of high gene flow between the two areas

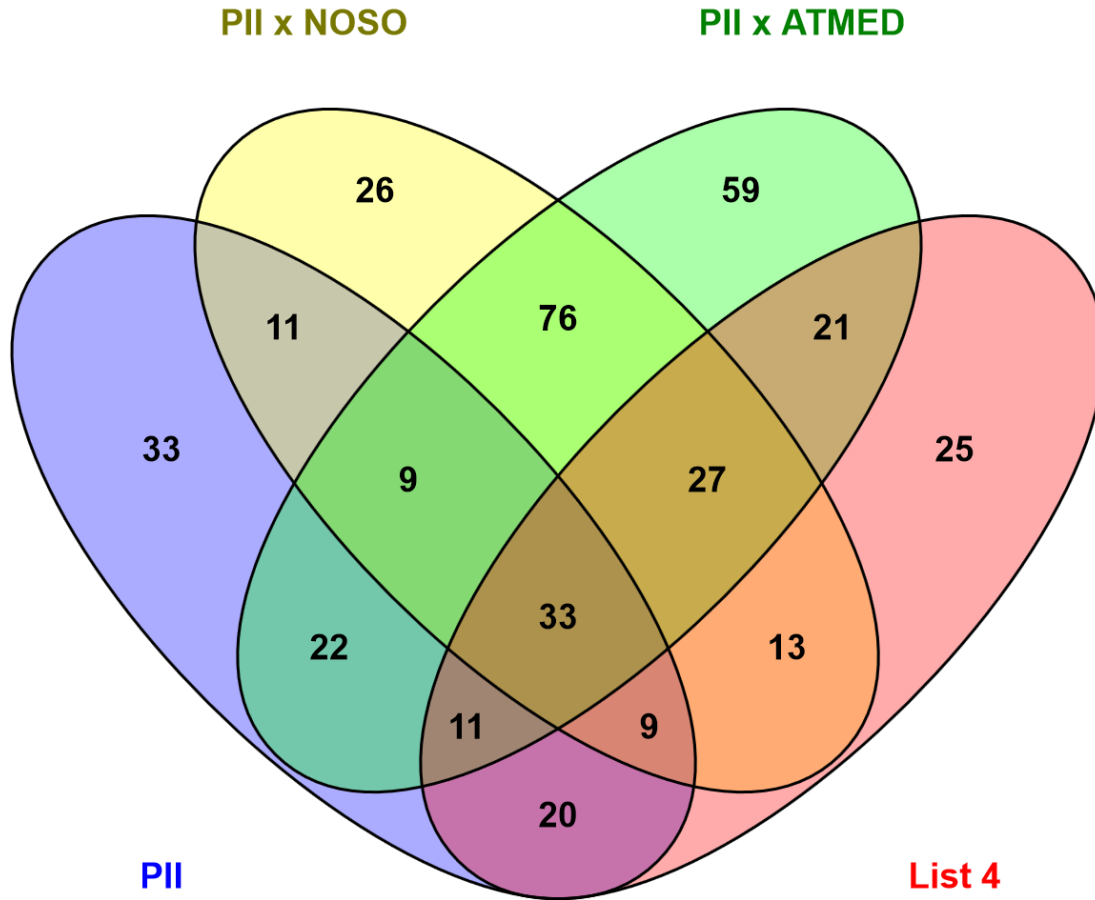
Phylogeographic Individual Index (PII)

Constructed from 3 intron markers with high F_{st} values



Multiple Linear Regression

Terms containing PII effect only



Functional characterization

- Gene ontology - Blast2GO
- KEGG pathways
- Enrichment of GO terms and KEGG – Fisher Exact Tests – DAVID

Term	Count	Fold Enrichment	2-way ANOVA		Multiple Linear Regression		
			ATMED	NOSO	PII	ATMED	NOSO
Biological Process							
GO:0001944~vasculature development (+1)	5	4,93	0,0143				
GO:0006412~translation	37	2,61	0,0011		0,0000	0,0000	0,0000
GO:0006754~ATP biosynthetic process (+1)	3	9,23		0,0367			
GO:0006811~ion transport (+1)	5	5,94		0,0070			
GO:0015986~ATP synthesis coupled proton transport (+ 12 similar)	3	9,67		0,0337			
GO:0048839~inner ear development (+1)	5	5,53				0,0085	0,0384
GO:0051726~regulation of cell cycle	6	5,39			0,0032	0,0077	
Cellular component							
GO:0005840~ribosome	33	2,68	0,0072		0,0000	0,0000	0,0000
GO:0015935~small ribosomal subunit	4	5,47	0,0289			0,0462	
GO:0030529~ribonucleoprotein complex	32	2,39	0,0214		0,0000	0,0000	0,0000
GO:0043232~intracellular non-membrane-bounded organelle (+1)	40	1,88			0,0000	0,0001	0,0002
GO:0045259~proton-transporting ATP synthase complex	3	11,26		0,0236			
Molecular function							
GO:0003723~RNA binding	13	1,83					0,0431
GO:0003735~structural constituent of ribosome	28	3,85	0,0026		0,0000	0,0000	0,0000
GO:0003743~translation initiation factor activity	6	3,17	0,0343				
GO:0003924~GTPase activity	6	3,36				0,0278	
GO:0004129~cytochrome-c oxidase activity	4	4,81	0,0435				
GO:0004197~cysteine-type endopeptidase activity	6	3,31	0,0292				
GO:0005198~structural molecule activity	32	3,40	0,0014		0,0000	0,0000	0,0000
GO:0015002~heme-copper terminal oxidase activity (+1)	4	4,81	0,0435				
GO:0016765~transferase activity, transferring alkyl or aryl (other than methyl)	4	4,96					0,0400
GO:0019843~rRNA binding	6	10,07			0,0003	0,0001	0,0002
KEGG pathways							
dre00270:Cysteine and methionine metabolism	4	5,74					0,0256
dre00980:Metabolism of xenobiotics by cytochrome P450	4	5,74					0,0256
dre00982:Drug metabolism	4	6,46					0,0181
dre03010:Ribosome	27	4,01	0,0004		0,0000	0,0000	0,0000

Term	Count	Fold Enrichment	2-way ANOVA		Multiple Linear Regression				
			ATMED	NOSO	PII	ATMED	NOSO		
				0,0143					
				0,0011		0,0000	0,0000	0,0000	
					0,0367				
					0,0070				
					0,0337				
						0,0085	0,0384		
						0,0032	0,0077		
					0,0072		0,0000	0,0000	0,0000
					0,0289		0,0462		
					0,0214		0,0000	0,0000	0,0000
							0,0000	0,0001	0,0002
						0,0236			
Molecular function									
GO:0003723~RNA binding	13	1,83						0,0431	
GO:0003735~structural constituent of ribosome	28	3,85		0,0026		0,0000	0,0000	0,0000	
GO:0003743~translation initiation factor activity	6	3,17		0,0343					
GO:0003924~GTPase activity	6	3,36					0,0278		
GO:0004129~cytochrome-c oxidase activity	4	4,81		0,0435					
GO:0004197~cysteine-type endopeptidase activity	6	3,31		0,0292					
GO:0005198~structural molecule activity	32	3,40		0,0014		0,0000	0,0000	0,0000	
GO:0015002~heme-copper terminal oxidase activity (+1)	4	4,81		0,0435					
GO:0016765~transferase activity, transferring alkyl or aryl (other than methyl)	4	4,96						0,0400	
GO:0019843~rRNA binding	6	10,07				0,0003	0,0001	0,0002	
KEGG pathways									
dre00270:Cysteine and methionine metabolism	4	5,74						0,0256	
dre00980:Metabolism of xenobiotics by cytochrome P450	4	5,74						0,0256	
dre00982:Drug metabolism	4	6,46						0,0181	
dre03010:Ribosome	27	4,01		0,0004		0,0000	0,0000	0,0000	

GO terms significant for the three factors:

- Protein biosynthesis
- Translation machinery
- Ribosomal activity

Term	Count	Fold Enrichment	2-way ANOVA		Multiple Linear Regression		
			ATMED	NOSO	PII	ATMED	NOSO
Biological Process							
					0,0000	0,0000	0,0000
GO terms significant for ATMED only:							
GO0004129: cytochrome c oxidase activity (3) - Respiration chain						0,0085	0,0384
					0,0032	0,0077	
GO0004197: cysteine-type endopeptidase activity - Protein degradation					0,0000	0,0000	0,0000
						0,0462	
					0,0000	0,0000	0,0000
					0,0000	0,0001	0,0002
GO0008924: GTPase activity - 3 tubulines and intracell transport proteins							
							0,0431
GO:0003735~structural constituent of ribosome	28	3,85	0,0026		0,0000	0,0000	0,0000
GO:0003743~translation initiation factor activity	6	3,17	0,0343				
GO:0003924~GTPase activity	6	3,36				0,0278	
GO:0004129~cytochrome-c oxidase activity	4	4,81	0,0435				
GO:0004197~cysteine-type endopeptidase activity	6	3,31	0,0292				
GO:0005198~structural molecule activity	32	3,40	0,0014		0,0000	0,0000	0,0000
GO:0015002~heme-copper terminal oxidase activity (+1)	4	4,81	0,0435				
GO:0016765~transferase activity, transferring alkyl or aryl (other than methyl)	4	4,96					0,0400
GO:0019843~rRNA binding	6	10,07			0,0003	0,0001	0,0002
KEGG pathways							
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dre00980:Metabolism of xenobiotics by cytochrome P450	4	5,74					0,0256
dre00982:Drug metabolism	4	6,46					0,0181
dre03010:Ribosome	27	4,01	0,0004		0,0000	0,0000	0,0000

Term	Count	Fold Enrichment	2-way ANOVA		Multiple Linear Regression		
			ATMED	NOSO	PII	ATMED	NOSO
143							
011					0,0000	0,0000	0,0000
					0,0367		
					0,0070		
					0,0337		
						0,0085	0,0384
					0,0032	0,0077	
072					0,0000	0,0000	0,0000
289						0,0462	
214					0,0000	0,0000	0,0000
					0,0000	0,0001	0,0002
					0,0236		
							0,0431
026					0,0000	0,0000	0,0000
343							
GO:0003924~GTPase activity	6	3,36				0,0278	
GO:0004129~cytochrome-c oxidase activity	4	4,81	0,0435				
GO:0004197~cysteine-type endopeptidase activity	6	3,31	0,0292				
GO:0005198~structural molecule activity	32	3,40	0,0014		0,0000	0,0000	0,0000
GO:0015002~heme-copper terminal oxidase activity (+1)	4	4,81	0,0435				
GO:0016765~transferase activity, transferring alkyl or aryl (other than methyl	4	4,96					0,0400
GO:0019843~rRNA binding	6	10,07			0,0003	0,0001	0,0002
KEGG pathways							
dre00270:Cysteine and methionine metabolism	4	5,74					0,0256
dre00980:Metabolism of xenobiotics by cytochrome P450	4	5,74					0,0256
dre00982:Drug metabolism	4	6,46					0,0181
dre03010:Ribosome	27	4,01	0,0004		0,0000	0,0000	0,0000

GO terms significant NOSO only:

* ATP synthesis in mitochondria

- ATP synthase sununit B1
- ATP synthase sununit delta
- ATP synthase sununit g

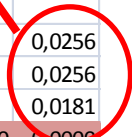
* Transferase activity

- Glutathione S transferase, subunit pi
- Glutathione S transferase, subunit omega 2
- RAB scort protein 1

Term	Count	Fold Enrichment	2-way ANOVA		Multiple Linear Regression		
			ATMED	NOSO	PII	ATMED	NOSO
Biological Process							
GO:0001944~vasculature development (+1)	5	4,93	0,0143				
GO:0006412~translation	37	2,61	0,0011		0,0000	0,0000	0,0000
GO:0006754~ATP biosynthetic process (+1)	3	9,23		0,0367			
GO:0006811~ion transport (+1)	5	5,94		0,0070			
GO:0015986~ATP synthesis coupled proton transport (+12 similar)	2	9,67		0,0337			
						0,0085	0,0384
					0,0032	0,0077	
			0,072		0,0000	0,0000	0,0000
			0,289			0,0462	
			0,214		0,0000	0,0000	0,0000
					0,0000	0,0001	0,0002
				0,0236			
							0,0431
			0,026		0,0000	0,0000	0,0000
			0,343				
						0,0278	
			0,435				
			0,292				
			0,014		0,0000	0,0000	0,0000
			0,435				
GO:0016765~transferase activity, transferring alkyl or aryl (other than methyl)	4	4,96					0,0400
GO:0019843~rRNA binding	6	10,07			0,0003	0,0001	0,0002
KEGG pathways							
dre00270:Cysteine and methionine metabolism	4	5,74					0,0256
dre00980:Metabolism of xenobiotics by cytochrome P450	4	5,74					0,0256
dre00982:Drug metabolism	4	6,46					0,0181
dre03010:Ribosome	27	4,01	0,0004		0,0000	0,0000	0,0000

KEGG pathways significant for NOSO only:

- * Cysteine and methionine metabolism
Cysteine is precursor of Glutathione
- * Metabolism of xenobiotics by cytochrome P450
- Glutathione transferases
- * Drug metabolism
- Glutathione transferases



Conclusions

- Differences in gene expression among populations appear at roughly 30% of the carpet shell clam genes
- Both the genetic background and the past and present environmental conditions experienced by the clams influence the patterns of gene expression.
- Protein biosynthesis is affected by both genetic and environmental factors in complex ways. This could be reflected in growth and reproduction.
- Other biological features affected by both genetic and environmental variables:
 - ATP synthesis in mitochondria
 - Protein degradation
 - Intracellular protein transport
- **We have to look at Genes and Environments together when dealing with restocking and spat transfers**

Acknowledgements

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F. Bonhomme (SMEL, France)

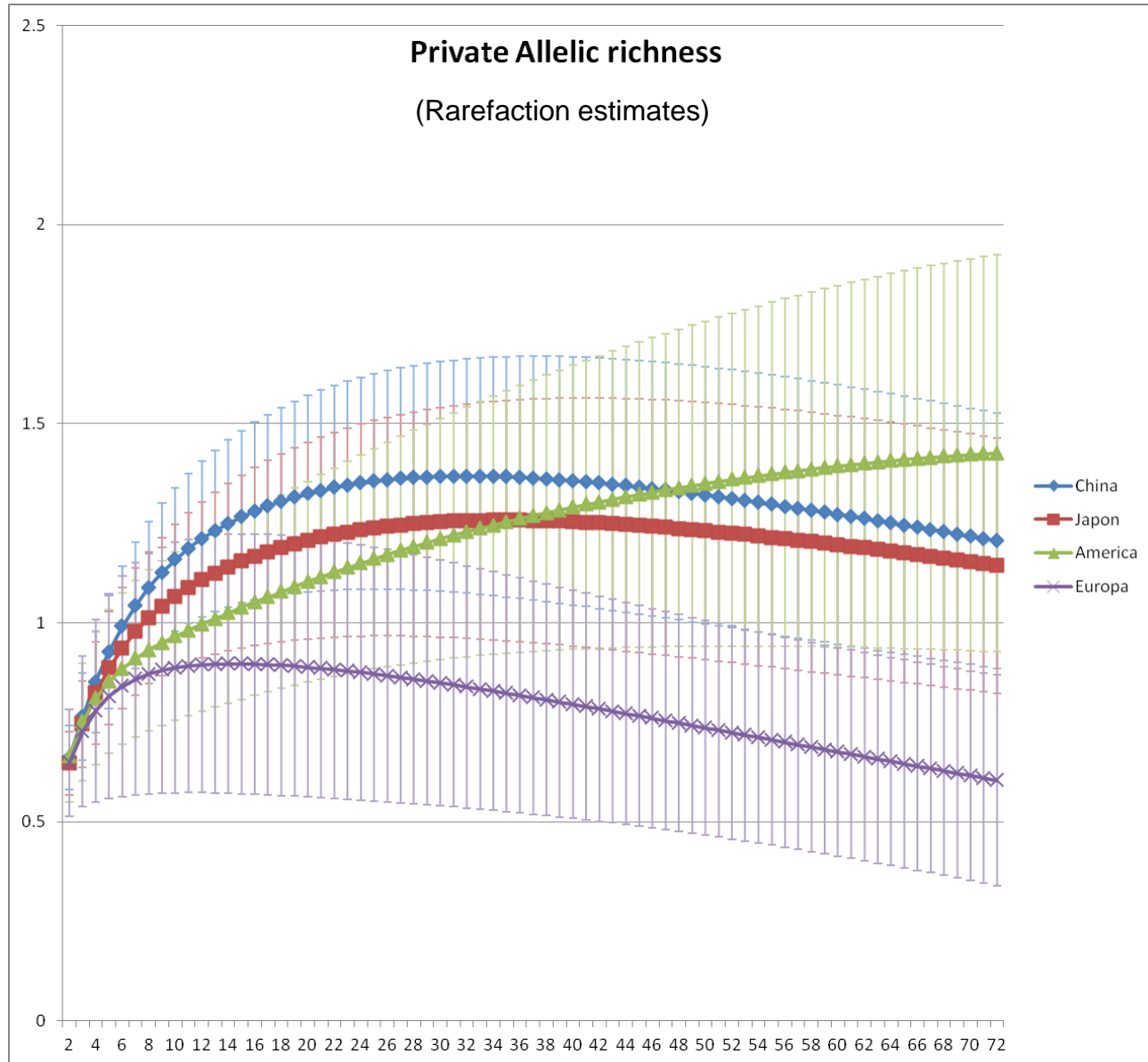
N. Bierne (SMEL, France)

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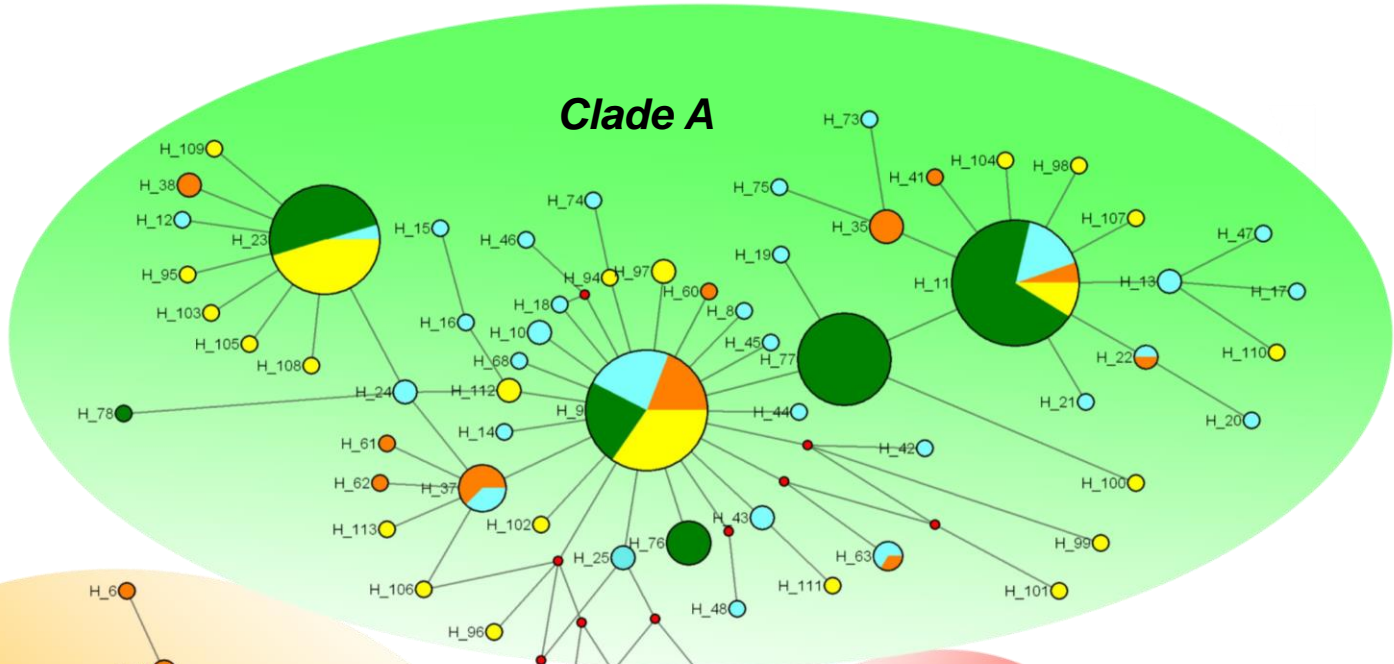




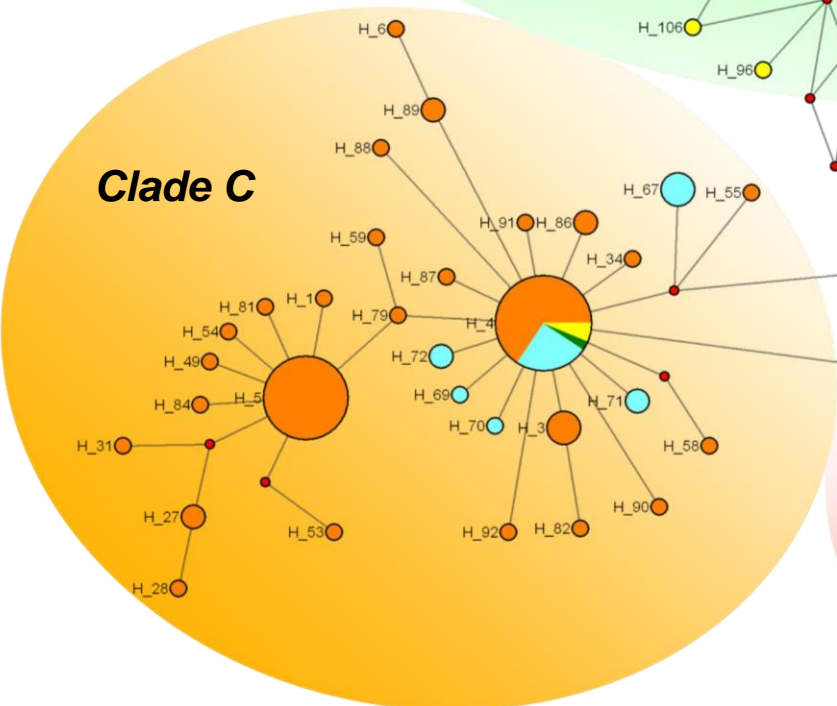
Genetic Variability



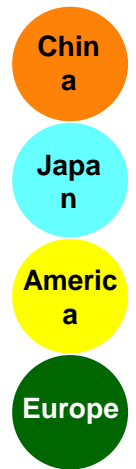
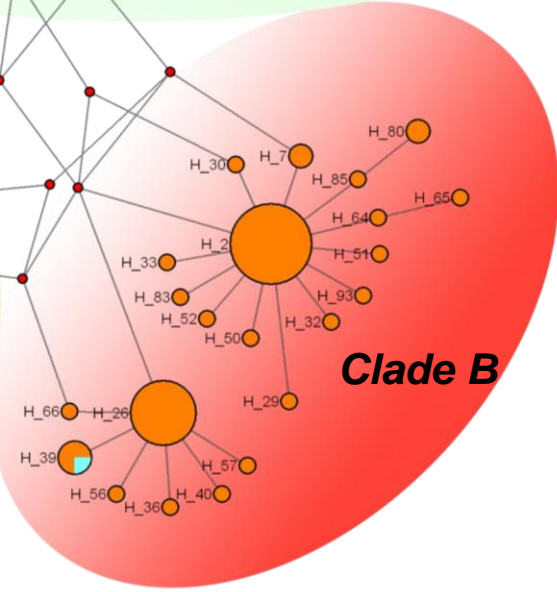
Clade A



Clade C

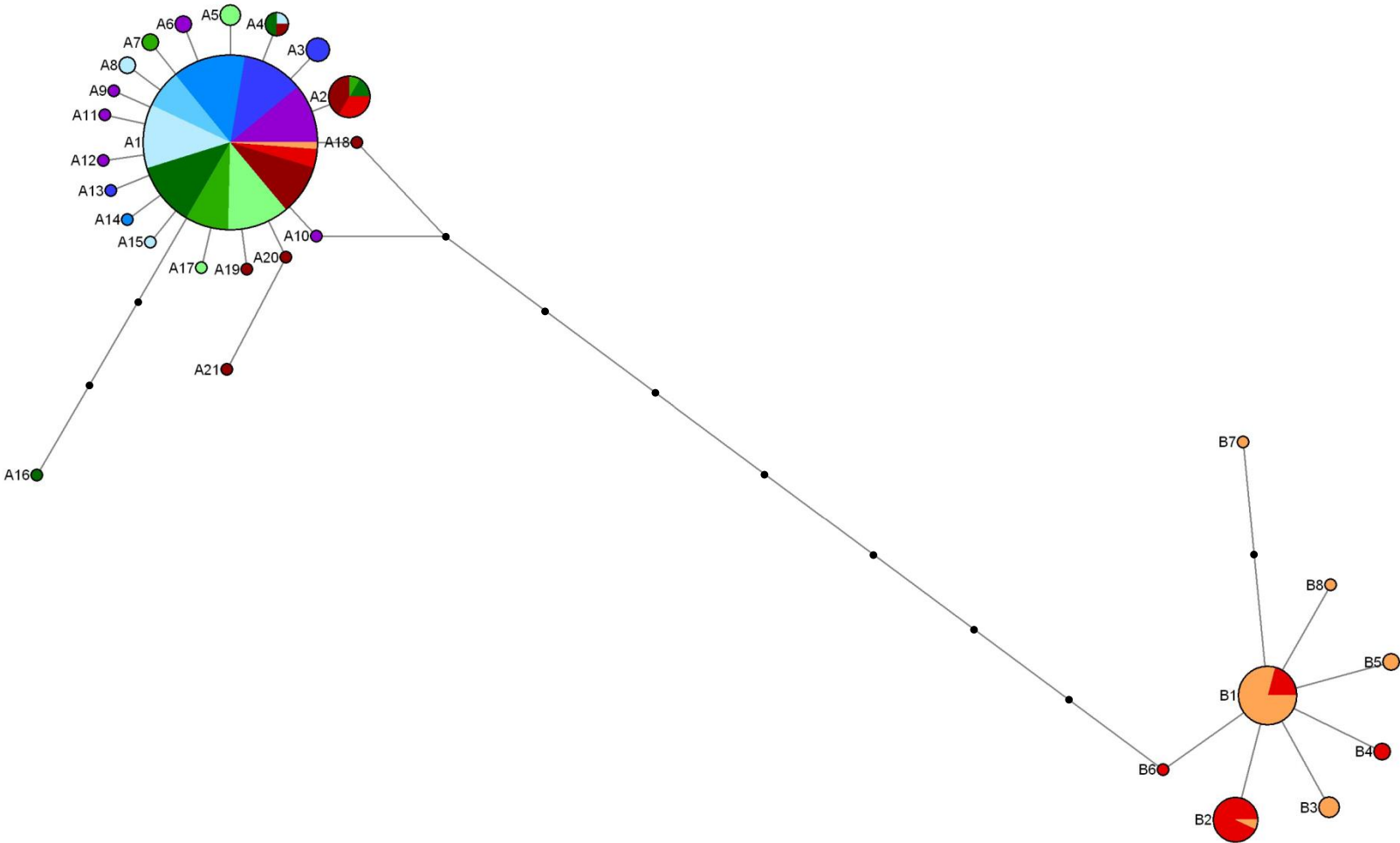


Clade B



Carpet-shell clam - Mitochondrial DNA – COI sequences (450 bp, 311 samples)

Haplotype Network



The Manila clam (*Ruditapes philppinarum*) is the most important clam species in world fisheries and aquaculture.

World production is MT, and European production is 30-60 KT, which is concentrated in Mediterranean countries and Ireland.

Manila clam is native from Pacific Asian coasts, and has been introduced in many localities along the world for aquaculture.

It was introduced in Europe in the 1970's due to its fast growth and disease resistance.

Introduction In Europe is well documented, and was carried out with adults and seed original from Canada (Vancouver region), where it had been introduced accidentally in the 1930's as a result of oyster imports from Japan.

Initial introduction in France in 1973 was followed by introduction in Italy, Spain and Ireland during the next decade, and natural expansion to UK, Portugal and Mediterranean countries,

No other introductions in Europe have been reported afterwards

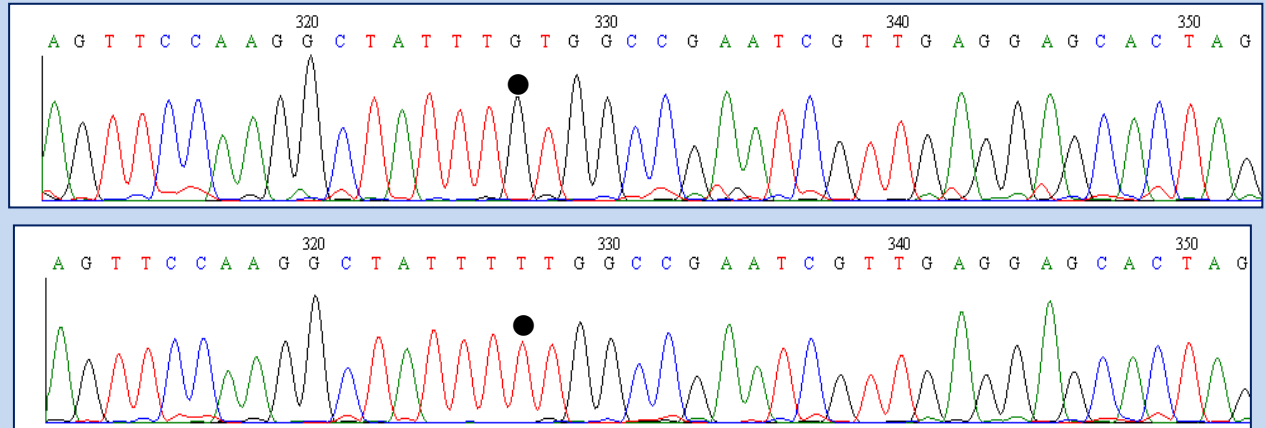
Genetic Markers

-Mitochondrial DNA sequences

COI gene

450 bp

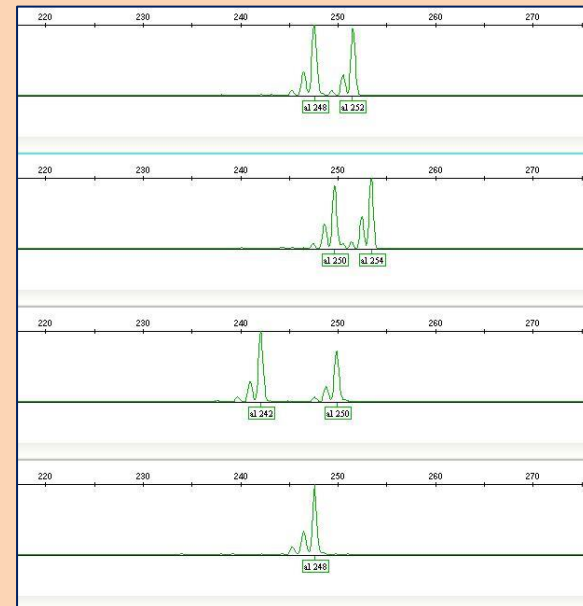
Our data pooled
with those of
Mao et al (2012)
Sekine et al (2006)

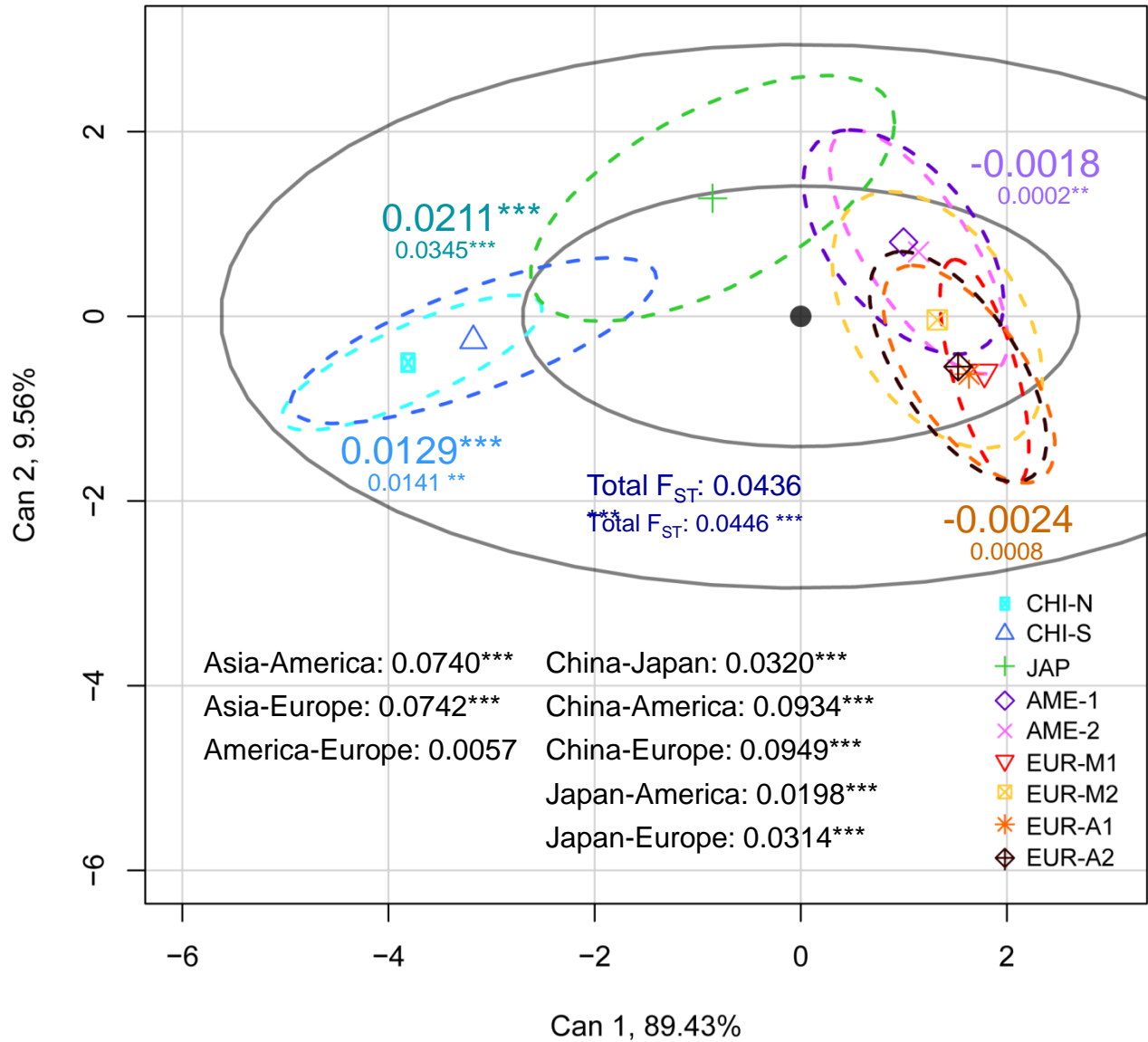


-Microsatellites (SSR)

-Yasuda et al (2007), An et al (2008)
Hu et al (2012), Nie et al (2012)

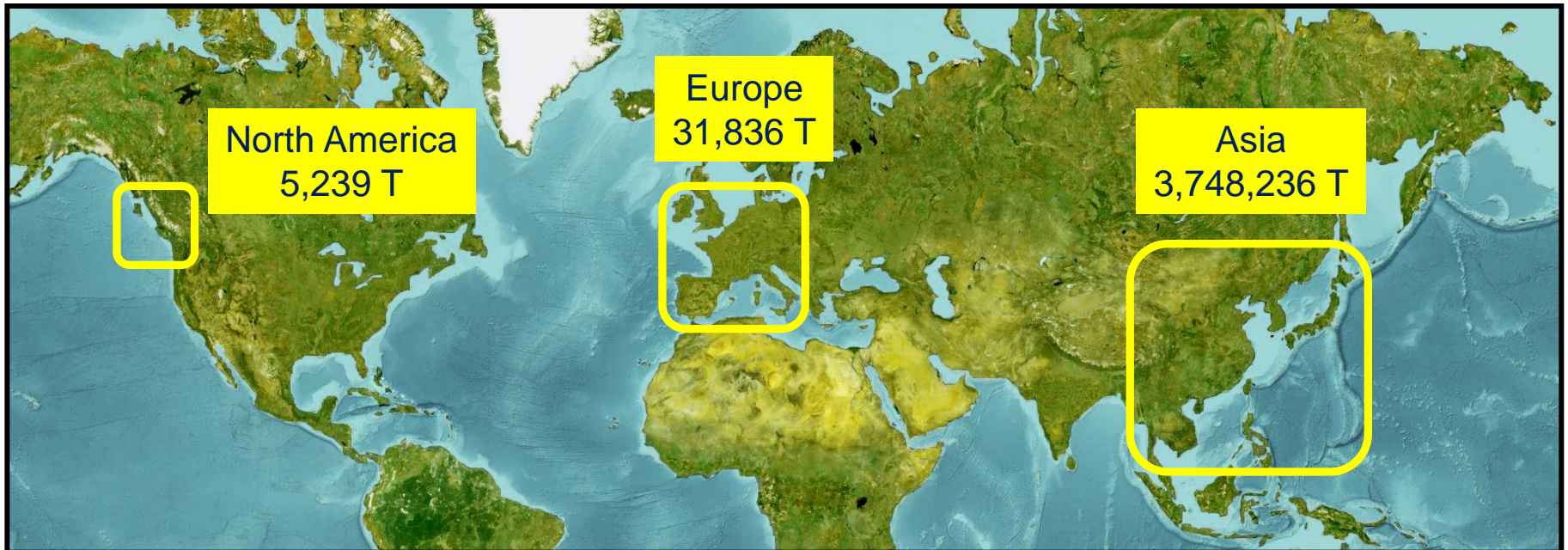
- 7 primer pairs with good results,
out of 21 tried



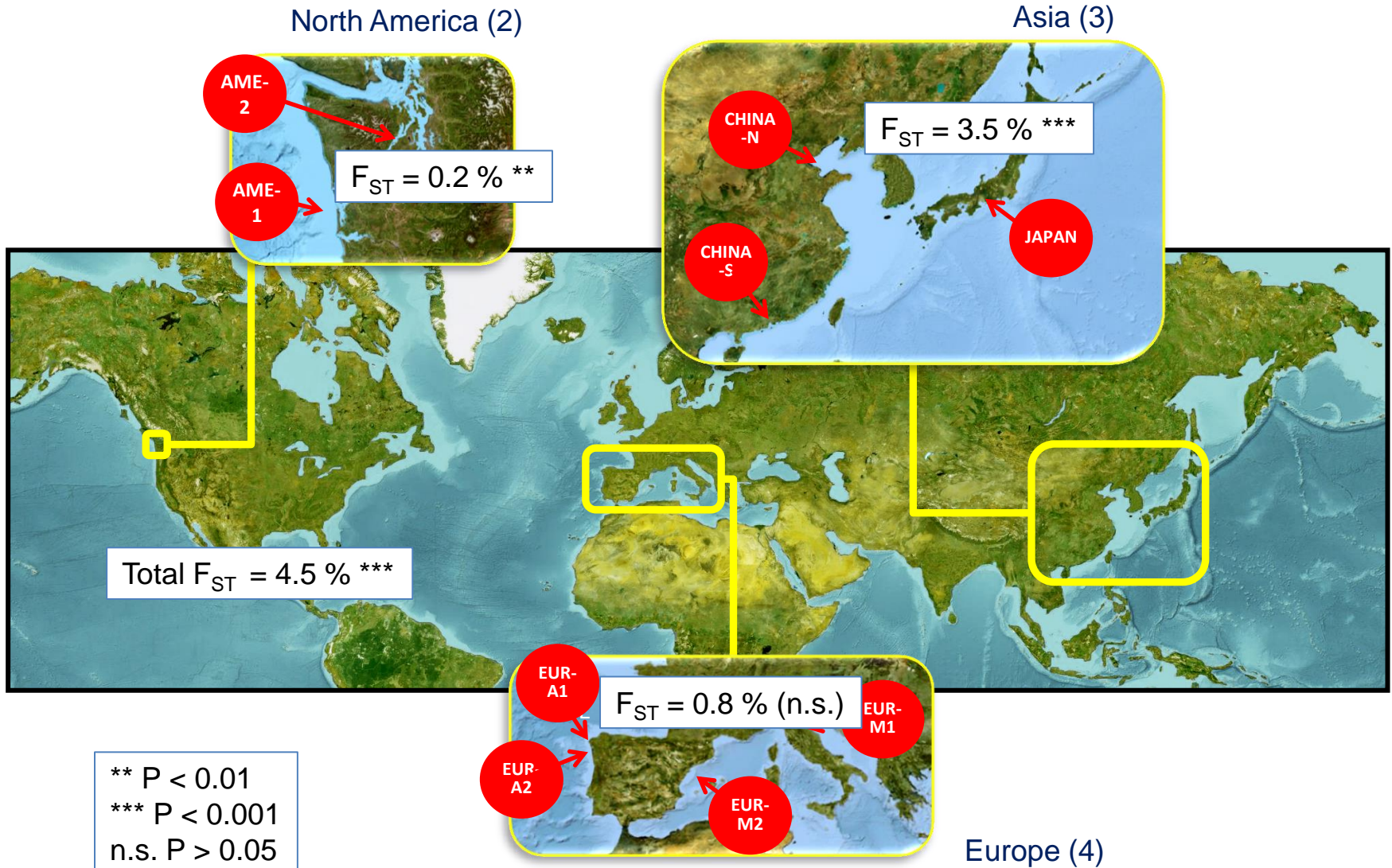


Manila Clam Global Production

(FAO data – 2012)



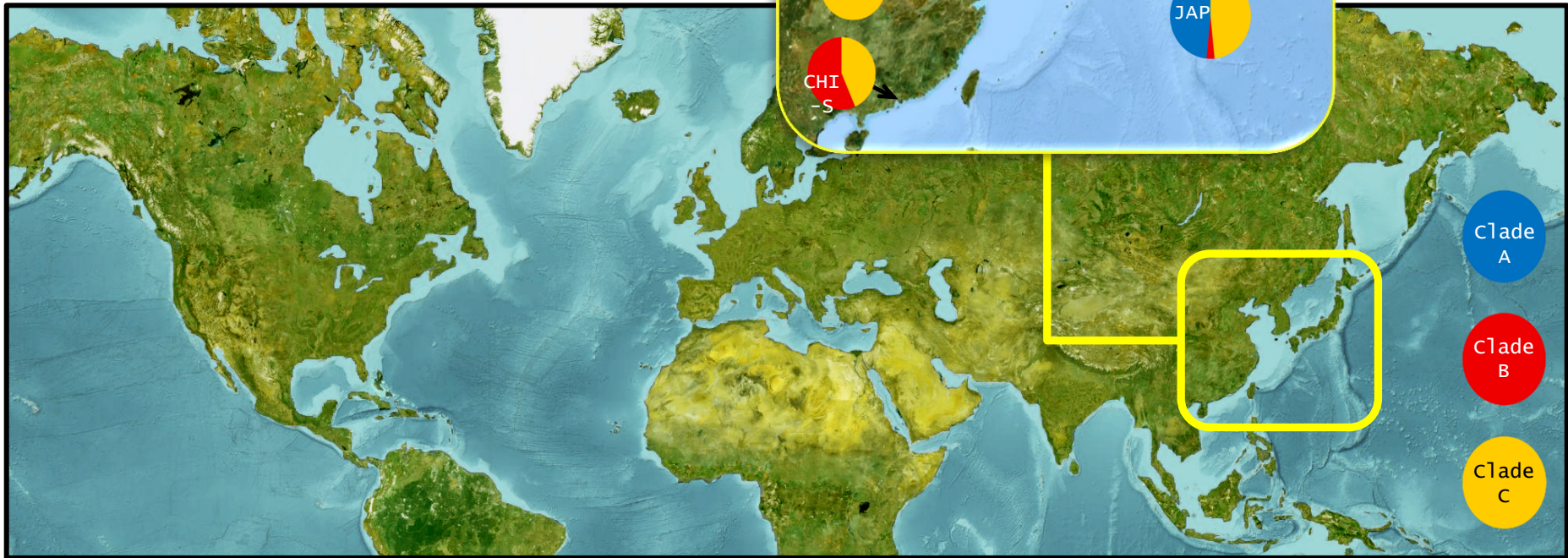
Genetic differences among Populations (regional)



Aims of this study:

1. Test the documented origin of the European populations of Manila Clam
 - ¿Have other undocumented introductions taken place after introduction?
2. ¿Has been genetic variability reduced due to founder effect and hatchery propagation?
3. Genetic differentiation – among European populations
 - with respect to other world regions

Distributi



CHI-N

CHI-S

JAP

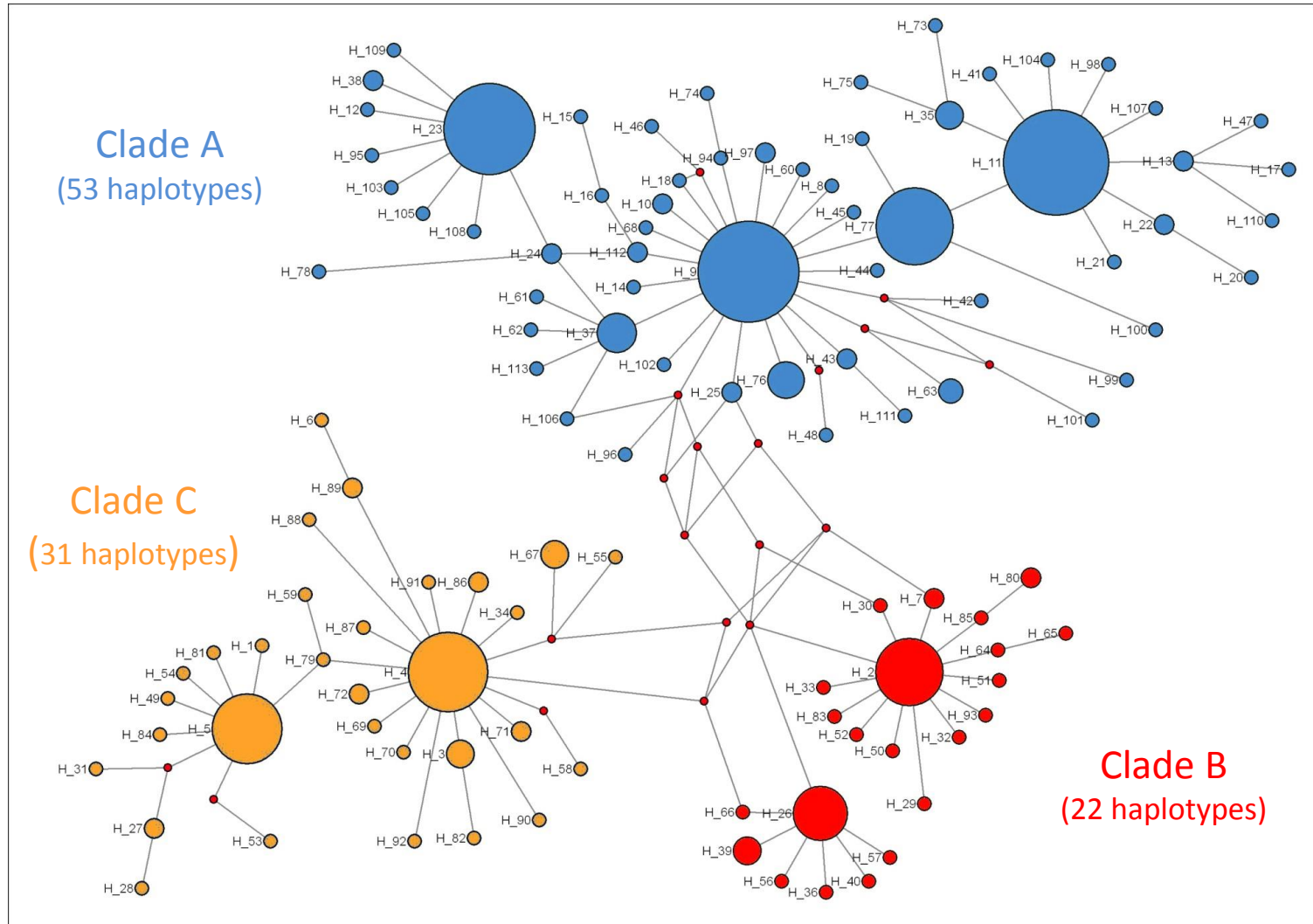
Clade A

Clade B

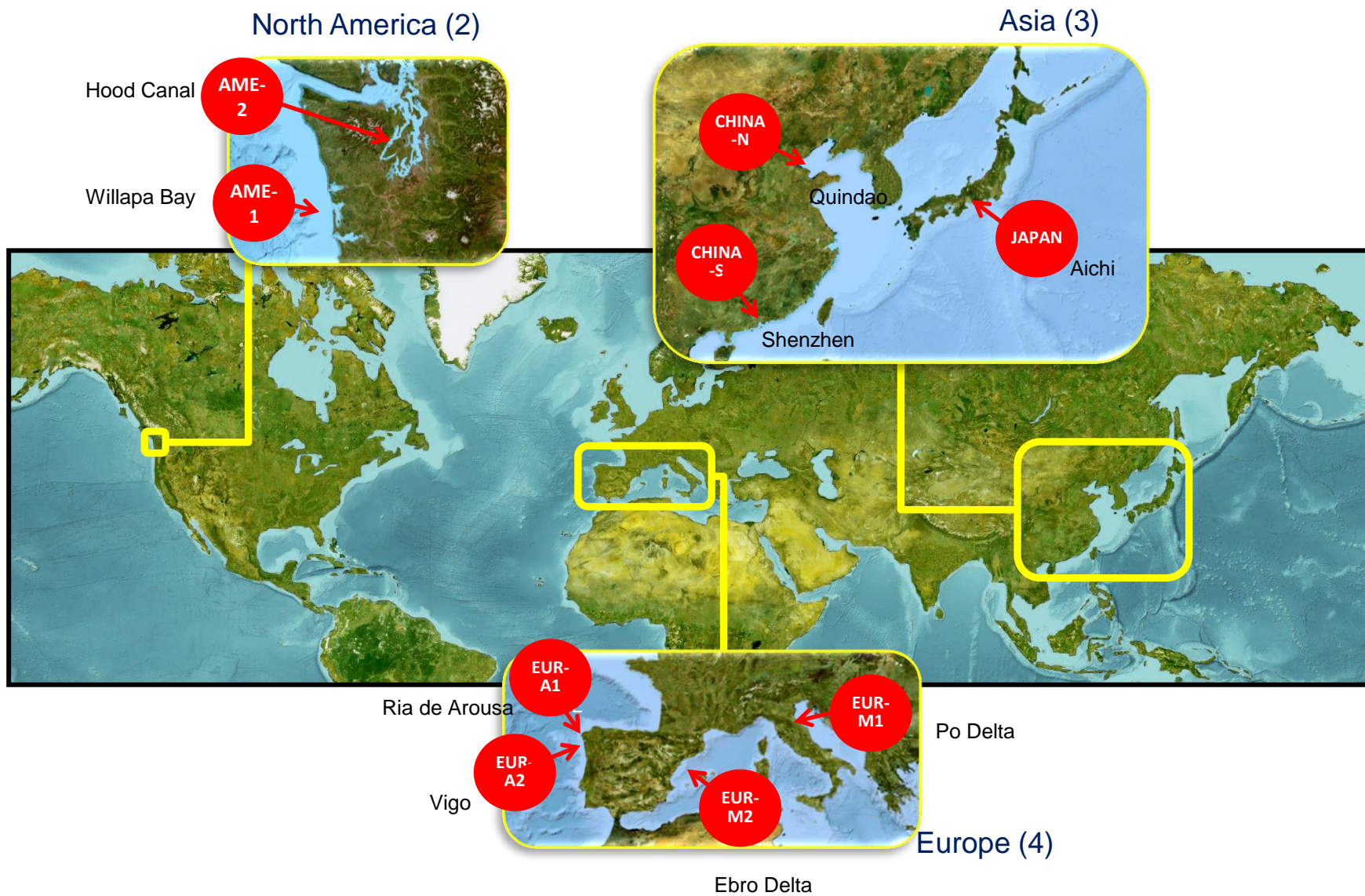
Clade C

Manila clam - Mitochondrial DNA – COI sequences (555 bp, XXX samples)

Haplotype Network: this study + Mao et al 2010 + Sekine et al 2007

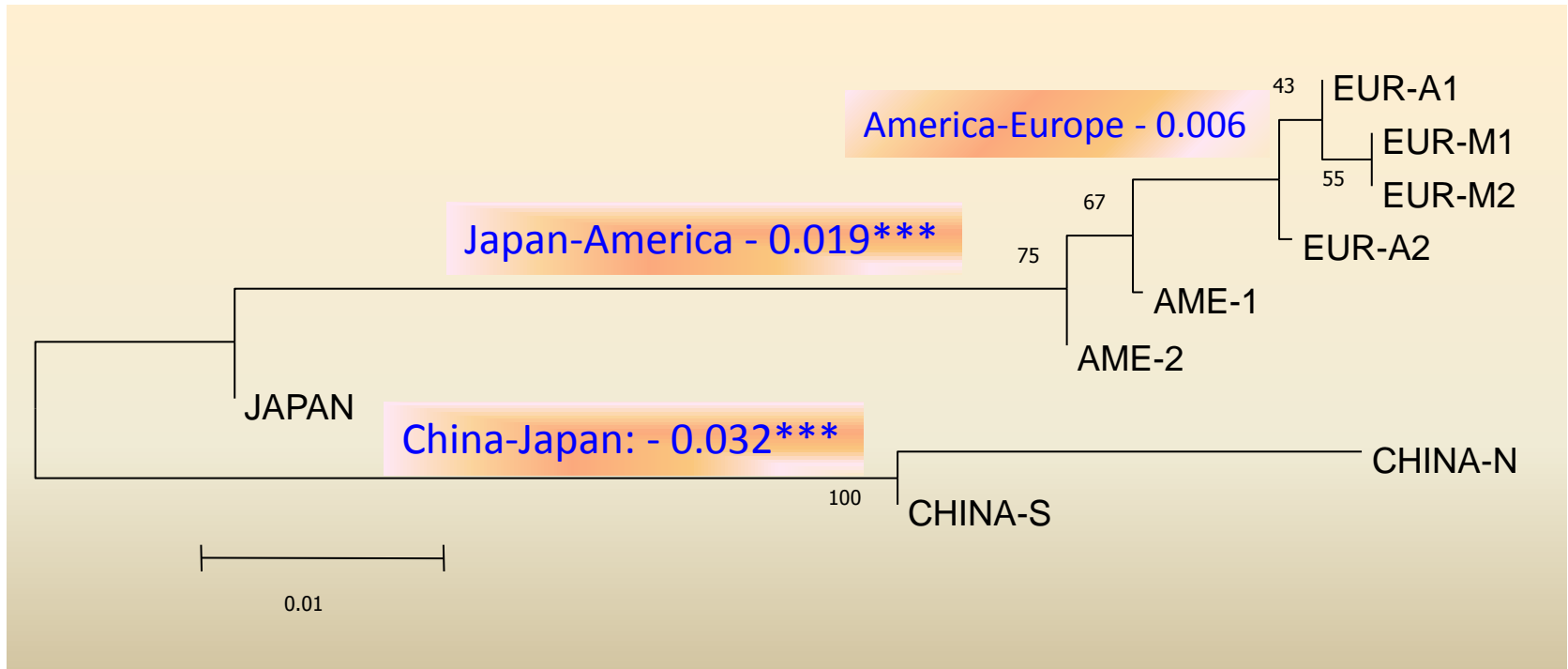


Population sampling



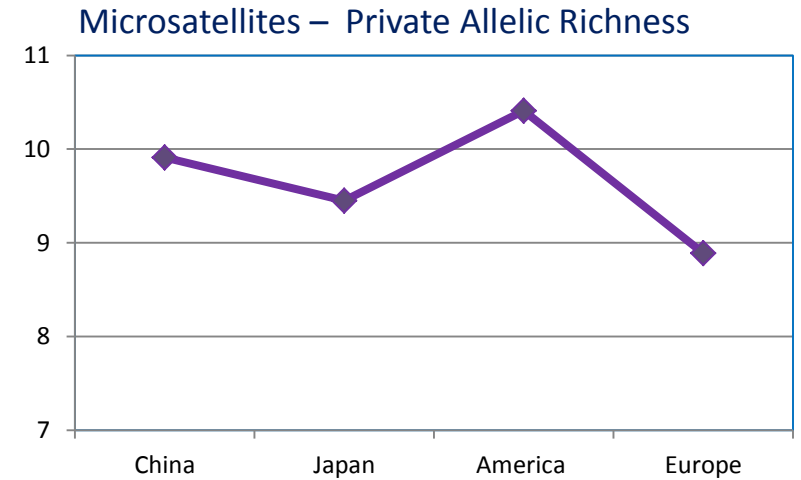
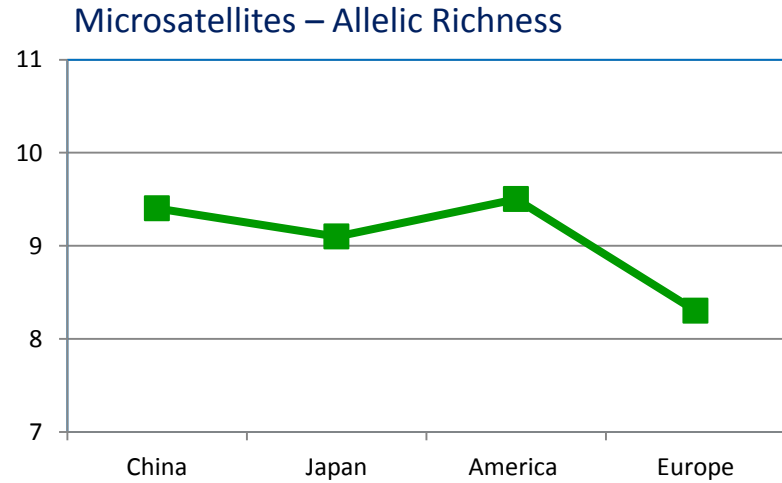
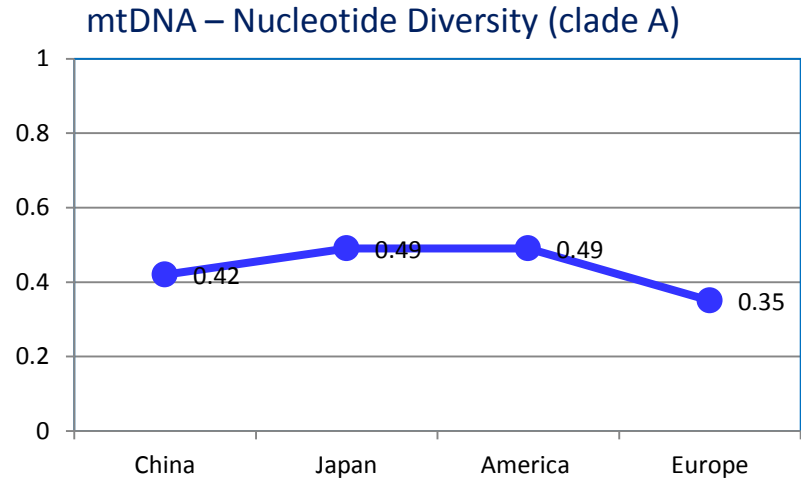
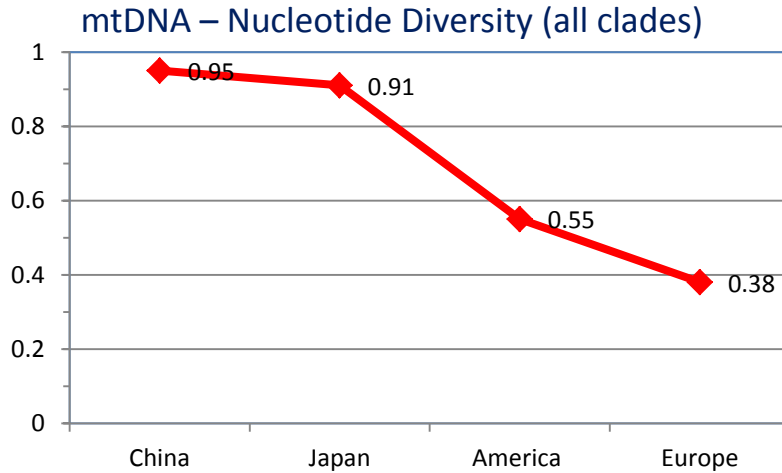
Manila clam – 7 Microsatellites

Population Tree , unrooted - NJ Algorithm - Pairwise F_{st}



Cordero et al, unpublished

Genetic Variability



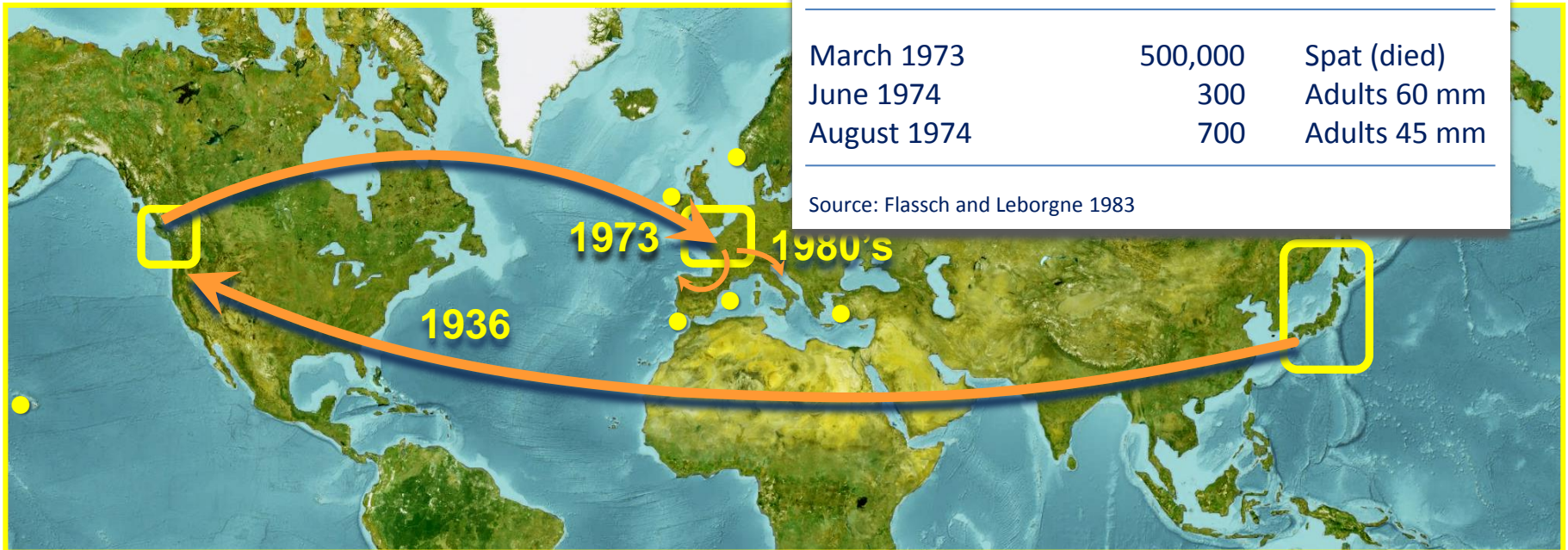
Cordero et al, unpublished

Manila Clam Introduction in Europe

Documented introduction in France

Date	Number	Type
March 1973	500,000	Spat (died)
June 1974	300	Adults 60 mm
August 1974	700	Adults 45 mm

Source: Flassch and Leborgne 1983



Sources: Quayle 1964; Flassch and Leborgne 1983; Ponurovsky and Yakovlev 1992; ; Breber 2002; Campos and Cachola 2006; Herbert et al 2012