FACULTAT DE FARMÀCIA

SEMINARIS DE RECERCA 2015

Universitat de Barcelona
Facultat de Farmàcia
Microbiologia i Parasitologia Sanitàries
Unitat de Microbiologia

Helicobacter pylori:

Migracions humanes,

càncer gàstric i perspectives

Dr. David Miñana i Galbis

http://www.madrimasd.org/blogs/microbiologia/2009/02/21/113252

Dr. David Miñana i Galbis, Dept. Microbiologia i Parasitologia Sanitàries, Facultat de Farmàcia, UB

Helicobacter pylori:

Migracions humanes, càncer gàstric i perspectives

- Introducció
- *Helicobacter pylori* i les malalties gàstriques
- *Helicobacter pylori* i les migracions humanes
- Perspectives

Dr. David Miñana i Galbis, Dept. Microbiologia i Parasitologia Sanitàries, Facultat de Farmàcia, UB

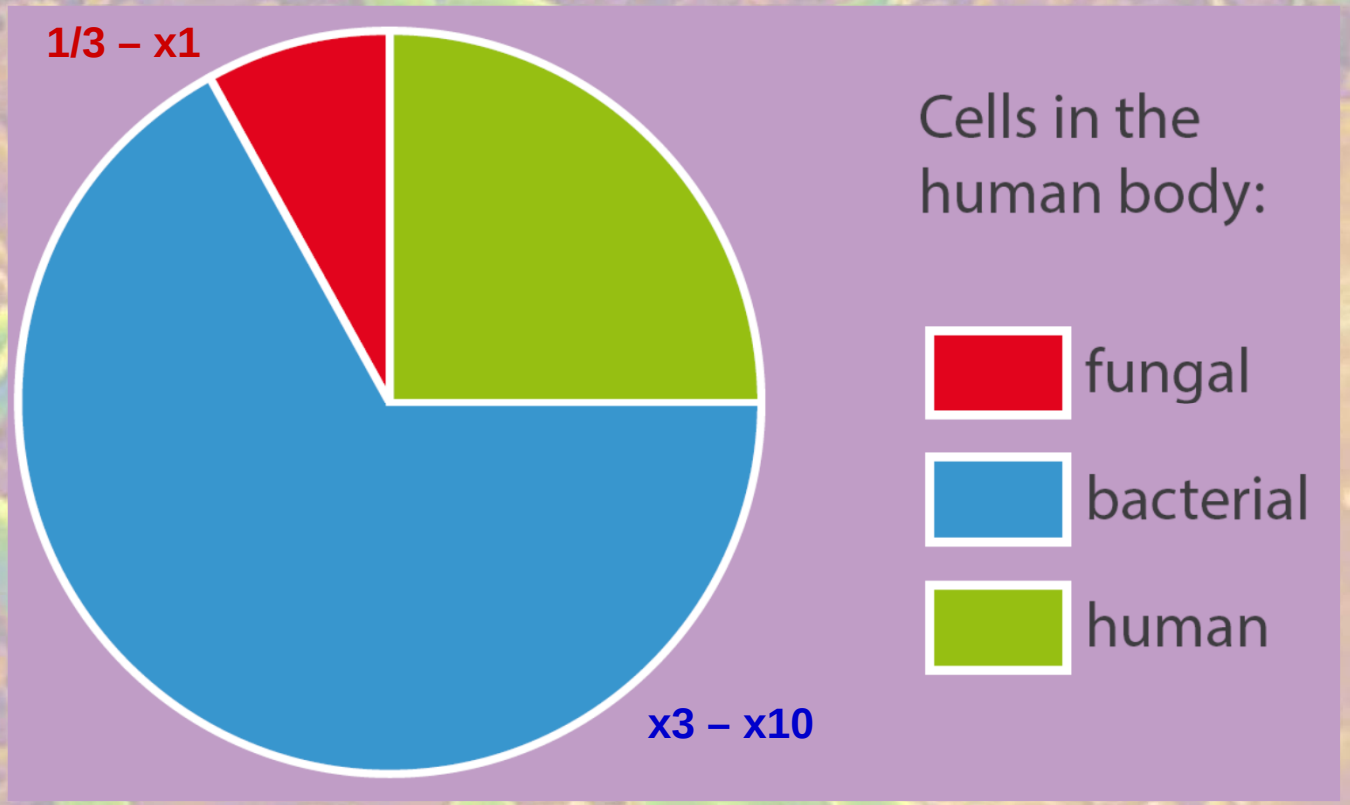
Helicobacter pylori:

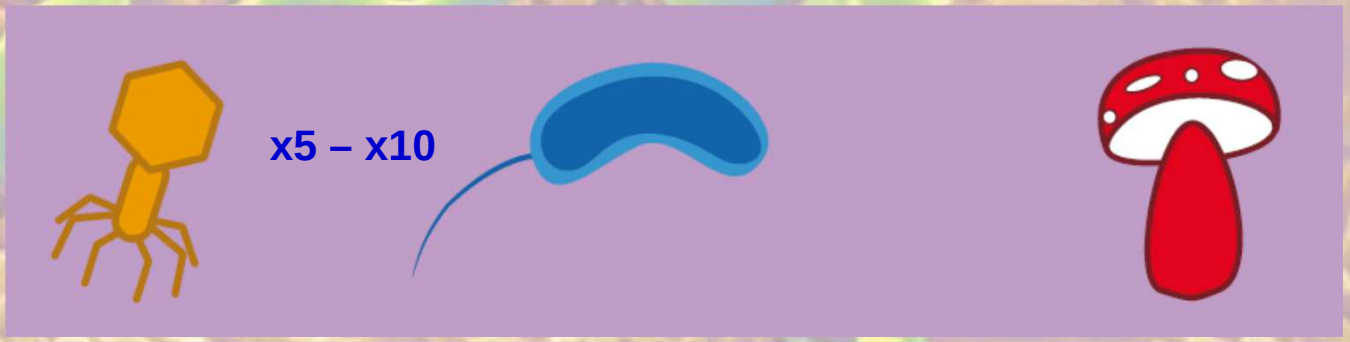
Migracions humanes, càncer gàstric i perspectives

- **Introducció**
- ***Helicobacter pylori* i les malalties gàstriques**
- ***Helicobacter pylori* i les migracions humanes**
- **Perspectives**

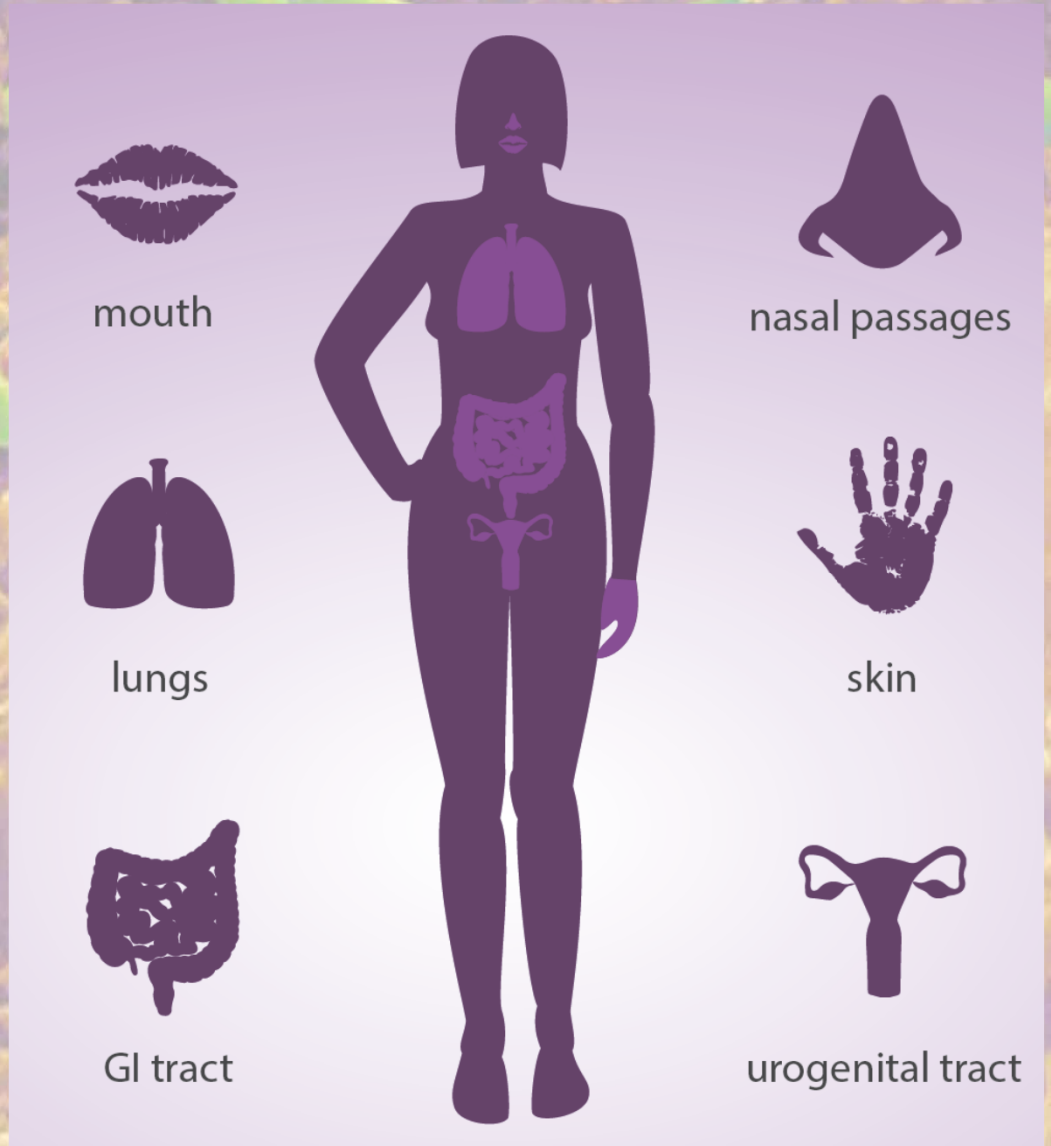
- **Microbiota**: tots els microorganismes que viuen en un ambient concret com, per exemple, el cos humà. No és correcte parlar de '**microflora**'.

- **Microbioma**: tota la col·lecció de gens que es troba en la microbiota d'un determinat hoste.

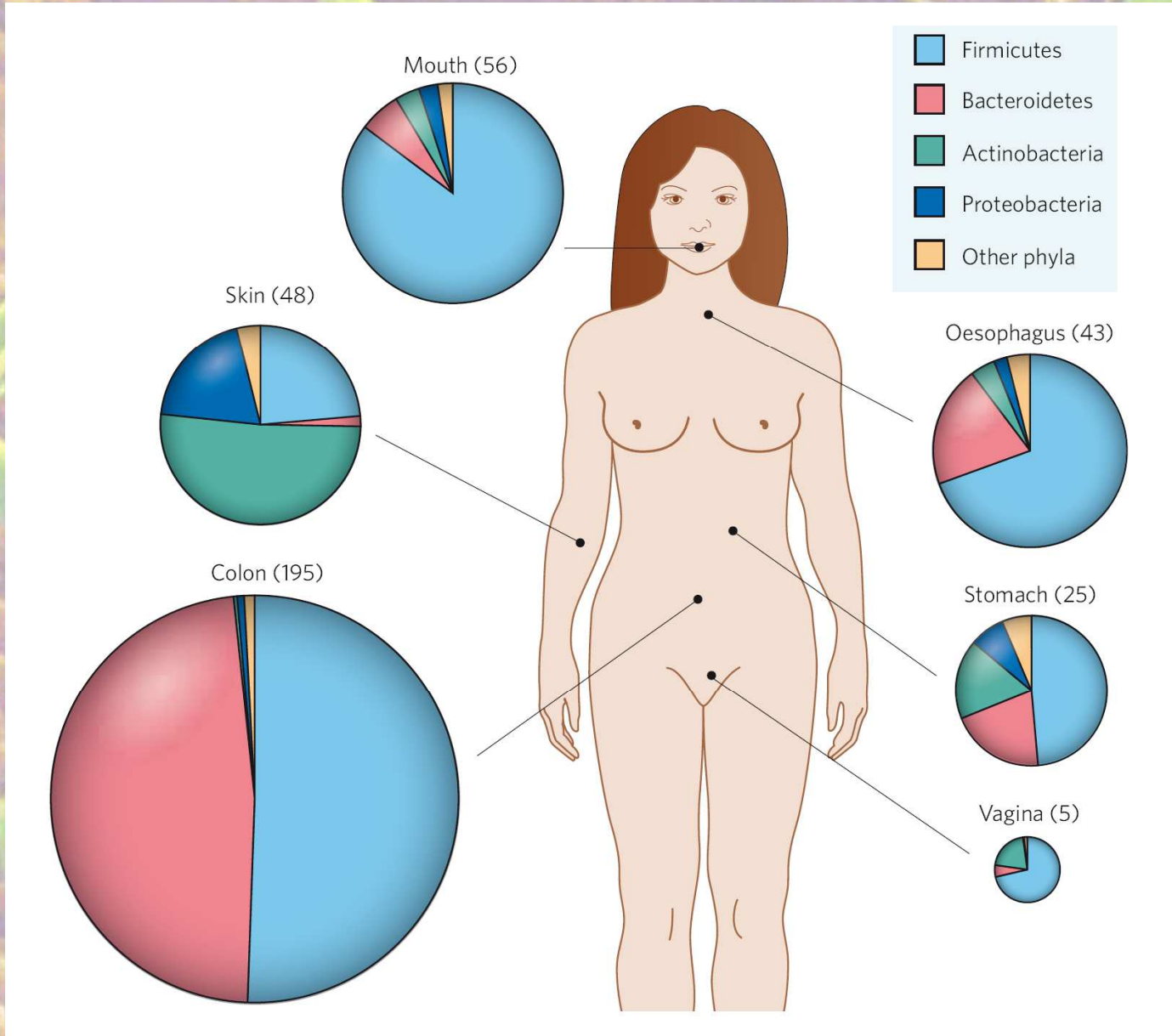




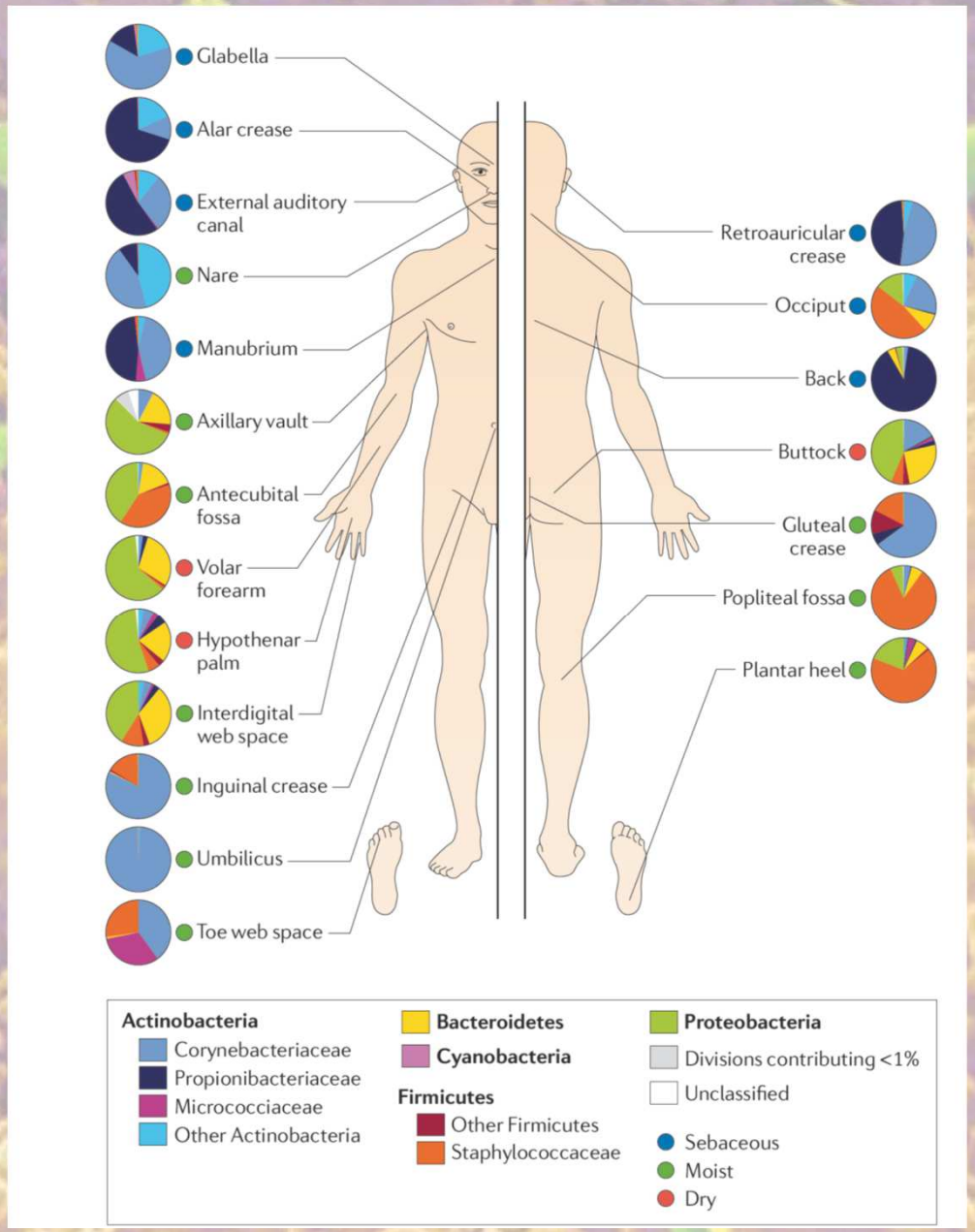
FAQ: Human Microbiome, ASM
http://academy.asm.org/index.php/faq-series/5122-humanmicrobiome



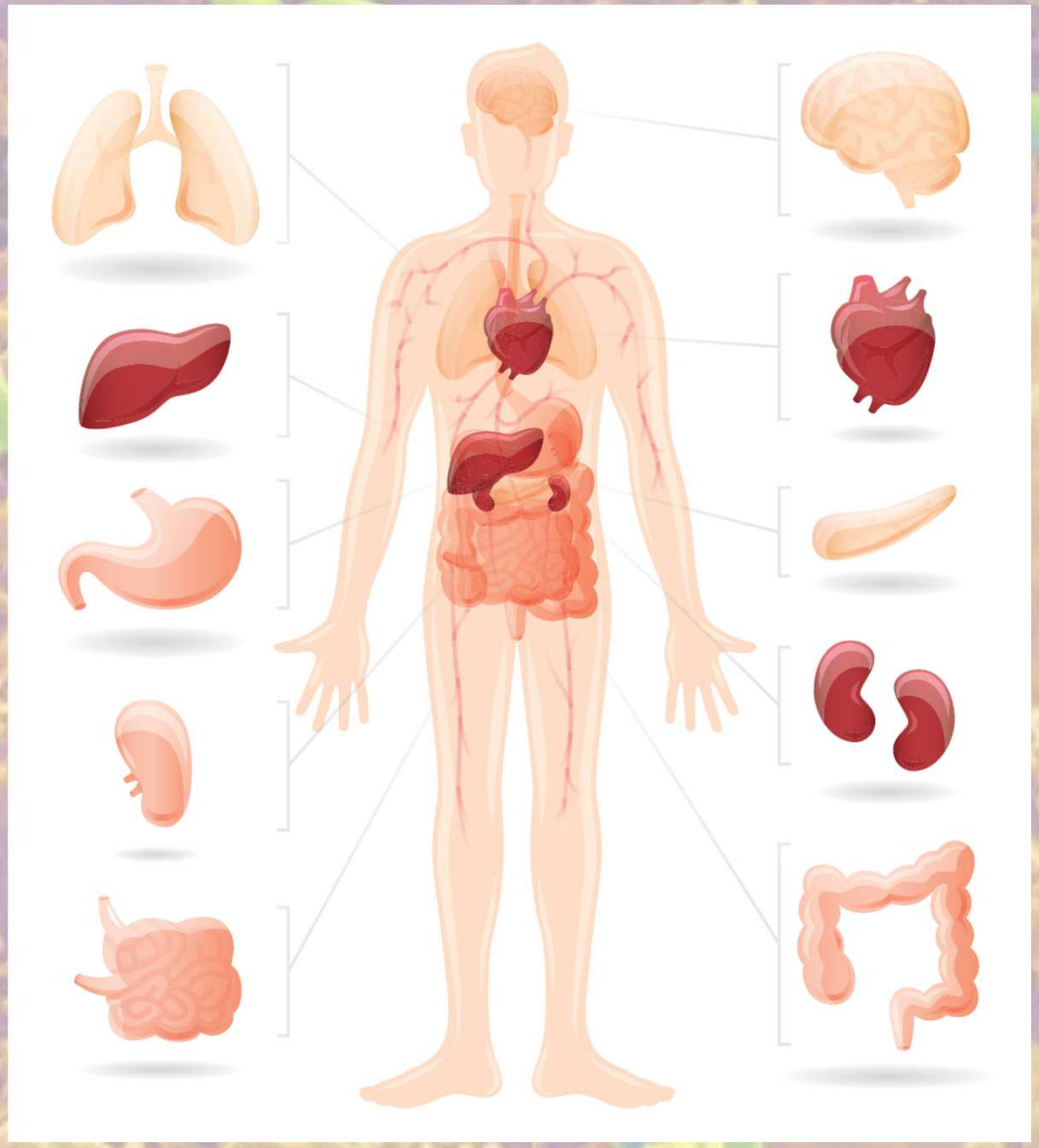
FAQ: Human Microbiome, ASM
http://academy.asm.org/index.php/faq-series/5122-humanmicrobiome



Dethlefsen L, McFall-Ngai M, Relman, DA. An ecological and evolutionary perspective on human-microbe mutualism and disease. Nature 2007; 449: 811-8.



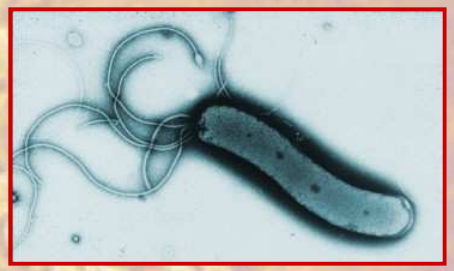
Grice EA, Segre JA. The skin microbiome. Nature Rev Microbiol. 2011; 9: 244-53.



FAQ: Human Microbiome, ASM
http://academy.asm.org/index.php/faq-series/5122-humanmicrobiome

Helicobacter pylori

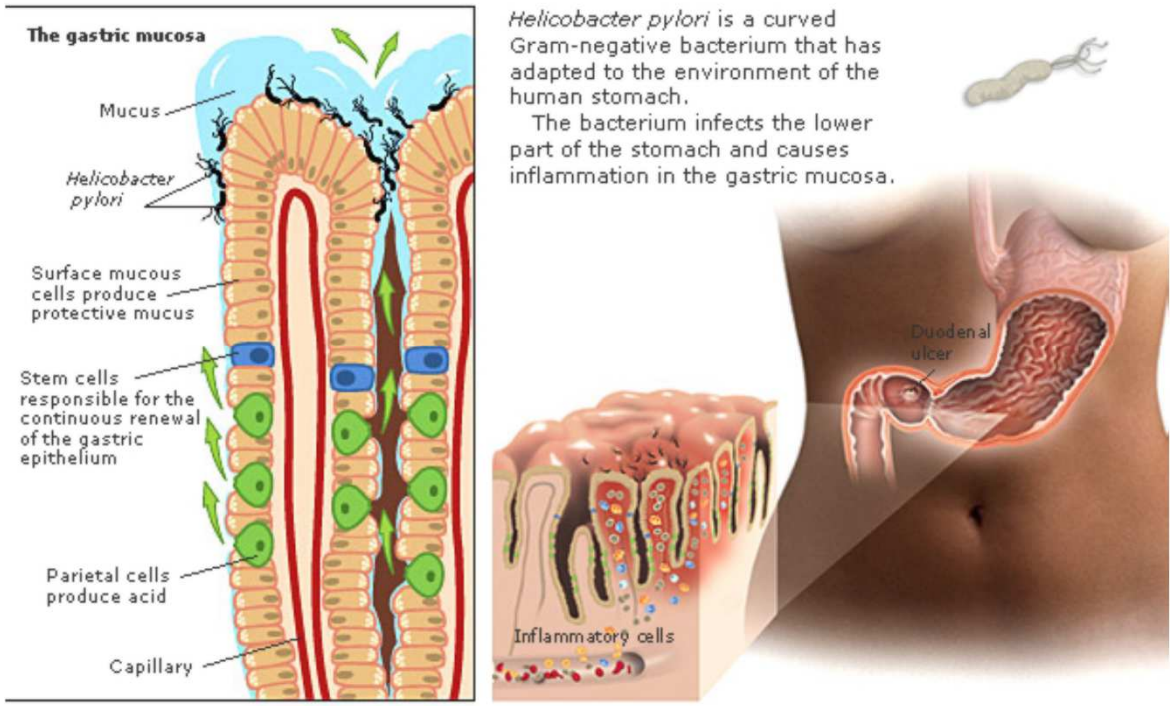
- **ε-Proteobacteri**
- **Bacteri gramnegatiu**
- **Forma d'espiril**
- **Mòbil per un feix de flagels polar**
- **Ureasa, oxidasa y catalasa +**
- **Cultiu exigent**
- **Microaeròfil**
- **37ºC**



- **Transmissió per contacte directe**

  (oral-oral, gastro-oral, fecal-oral)

- **Colonitza l'estómac humà**



'*The Nobel Prize in Physiology or Medicine 2005*'
http://www.nobelprize.org/nobel_prizes/medicine/laureates/2005/

- **La meitat de la població mundial està infectada**

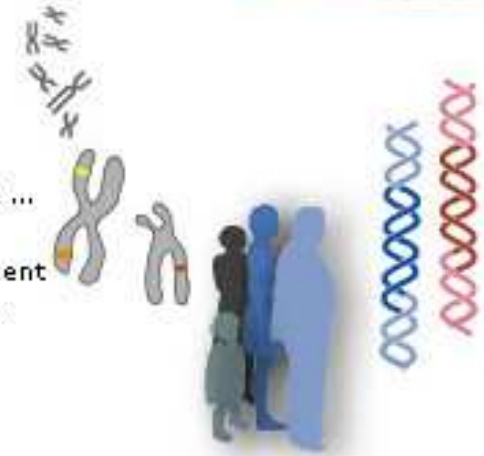
Lifelong infection

Helicobacter pylori colonizes the stomach in about 50% of all humans with great differences among countries.

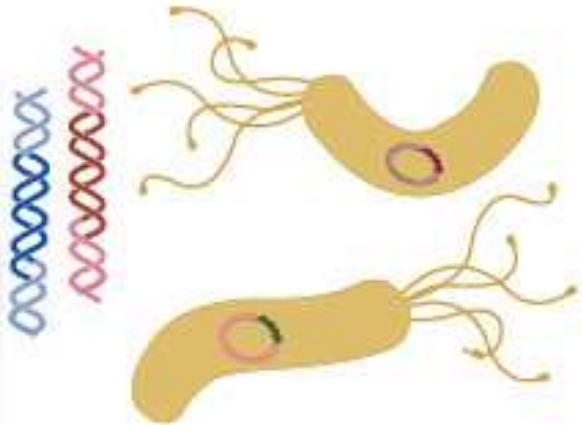
Infection is typically contracted in early childhood, frequently by transmission from mother to child. The bacteria may remain in the stomach for the rest of the person's life.

Disease or not?

Only a minority of infected individuals develop stomach disease. The bacterium itself is extremely variable and the variants confer different risks of disease. Genetic variation among humans may also affect the susceptibility to disease caused by *Helicobacter pylori*.

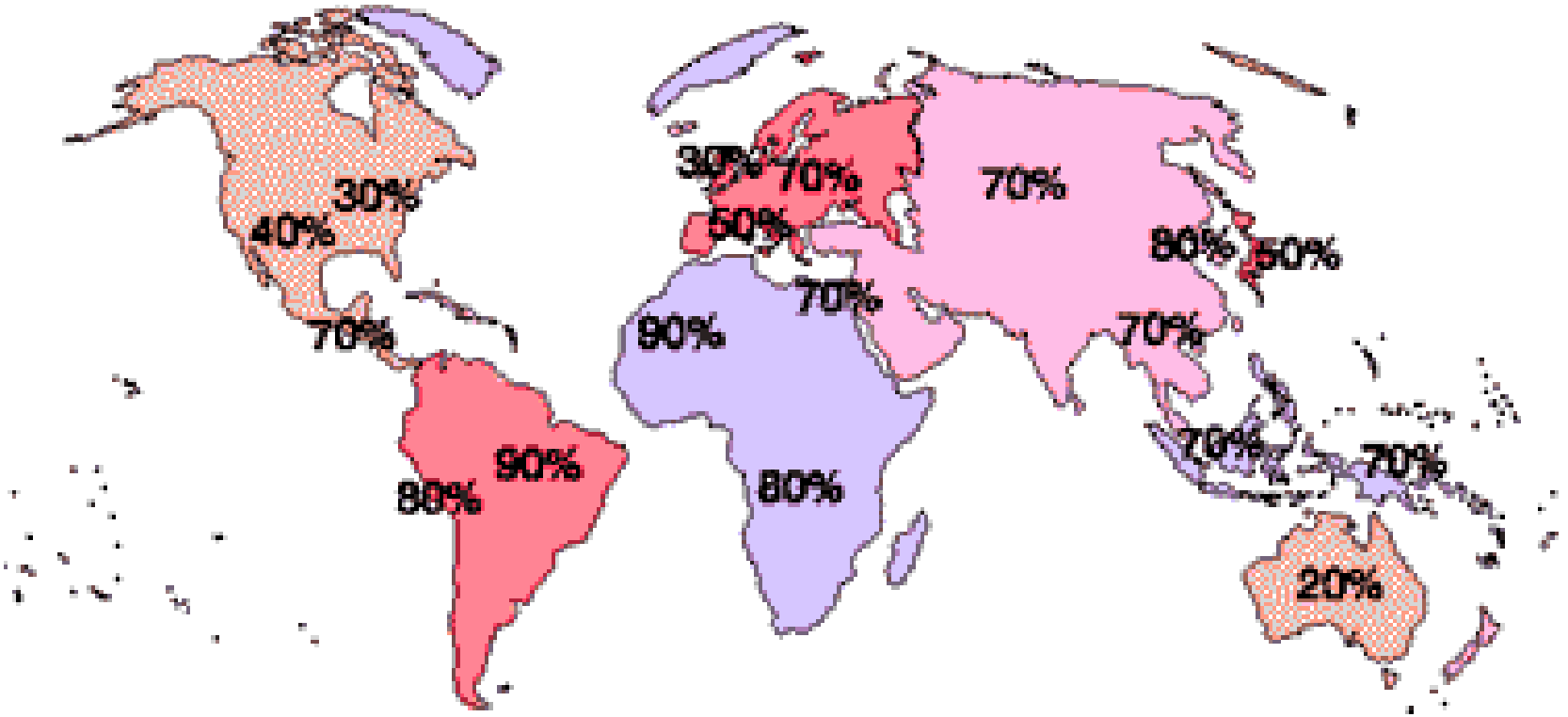


Genetic variation among humans.



Genetic variation among bacteria.

'*The Nobel Prize in Physiology or Medicine 2005*'
http://www.nobelprize.org/nobel_prizes/medicine/laureates/2005/



The 'Helicobacter Foundation'
http://www.helico.com/

Dr. David Miñana i Galbis, Dept. Microbiologia i Parasitologia Sanitàries, Facultat de Farmàcia, UB

Helicobacter pylori:

Migracions humanes, càncer gàstric i perspectives

- Introducció
- *Helicobacter pylori* i les malalties gàstriques
- *Helicobacter pylori* i les migracions humanes
- Perspectives

The Nobel Prize in Physiology or Medicine 2005
Barry J. Marshall, J. Robin Warren

Share this: 15

The Nobel Prize in Physiology or Medicine 2005

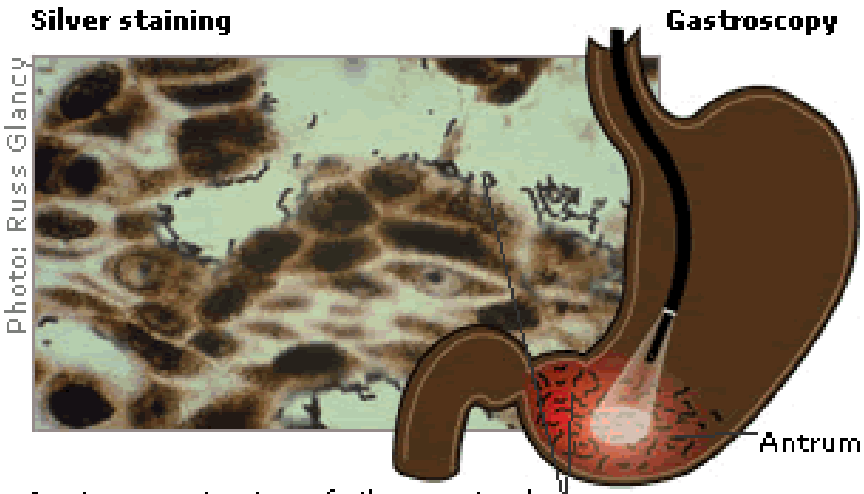
Photo: C. Northcott
Barry J. Marshall
Prize share: 1/2

Photo: U. Montan
J. Robin Warren
Prize share: 1/2

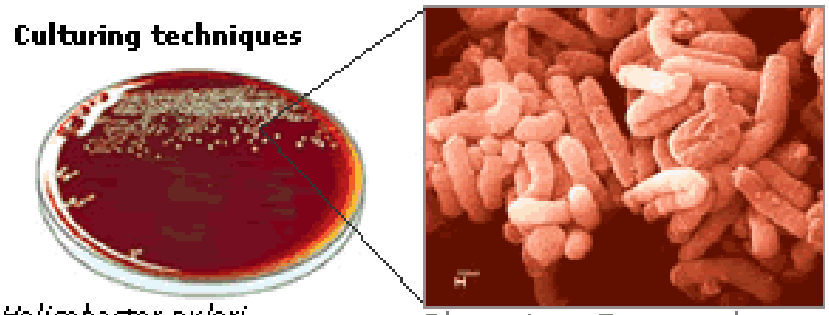
The Nobel Prize in Physiology or Medicine 2005 was awarded jointly to Barry J. Marshall and J. Robin Warren *"for their discovery of the bacterium Helicobacter pylori and its role in gastritis and peptic ulcer disease"*

Photos: Copyright © The Nobel Foundation

1981-2



A microscopic view of silver stained *Helicobacter pylori* attached to the gastric epithelium.



Helicobacter pylori forms colonies visible after 3 to 5 days.

Photo: Lars Engstrand, Christina Nilsson

1984 *Campylobacter pyloridis* → 1987 *Campylobacter pylori* → 1989 *Helicobacter pylori*

'*The Nobel Prize in Physiology or Medicine 2005*'
http://www.nobelprize.org/nobel_prizes/medicine/laureates/2005/

1984

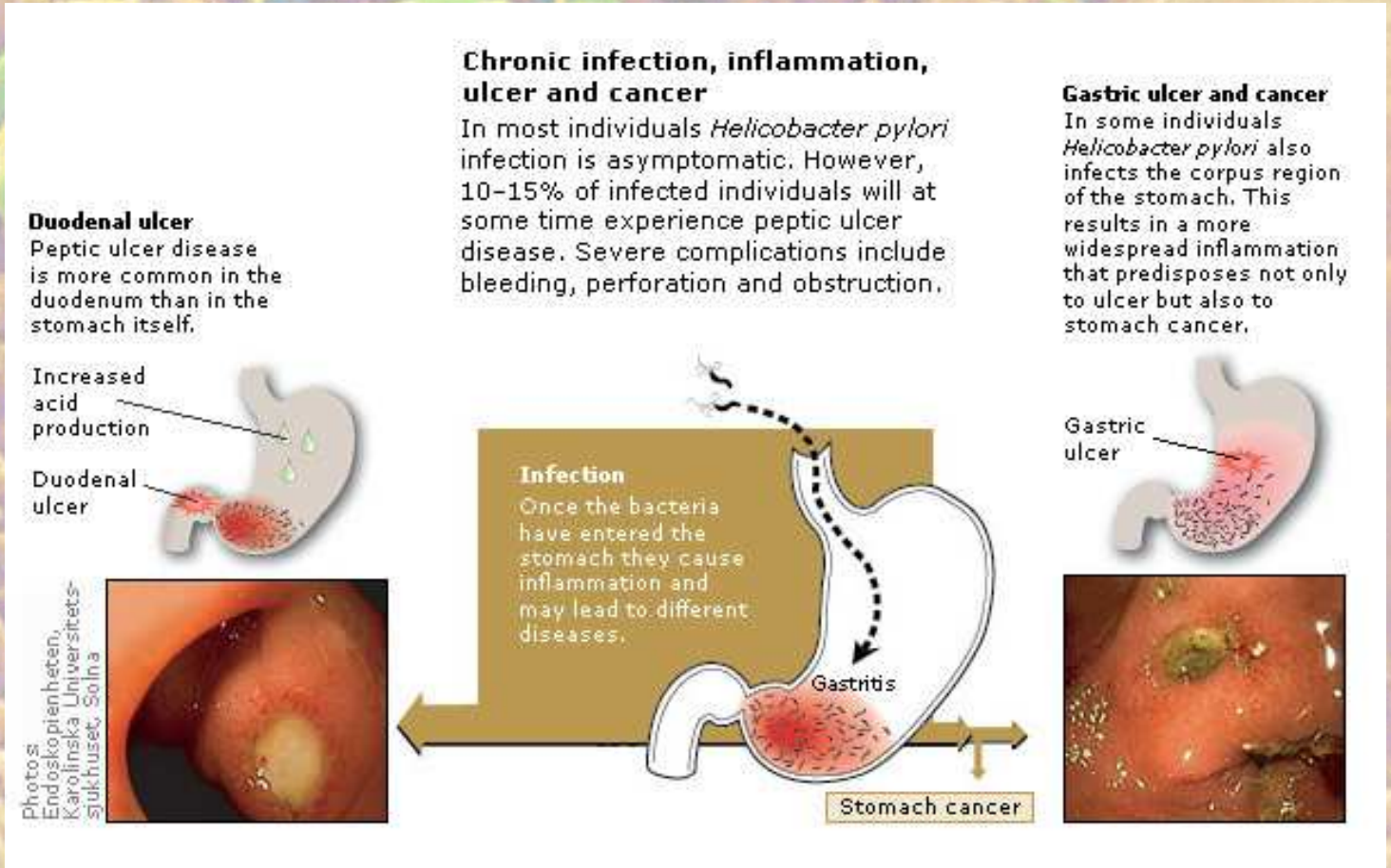
'*The Nobel Prize in Physiology or Medicine 2005*'
http://www.nobelprize.org/nobel_prizes/medicine/laureates/2005/

1994 **S'aprova el tractament de les úlceres per l'FDA**

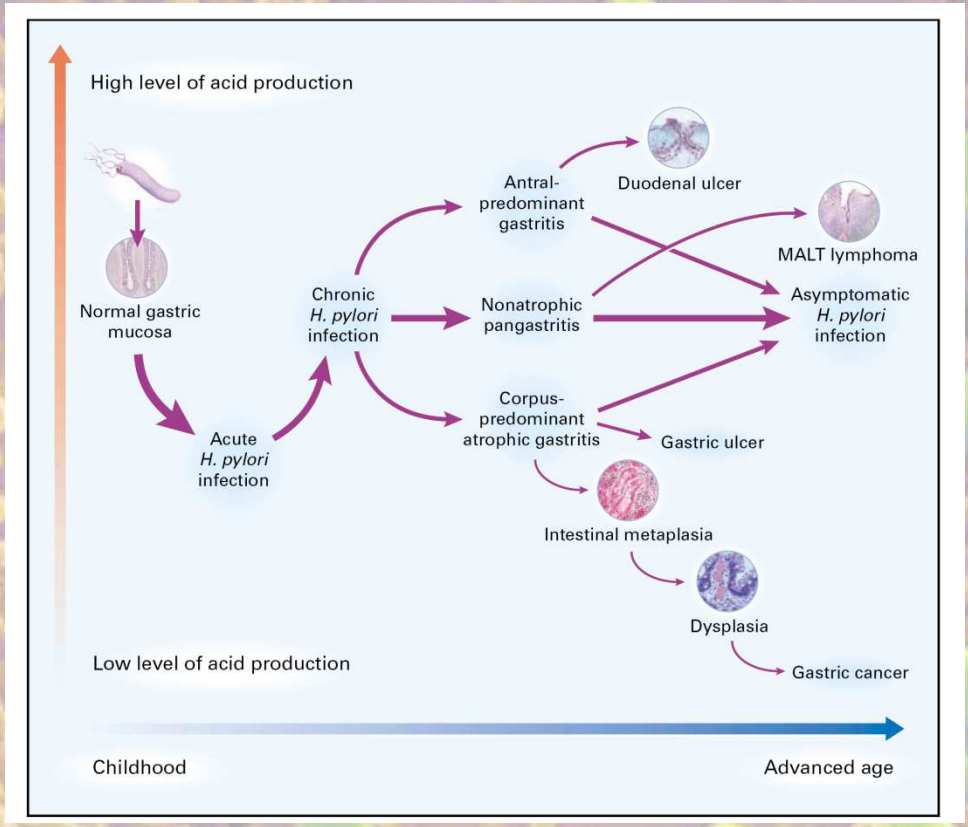
(*US Food and Drug Administration*)

Es classifica *H. pylori* com a carcinogen de classe I per la IARC

(WHO *International Agency for Research on Cancer*)



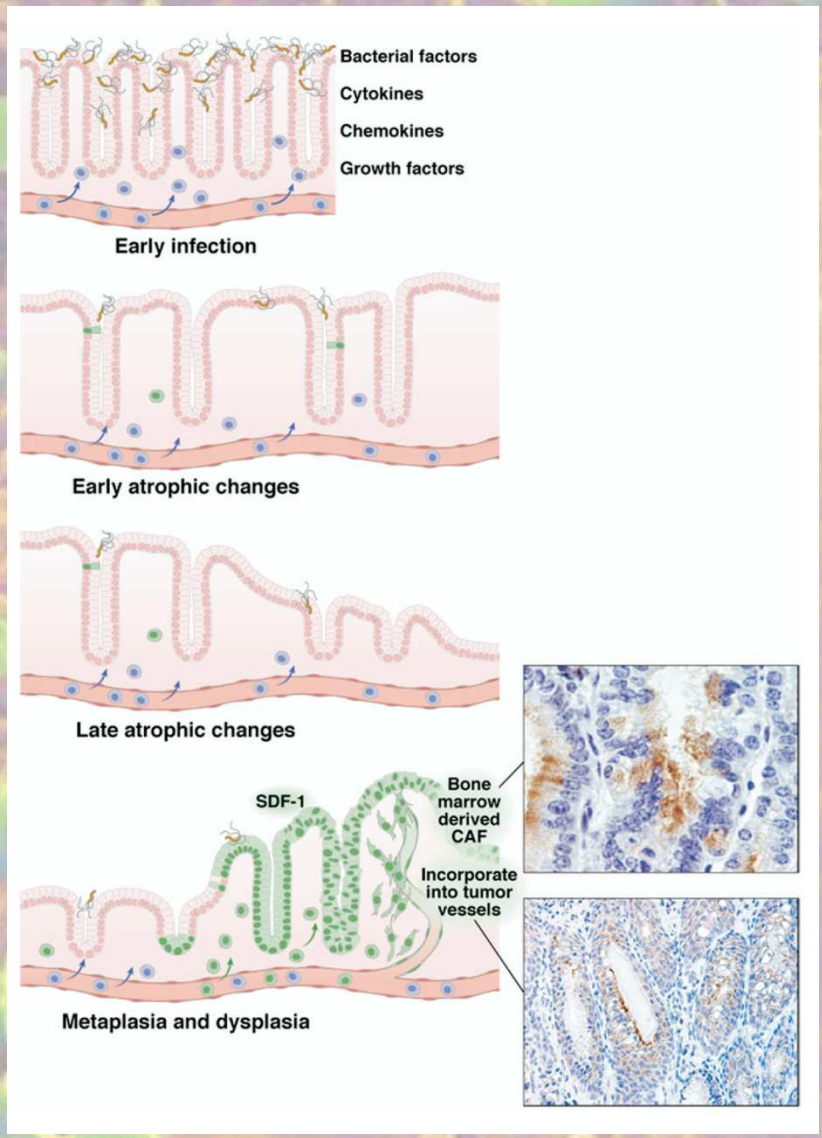
'*The Nobel Prize in Physiology or Medicine 2005*'
http://www.nobelprize.org/nobel_prizes/medicine/laureates/2005/



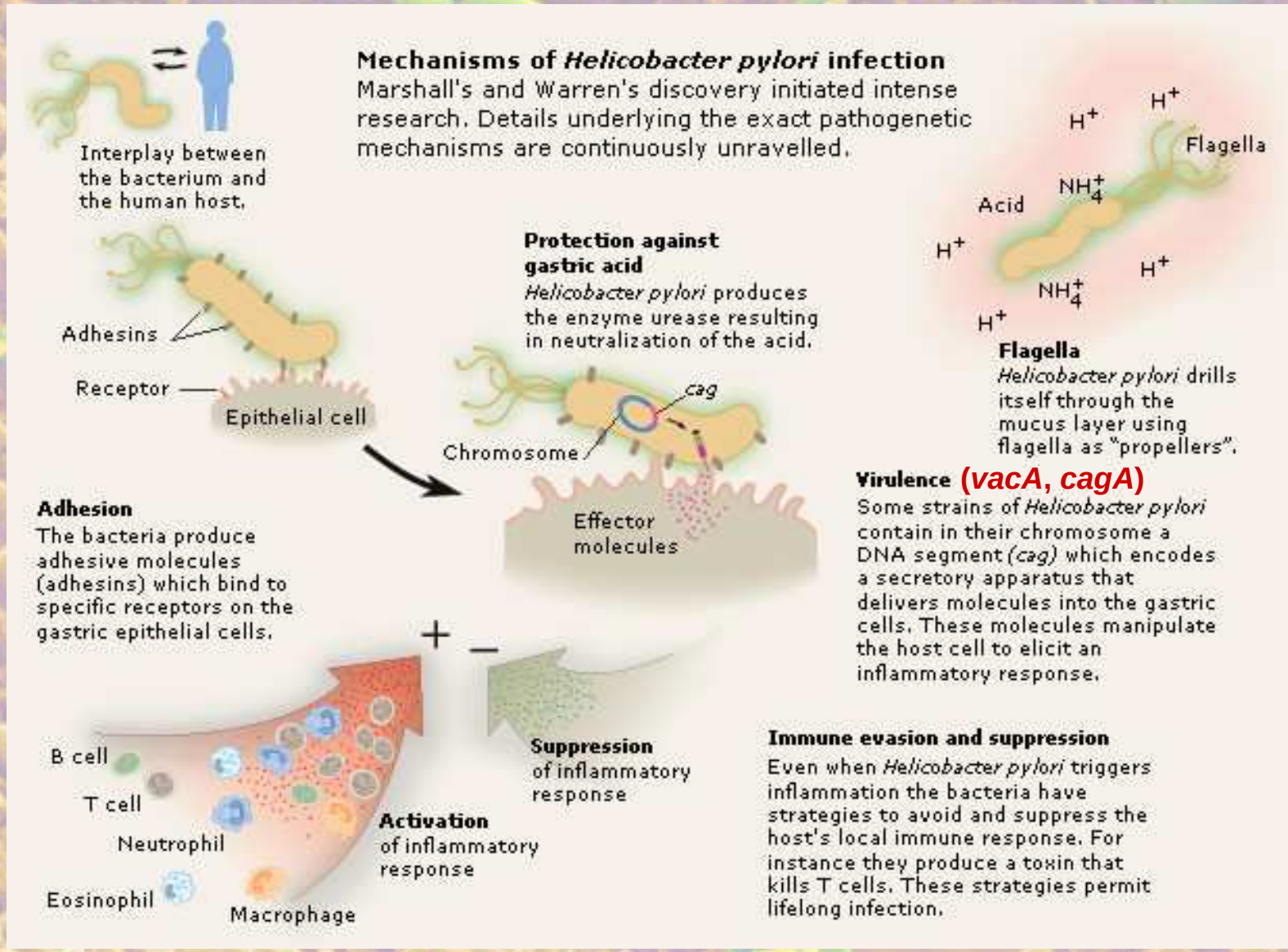
Suerbaum S, Michetti P. *Helicobacter pylori* infection.
N Engl J Med. 2002; 347 (15): 1175-86.

4t càncer més comú

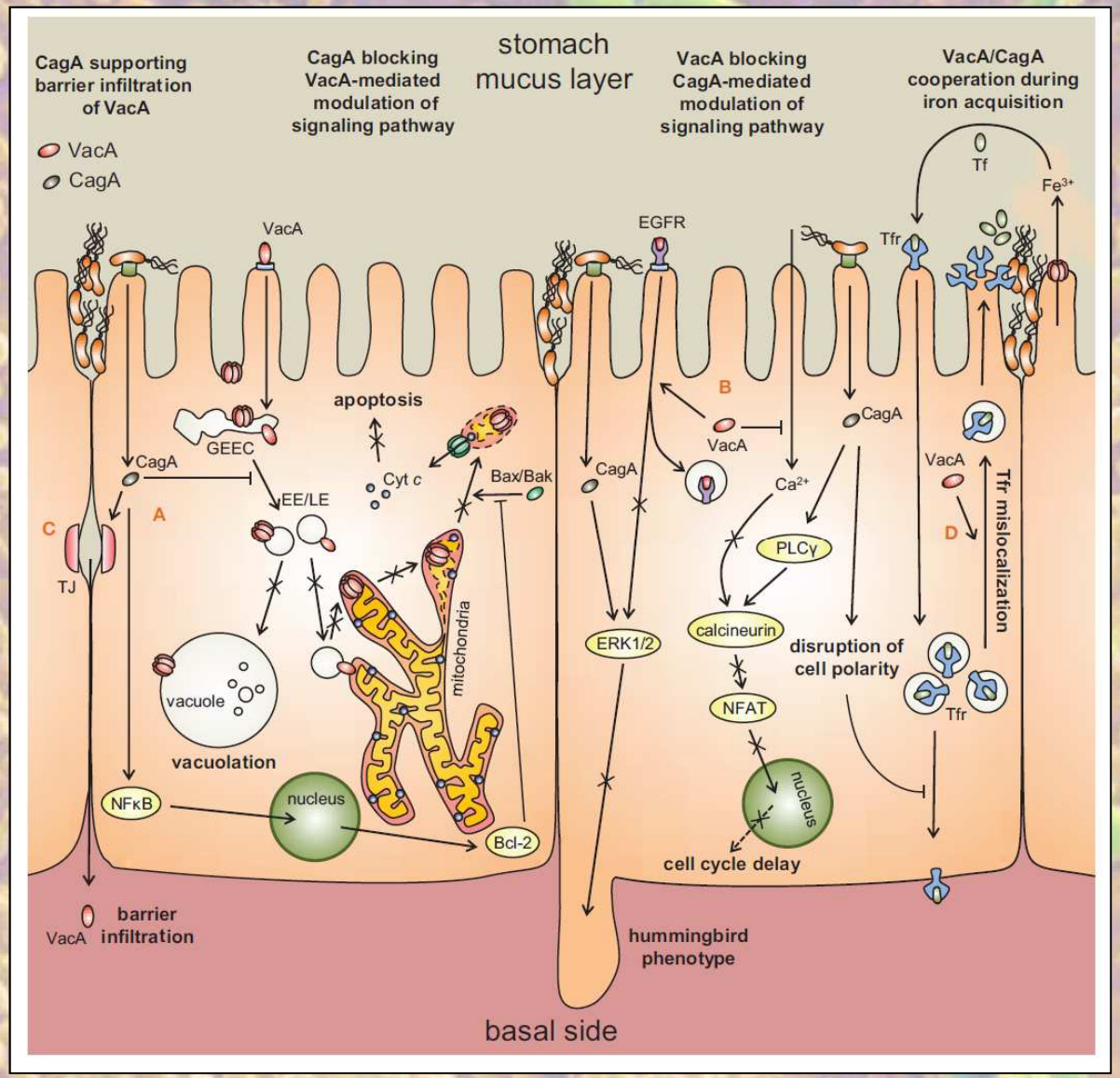
2a causa de mort per càncer

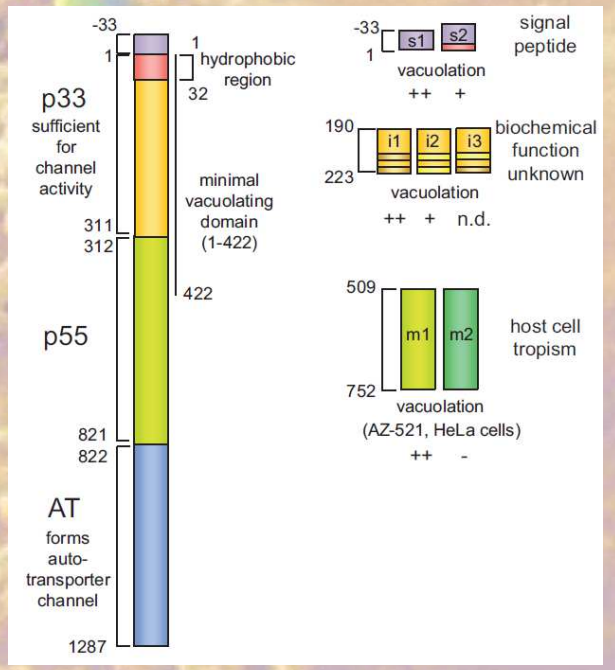


Correa P, Houghton J. Carcinogenesis of *Helicobacter pylori*.
Gastroenterology 2007; 133 (2): 659-72.



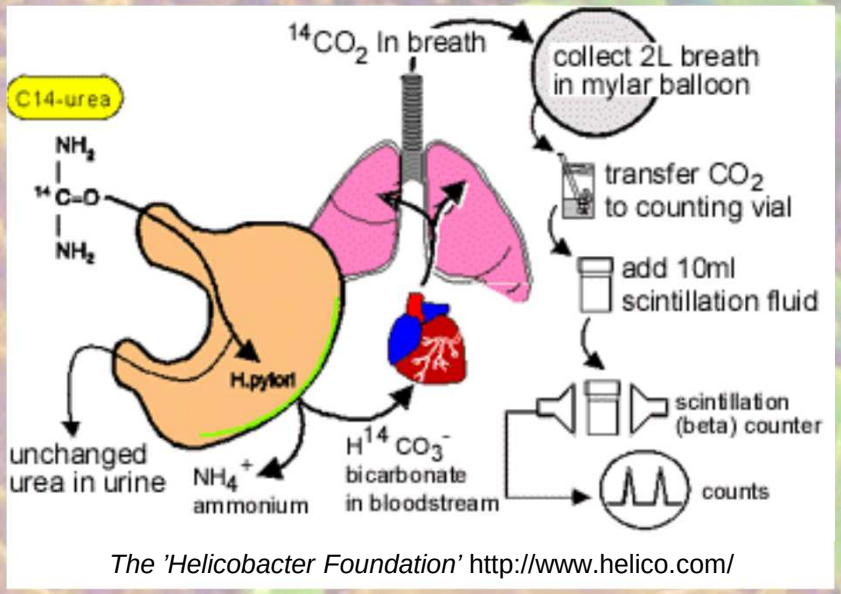
'*The Nobel Prize in Physiology or Medicine 2005*'
http://www.nobelprize.org/nobel_prizes/medicine/laureates/2005/



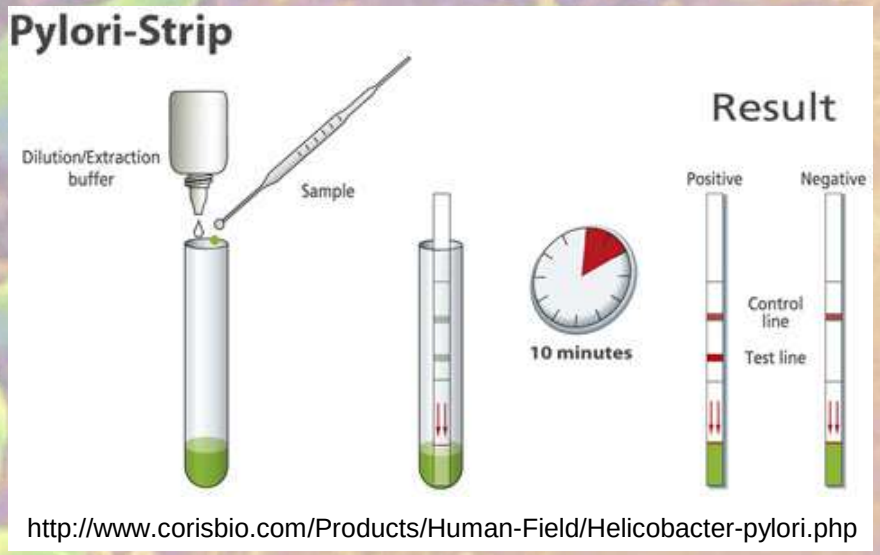


Kim IJ, Blanke SR. Remodeling the host environment: modulation of the gastric epithelium by the *Helicobacter pylori* vacuolating toxin (VacA). Front Cell Infect Microbiol. 2012; 2: 37.

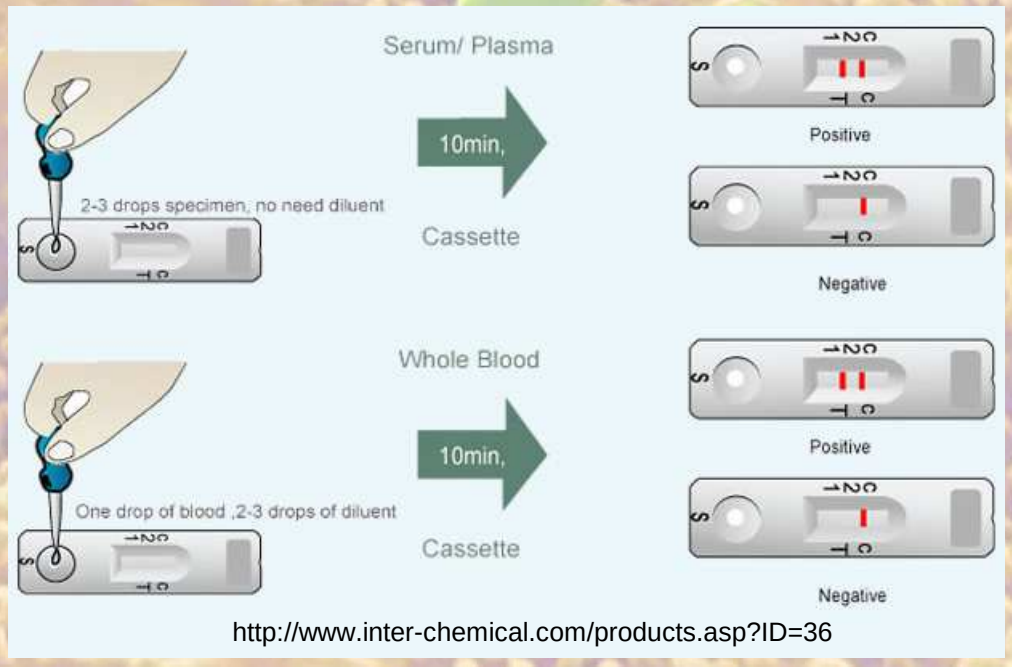
DIAGNÒSTIC PER MÈTODES NO INVASIUS



The 'Helicobacter Foundation' http://www.helico.com/



http://www.corisbio.com/Products/Human-Field/Helicobacter-pylori.php



http://www.inter-chemical.com/products.asp?ID=36

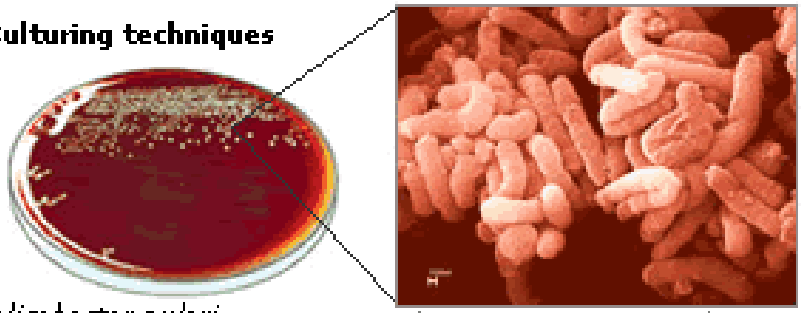
Dr. David Miñana i Galbis, Dept. Microbiologia i Parasitologia Sanitàries, Facultat de Farmàcia, UB

DIAGNÒSTIC POR MÈTODES INVASIUS

Silver staining **Gastroscopy**

A microscopic view of silver stained *Helicobacter pylori* attached to the gastric epithelium.

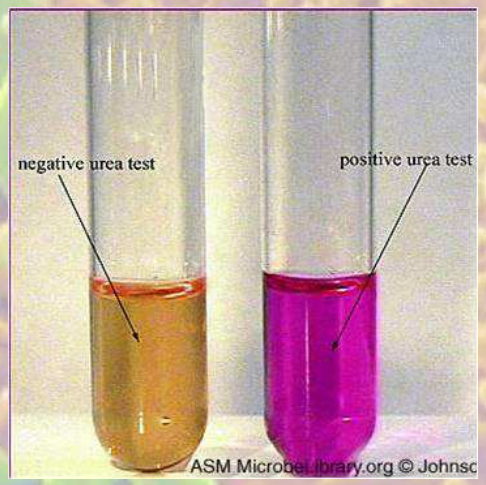
Culturing techniques



Helicobacter pylori forms colonies visible after 3 to 5 days.

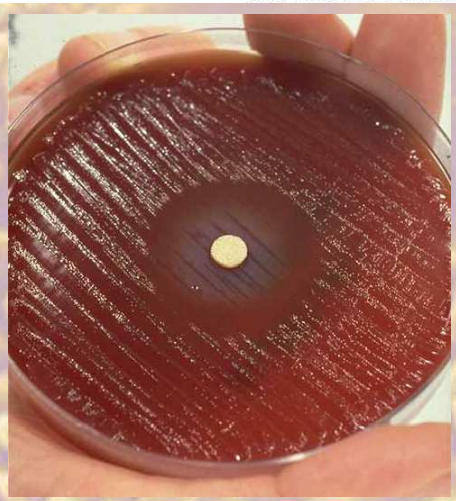
Photo: Lars Engstrand, Christina Nilsson

Large benign gastric ulcer. Ulcers are the white areas (Images.MD)



PCR

Genta stain of aa biopsy specimen (Images.MD)



'*The Nobel Prize in Physiology or Medicine 2005*'
http://www.nobelprize.org/nobel_prizes/medicine/laureates/2005/

http://www.angelfire.com/planet/hpylori/symptoms.htm

Table 4. First-Line Regimens for *Helicobacter pylori* Eradication

Regimen	Duration	Eradication Rates	Comments
Standard dose PPI b.i.d. (esomeprazole is q.d.), clarithromycin 500 mg b.i.d., amoxicillin 1,000 mg b.i.d.	10–14	70–85%	Consider in nonpenicillin allergic patients who have not previously received a macrolide
Standard dose PPI b.i.d., clarithromycin 500 mg b.i.d. metronidazole 500 mg b.i.d.	10–14	70–85%	Consider in penicillin allergic patients who have not previously received a macrolide or are unable to tolerate bismuth quadruple therapy
Bismuth subsalicylate 525 mg p.o. q.i.d. metronidazole 250 mg p.o. q.i.d., tetracycline 500 mg p.o. q.i.d., ranitidine 150 mg p.o. b.i.d. or standard dose PPI q.d. to b.i.d.	10–14	75–90%	Consider in penicillin allergic patients
PPI + amoxicillin 1 g b.i.d. followed by: PPI, clarithromycin 500 mg, tinidazole 500 mg b.i.d.	5 5	>90%	Requires validation in North America

PPI = proton pump inhibitor; pcn = penicillin; p.o. = orally; q.d. = daily; b.i.d. = twice daily; t.i.d. = three times daily; q.i.d. = four times daily.
*Standard dosages for PPIs are as follows:
lansoprazole 30 mg p.o., omeprazole 20 mg p.o., pantoprazole 40 mg p.o., rabeprazole 20 mg p.o., esomeprazole 40 mg p.o.
Note: the above recommended treatments are not all FDA approved. The FDA approved regimens are as follows:
1. Bismuth 525 mg q.i.d. + metronidazole 250 mg q.i.d. + tetracycline 500 mg q.i.d. × 2 wk + H_2RA as directed × 4 wk.
2. Lansoprazole 30 mg b.i.d. + clarithromycin 500 mg b.i.d. + amoxicillin 1 g b.i.d. × 10 days.
3. Omeprazole 20 mg b.i.d. + clarithromycin 500 mg b.i.d. + amoxicillin 1 g b.i.d. × 10 days.
4. esomeprazole 40 mg q.d. + clarithromycin 500 mg b.i.d. + amoxicillin 1 g b.i.d. × 10 days.
5. Rabeprazole 20 mg b.i.d. + clarithromycin 500 mg b.i.d. + amoxicillin 1 g b.i.d. × 7 days.

Table 5. Salvage Therapies for Persistent *H. pylori* Infection (164)

Regimen	Duration	Eradication Rates	Comments
Bismuth quadruple therapy PPI q.d. tetracycline, Pepto Bismol, metronidazole q.i.d.	7	68% (95% CI 62–74%)	Accessible, cheap but high pill count and frequent mild side effects
Levofloxacin triple therapy PPI, amoxicillin 1 g b.i.d., levofloxacin 500 mg q.d.	10	87% (95% CI 82–92%)	Requires validation in North America

For recommendations regarding rifabutin and furazolidone, please refer to the text.
PPI = proton pump inhibitor; q.d. = daily; q.i.d. = four times daily; b.i.d. = twice daily.

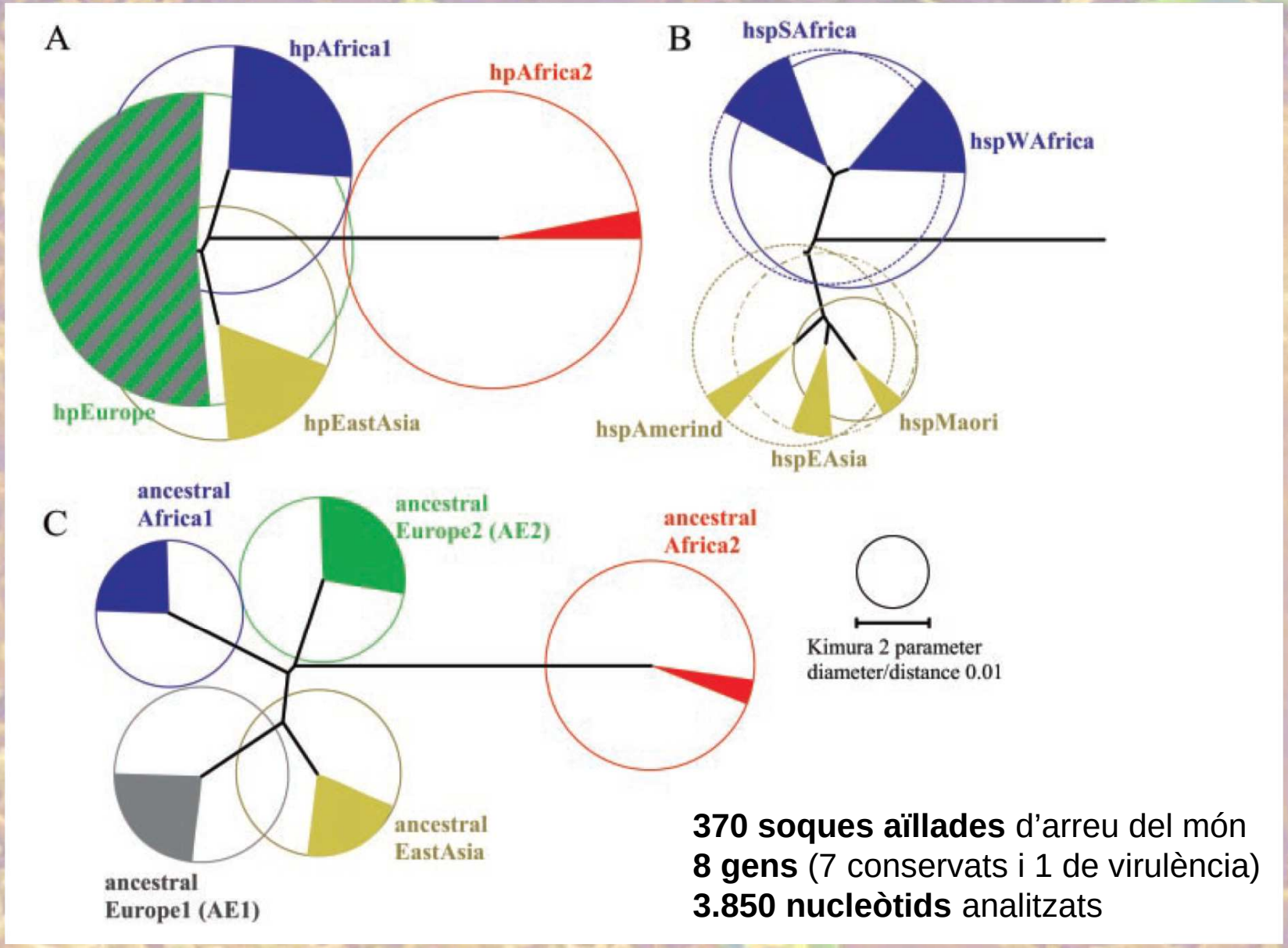
Chey WD, et al. American College of Gastroenterology guideline on the management of *Helicobacter pylori* infection. Am J Gastroenterol. 2007; 102 (8): 1808-25.

Dr. David Miñana i Galbis, Dept. Microbiologia i Parasitologia Sanitàries, Facultat de Farmàcia, UB

Helicobacter pylori:

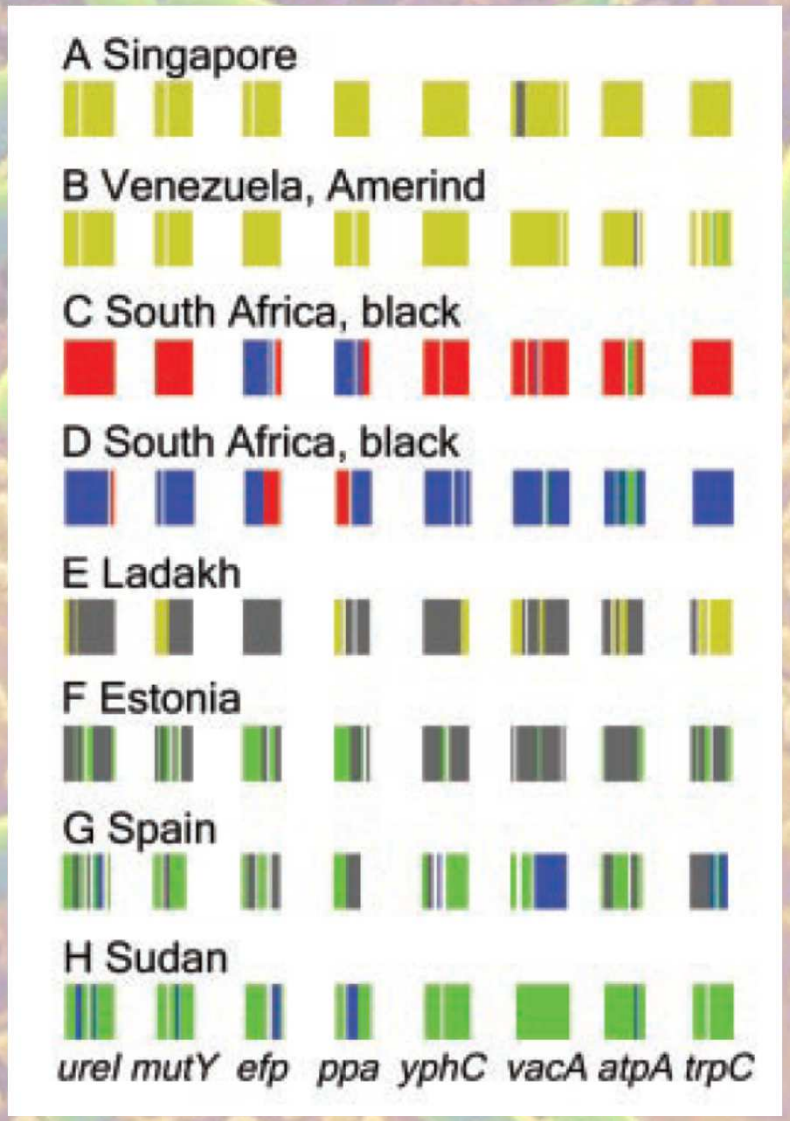
Migracions humanes, càncer gàstric i perspectives

- Introducció
- *Helicobacter pylori* i les malalties gàstriques
- *Helicobacter pylori* i les migracions humanes
- Perspectives

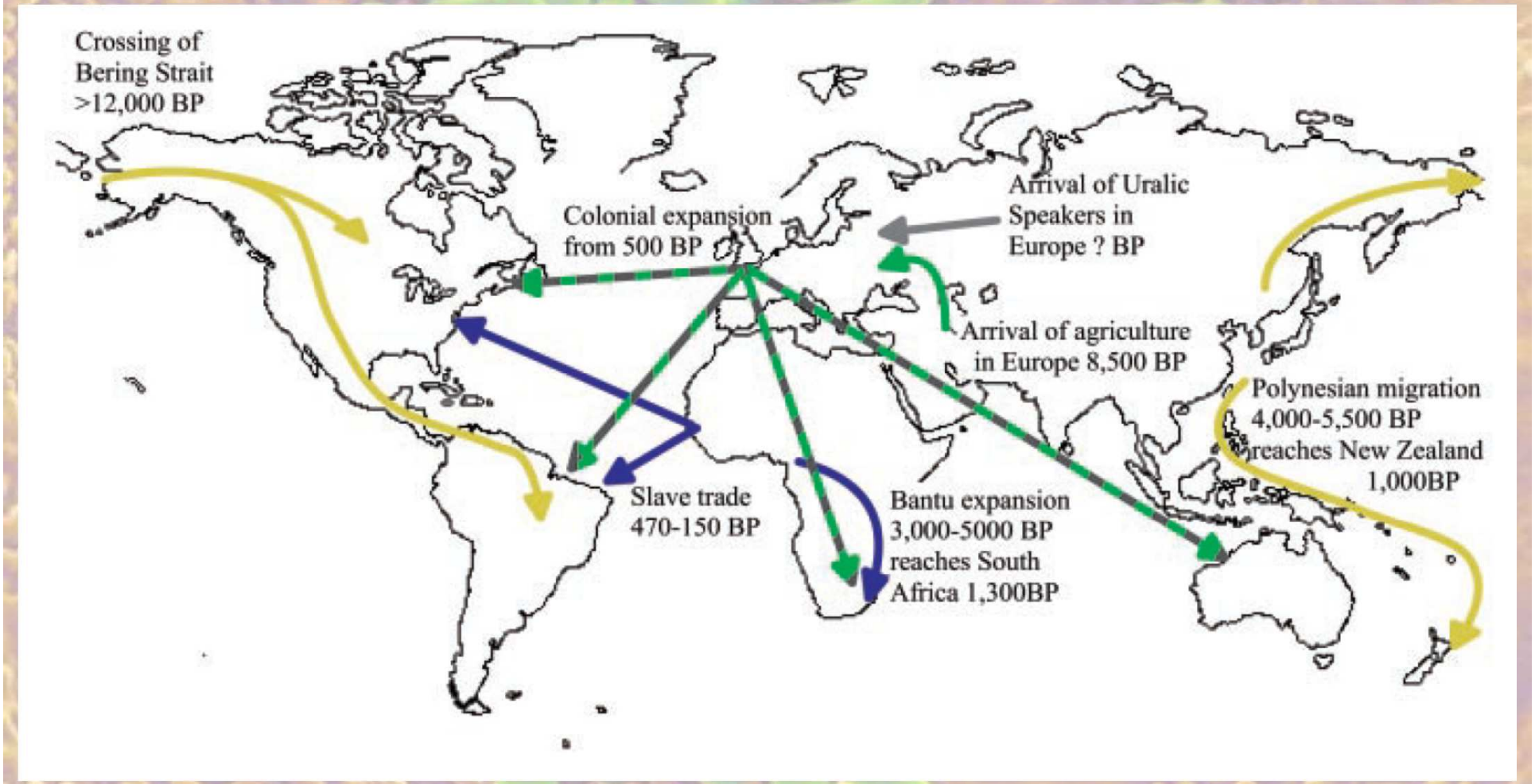


370 soques aïllades d'arreu del món
8 gens (7 conservats i 1 de virulència)
3.850 nucleòtids analitzats

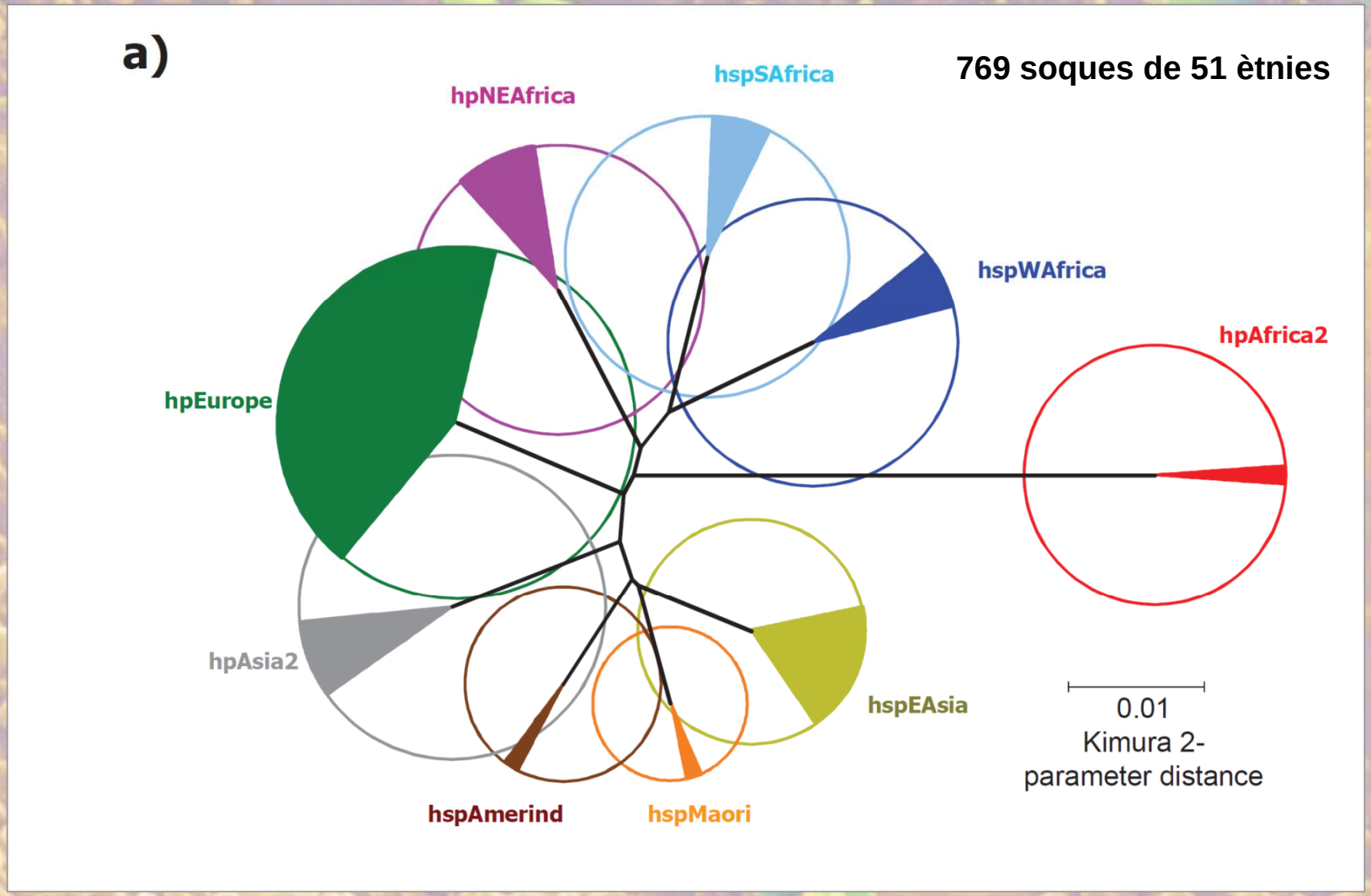
Falush D, et al. Traces of human migrations in *Helicobacter pylori* populations. Science 2003; 299 (5612): 1582-5.



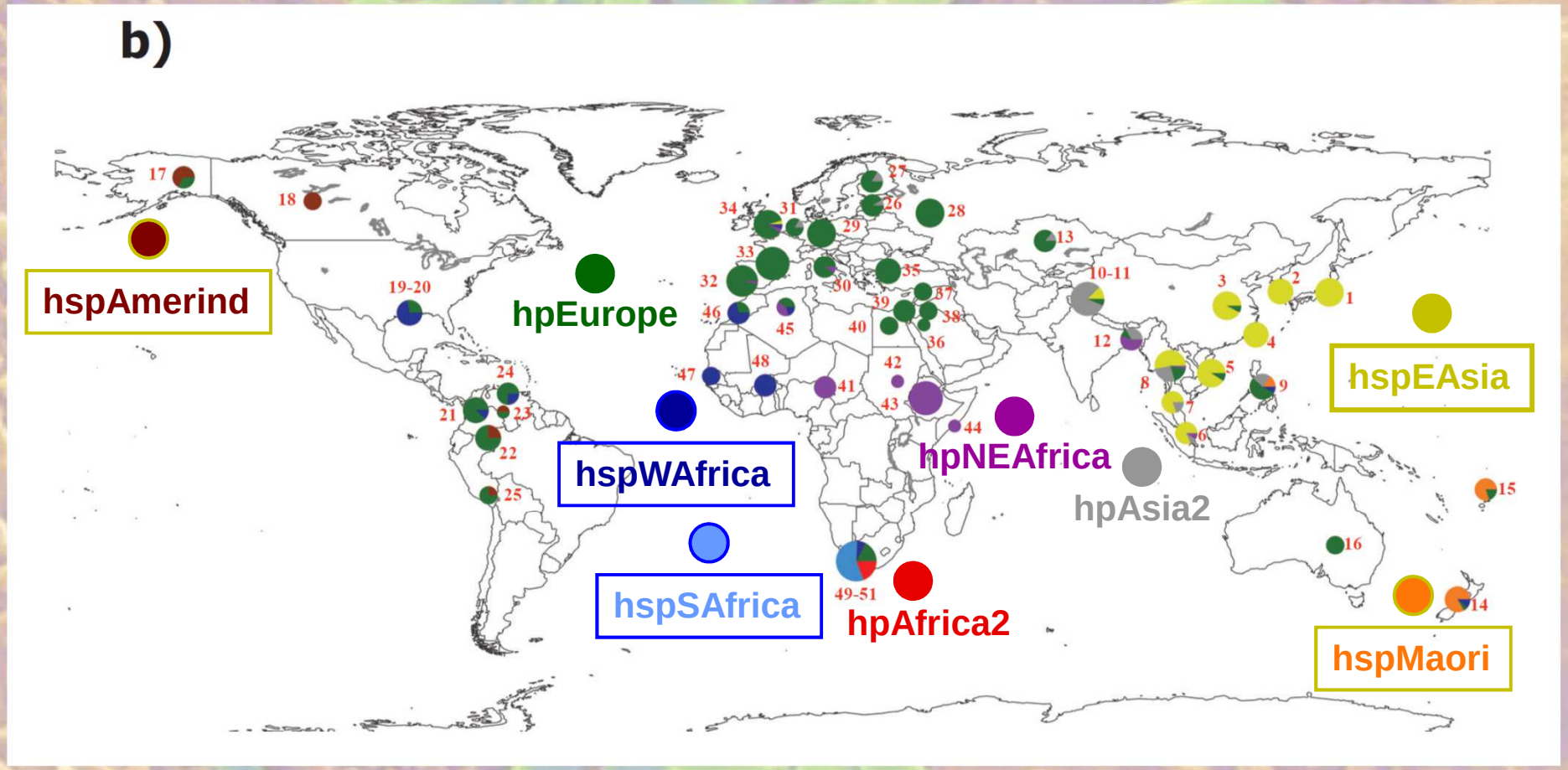
Falush D, et al. Traces of human migrations in *Helicobacter pylori* populations. Science 2003; 299 (5612): 1582-5.



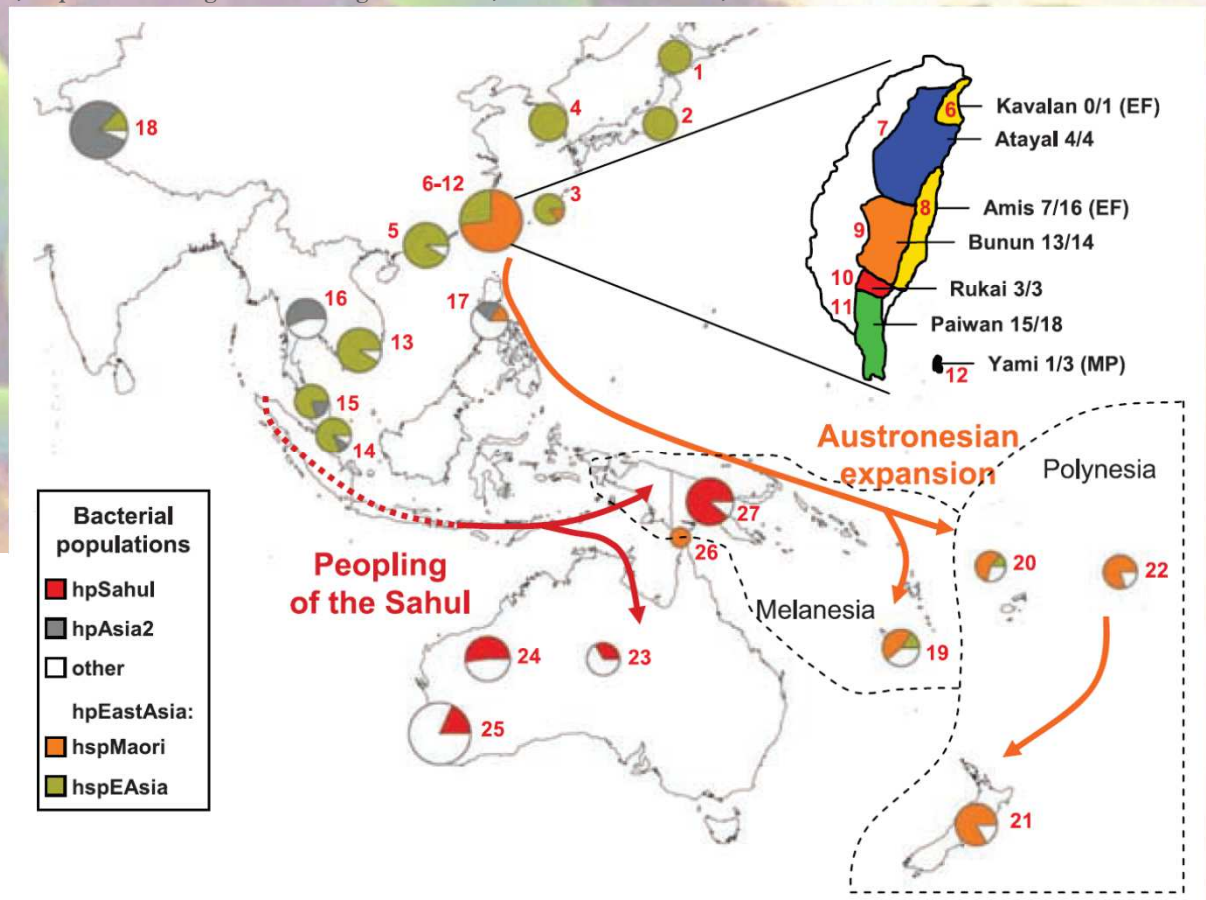
Falush D, et al. Traces of human migrations in *Helicobacter pylori* populations. Science 2003; 299 (5612): 1582-5.

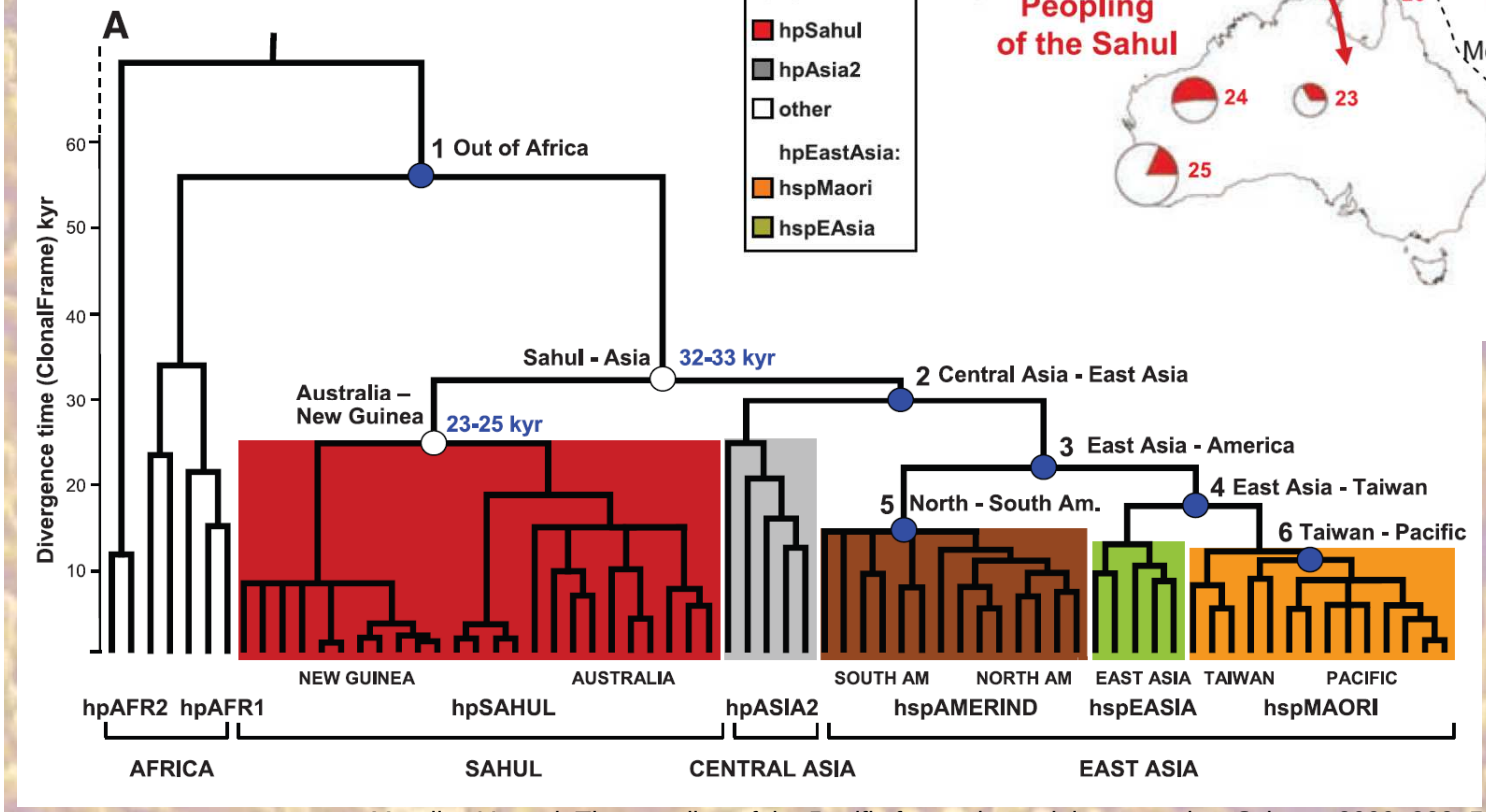


Linz B, et al. An African origin for the intimate association between humans and *Helicobacter pylori*. Nature 2007; 445: 915-8.

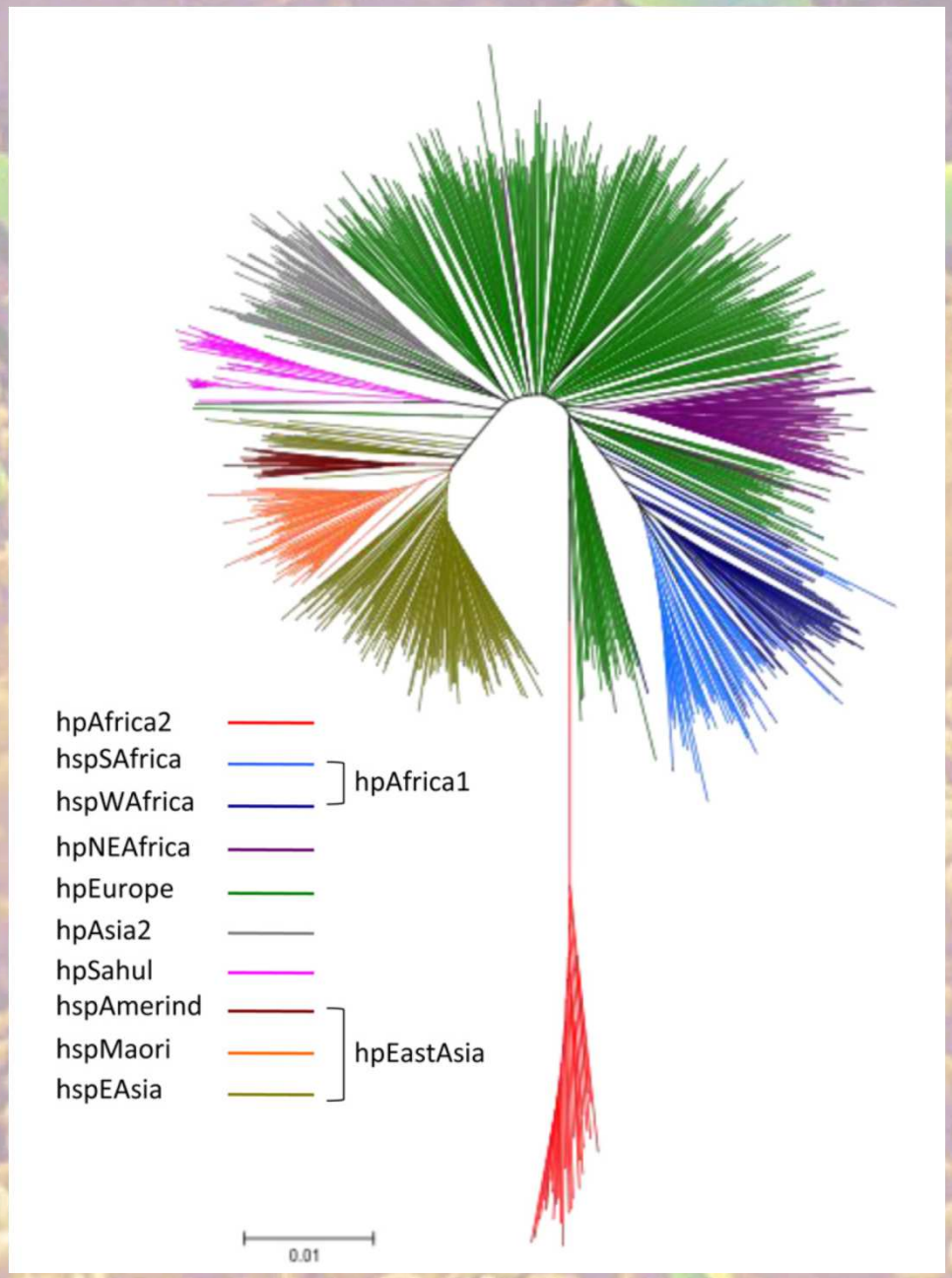


Linz B, et al. An African origin for the intimate association between humans and *Helicobacter pylori*. Nature 2007; 445: 915-8.

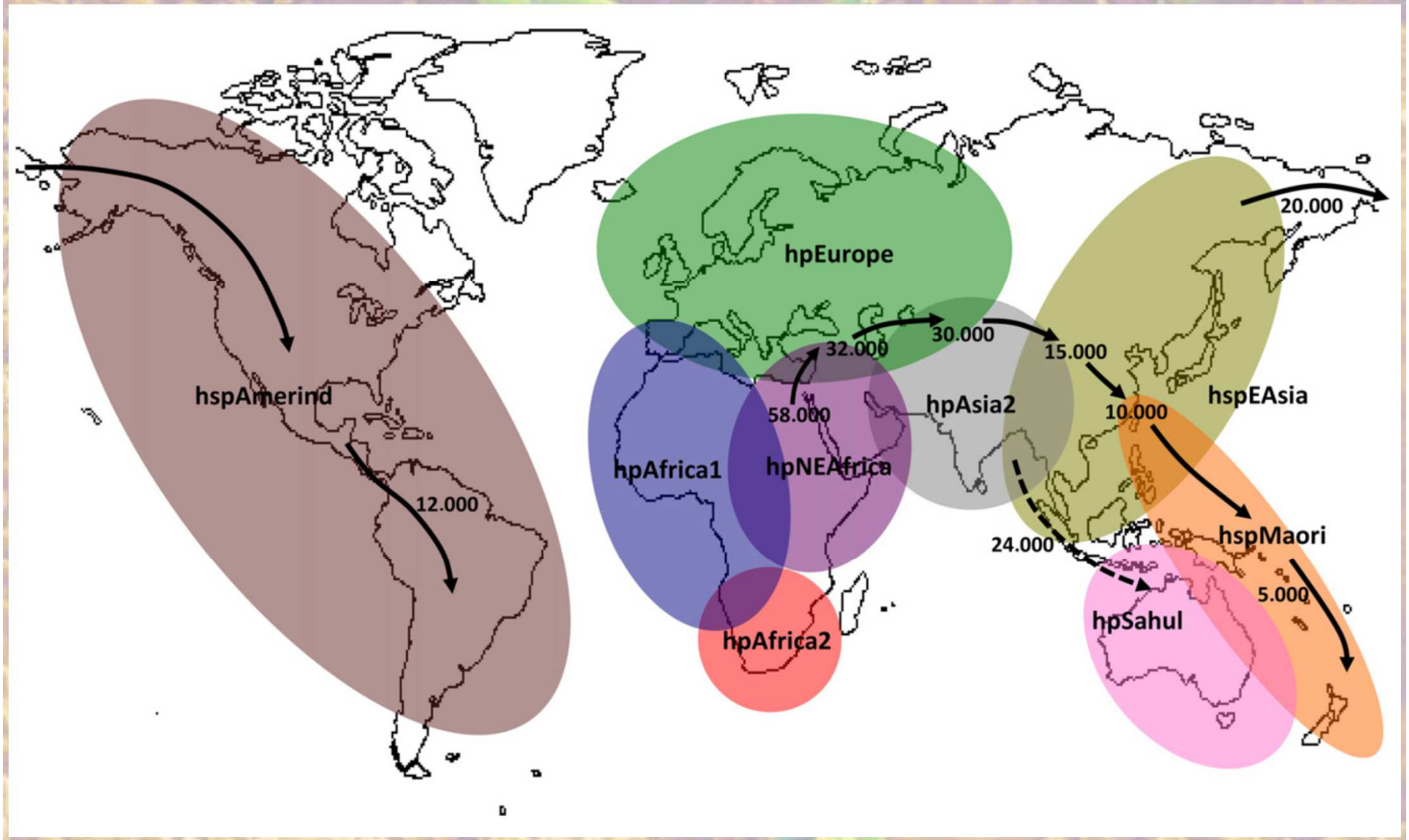




Moodley Y, et al. The peopling of the Pacific from a bacterial perspective. Science 2009; 323: 527-30.



Suzuki R, Shiota S, Yamaoka Y. Molecular epidemiology, population genetics, and pathogenic role of *Helicobacter pylori*. Infect Genet Evol. 2012; 12 (2): 203-13.



Suzuki R, Shiota S, Yamaoka Y. Molecular epidemiology, population genetics, and pathogenic role of *Helicobacter pylori*. Infect Genet Evol. 2012; 12 (2): 203-13.

Dr. David Miñana i Galbis, Dept. Microbiologia i Parasitologia Sanitàries, Facultat de Farmàcia, UB

Helicobacter pylori:

Migracions humanes, càncer gàstric i perspectives

- Introducció
- *Helicobacter pylori* i les malalties gàstriques
- *Helicobacter pylori* i les migracions humanes
- Perspectives

Dr. David Miñana i Galbis, Dept. Microbiologia i Parasitologia Sanitàries, Facultat de Farmàcia, UB

Fundació La Marató de TV3 1007/C/2013

NOVES TECNOLOGIES COM A EINES NO INVASIVES PER A LA PROGNOSI / DIAGNOSI DEL CÀNCER GÀSTRIC

- **Obtenció de mostres de plasma i de biòpsies gàstriques representatives de les diferents fases de les malalties gàstriques (asimptomàtics → → → càncer gàstric).**

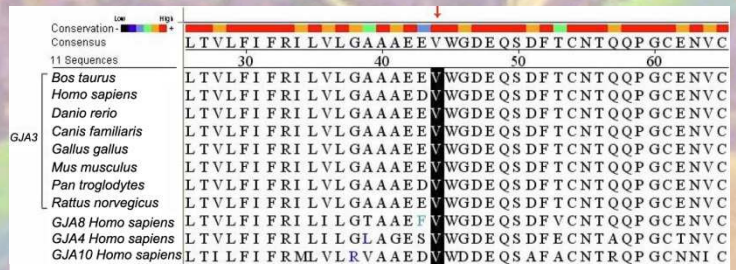
- **Estudi dels perfils de microRNA en plasma: biomarcadors d'evolució a càncer gàstric.**

- **Metabolòmica del plasma: biomarcadors d'evolució a càncer gàstric.**

- **Metagenòmica de biòpsies: multiinfecció i nous marcadors de patogenicitat d'*Helicobacter pylori* (soques comensals *versus* soques virulentes).**

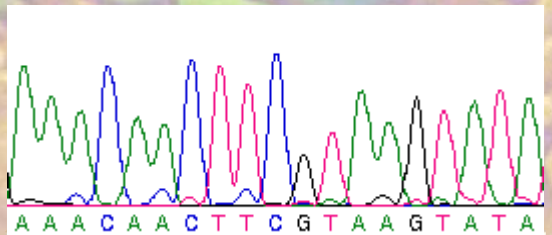
Dr. David Miñana i Galbis, Dept. Microbiologia i Parasitologia Sanitàries, Facultat de Farmàcia, UB

Soques



Alineament

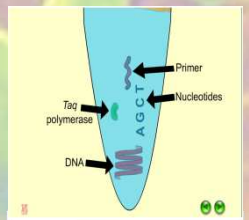
Cultiu pur



Seqüenciació

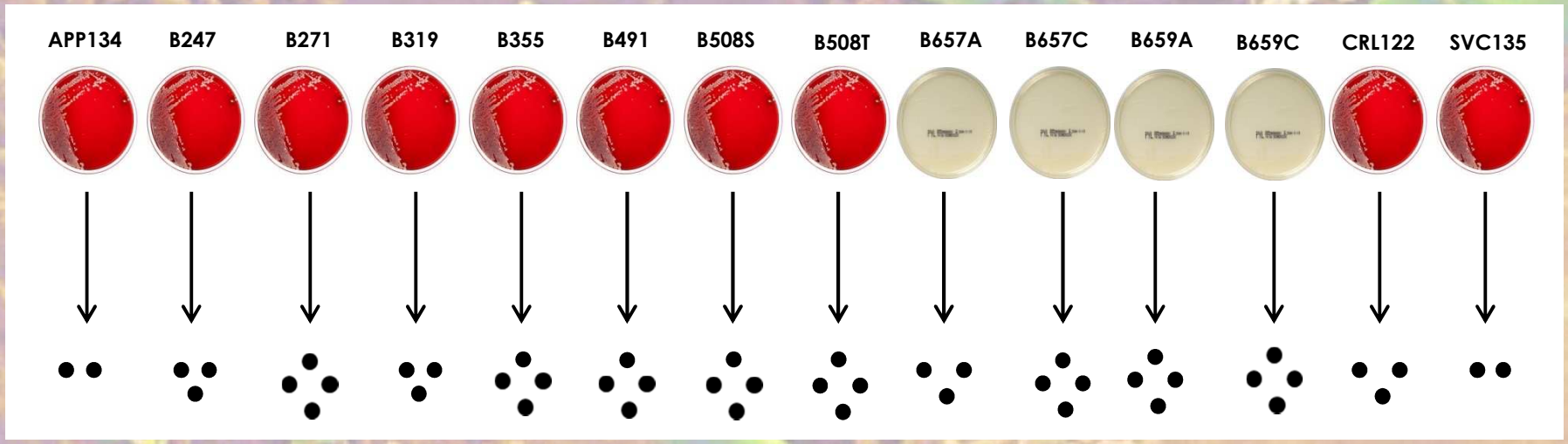
Anàlisi filogenètic

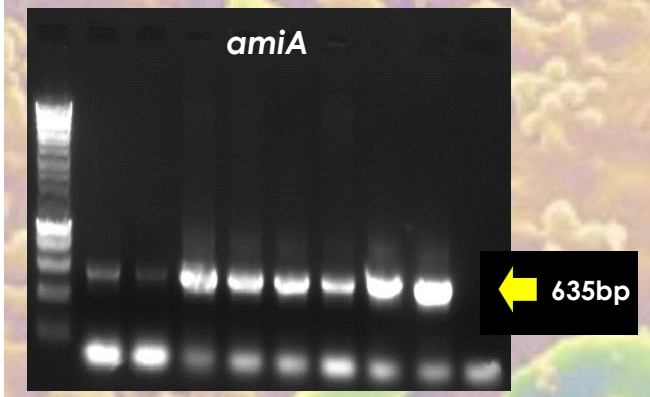
Extracció de DNA

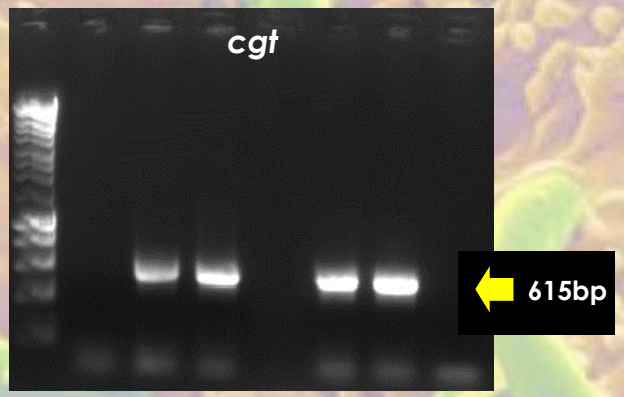


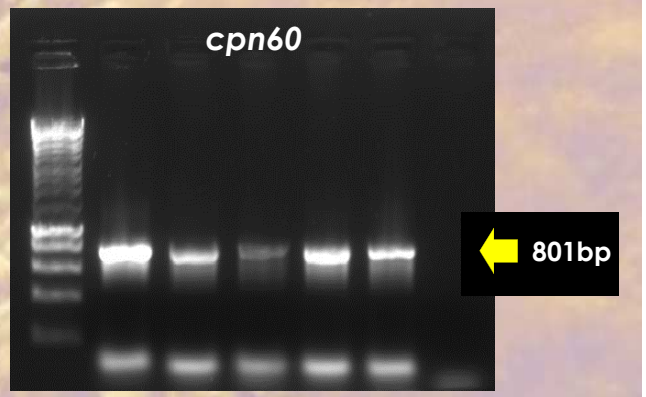
PCR

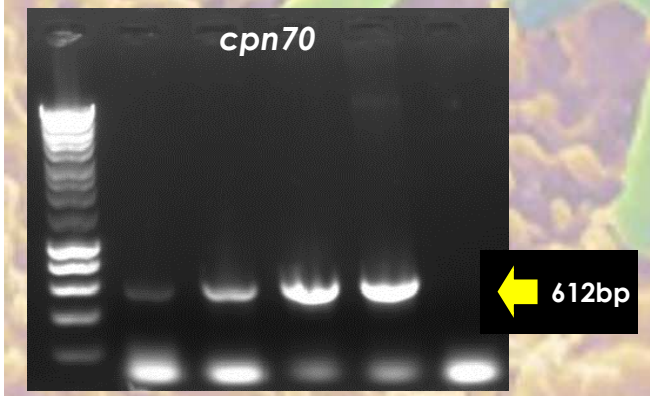
Dr. David Miñana i Galbis, Dept. Microbiologia i Parasitologia Sanitàries, Facultat de Farmàcia, UB

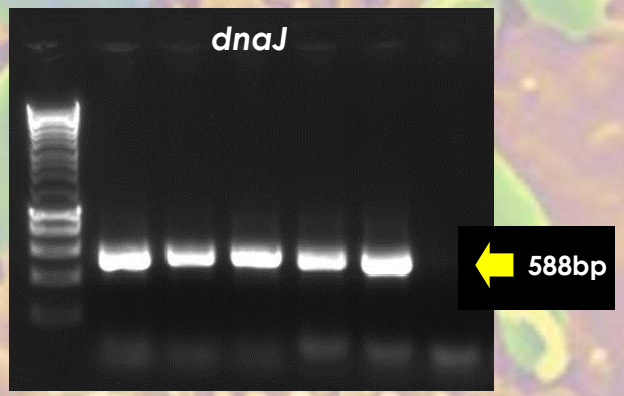


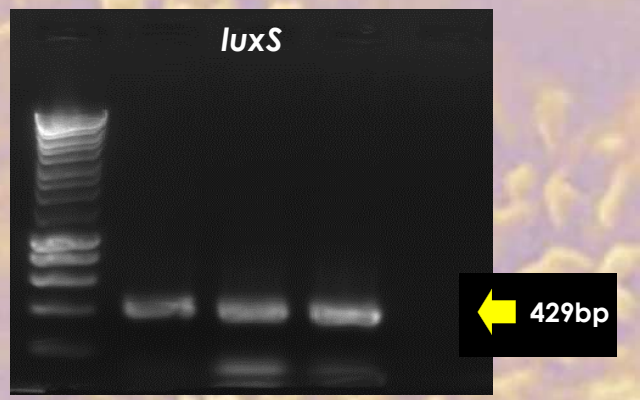






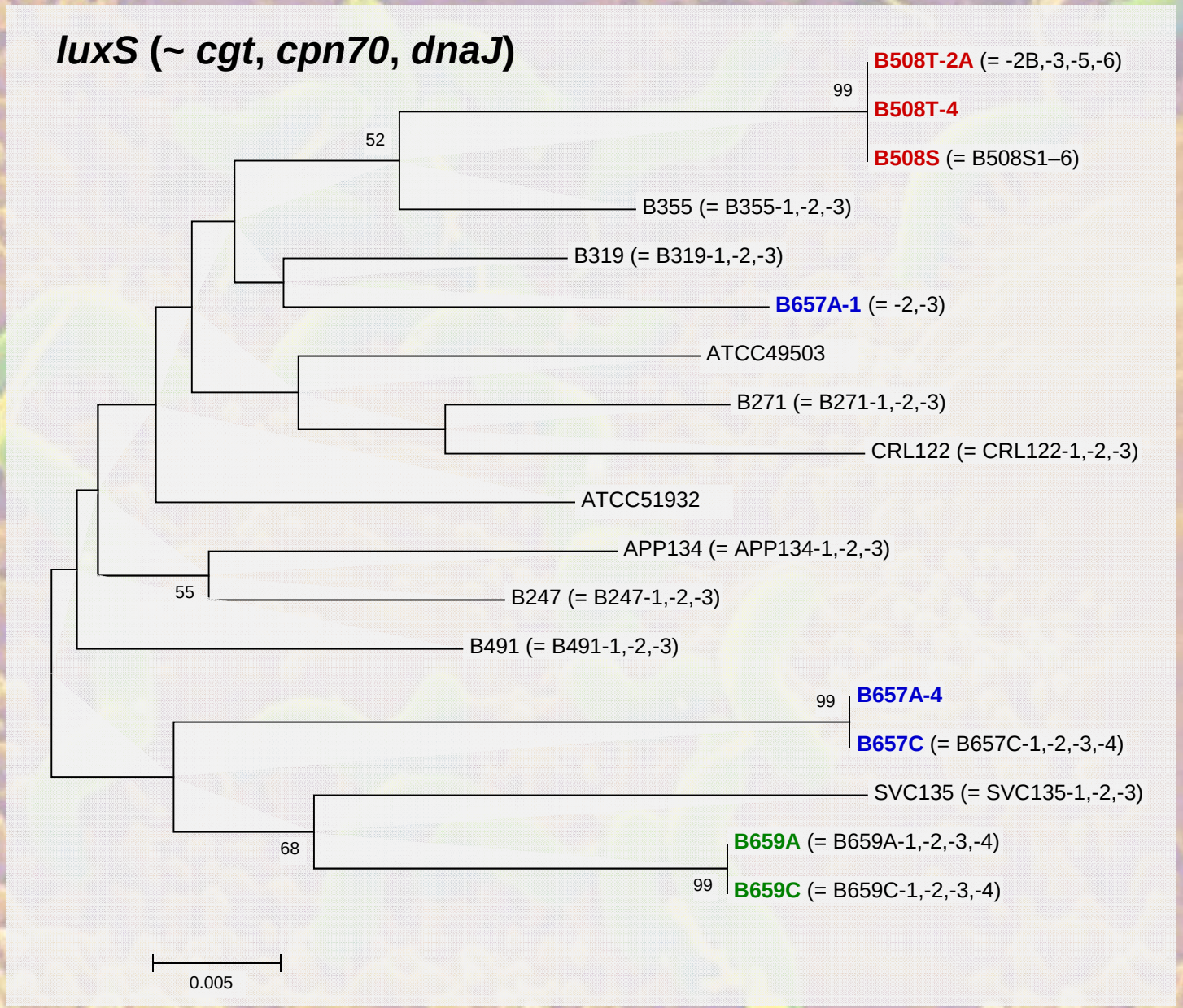


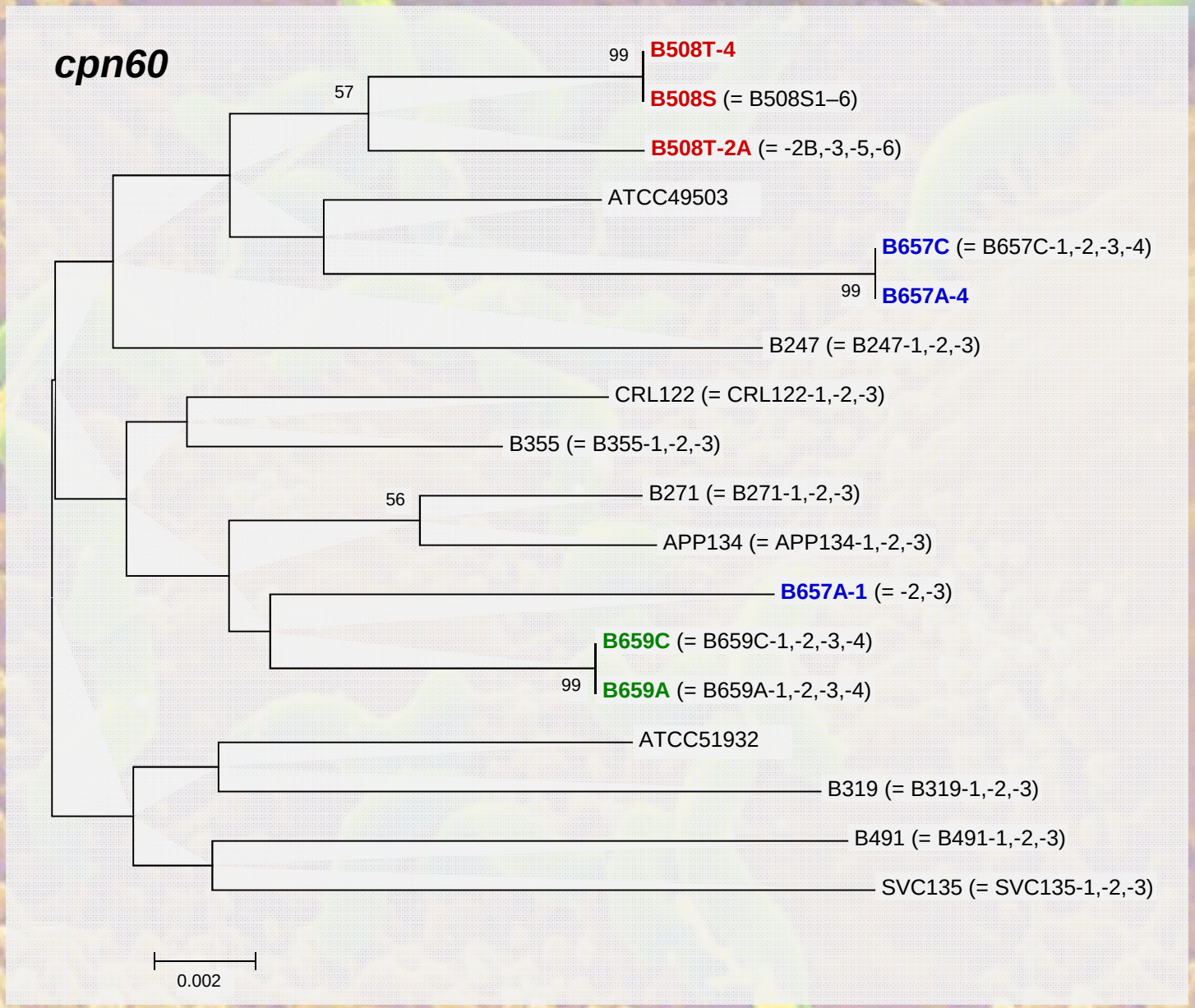


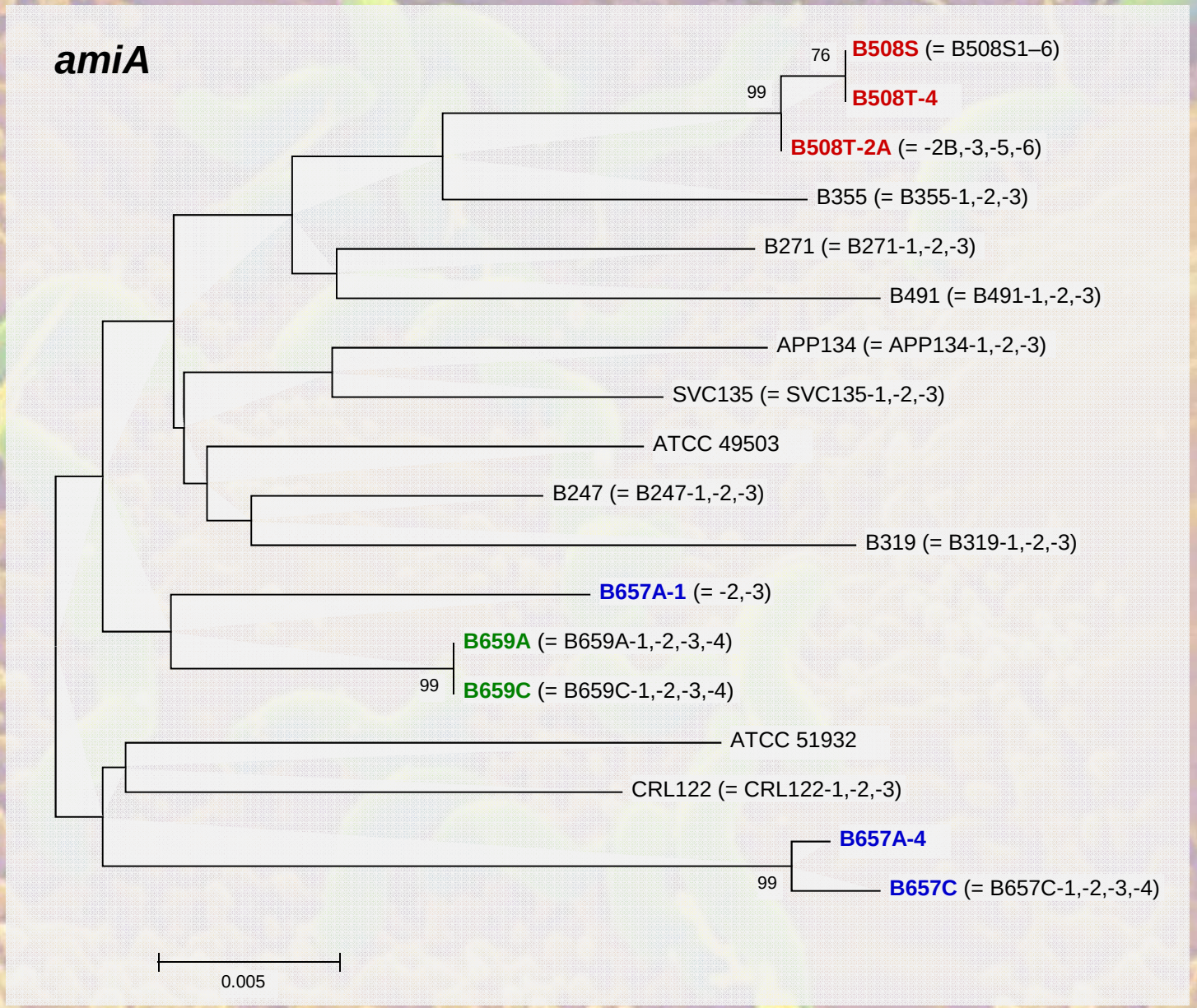


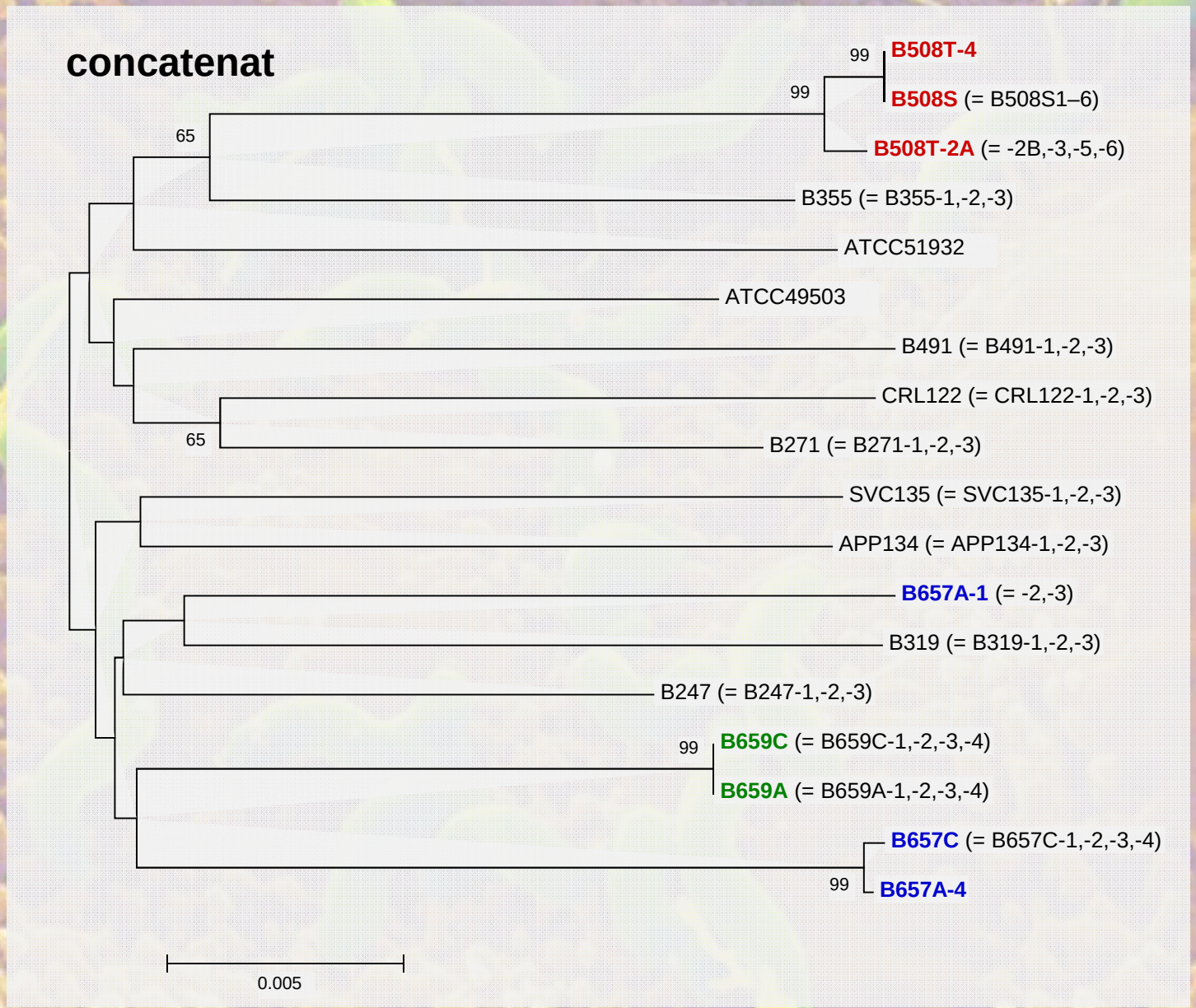
Gastric biopsies (n = 14)	Histopathological diagnostic	Clones analysed (n = 52)
APP134	moderate gastritis	APP134-1, -2, -3
B247*	adenocarcinoma	B247-1, -2, -3
B271	moderate gastritis	B271-1, -2, -3
B319	mild gastritis	B319-1, -2, -3
B355	mild gastritis	B355-1, -2, -3
B491	adenocarcinoma	B491-1, -2, -3
B508S*	adenocarcinoma	B508S-1, -2, -3, -4, -5, -6
B508T	adenocarcinoma	B508T-2A, -2B, -3, **-4**, -5, -6
B657A	mild gastritis	B657A-1, -2, -3, **-4**
B657C	mild gastritis	B657C-1, -2, -3, -4
B659A	moderate gastritis	B659A-1, -2, -3, -4
B659C	moderate gastritis	B659C-1, -2, -3, -4
CRL122	mild gastritis	CRL122-1, -2, -3
SVC135	moderate gastritis	SVC135-1, -2, -3

*Gastric biopsies obtained from normal tissue of patients with adenocarcinoma


luxS (~ cgt, cpn70, dnaJ)
B508T-2A (= -2B,-3,-5,-6)
99
B508T-4
52
B508S (= B508S1–6)
B355 (= B355-1,-2,-3)
B319 (= B319-1,-2,-3)
B657A-1 (= -2,-3)
ATCC49503
B271 (= B271-1,-2,-3)
CRL122 (= CRL122-1,-2,-3)
ATCC51932
APP134 (= APP134-1,-2,-3)
55
B247 (= B247-1,-2,-3)
B491 (= B491-1,-2,-3)
99
B657A-4
B657C (= B657C-1,-2,-3,-4)
SVC135 (= SVC135-1,-2,-3)
68
B659A (= B659A-1,-2,-3,-4)
99
B659C (= B659C-1,-2,-3,-4)
0.005


cpn60
99
B508T-4
57
B508S (= B508S1–6)
B508T-2A (= -2B,-3,-5,-6)
ATCC49503
B657C (= B657C-1,-2,-3,-4)
99
B657A-4
B247 (= B247-1,-2,-3)
CRL122 (= CRL122-1,-2,-3)
B355 (= B355-1,-2,-3)
56
B271 (= B271-1,-2,-3)
APP134 (= APP134-1,-2,-3)
B657A-1 (= -2,-3)
B659C (= B659C-1,-2,-3,-4)
99
B659A (= B659A-1,-2,-3,-4)
ATCC51932
B319 (= B319-1,-2,-3)
B491 (= B491-1,-2,-3)
SVC135 (= SVC135-1,-2,-3)
0.002


amiA
76
99
B508S (= B508S1–6)
B508T-4
B508T-2A (= -2B,-3,-5,-6)
B355 (= B355-1,-2,-3)
B271 (= B271-1,-2,-3)
B491 (= B491-1,-2,-3)
APP134 (= APP134-1,-2,-3)
SVC135 (= SVC135-1,-2,-3)
ATCC 49503
B247 (= B247-1,-2,-3)
B319 (= B319-1,-2,-3)
B657A-1 (= -2,-3)
B659A (= B659A-1,-2,-3,-4)
99
B659C (= B659C-1,-2,-3,-4)
ATCC 51932
CRL122 (= CRL122-1,-2,-3)
B657A-4
99
B657C (= B657C-1,-2,-3,-4)
0.005


concatenat
99
B508T-4
99
B508S (= B508S1–6)
65
B508T-2A (= -2B,-3,-5,-6)
B355 (= B355-1,-2,-3)
ATCC51932
ATCC49503
B491 (= B491-1,-2,-3)
CRL122 (= CRL122-1,-2,-3)
65
B271 (= B271-1,-2,-3)
SVC135 (= SVC135-1,-2,-3)
APP134 (= APP134-1,-2,-3)
B657A-1 (= -2,-3)
B319 (= B319-1,-2,-3)
B247 (= B247-1,-2,-3)
99
B659C (= B659C-1,-2,-3,-4)
B659A (= B659A-1,-2,-3,-4)
B657C (= B657C-1,-2,-3,-4)
99
B657A-4
0.005

Gene	Sequence (nt)	Distances values		
		Range	Minimum*	Mean ± SE
amiA	576	0–0,052	0,019	0,031 ± 0,004
cgt	558	0–0,044	0,009	0,025 ± 0,004
cpn60	555	0–0,039	0,009	0,023 ± 0,004
cpn70	588	0–0,040	0,015	0,027 ± 0,004
dnaJ	564	0–0,061	0,013	0,032 ± 0,004
luxS	**405**	**0–0,078**	**0,028**	**0,046 ± 0,006**
concatenated	3246	0–0,036	0,024	0,030 ± 0,002

*minimum distances values between sequences from clones isolated from biopsies of different patients.

- **Detecció específica d'*H. pylori***
- **Discriminació de les soques provinents de biòpsies gàstriques de pacients diferents**
- **Detecció de microevolució i multiinfecció**

- **Detecció específica d'*H. pylori* en diferents tipus de mostra.**
- **Dinàmica poblacional de la microevolució i / o multiinfecció (metagenòmica).**
- **Vies de transmissió.**
- **Comensalisme v. virulència.**
- **Nous marcadors de patogenicitat.**

Universitat de Barcelona
Facultat de Farmàcia
Microbiologia i Parasitologia Sanitàries
Unitat de Microbiologia

Montserrat Palau de Miguel

Marcos Iuri Roos Kulmann

Ariadna Flaqué Espaulella

Dra. Núria Piqué i Clusella

Dr. David Miñana i Galbis

LEITAT Technological Center
managing your technologies
member of TECNIO

Dra. Lourdes Gombau Suárez

Dr. Guillermo Quintás Soriano

Parc Taulí Sabadell
Hospital Universitari
Fundació Parc Taulí

UAB
Universitat Autònoma de Barcelona

ciberehd
Centro de Investigación Biomédica en Red: Enfermedades Hepáticas y Digestivas

Mª Elisa Quilez

Dr. Carles Pericay Pijaume

Dra. Anna Brunet Vega

Dr. Félix Junquera Flórez

Dra. Mª José Ramírez Lázaro

Dr. Sergio Lario

Dr. Xavier Calvet