The results of the EU project QBOL deposited in the Q-bank database to support Plant Health Diagnostics

Peter Bonants, Plant Research International, Wageningen, NL
Mariëtte Edema, Plant Protection Service, Wageningen, NL

QBOL:

Development of a new diagnostic tool using DNA barcoding to identify quarantine organisms in support of plant health

2009-2012

www.qbol.org
peter.bonants@wur.nl

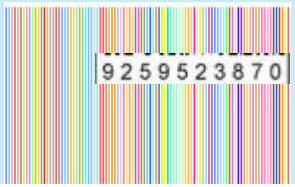
SEVENTH FRAMEWORK PROGRAMME

Why DNA barcoding?

- Increasing world wide trading of plants enhances risk of spreading harmful organisms
- Result in significant possible economic damage
- Decreasing taxonomic knowledge to identify Q-organisms
- DNA barcoding offers accurate identification and focuses on strengthening the link between traditional and molecular taxonomy

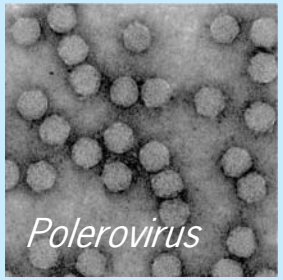
Three principle QBOL Objectives

- *to DNA barcode relevant Q-organisms + morphologically and/or taxonomically related organisms*
- *to develop a database of DNA barcode sequences plus relevant taxonomic/geographic/host data*
- *to develop a DNA bank for the selected set of Q-organisms + morphologically and/or taxonomically related organisms*



Targets Quarantine

- Which?
  - Fungi
  - Arthropods
  - Bacteria
  - Nematodes
  - Viruses
  - Phytoplasmas



- Council Directive 2000/29/EC
- EPPO list A1 and A2

Partners QBOL

PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

fera
The Food and Environment Research Agency

ILVO

INRA

CENTRO INTERNACIONAL DE LA PAPA
CIP

Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

CBS

landbouw, natuur en voedselkwaliteit

Dipartimento di Scienze Agrarie e degli Alimenti
Università degli Studi di Modena e Reggio Emilia

INIA
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria

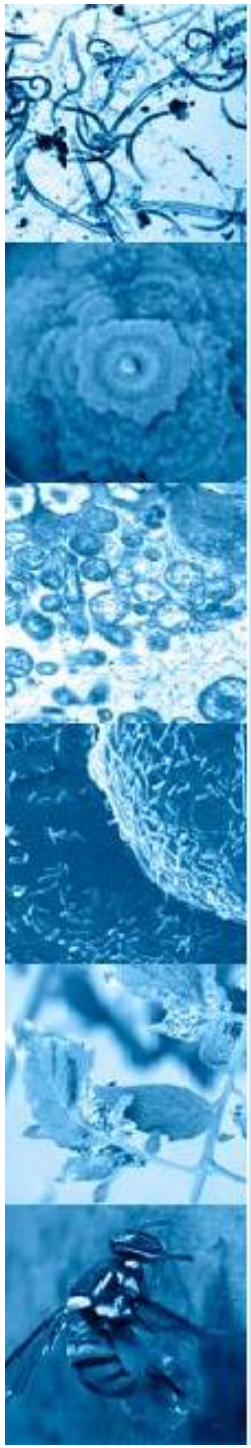
Embrapa

Bio-Protection
Bioprotection science for New Zealand

MINISTÈRE DE L'ALIMENTATION DE L'AGRICULTURE ET DE LA PÊCHE

NIB
NATIONAL INSTITUTE OF BIOLOGY

QBOL

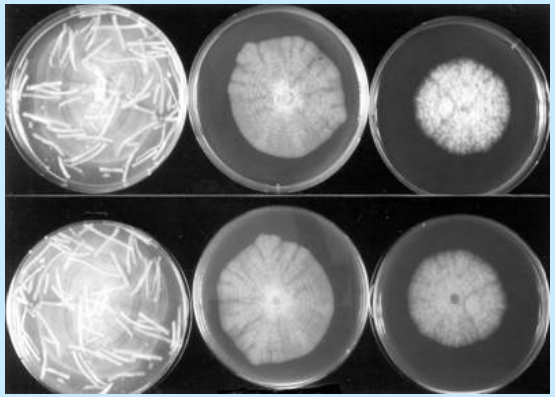


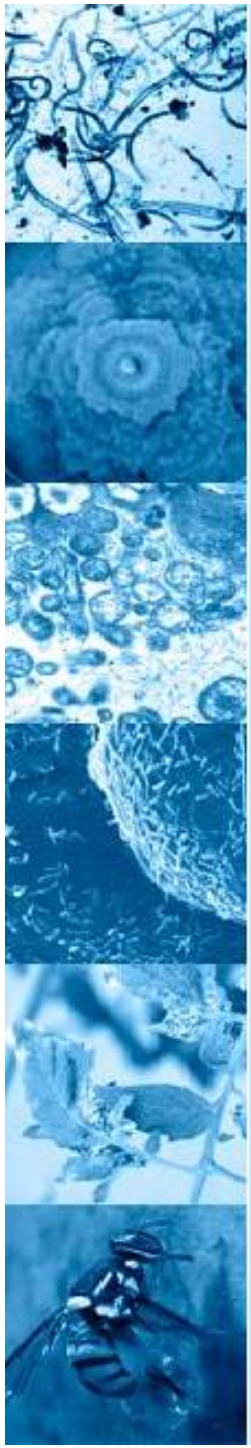
WP2 leader: Ewald Groenewald (KNAW-CBS)

WP2: Fungi

Which:

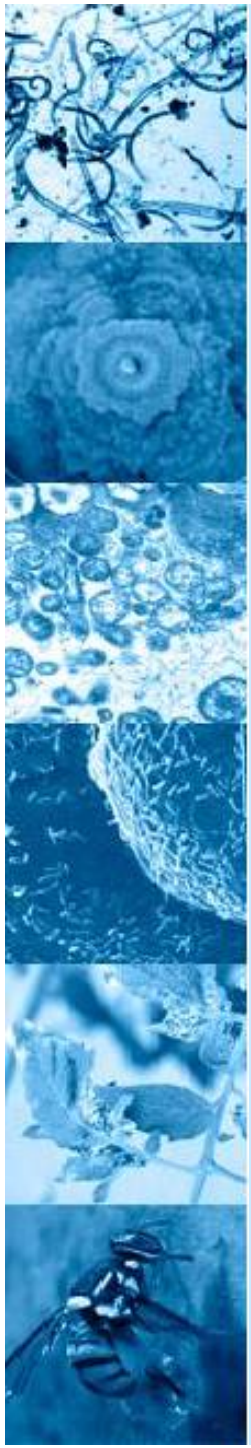
Monilinia, Ceratocystis, Melampsora, Puccinia, Thecaphora and Mycosphaerella





WP2: Fungi: potential barcode loci

- **Internal transcribed spacers of rDNA operon (ITS):**
  V9G, ITS1, ITS1F / ITS4, LR6
- **Beta-tubulin (TUB):** **T1, Bt1a / Bt1b, Bt2b**
- **Cytochrome oxidase I (COI):** **PenF1 / PenR1, AspR1**
- **Histone H3 (HIS):** **CYLH3F / CYLH3R**
- **Translation elongation factor 1-alpha (TEF):**
  EF1-728F / EF1-986R, EF-2
- **Calmodulin (CAL):** **CAL-228F / CAL-737R**
- **Actin (ACT):** **ACT-512F / ACT-783R**



WP3 leader: Jean-Yves Rasplus (INRA)

WP3: Arthropods

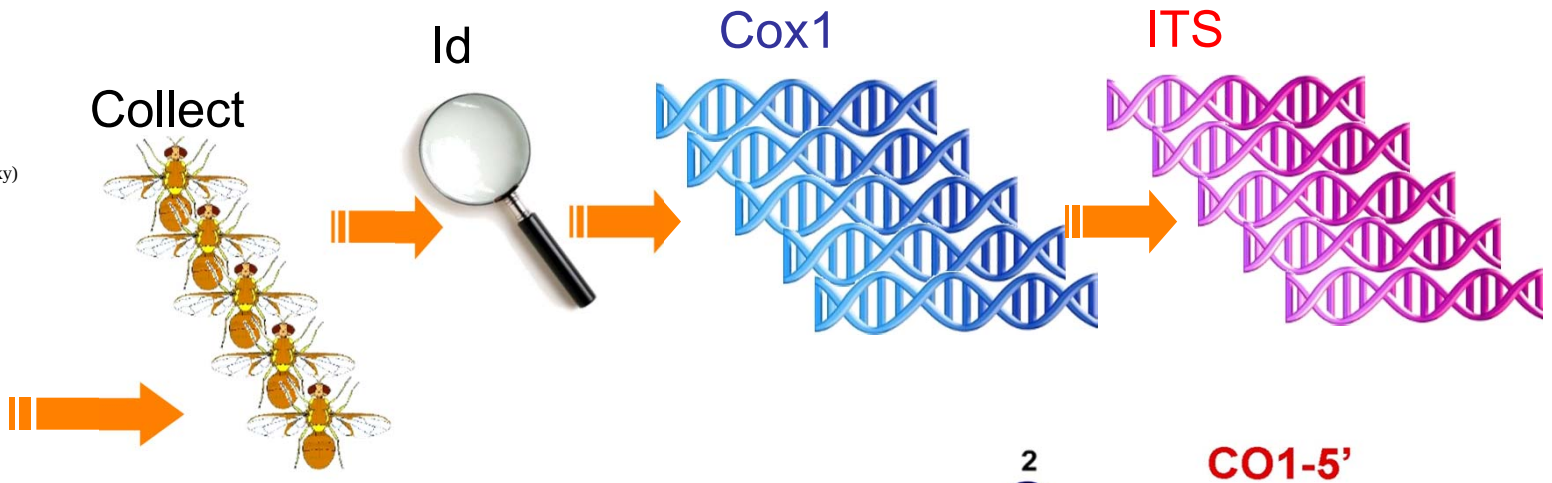
- Which?

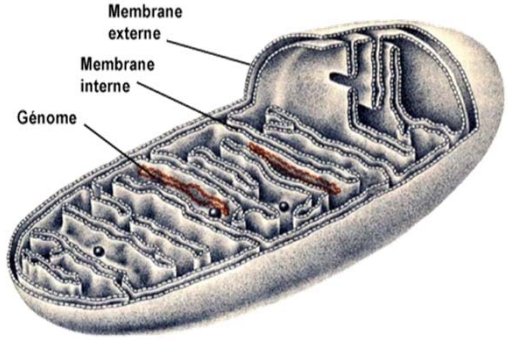
1. Number of species (198), multiple target crops (Agriculture also forests, ornamentals etc.)
2. Example : *Diabrotica* spp. on Maize
3. Billion $ cost in US, introduced in Europe
4. Pesticide use (20 to 25 million acres in US) ➔ indirect costs hardly estimated
5. Species complex (i.e. Ips, *Gonipterus*, *Epitrix*, *Bemisia* globaly poorly known)

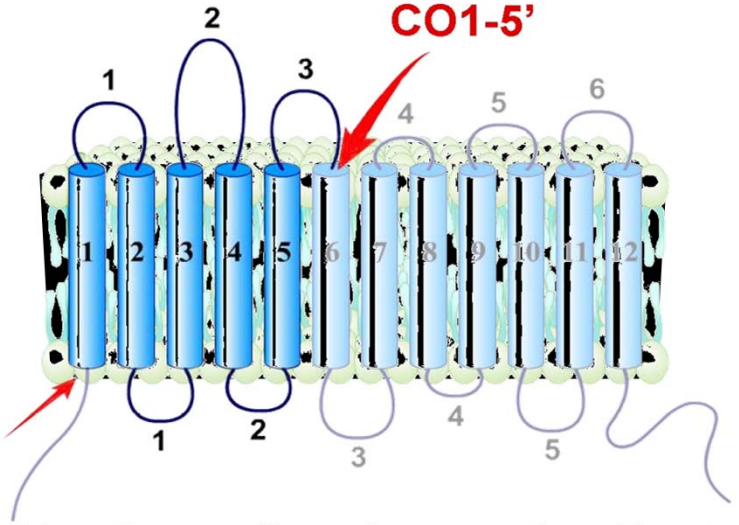
Species list

WP3 Barcoding Arthropods

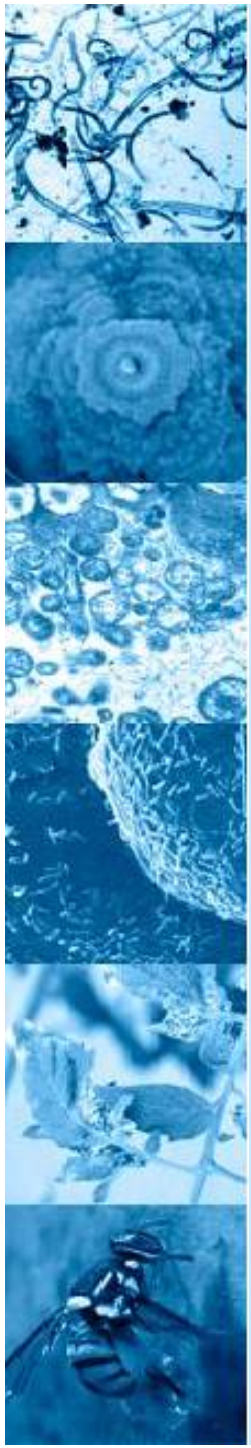
Acleris gloverana (Walsingham)
Acleris variana (Fernie)
Aculops fuchsiae Keifer
Aeolesthes sarta (Solsky)
Agrilus planipennis Fairmaire
Aleurocanthus spiniferus (Quaintance)
Aleurocanthus woglumi Ashby
Anastrepha obliqua (Macquart)
Anastrepha fraterculus Wiedmann
Anastrepha ludens (Loew)
Anastrepha suspensa Loew
Blitopertha orientalis (Waterhouse)
Anoplophora chinensis (Thomson)
Anoplophora glabripennis (Motschulsky)
Anthonomus bisignifer Schenkling
Anthonomus eugenii Cano
Anthonomus grandis Boheman
Anthonomus quadrigibbus Say
Anthonomus signatus Say
Aonidiella citrina (Coquillett)
Arrhenodes minutus (Drury)
Aschistonyx eppoi Inouye
Aulacaspis yasumatsui Takagi
Bactrocera cucumis (French)
Bactrocera cucurbitae Coquillett
Bactrocera dorsalis (Hendel)
Bactrocera invadens (Hendel)
Bactrocera minax (Enderlein)
Bactrocera tryoni (Froggatt)
Bactrocera tsuneonis (Miyake)
Bactrocera zonata (Saunders)
Bemisia tabaci (Gennadius) & biotype B
Cacoecimorpha pronubana Hübner
Cacyreus marshalli Butler
Carneocephala fulgida Nottingham
Carposina niponensis (Walsingham)
Cephalcia lariciphila (Wachtl)
Ceratitis capitata Wiedemann
Ceratitis quinaria (Bezzi)
Ceratitis rosa Karsch
Ceratitis cosyra (Walker)
Choristoneura conflictana (Walker)
Choristoneura fumiferana (Clemens)
Choristoneura occidentalis Freeman
Choristoneura rosaceana (Harris)
Circulifer tenellus (Baker)







QBOL : Barcoding Quarantine Pests



WP4: Bacteria

WP4 leader: Martine Maes (ILVO)

	QBOL Priority group 1	QBOL Priority group 2
Xylella fastidiosa	X	
Clavibacter michiganensis subsp. *sepedonicus*	X	
Pseudomonas solanacearum = *Ralstonia solanacearum*	X 3 phylotypes	
Xanthomonas strains pathogenic to Citrus	X X X 3 subspp.	
Xanthomonas oryzae pv. *oryzae* & *oryzicola*	X X 2 pvs	
Clavibacter michiganensis subsp. *insidiosus*	X	
Clavibacter michiganensis subsp. *michiganensis*	X	
Xanthomonas axonopodis pv. phaseoli		X
Xanthomonas vesicatoria & *X. axonopodis* pv. *vesicatoria*		X X
Xanthomonas fragariae		X
Xanthomonas translucens		X
Xanthomonas axonopodis pv. *dieffenbachiae*		X
Xanthomonas axonopodis pv. *allii*		X

ILVO

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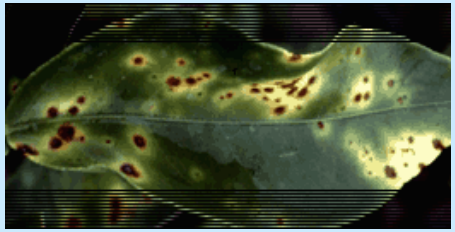
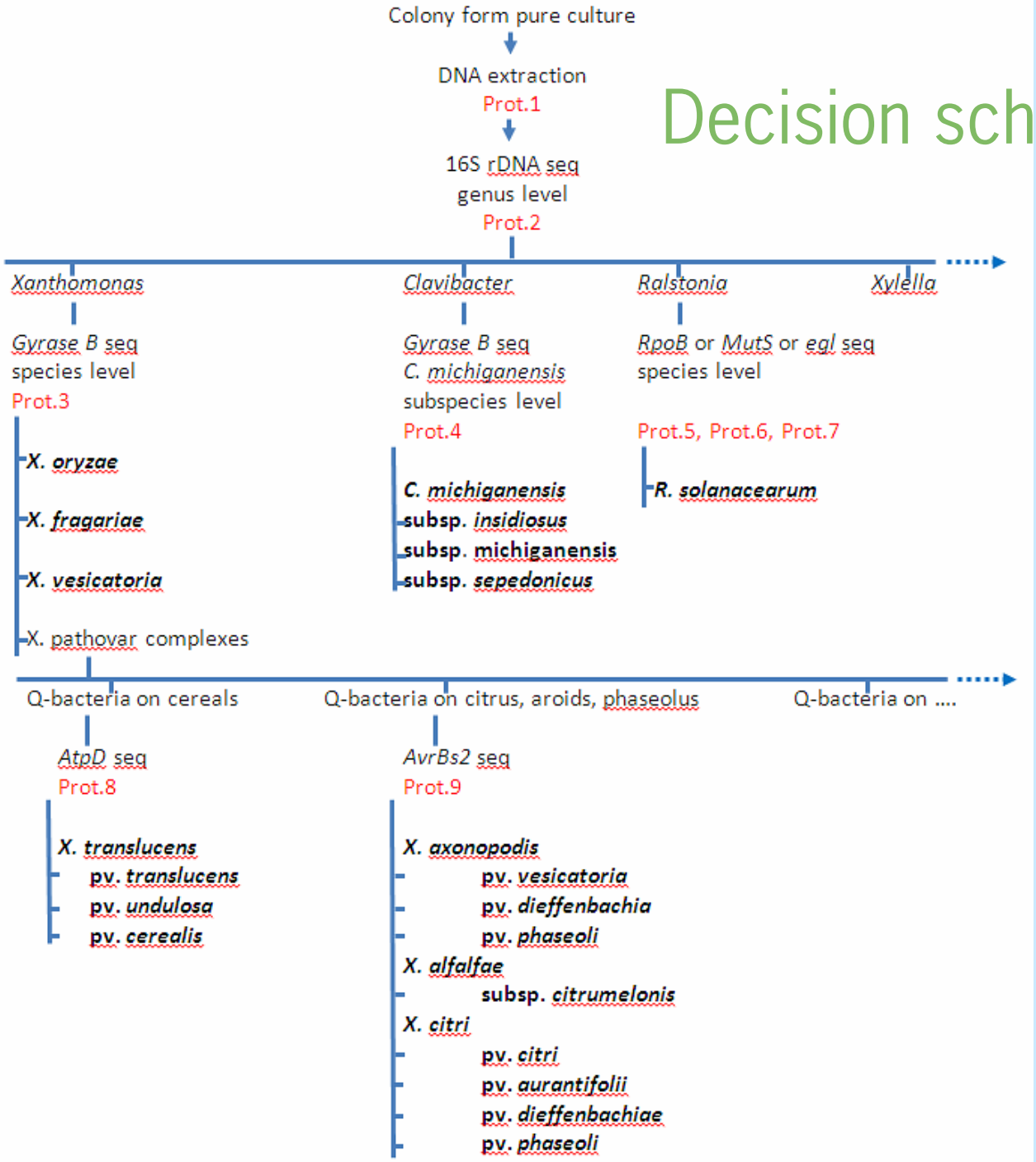
WP4: Bacteria: potential barcode loci

- 16S
- GyrB : DNA gyrase, subunit β, which unwinds double stranded DNA
- rpoB : RNA polymerase, subunit β, involved in RNA biosynthesis
- accessory genes

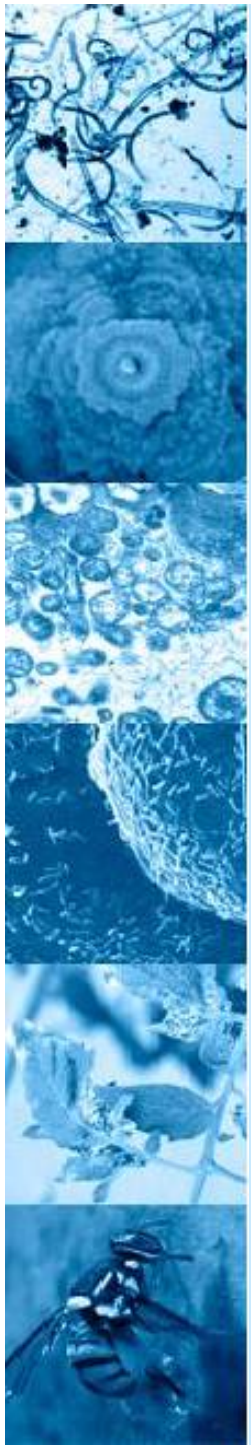


Decision scheme bacteria



WP5: Nematodes

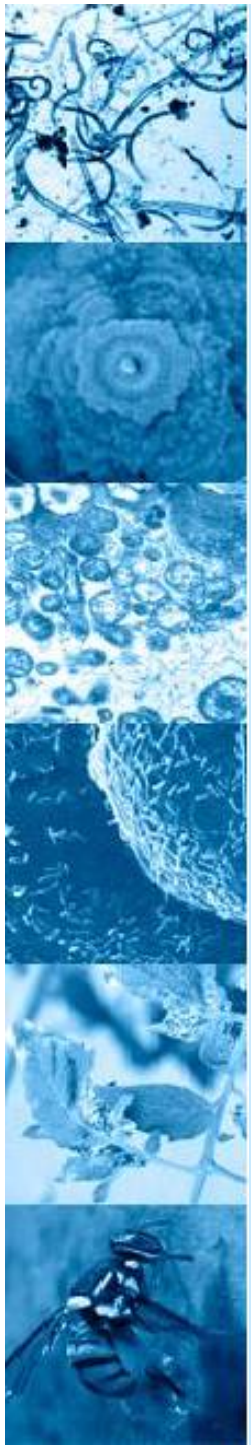
WP5 leader: Juerg Frey (ACW)



WP5: Nematodes

Seven barcode regions were screened in Priority Group 1:

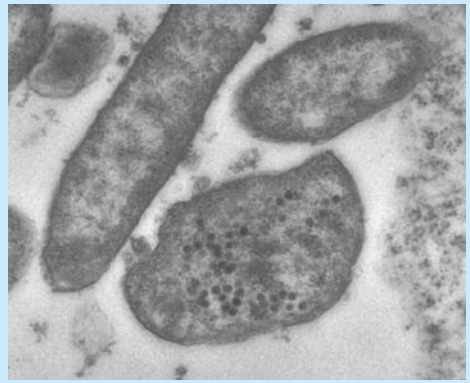
- SSU rDNA,
- LSU rDNA, D1-D2 and D2-D3 region
- IGS2
- COI
- COII
- RNA polymerase II

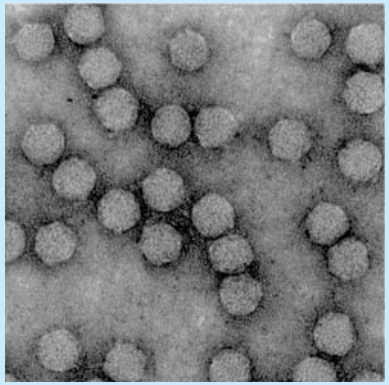


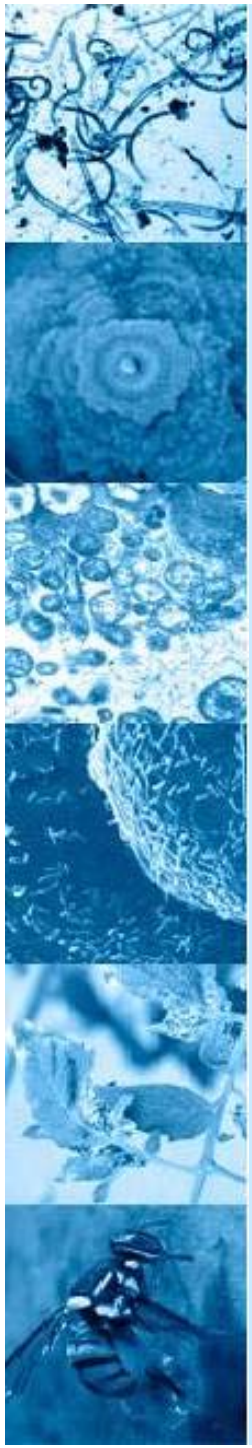
WP6: Viruses

WP6 leader: Neil Boonham (Fera)

- No standard region
- Not culturable
- Relative small genomes
- 90% RNA viruses
- Barcodes generated by Next Generation Sequencing (NGS)

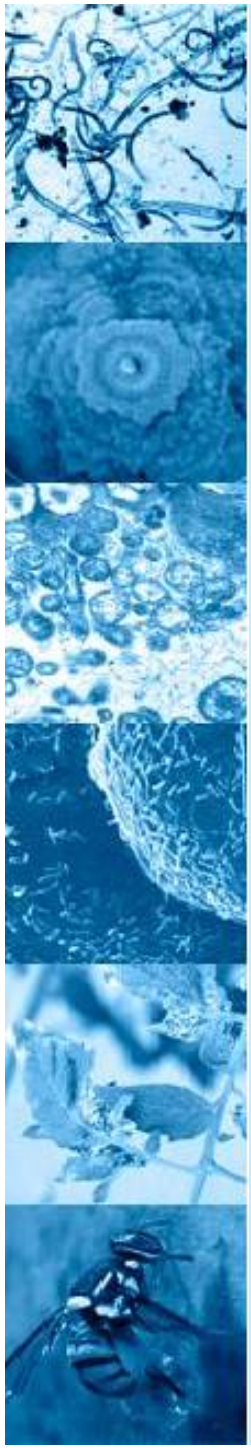






WP6: Viruses

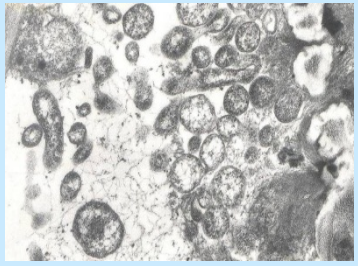
Species	Genus	List	Genome sequence available	Partner responsible	Status
Arracacha virus B, oca strain	Nepovirus	IAI	No	Fera	Complete
Potato black ringspot virus	Nepovirus	IAI / EPPO A1	No	Fera	Complete
Potato virus T	Trichovirus	IAI /EPPO A1	Yes x2	CIP	Complete
Chrysanthemum stem necrosis virus	Tospovirus	EPPO A1	No	PRI	Complete
Potato yellow dwarf virus	Rhabdovirus	EPPO A1	No		Completed (not by consortium)
Potato yellowing virus	Alphamovirus	EPPO A1	No	CIP	Partial (3' and 5' end not confirmed by RACE)
Tomato infectious chlorosis virus	Crinivirus	EPPO A2	No	Fera	Complete
Iris yellow spot virus	Tospovirus	EPPO Alert	No	PRI	Partial (few small gaps)
Tomato torrado virus	Toradovirus	N/A[1]	Yes	PRI	Complete
Tomato marchitez virus	Toradovirus	N/A[1]	Yes	PRI	Complete

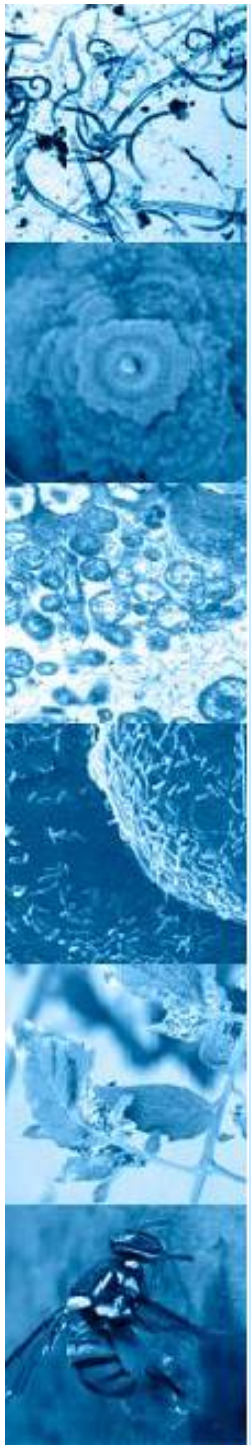


WP7 leader: Mogens Nicolaisen (UA)

WP7: Phytoplasmas

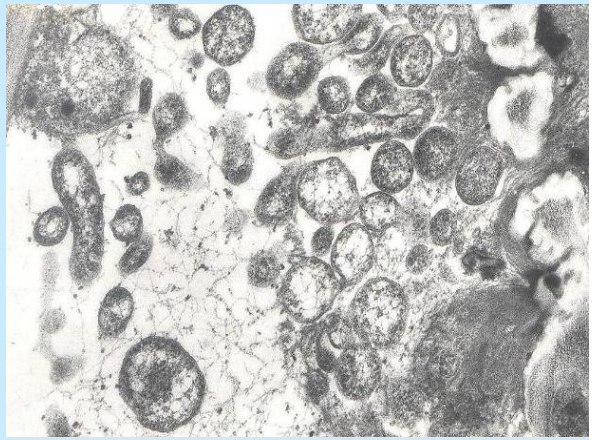
- Cell wall-less bacteria
- Inhabits the plant phloem
- Small genomes (~500-1200 kbp)
- Transmitted by insect vectors
- Obligate parasites
- Symptoms include witches broom, phyllody, virescence, dwarfing, yellowing

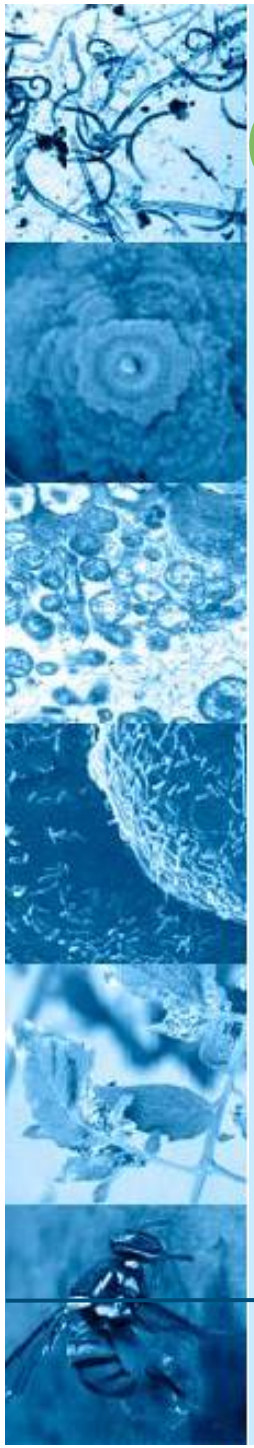




WP7: Phytoplasmas: potential barcode loci

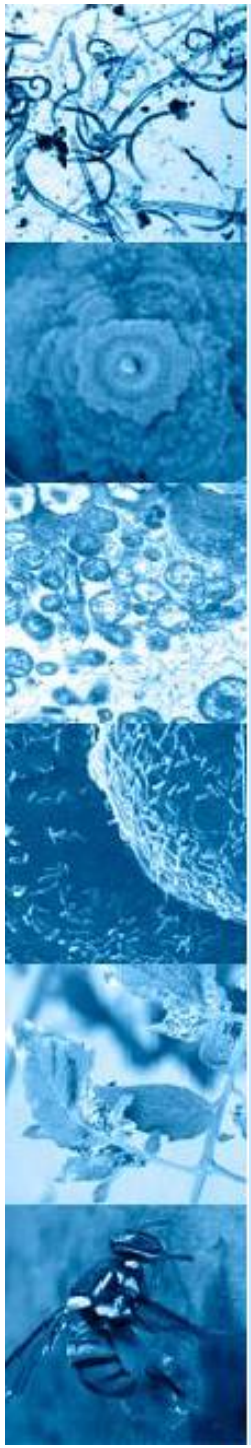
- 16S
- Tuf
- SecA
- 16S-23S spacer
- Ribosomal proteins
- SecY
- rpoC





QBOL: DNA Barcode sequences obtained now

QBOL	sequences obtained		19-5-2012
		# sequences	Remark
WP2	FUNGI	6725	up to 11 loci tested (ITS is predominant)
WP3	ARTHROPODS	4103	2 loci
WP4	BACTERIA	3312	20 loci
WP5	NEMATODES	1641	6 loci
WP6	VIRUSES	46	whole genome and almost complete genome seq
WP7	PHYTOPLASMS	472	3 loci
	TOTAL	16299	



WP8 leader: Peter Bonants (PRI)

WP8. DNA bank:

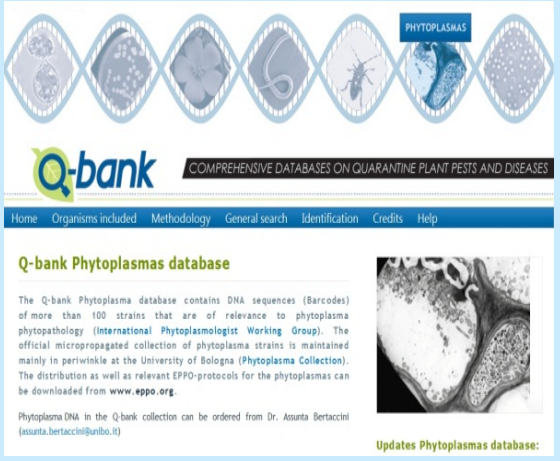
Protocols developed for:

- Whole Genome Amplification (WGA)
- Storage
- Transport

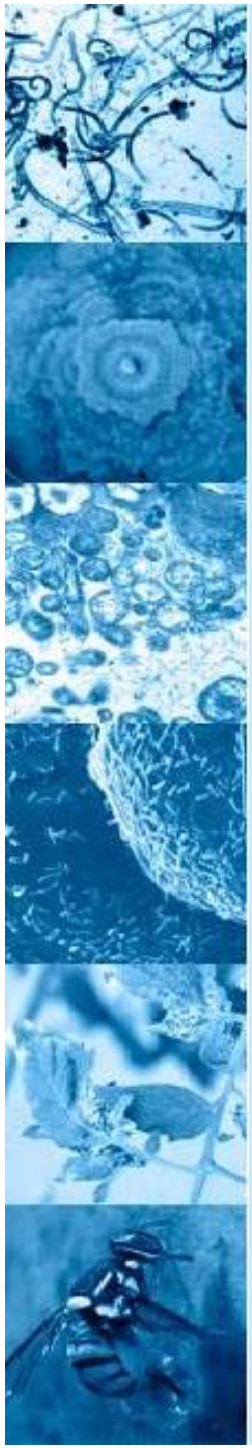
Prototype DNA bank of quarantine and regulated plant pathogens and their taxonomically closely related species.

WP9 leader: Vincent Robert (KNAW-CBS)

WP9. Database: BioLomics



Q-bank: www.q-bank.eu



WP10 leader: Bart van den Vossenberg (PPS)

WP 10. Validation

Netherlands Food and Consumer Product Safety Authority
Ministry of Economic Affairs, Agriculture and Innovation

INIA
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria

MINISTÈRE DE L'ALIMENTATION DE L'AGRICULTURE ET DE LA PÊCHE

Embrapa

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Bioprotection science for New Zealand

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Dipartimento di Scienze Agrarie e degli Alimenti
Università degli Studi di Modena e Reggio Emilia

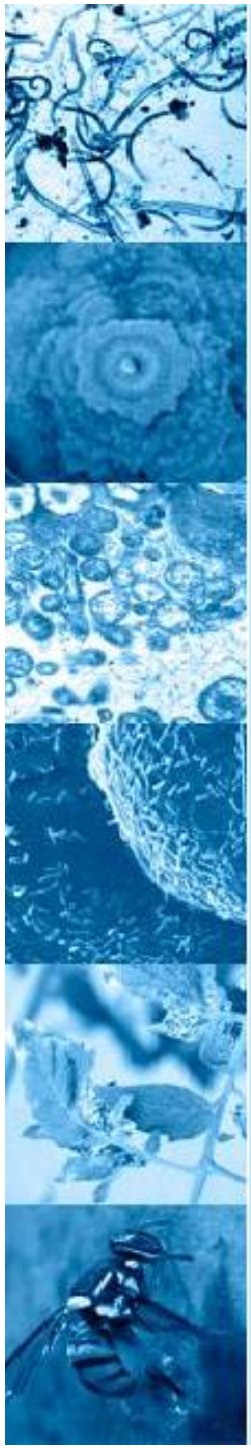
QBOL

Task: Validation of barcoding protocols for diagnostic use

Proficiency test:

DNA/RNA extraction, amplification, sequencing and database searching according to developed protocols

Endusers: NPPO's



WP11. Dissemination:

- Website, www.qbol.org, >10,000 visitors
- Flyers, posters, E-Newsletters
- Training
- Final QBOL Meeting / EPPO Diagnostic symposium in The Netherlands (21-25 May 2012)
    - presentations on www.eppo.int

WP11 Dissemination: Training

- China

- South Africa
- Kenya

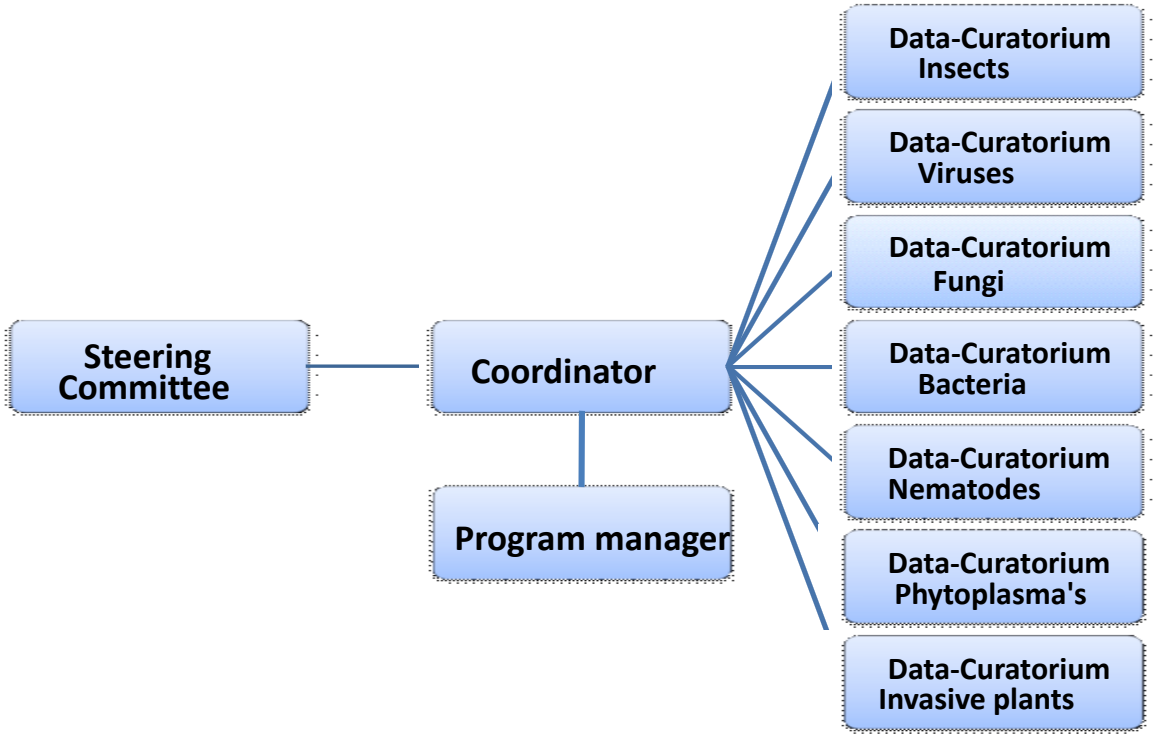
- Peru

- Honduras

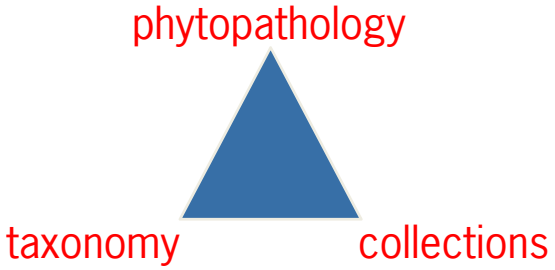
- The Netherlands

- India

Structure


Steering Committee
Coordinator
Program manager
Data-Curatorium Insects
Data-Curatorium Viruses
Data-Curatorium Fungi
Data-Curatorium Bacteria
Data-Curatorium Nematodes
Data-Curatorium Phytoplasma's
Data-Curatorium Invasive plants


Q-bank


phytopathology
taxonomy
collections

Q-bank

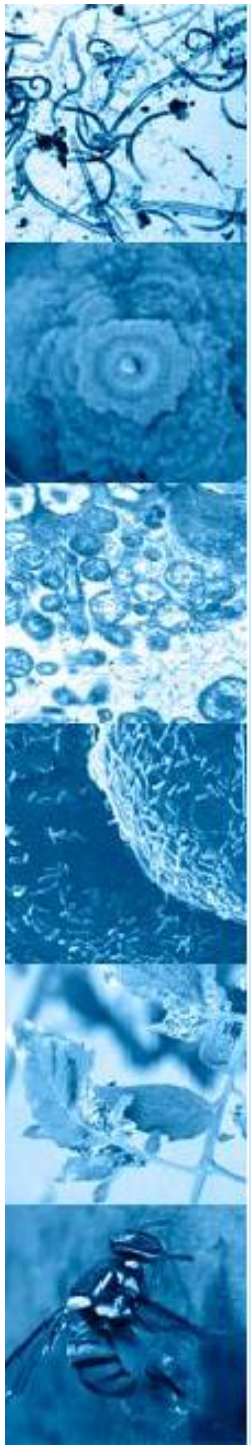
COMPREHENSIVE DATABASES ON QUARANTINE PLANT PESTS AND DISEASES

Website

http://www.q-bank.eu/

Videolink

Q-bank, The Movie:
on the homepage of the Q-bank website



QBOL: Acknowledgements

Ewald Groenewald, William Quaedvlieg, Els Verstappen, Annette Dullemans, James Woodhall, Martin D'Ágostino, Willmer Pérez Barrera, Soledad Gamboa

Jean-Yves Rasplus, Marie-Anne Auger-Rozenberg, Beatrice Courtial, Isabelle Meusnier, Emma Artige, Alain Roques

Martine Maes, Liselot Detemmerman, Kim Heylen, Joanna Zaluga, Paul de Vos

Juerg Frey, Martijn Holterman, Sebastian Kiewnick, Hans Helder, Paul Mooyman

Neil Boonham, Ian Adams, Rachel Glover, Jennifer Hodgetts, Rene vd Vlugt

Assunta Bertaccini, Mogens Nicolaisen, Olga Makarova, Nicoletta Contaldo, Samanta Paltrinieri

Bart vd Vossenberg, Marcel Westenberg, Linda Kox

Vincent Robert, Pedro Crous

Jan Kreuze, Giovanna Muller, Wilmer Cuellar

Validation Partners: NIB (Maja Ravnikar and Tanja Dreo), SPA (Hana Orsagova), Anses (Francoise Poliakoff), CAIQ (Mingfu Li), LU (Karen Armstrong), CNPMF (Juliana Freitas-Astua, DAFS (Emilio Stefani), INIA (Jaime Cubero), US (Lizel Mostert)

Advisory Board: Paul Hebert, Francoise Petter, André Levesque

Q-bank: Mariette Edema, steering committee, curators

All contributors for QBOL and Q-bank

Thank you for your attention!
To be or not to be barcoded?
That is the question

www.qbol.org
peter.bonants@wur.nl