THE WASTEWATER MICROBIOTA

THE COMPLEXITY OF THE TASK

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- **Pollutants complexity**
- **Wastewater microbiota complexity**
- **Conclusion and perspectives**

WASTEWATER COMPOSITION

- Carbon (sugars, proteins, lipids)
- Nitrogen (proteins, urea, ammonia)
- Phosphorus (organic, phosphates)

- Biodegradable compounds
- Non-biodegradable compounds

- Microbes : pathogens or not

- **Micropollutants (organics, metals, etc)**

PROCESS DESCRIPTION

- **If discharged in the natural streams, lakes and rivers**

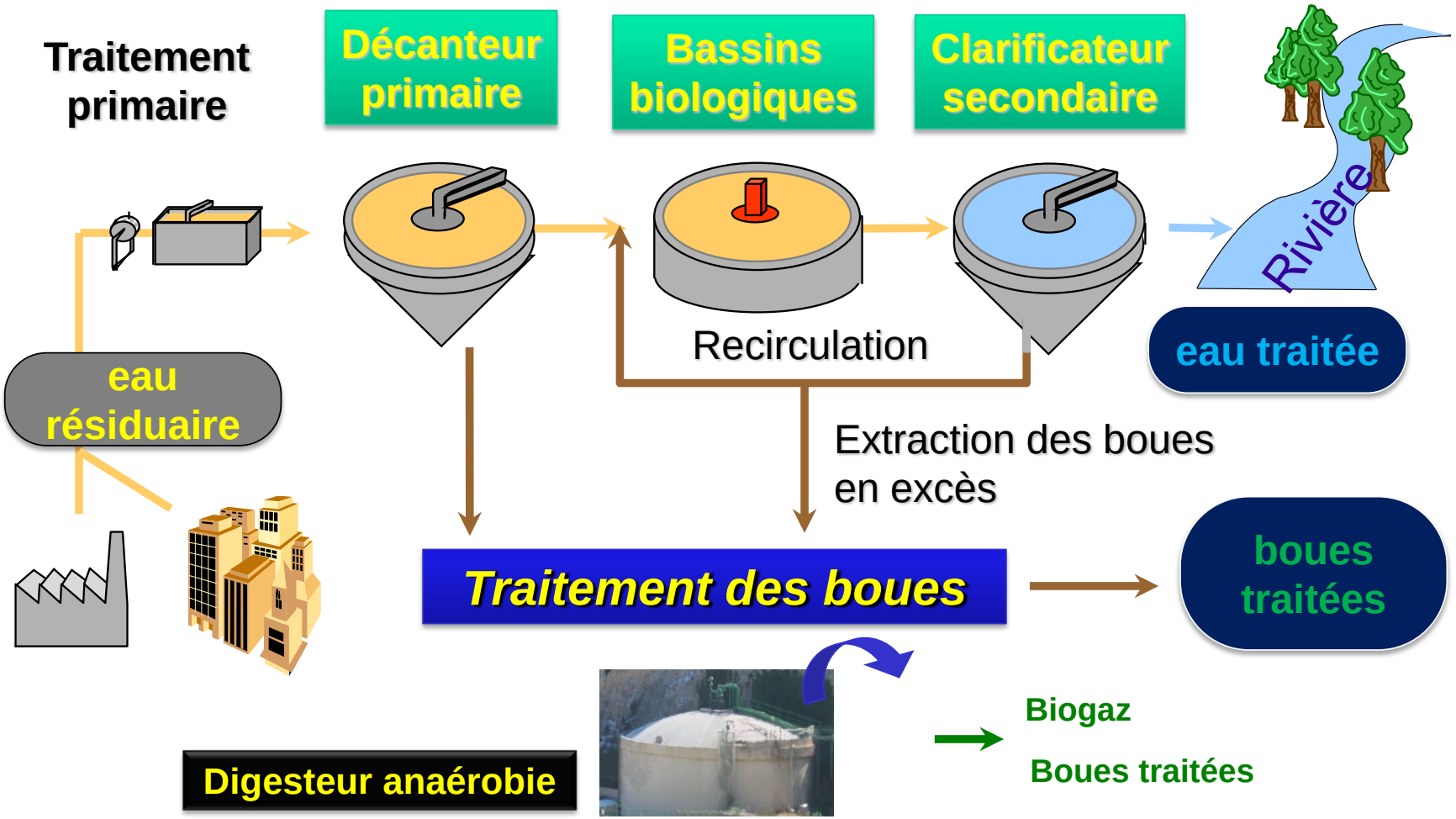
  - Harm to biological resources and ecological systems,
  - Interfere with other legitimate uses of the environment,
  - Endanger human health,

- The treatment of domestic and industrial wastewater is a very important **biotechnological process**

The main objective : the elimination of carbon, nitrogen and phosphorus, to avoid eutrophication and deterioration of the receiving environment

A major public concern in almost all parts of the world

Traitement des eaux



- **Priority Substances** **are chemical pollutants that pose a significant risk to (or via) the aquatic environment**

- **Priority Hazardous Substances** are **a subset of Priority Substances, of which they are the most dangerous.**
  - characterized **by their persistence, bioaccumulation and toxicity.**

- **Emerging Substances** : These are the **pollutants whose impacts are still poorly identified**

http://europa.eu/rapid/press-release_MEMO-12-59_en.htm

Priority substances

		Substance prioritaire	Substance prioritaire à l'examen	Substance prioritaire dangereuse
Métaux		Nickel	Plomb	Cadmium
				Mercure
Composés Organiques	HAP	Fluoroanthène	Anthracène	Benzo(a)pyrène,
			Naphthalène	Benzo(b)fluoroanthène,
				Benzo(g,h,i)perylène,
				Benzo(k)fluoroanthène,
				Indeno(1,2,3-cd)pyrene
	Pesticides	Alachlore	Atrazine	Hexachlorocyclohexane
		Chlorfenvinphos	Chlorpyriphos	Tributyltin compounds
		Diuron	Endosulfan	
		Isoproturon	Trifluraline	
		Simazine	Pentachlorophenol	
	Industries	Benzene	DEHP	Hexachlorobutadiène
			Trichlorobenzenes	C10-13-chloroalkanes
				Pentachlorobenzène
				PBDE
				Hexachlorobenzène
	Solvants, Détergents	1,2-Dichloroéthane	Octylphenols	Nonylphenols
		Dichlorométhane		
		Chloroforme		

Priority Hazardous Substances
Dans les boues

Composés organiques	HAP	Acénapthène
		Phénanthrène
		Fluorène
		Fluoranthène
		Pyrène
		Benzo(b) fluoranthène
		Benzo(b) fluoranthène
		Benzo(b) fluoranthène
		Benzo(a) pyrène
		Benzo(g) pépylène
		Benzo(h) pépylène
		Benzo(i) pépylène
		Indeno(1,2,3-cd)pyrene
	PCB	Polychlorophényle 28
		Polychlorophényle 52
		Polychlorophényle 101
		Polychlorophényle 118
		Polychlorophényle 138
		Polychlorophényle 153
		Polychlorophényle 180
		Polychlorophényle 180
	AOX	Organohalogénés
	Industrie	Alkylbenzènesulfonates à chaîne linéaire (LAS)
		Di(2-ethylhexyl)phtalate (DEHP)
	Solvants/détergents	Nonylphénol
		Ethoxylates de nonylphénol à 1 ou 2 groupes éthoxy

Dioxines	Polychlorodibenzodioxines
	Polychlorodibenzofirannes

Métaux lourds	Cadmium
	Chrome
	Cuivre
	Mercure
	Nickel
	Plomb

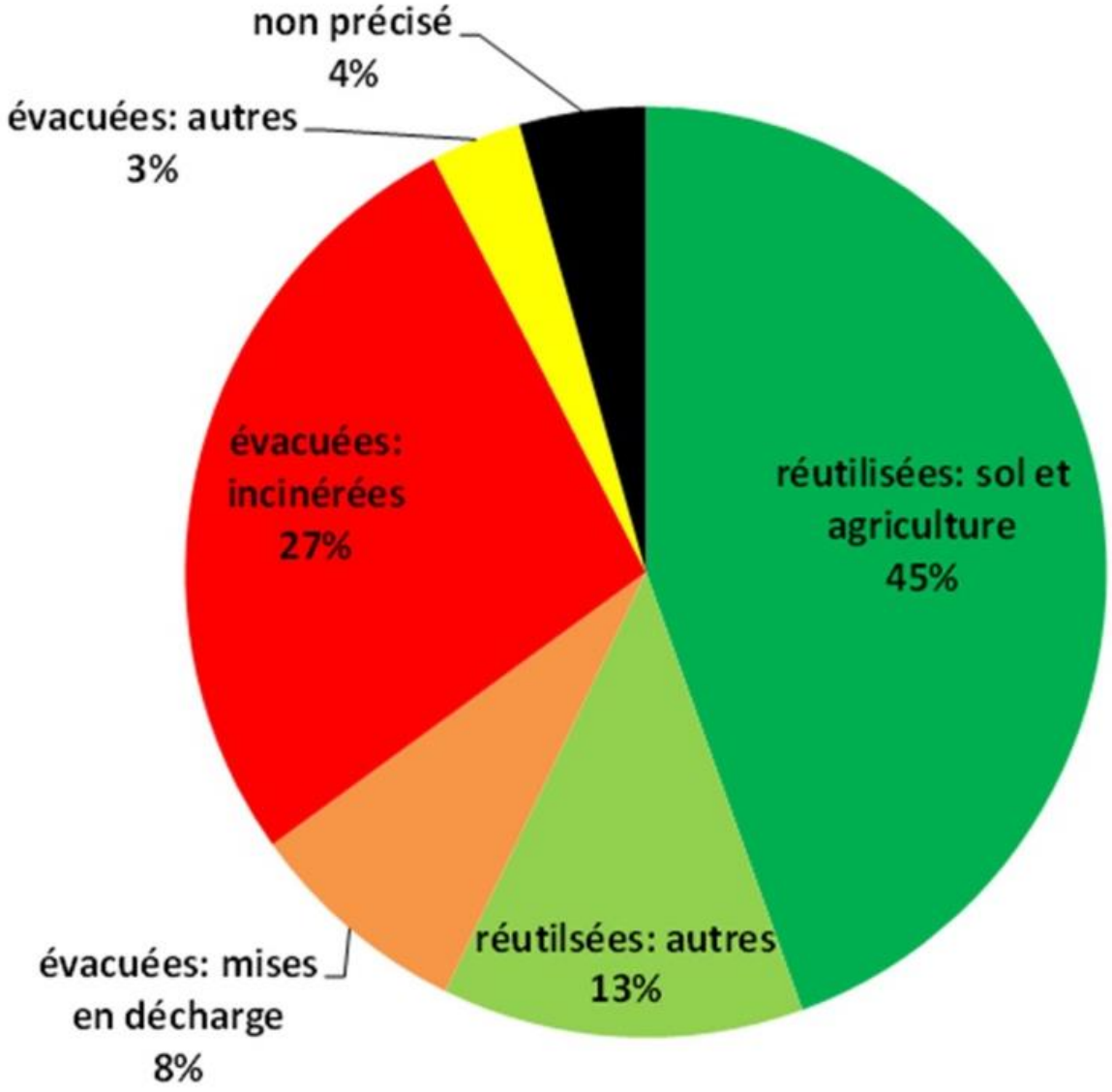
Emerging Substances

Substances pharmaceutiques
Hormones (5)
17beta-estradiol
17alpha- estradiol
estrone
estriol
éthinylestradiol
Betabloquants (10)
oxprénolol
métoprolol
timolol
propranolol
nadolol
bétaxolol
bisoprolol
acébutolol
aténolol
sotalol
Antibiotiques
sulfaméthoxazole
roxythromycine

Antidépresseur
amitryptiline
doxépine
imapramine
carbamazépine
diazepam
nordiazepam
analgésique, anti inflammatoire
ibuprofène
paracétamol
kétoprofene
aspirine
diclofénac
hypolipémiant
gemfibrozil
bronchodilatateur
clenbutérol
salbutamol
terbutaline

- **Not biodegraded: carbamazepine**
- **Moderately degraded: Triclosan, triclocarban**
- **Substances eliminated at <30%**
  - Pesticides: glyphosate, AMPA, diuron, Atrazine, isoproturon, simazine.
  - Analgesics: Diclofenac,
  - Antidepressants: Carbamazepine, Diazepam, Doxipine, Alhylphenol Carboxylates
  - Bronchodilators: Salbutamol, terbutaline
  - Betabloquants: oxprenolol, propranonlol, sotalol
  - Metals,

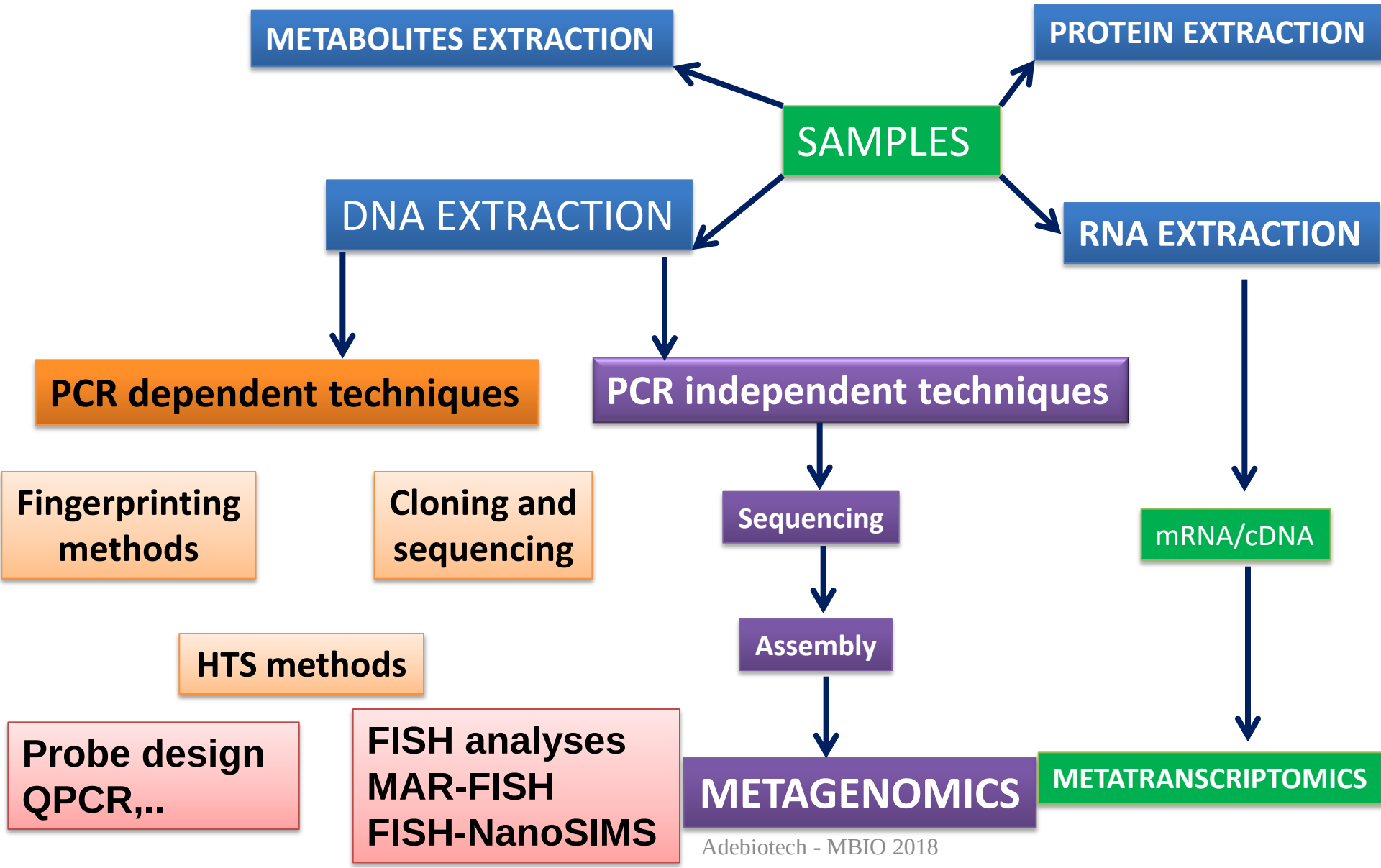
90% of the pharmaceutical substances are found in STEPs at concentrations> 100ng / L



Destination of sewage sludge from reported urban wastewater

Enormous advances in understanding the identity and the function of wastewater microbiota

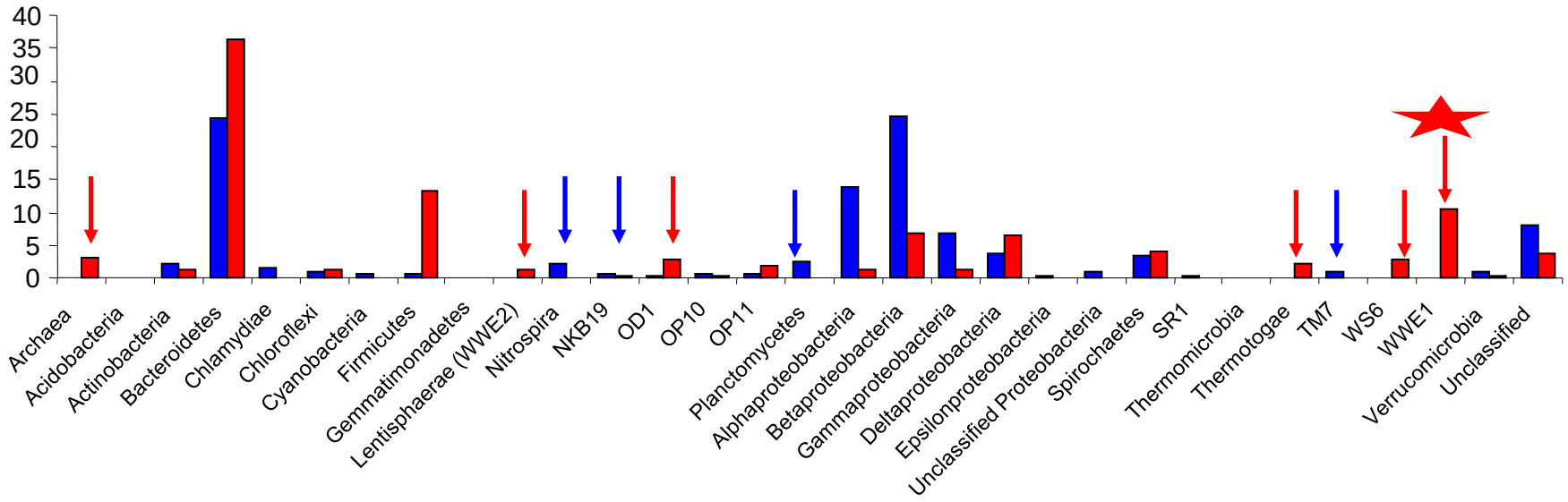
MOLECULAR APPROACHES
PHYLOGENETICS AND FUNCTIONNAL COMMUNITY ANALYSES



DISTRIBUTION DES rDNAs DANS LES CLONES DE FOSMIDES/BACS

Bassin aérobie 2979 BACs

Digesteur anaérobie 558 fosmids



- 23 divisions bactériennes représentées dans les BACs et fosmides y compris celles sans représentant cultivable

- Profil comparable à celui obtenu par PCR

- Accès à des génomes (fractions) de bactéries non encore cultivées

NITROGEN REMOVAL

Oxic zone

Nitrification

NH_4^+ → NO_2^- → NO_3^-

AOB : *Nitrosomonas, Nitrosospira, Nitrosococcus, Nitrosovibrio*

AOA: *Nitrosopumilus Nitrososphaera*

*Nirobacter Nitrococcus, Nitrospira, Cand. "**Nitrotoga**"*

Anammox bacteria

Cand. Brocadia, Kuenenia, Scalindua, Jettania, etc

Anammox

NO_2^- → N_2

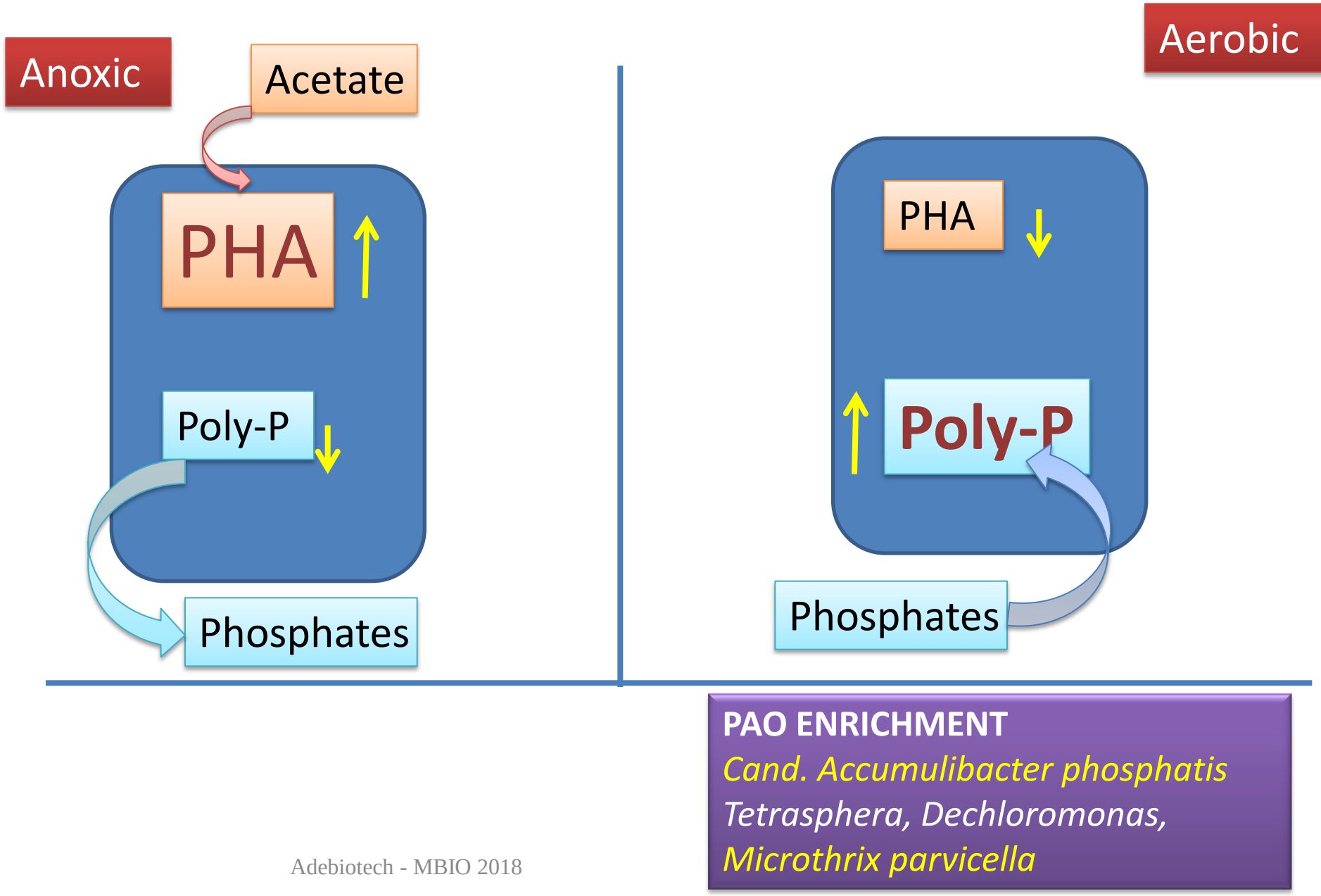
Cand. "Methylomirabilis oxyfera"

NO_2^- → NO → N_2O → N_2

Denitrification

Anoxic zone

PHOSPHORUS REMOVAL



PAO ENRICHMENT

Cand. Accumulibacter phosphatis

Tetrasphera, Dechloromonas,

Microthrix parvicella

Carbon and toxic compounds removal

Aminomonas paucivorans
Pitluck et al., 2010

Anaerobaculum mobile
Mavromatis et al., 2013

Arthrobacter nitroguajacolicus
Niewerth et al., 2012

Cloacibacillus evryensis
Comamonas testosteroni
Fukuda et al., 2014

Cand. Competibacter denitrificans

Can. Contendobacter odensis

Exiguobacterium alkaliphilum

Methanocorpusculum Labreanum
Anderson et al., 2009

Methanofollis liminatans

Methanolinea tarda
Yamamoto *et al., 2014*

Pseudomonas moraviensis
Hunter *et al., 2014*

Pseudomonas otitidis

Pseudomonas stutzeri
Busquets *et al., 2013*

Rhodococcus ruber
Shumkova *et al., 2015*

Sediminibacterium sp.
Ayarza et al., 2014

Sphingomonas sp.
Chen *et al., 2014*

Thauera sp.
Dichosa *et al., 2015*

Nitrogen removal

Ammonia oxidation

Nitrosomonas europaea
Chain *et al., 2003*

Nitrosomonas eutropha
Stein *et al., 2007*

Nitrite oxidation

Nitrobacter hamburgensis
Starkenburg *et al., 2008*

Nitrobacter winogradskyi
Starkenburg *et al., 200*

Nitrospina gracilis
Lücker et al., 2013
‘

Cand. Nitrospira defluvii’
Lücker *et al., 2010*

Denitrification

Paracoccus denitrificans
Siddavattam et al., 2011

Thiobacillus denitrificans
Beller et al., 2006

Anammox

‘Cand. Kuenenia stuttgartiensis’
Strous et al., 2006

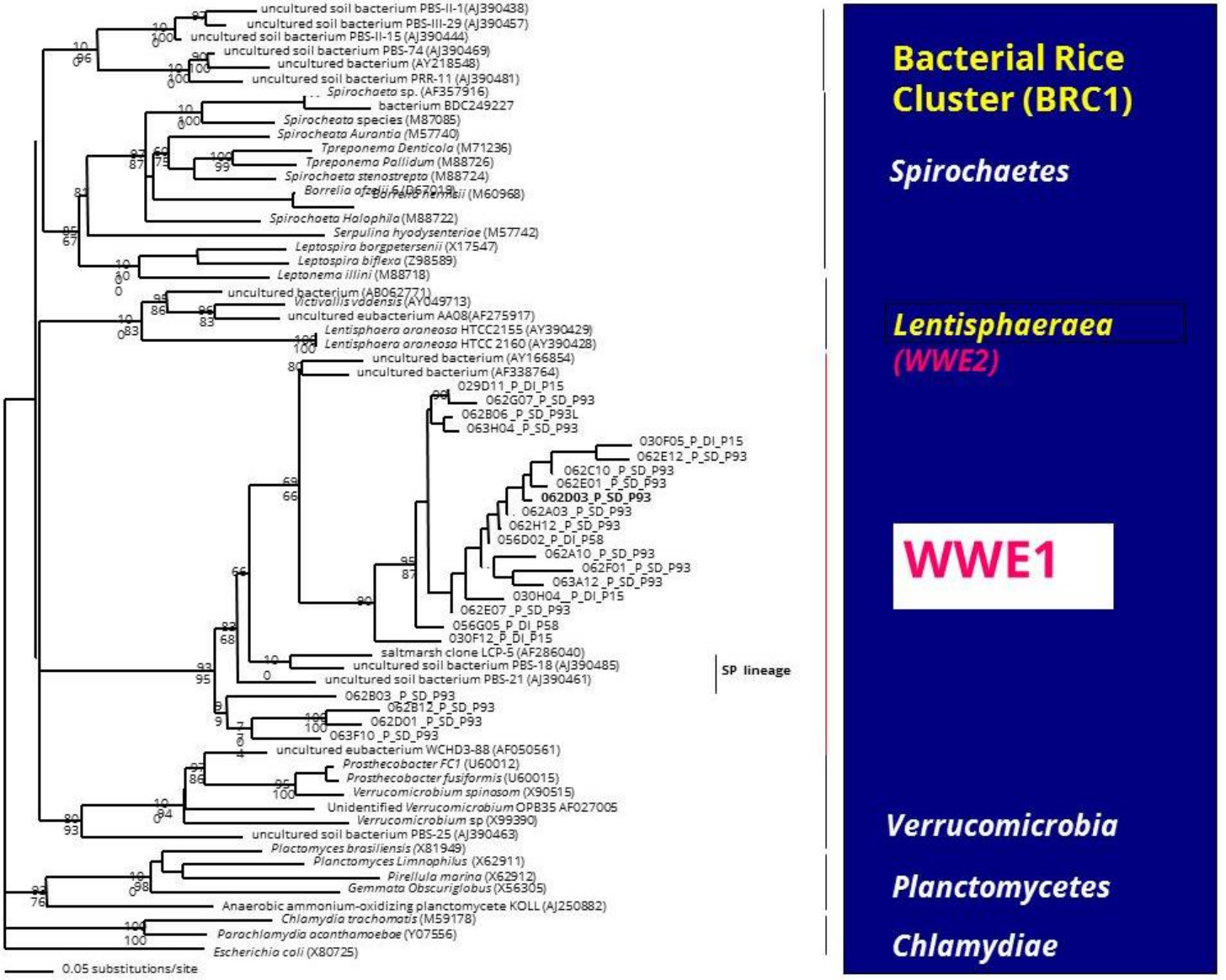
Phosphorus removal

Gemmatimonas aurantiaca

‘Cand. *Accumulibacter phosphatis’*
Mao et al., 2014

Tetrasphaera jenkinsii
Kristiansen et al., 2013

Novel Bacterial Candidate Divisions



Adebiotech - MBIO 2018

Chouari ey al., 2005

GENOME ANALYSES OF A VIRTUAL CANDIDATE DIVISION

(*Candidatus* Cloacamons acidaminovorans)

Gram negative, 2 membranes, fermentative

1818 CDS

44.3% HP & 7.9 CHP

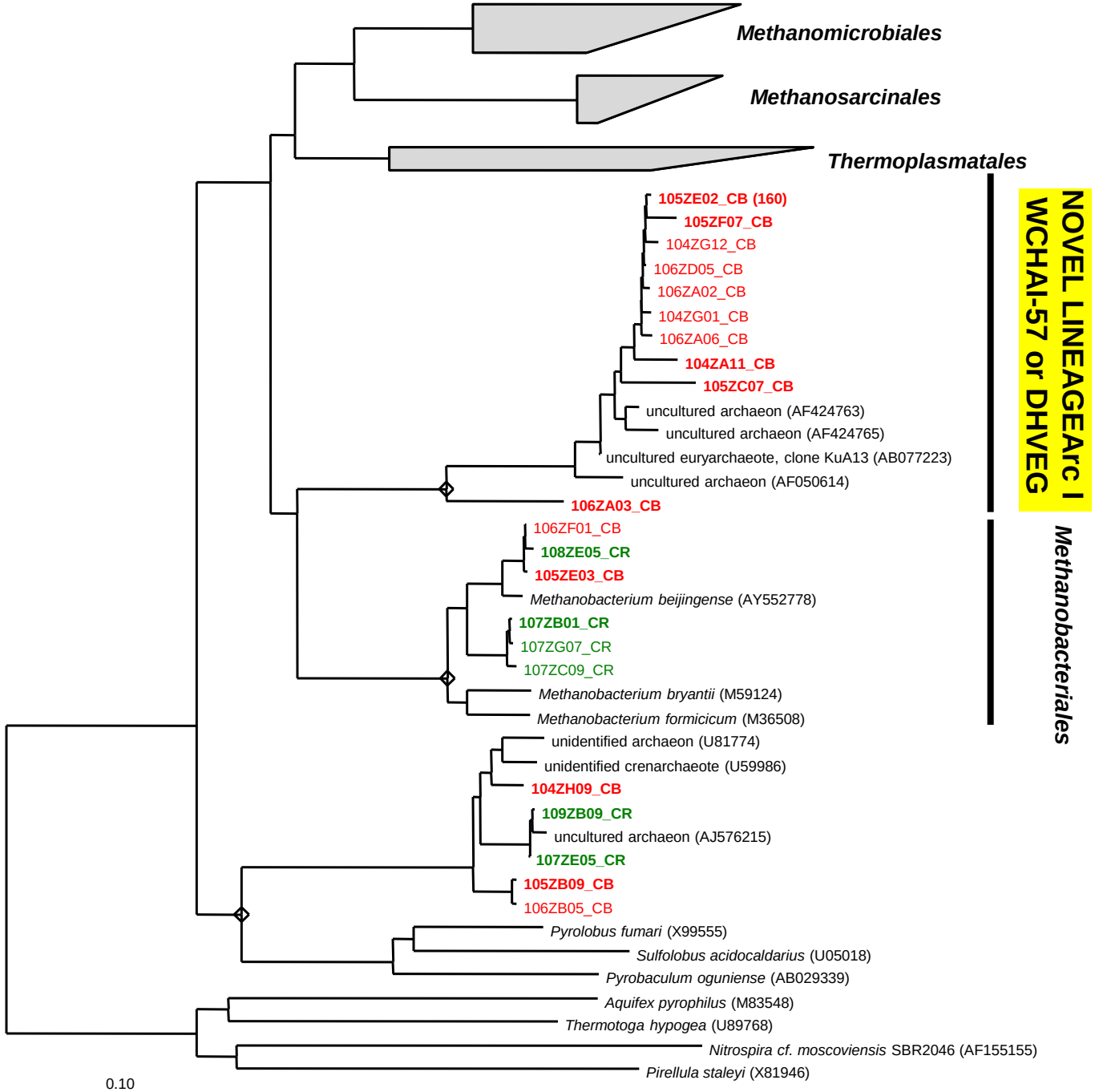
2.25 Mb
38 GC%

2 rDNA operons
45 tRNAs

Thermoanaerobacter tengcongensis
Geobacter sulfurreducens
Desulfovibrio vulgaris
Candidatus Kuenenia stuttgartiensis

- **Many proteases, aminopeptidases & carboxypeptidases,**
- **Hydrogene production, syntrophy with H_2 scavenger ?**

Pelletier et al.,2008



Chouari et al., 2005

Guermazi et al., 2008

- **Third type of methanogenesis pathway: The methylotrophic pathway beside the acetoclastic and the hydrogenotrophic ones**

- Genome reconstruction of '***Candidatus* Methanofastidiosa**' within **ARC 1/WCHAI-57or DHVEG WSA2 lineage** a methanogen of **the new proposed phylum *Verstraetearchaeota* shown to have specific genes for carrying methylotrophic methanogenesis**

Nobu et al., 2016
Vanwonterghem et *al.*, 2016)
Hugneholtz, 2002; Chouari *et al*., 2005

- Yet, the vast amount of genetic information generated has so far **not resulted in a significant improvement in our understanding of the functioning of these systems**

- **Our knowledge of the population dynamics, complexity and stability of the microbial community, remains very limited**

Current microbial genomic data are heavily biased towards bacteria

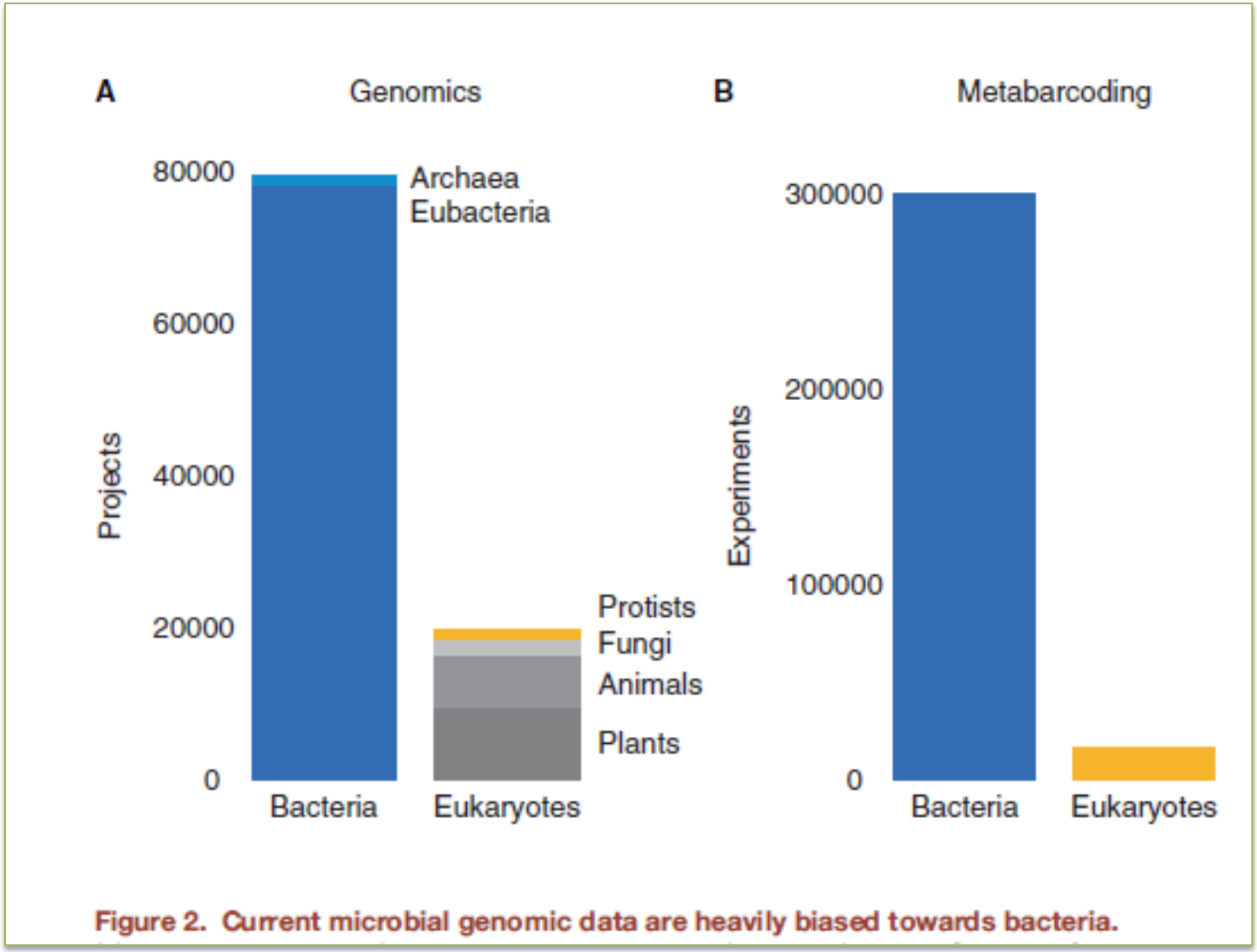


Figure 2. Current microbial genomic data are heavily biased towards bacteria.

https://omictools.com/imicrobe-tool

A significant part of the Wastewater microbiota is still almost completely ignored !!

Whose role is underestimated

PEGASUS PROJECT

Phylogeny of Eukaryotic Genomes in Activated Sludge and Untreated Sewage

We investigated the microbial eukaryotic communities of

12 domestic and Industrial activated, oxic/anoxic,

anaerobic sludge samples

Valenton and Noisy-Le Grand

La Morée

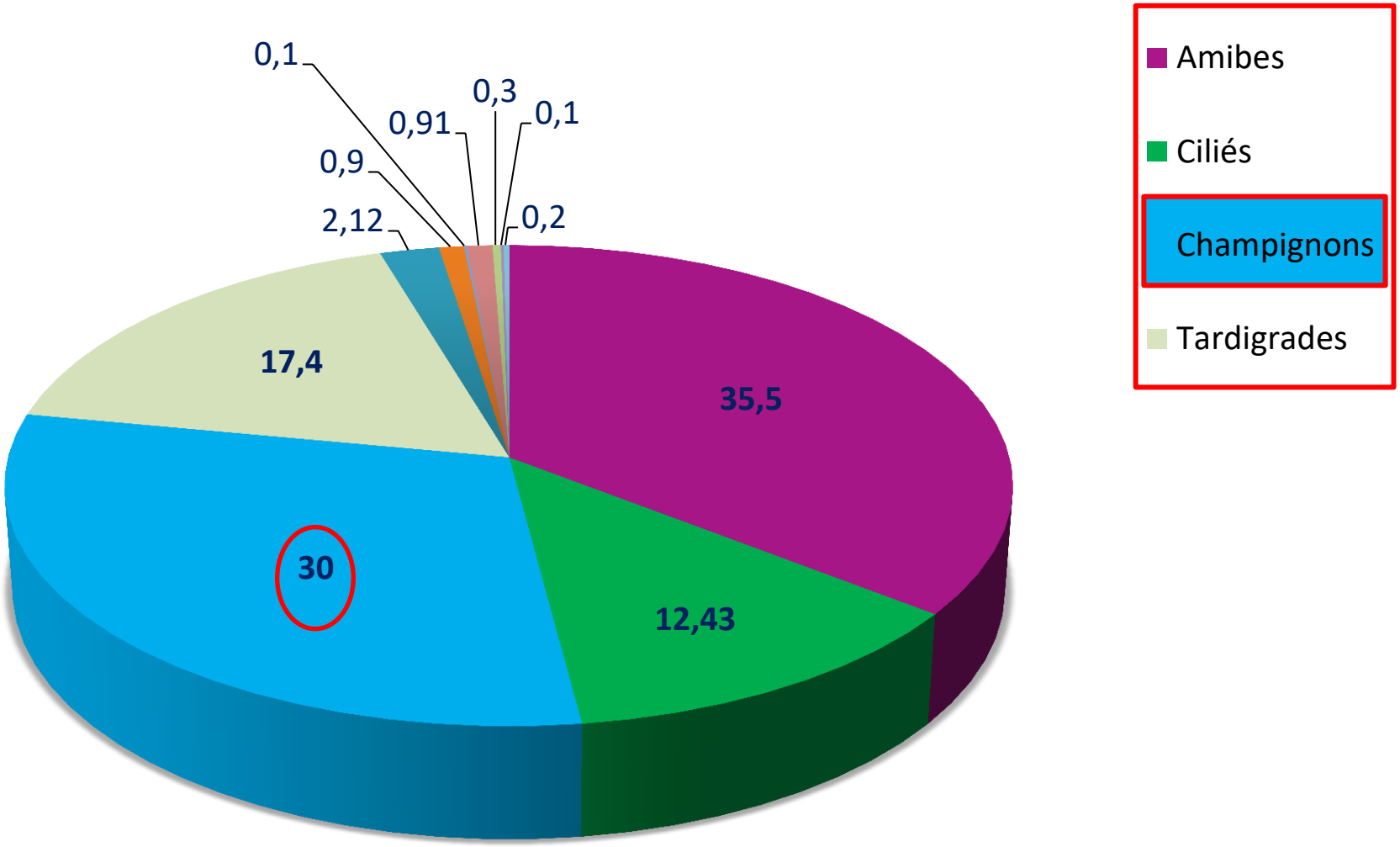
1600 Sequences → **989 Contigs**

Blast Analysis

GenBank, PR2, Silva databases

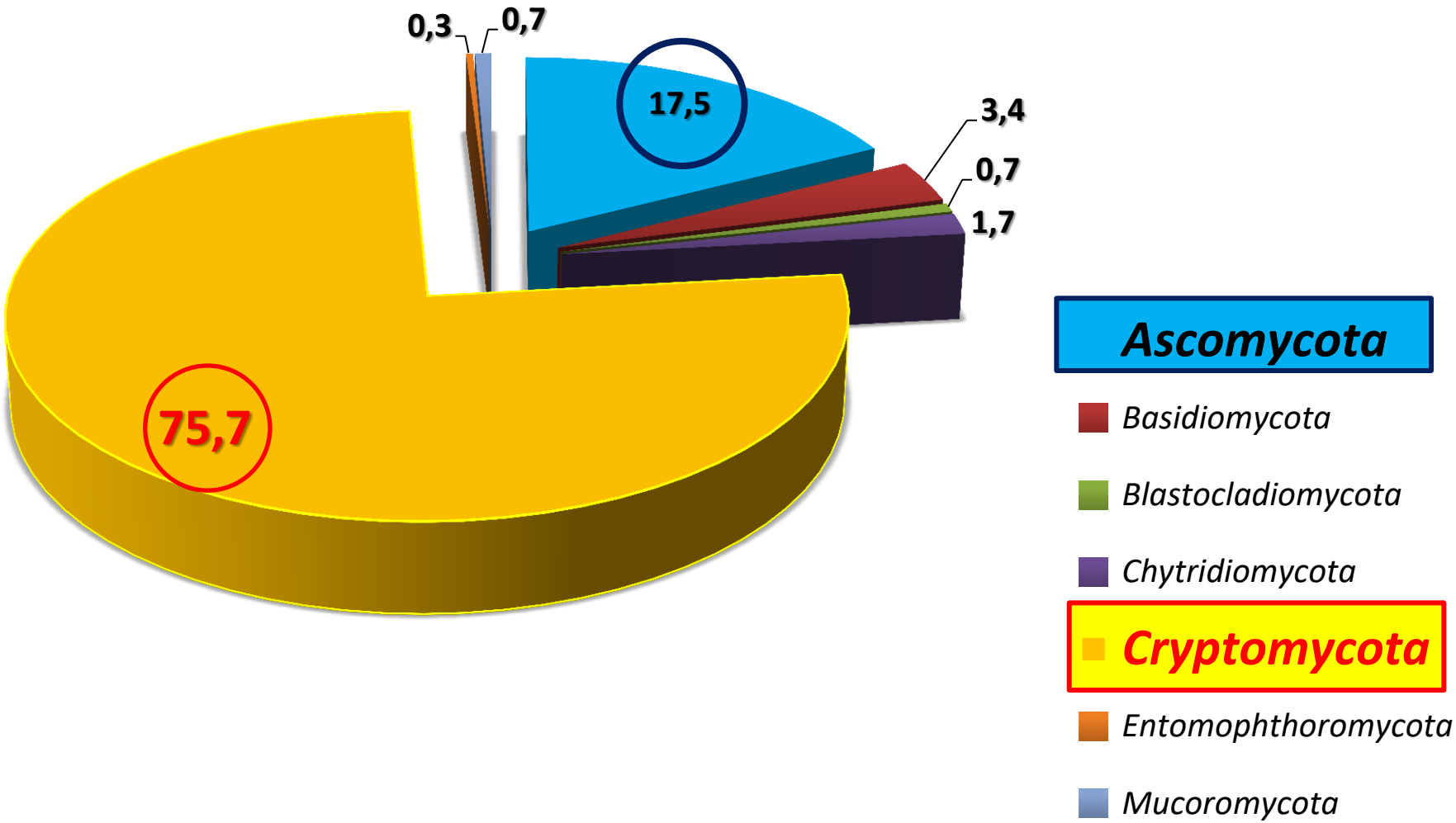
Amoeba	*35,5*
Fungi	*30*
Tardigrades	*17,4*
Ciliates	*12,43*
Stramenopiles	**2,12**
Rotifères	**0,9**
Nematodes	**0,1**
Mesomycetozoa	**0,91**
Annélides	**0,3**
Embryophytes	**0,1**
Chlorophytes	**0,2**

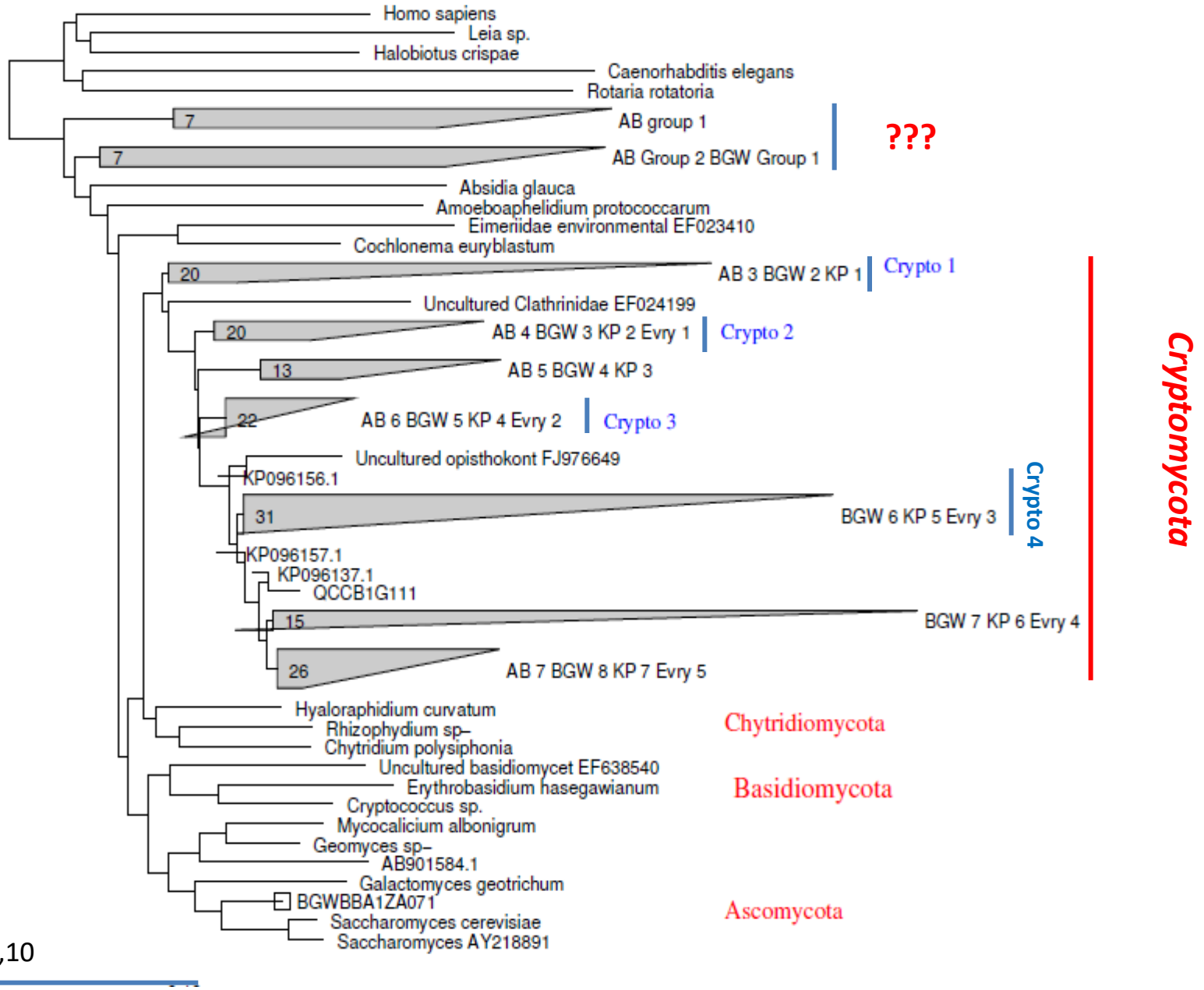
Blast : Four groups > 95% of the contigs



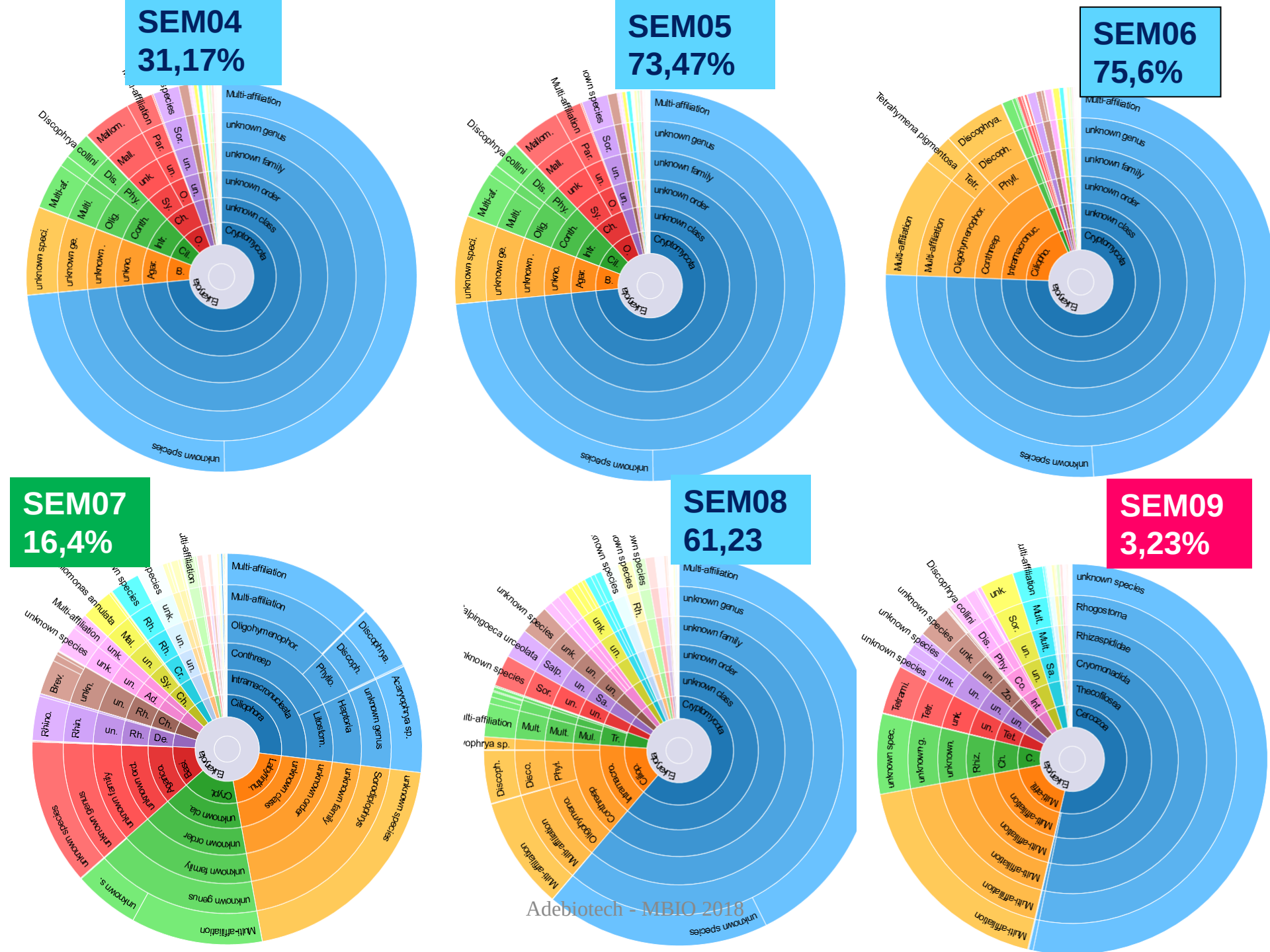
Two groups (Ascomycota and Cryptomycota) made up > 93% of the Fungi contigs

> 75% of *Cryptomycota*





Neighbour-joining tree of the eukaryal 18S rRNA gene sequences showing the position of Cryptomycota among the *Fungi*



Phylogenetic group	%
Cercozoa	18,92
Chrysophyceae	12,23
Rotifera	11,95
Cryptomycota	8,34
Ichthyosporea	7,32
Euglenozoa	5,55
Arthropoda	5,02
Discosea	2,94
Heterolobosea	2,21
Oomycetes	2,11
Hyphochytriomycetes	2,11

CONCLUSIONS

AND

PERSPECTIVES

- We ask for the impossible to a Microbiota that we still do not know enough !

- **We do not know who to contact !**

- **A microbiota that must fight on all fronts** : Resist toxic stresses and at the same time degrade the most recalcitrant substances !!

- **Minimize introduction of critical pollutants into the aquatic environment, by the adoption environmentally friendly products and processes.**

- **Improve existing unit processes and design new ones to optimize conventional processes for removal of compounds through adsorption and biodegradation in a broad range of water matrices.**

A combination of all approaches on an « eco-system biology basis » for a holistic interactions and characterization of microbial consortia is more than needed

AKNOWLEDGEMENTS

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