

Microbial ecology of the rumen

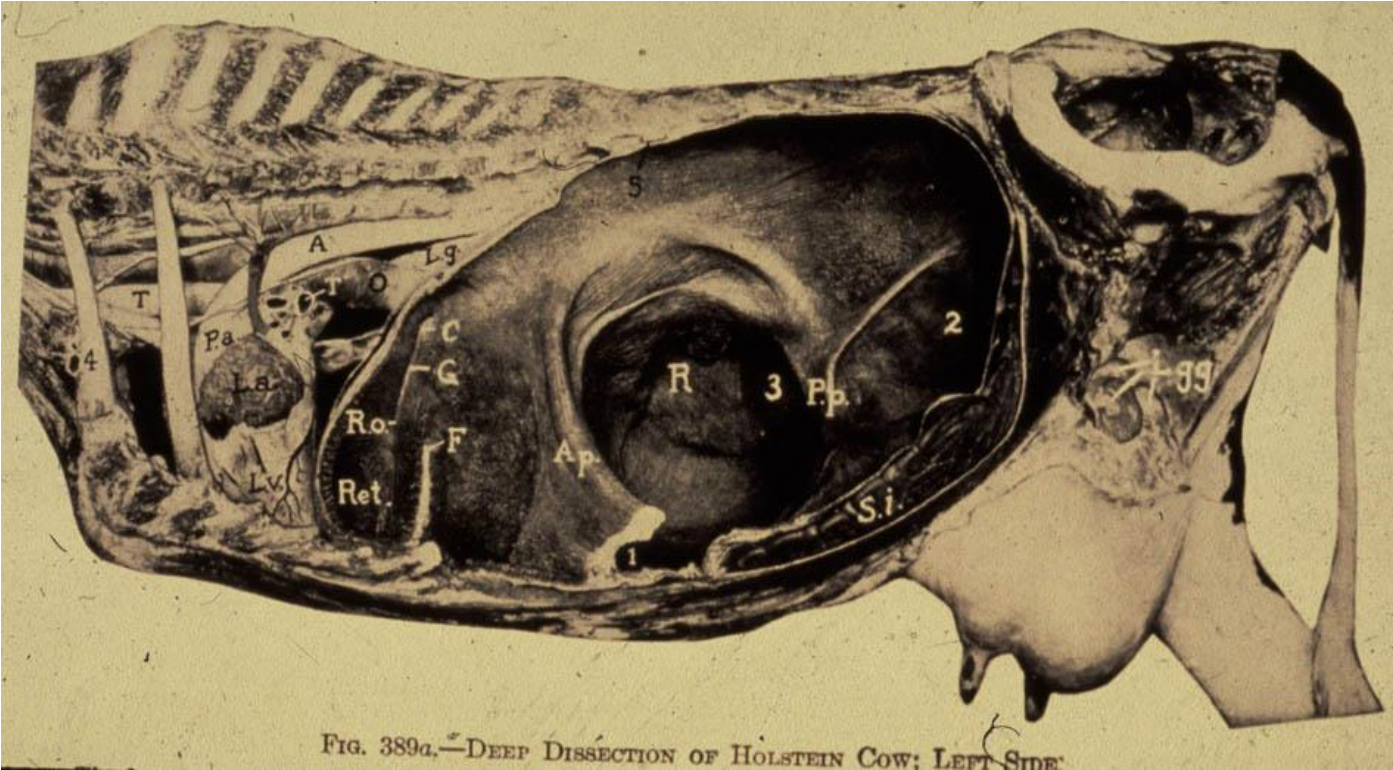


**John
Wallace**

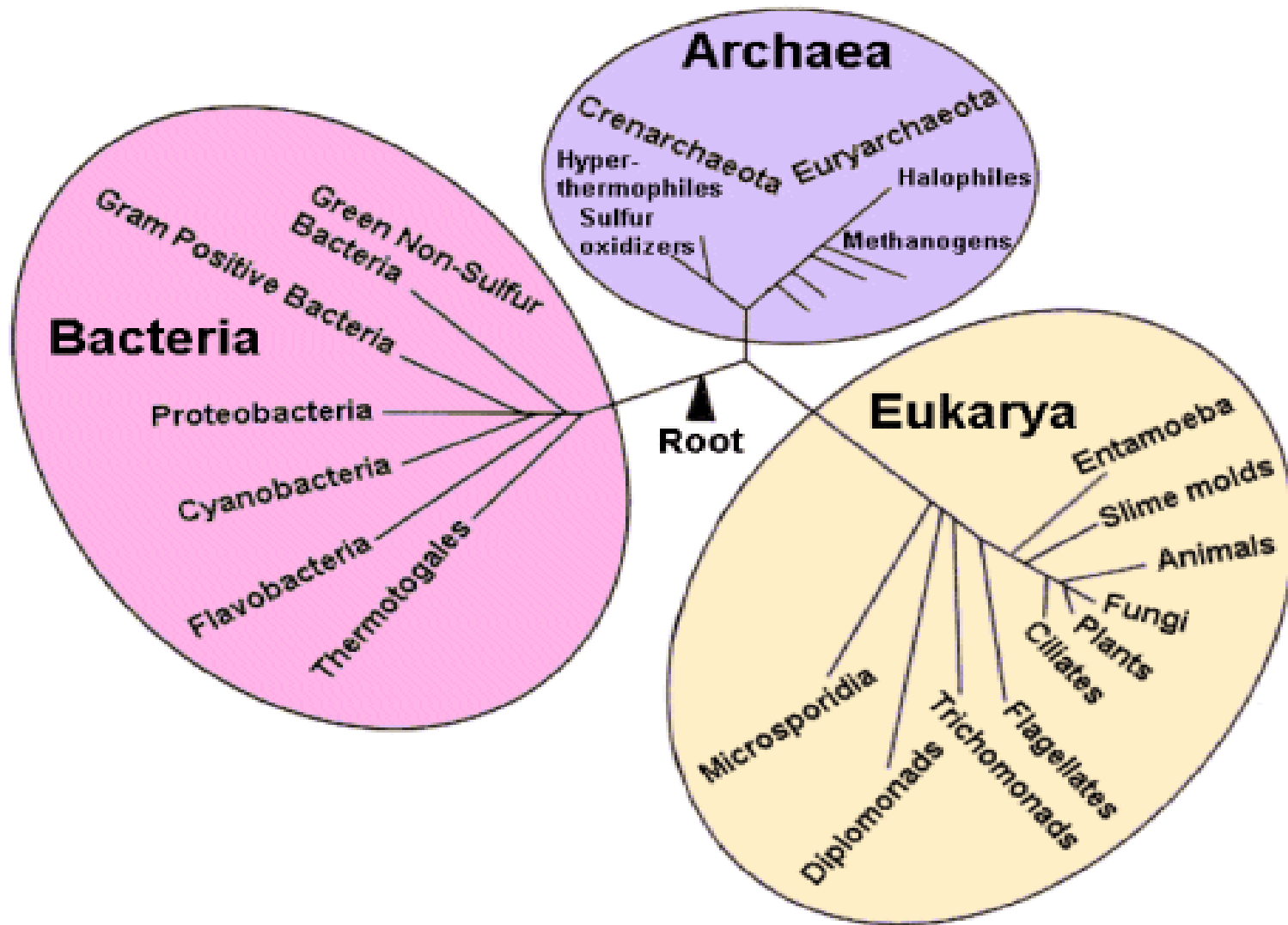


**Rowett Institute
of Nutrition and Health**
University of Aberdeen

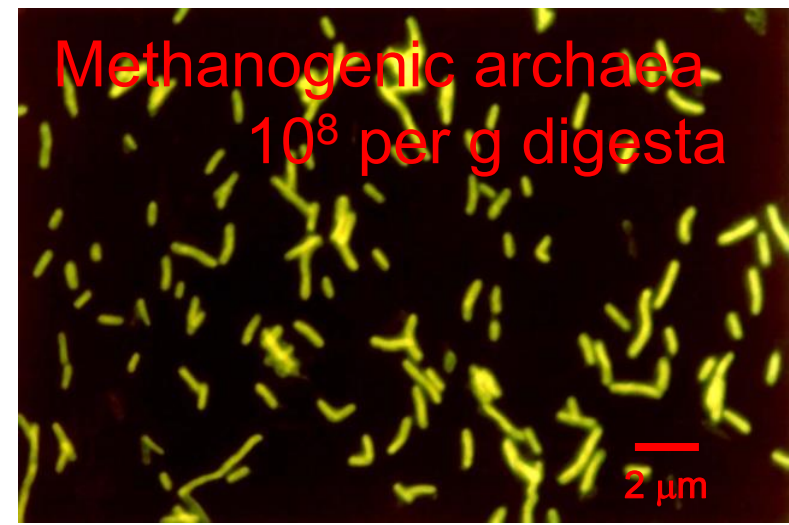
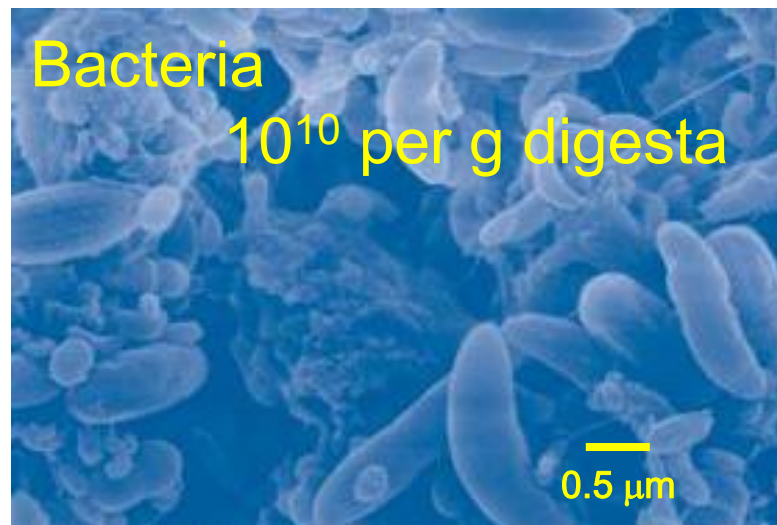
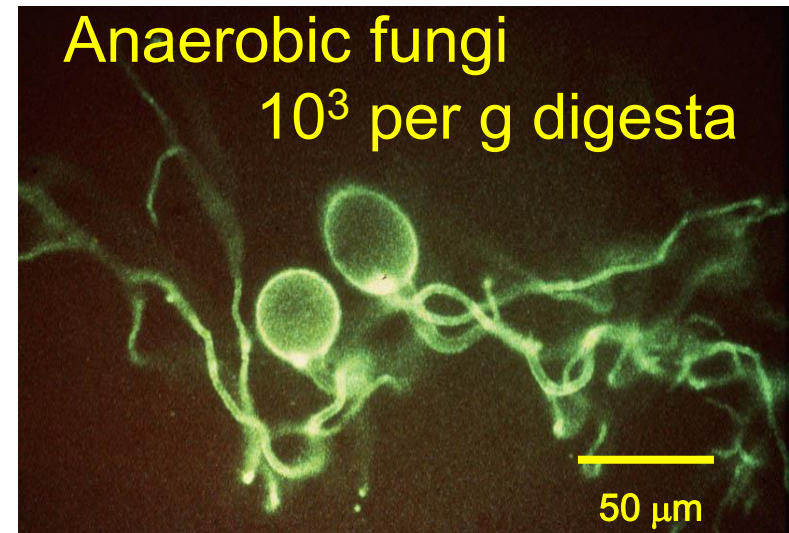
The rumen



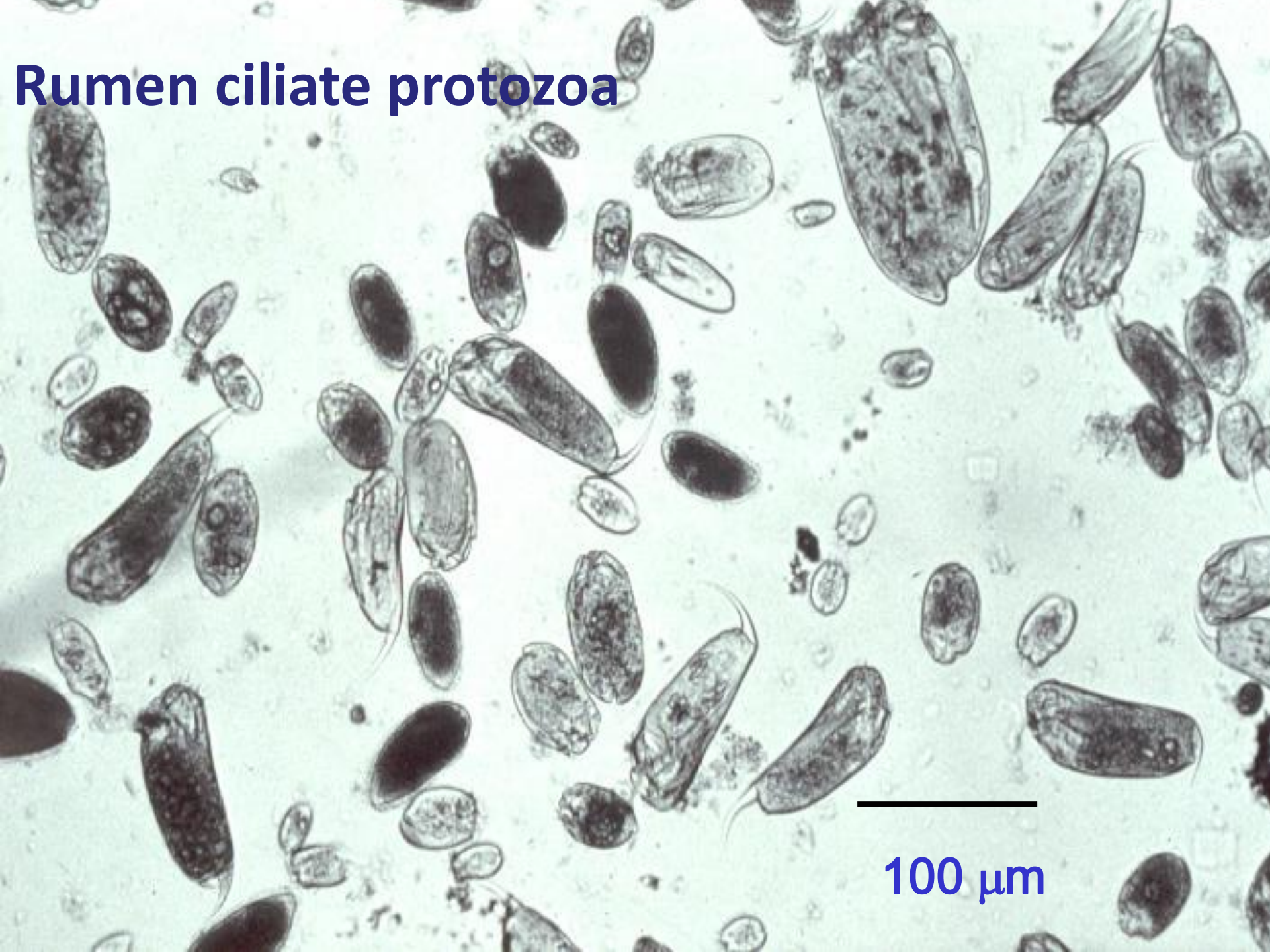
The three domains of life



Ruminal microorganisms



Rumen ciliate protozoa

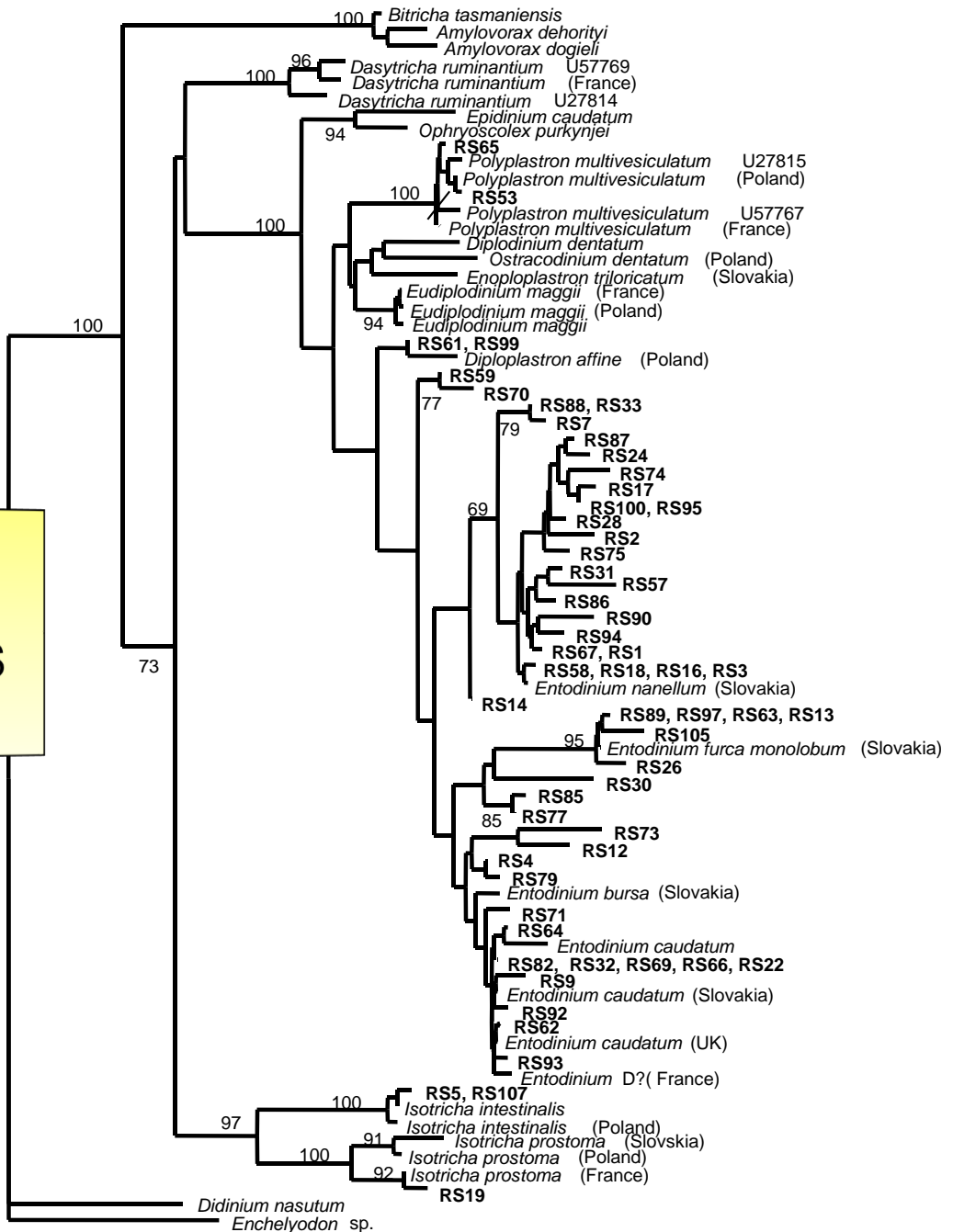


100 μm



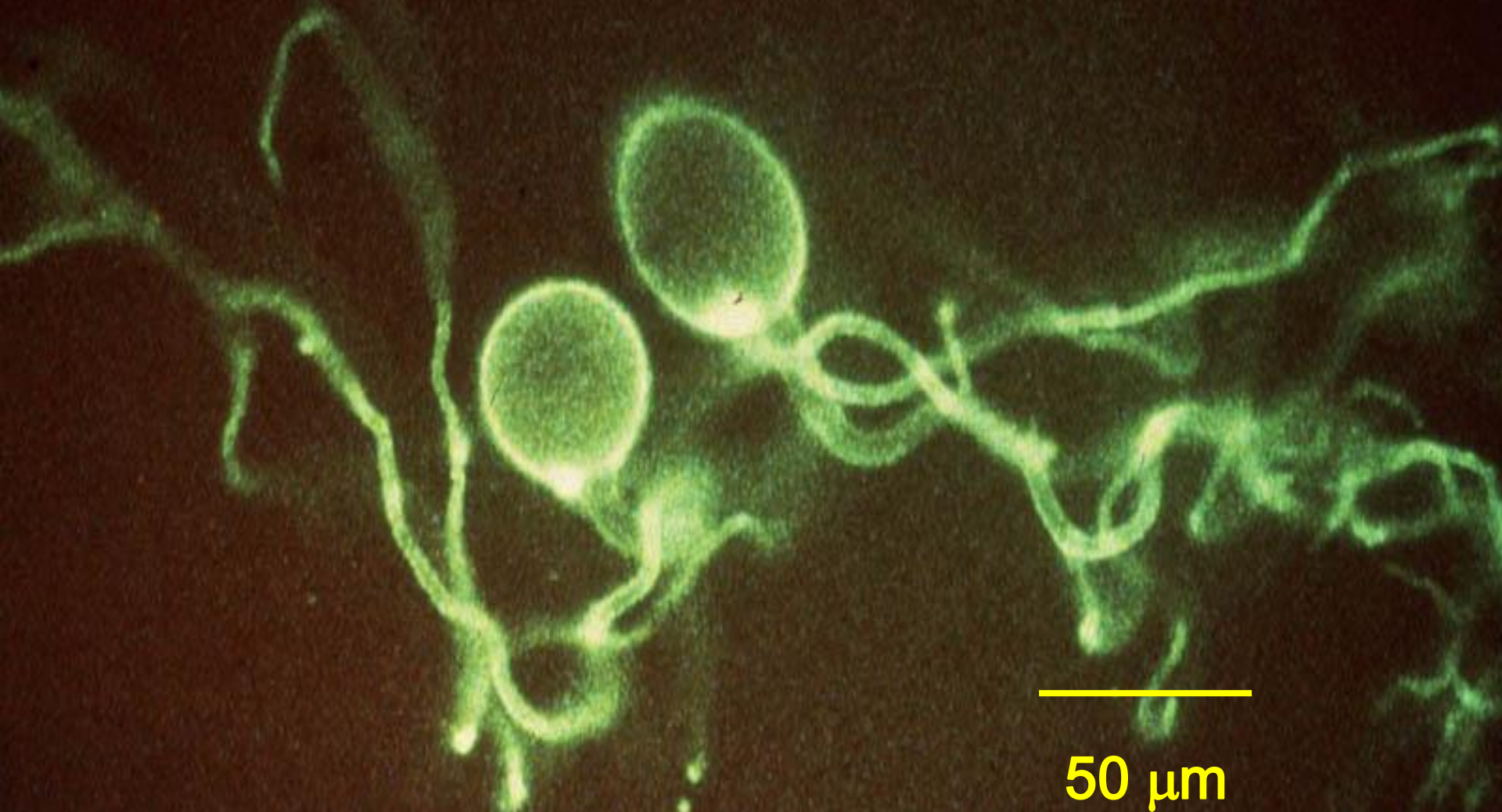
Unexpected protozoal diversity

Phylogenetic tree of rumen ciliate protozoa, based on cloning and sequencing of 18S rRNA genes



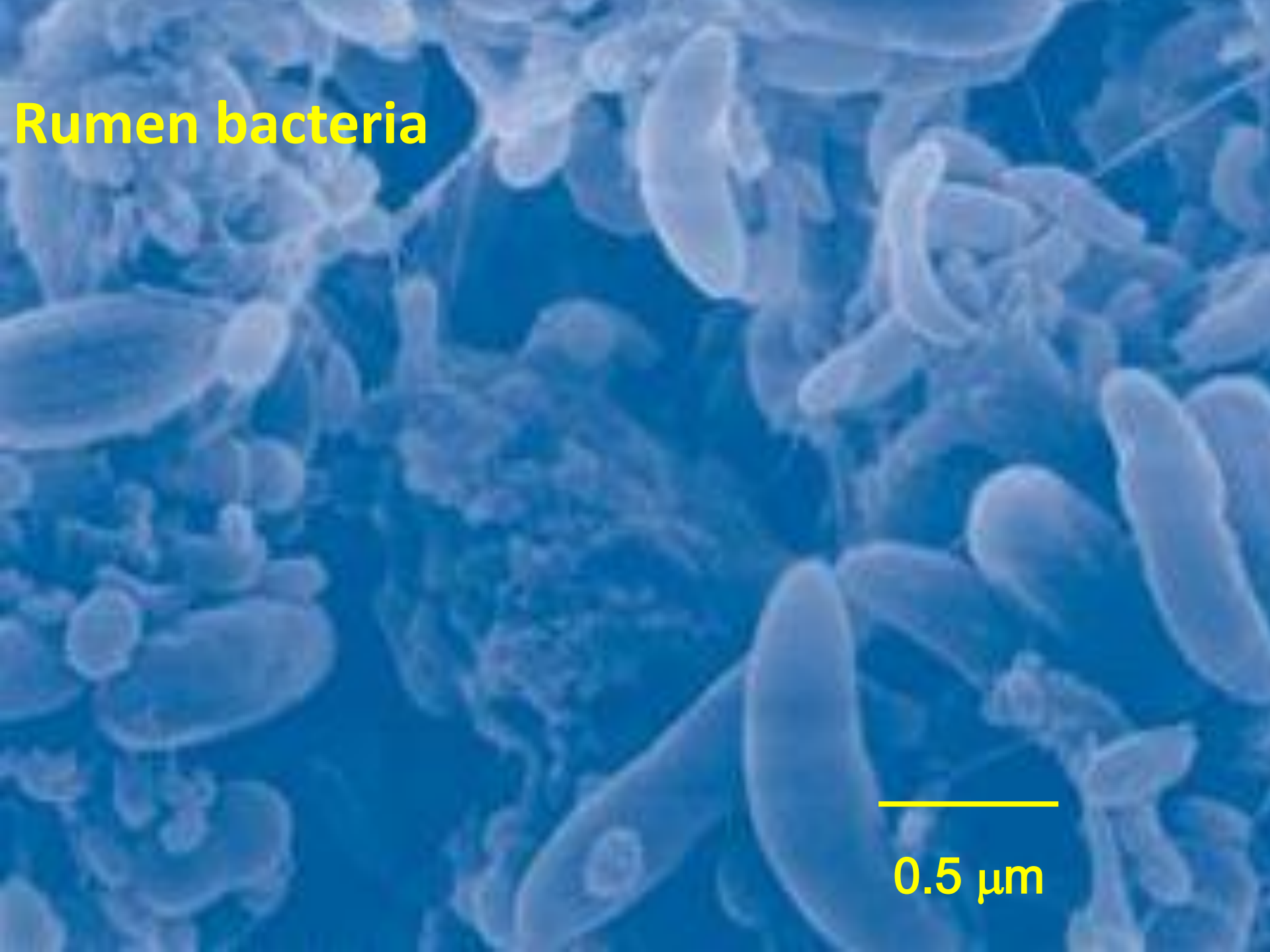
Wright et al (1997). J Eukar Microbiol 44, 61-67

Rumen anaerobic fungi

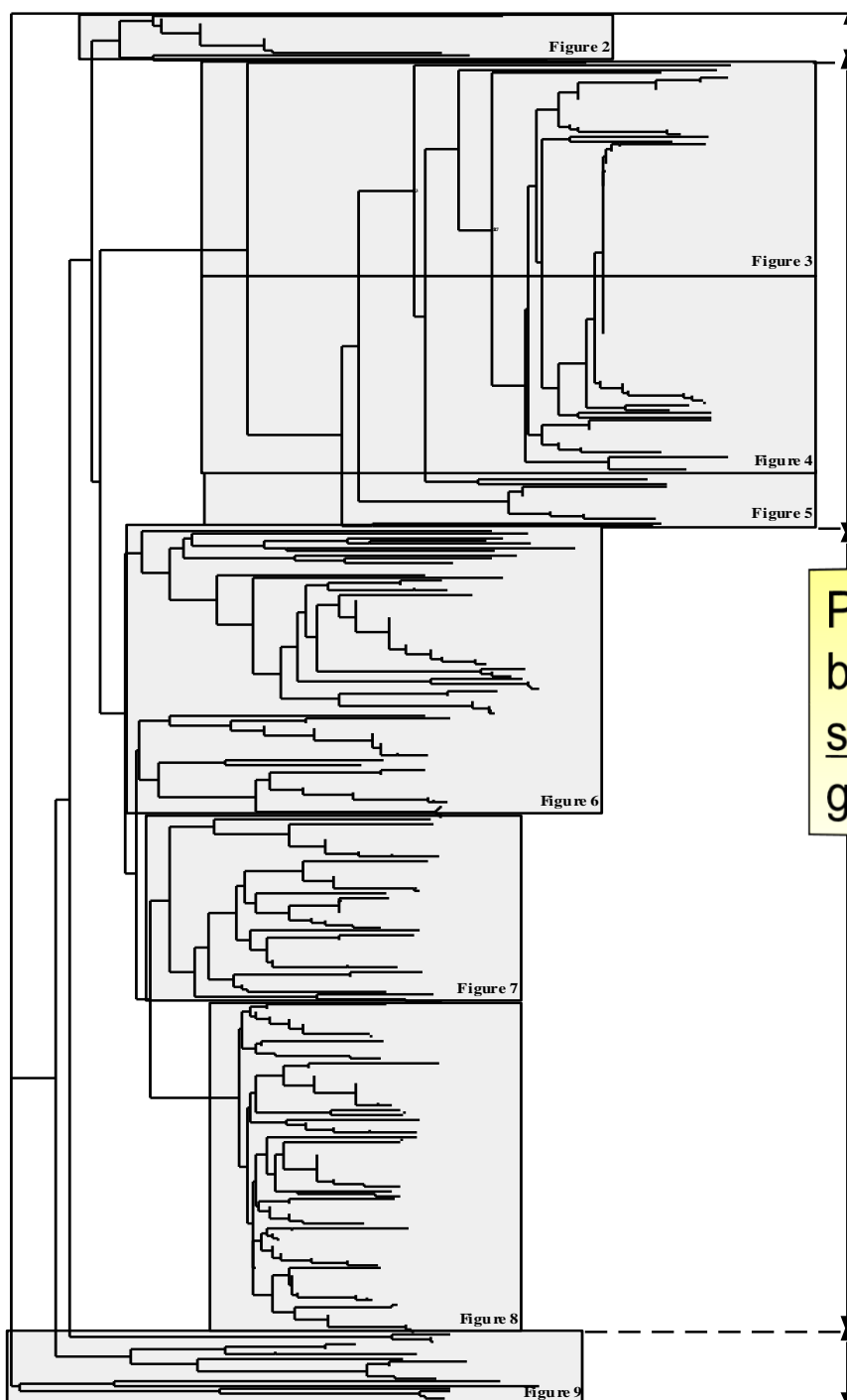


50 μm

Rumen bacteria



0.5 μm



Proteobacteria

Bacteroidetes

Phylogenetic tree of ruminal bacteria, based on cloning and sequencing of 16S rRNA genes, >1000 nt length

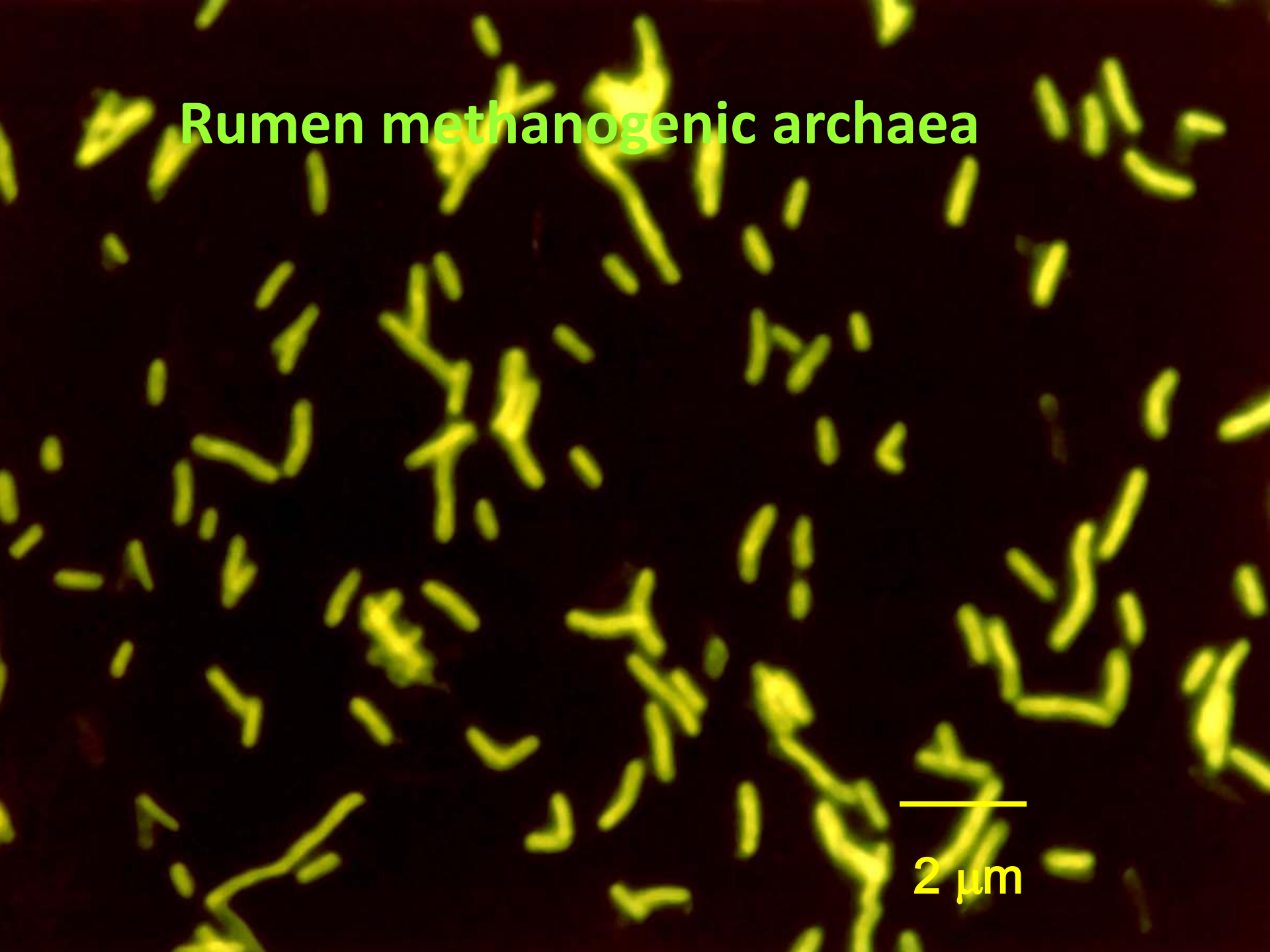
Firmicutes

Edwards et al. (2004) Anton v Leeuwen 86, 263-281

Fibrobacteraceae

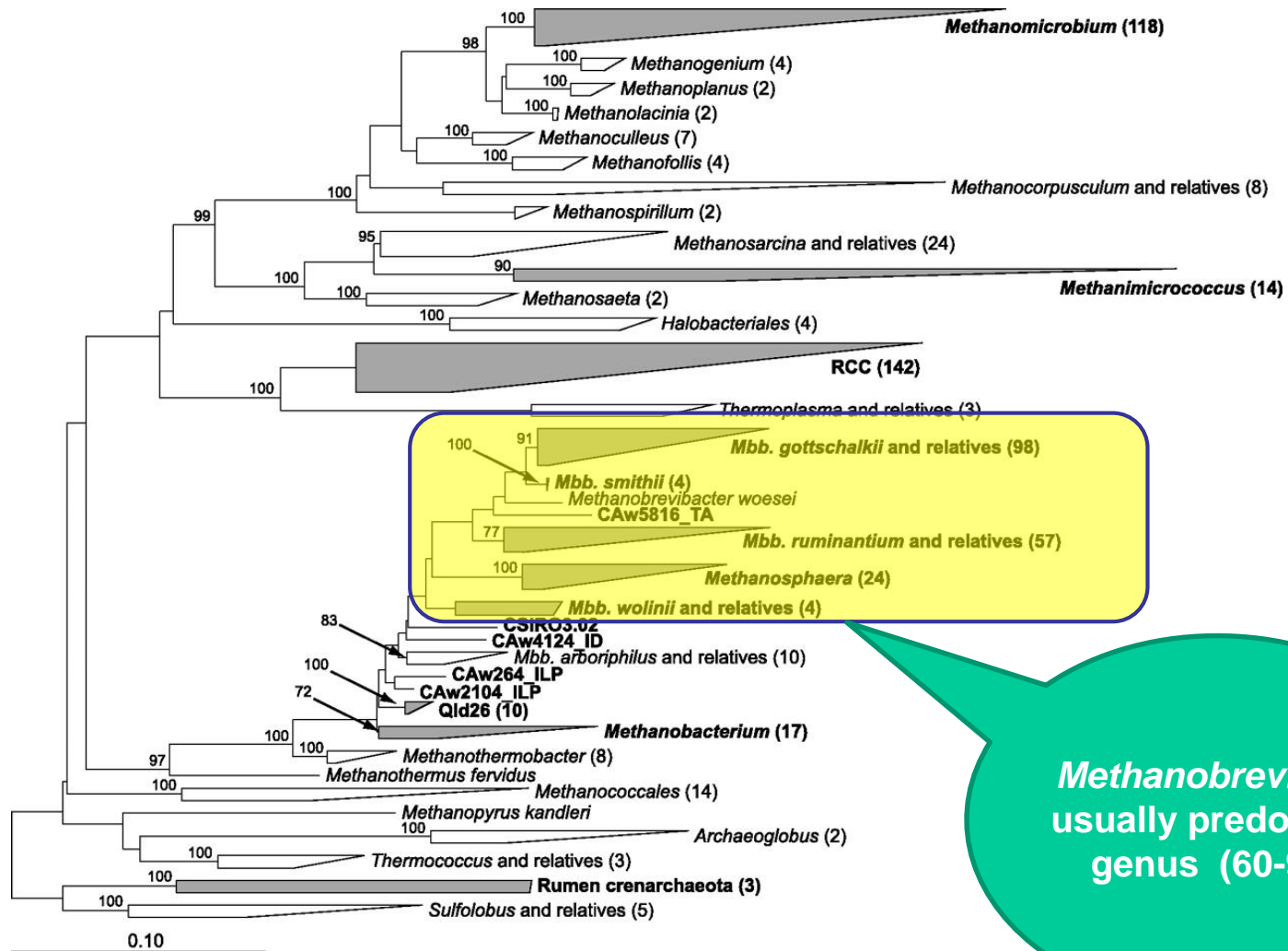
Spirochaetes

Rumen methanogenic archaea



—
2 μm

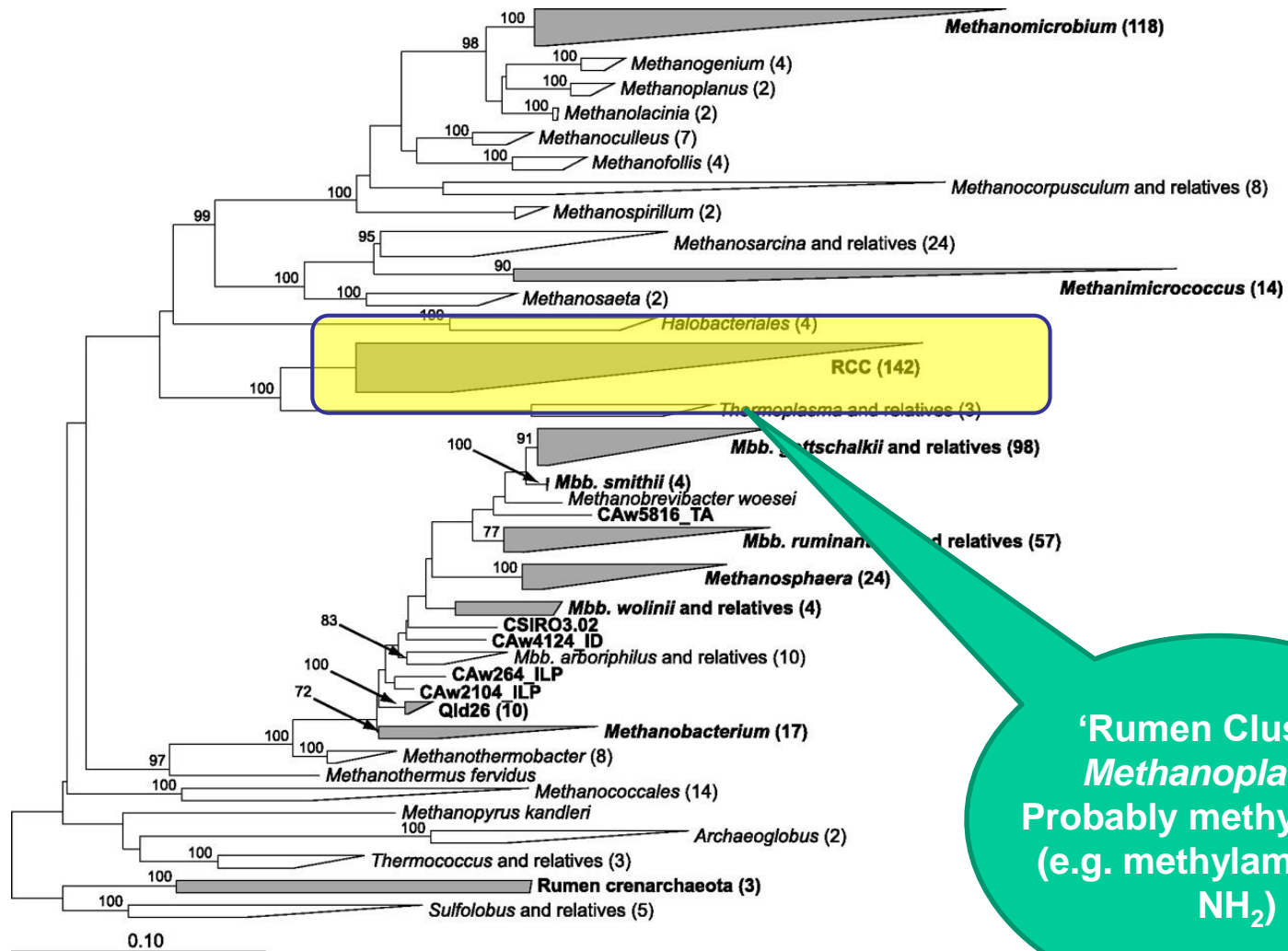
Phylogenetic dendrogram of total rumen and rumen protozoan-associated archaea and selected reference sequences.



Janssen P H , and Kirs M Appl. Environ. Microbiol.
2008;74:3619-3625

Applied and Environmental Microbiology

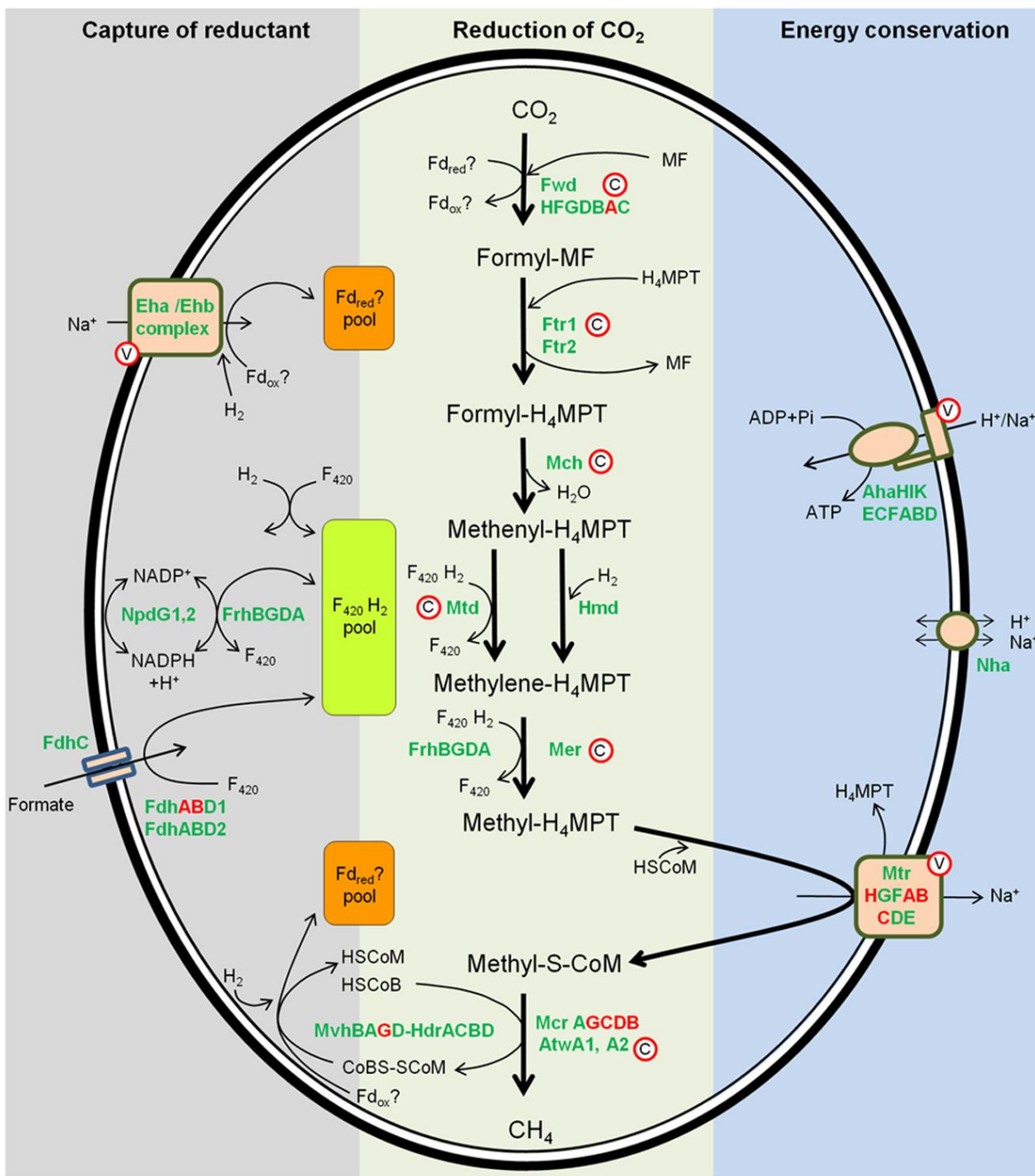
Phylogenetic dendrogram of total rumen and rumen protozoan-associated archaea and selected reference sequences.



'Rumen Cluster C'
Methanoplasmata
 Probably methylotrophic
 (e.g. methylamine CH₃-NH₂)

Janssen P H , and Kirs M Appl. Environ. Microbiol. 2008;74:3619-3625

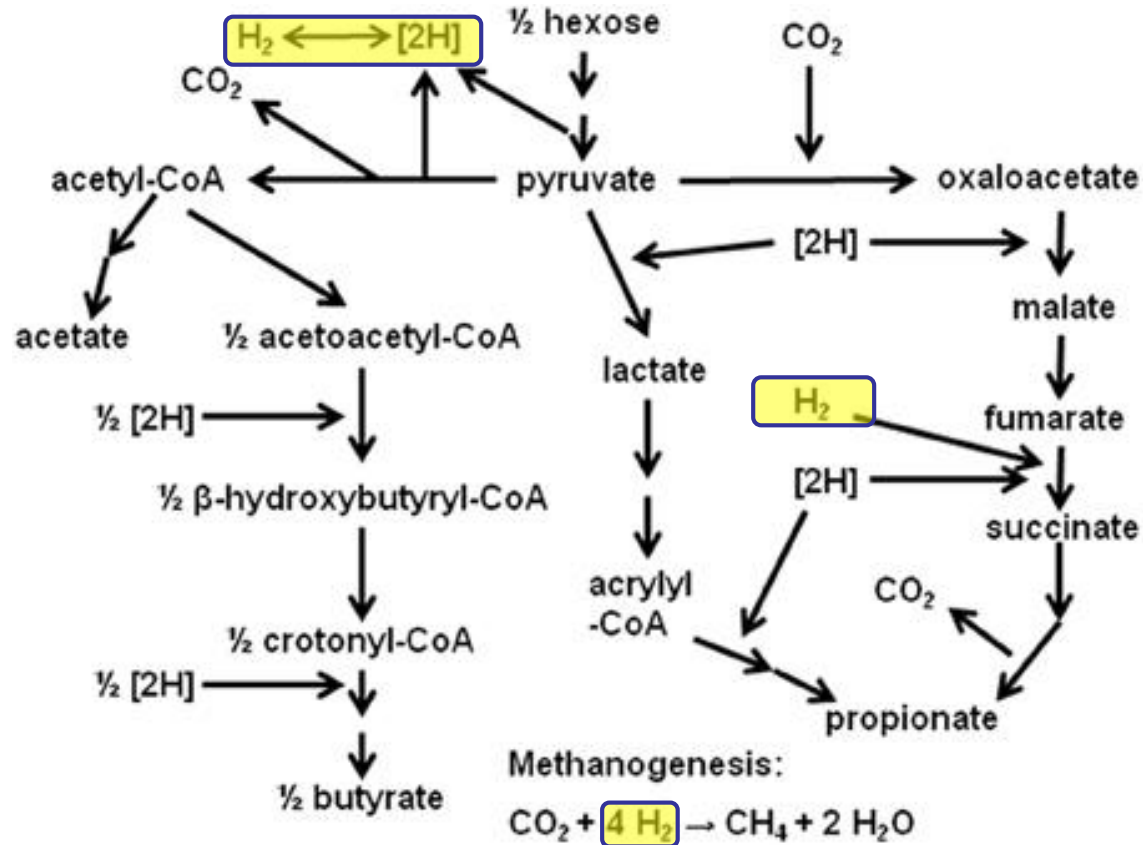
Applied and Environmental Microbiology



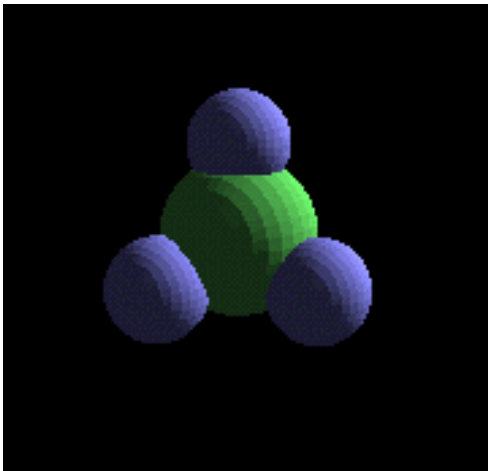
- Cofactor biosynthesis** ©
- Methanofuran
 - MfnA
 - Methanopterin
 - MptGA, CitG, ArfB
 - Coenzyme F₄₂₀
 - CofACHEDG, FucA
 - Coenzyme M
 - ComBC
 - Coenzyme B
 - AksDAFE

- Methanogenesis-associated** ©
- Hydrogenase helper genes
- HypABCDEFGF, Hycl
- Methanogenesis marker proteins
- 1 to 17

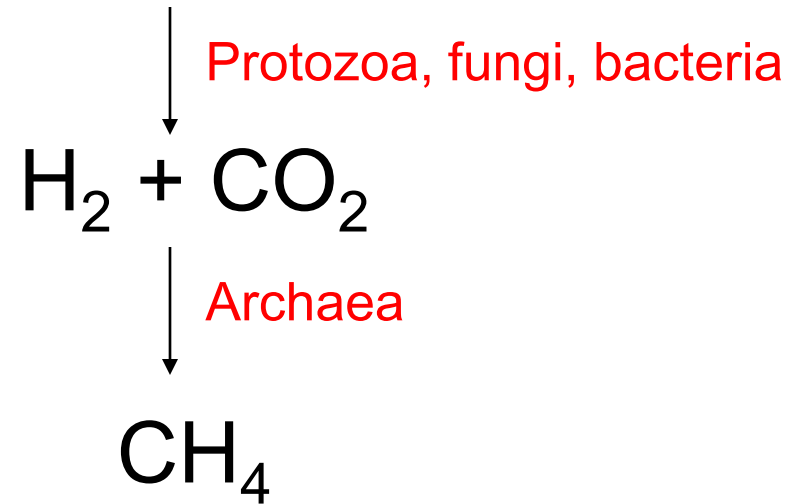
Methane production in the rumen



Methane production in ruminants



Fermentation



Respiration chambers SRUC, Edinburgh



The Extreme 8 experiment

- 36 Aberdeen Angus, 36 Limousin cross steers, 1-2 years
- Two diets: Forage diet 50:50, forage (grass silage / whole crop silage) : concentrate (barley / barley dark grains)
(n=36) Concentrate diet 8:92, forage (straw) : concentrate

Selected the 4 highest and 4 lowest methane emitters for full microbiome/metagenome analysis

Animals slaughtered 10 weeks later, ruminal digesta taken again

- Archaea and bacterial 16S rRNA genes measured by qPCR
- Data analysis, GLM procedure of SAS

The Extreme 8 analysis

Microbiome

Microbiota

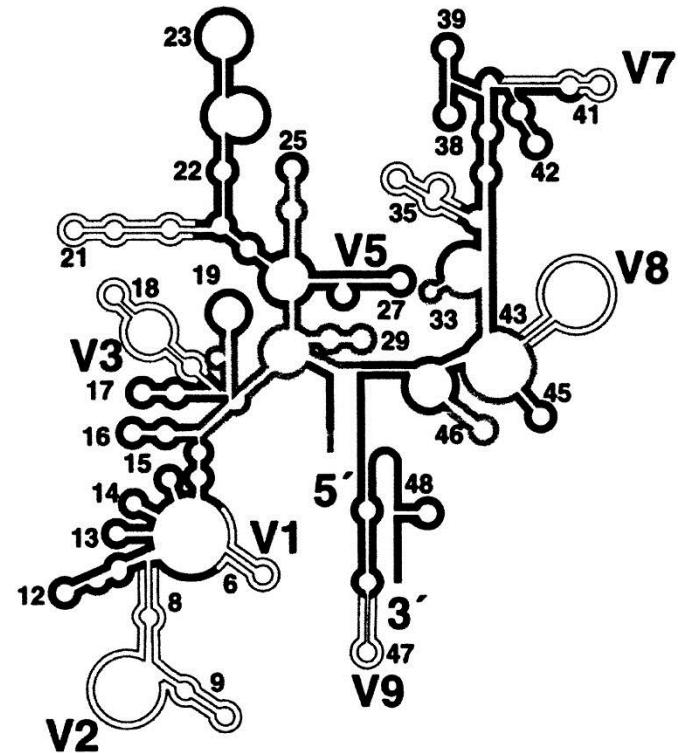
What species are different?

Metagenome

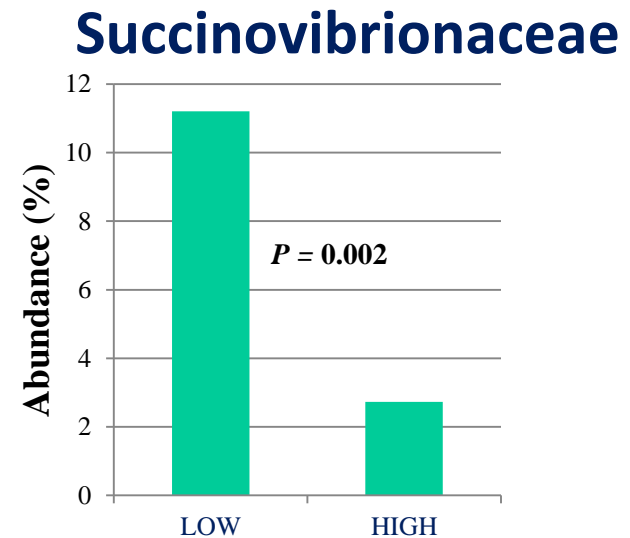
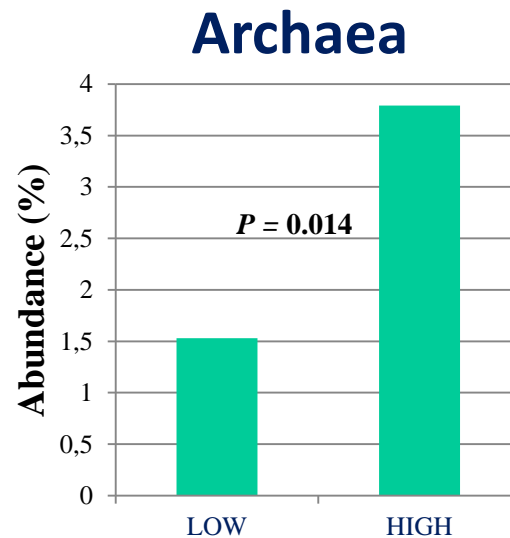
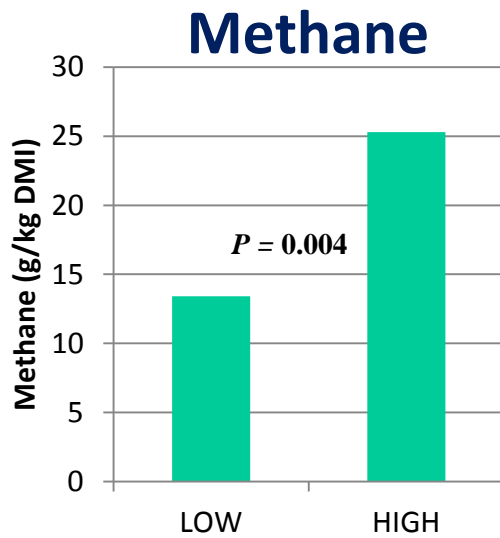
What genes are different?

The Extreme 8 microbiota analysis

Secondary structure
of small
subunit ribosomal
RNA



Variation in methane production: microbiota analysis



Methane emission status of beef cattle
Four highest and four lowest from 72 beef cattle

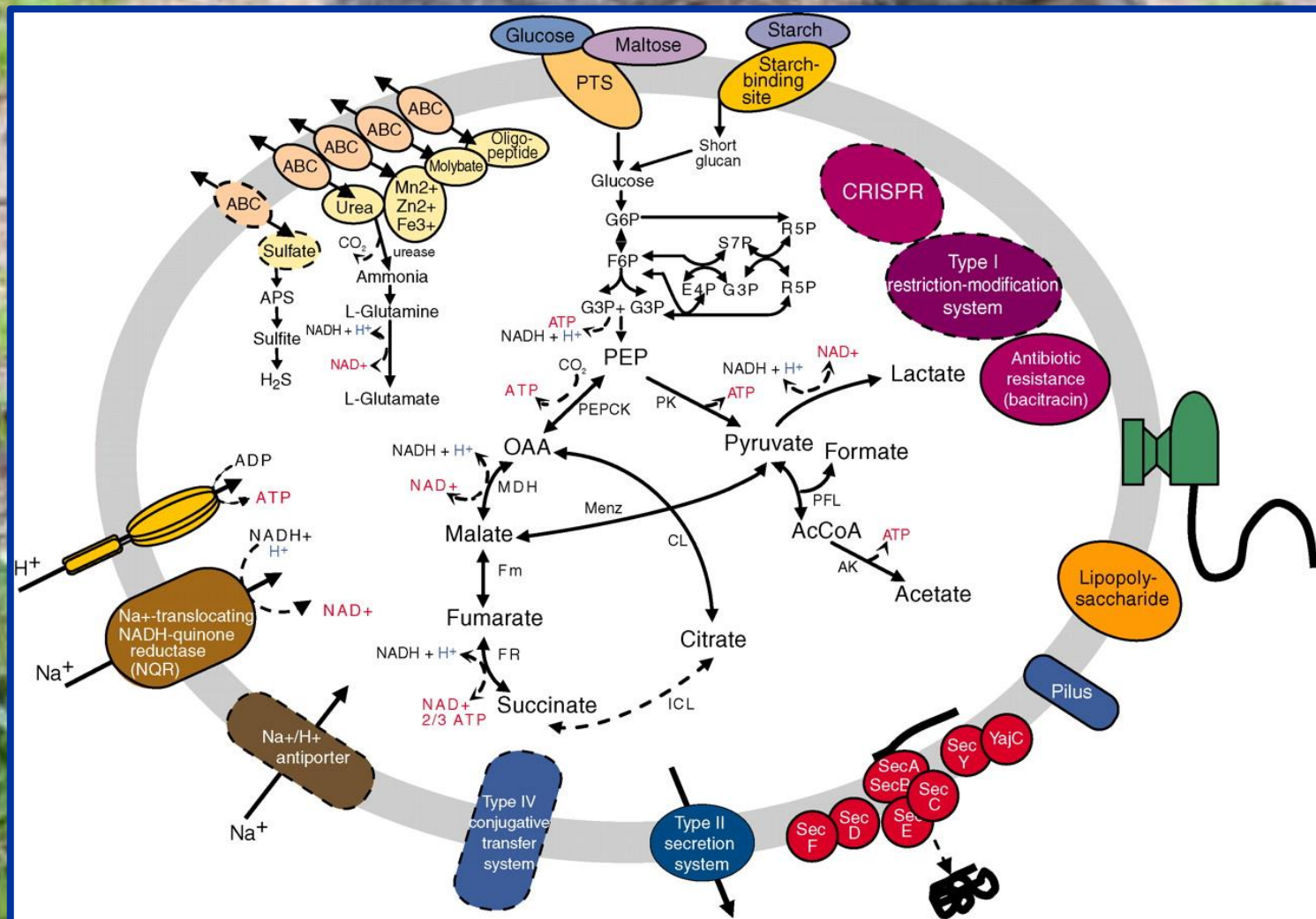
Herbivore

Foregut fermenter

Produces 20% of methane per DMI
compared to cattle



Herbivore
 Foregut fermenter
 Has large numbers of
 Succinivibrionaceae



The Extreme 8 analysis

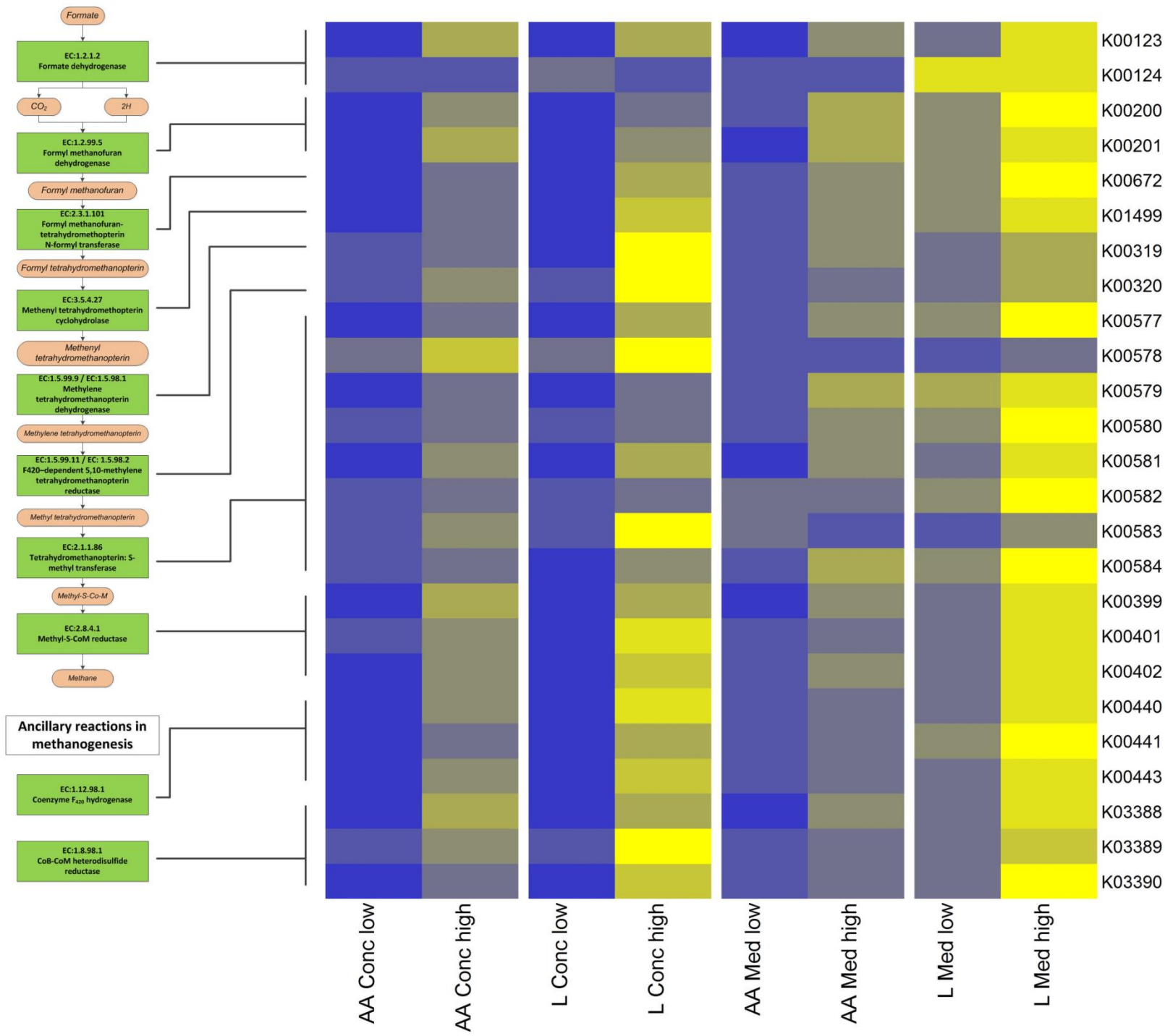
Microbiome

Microbiota

What species are different?

Metagenome

What genes are different?



Variation in methane production: the geneticist's slant



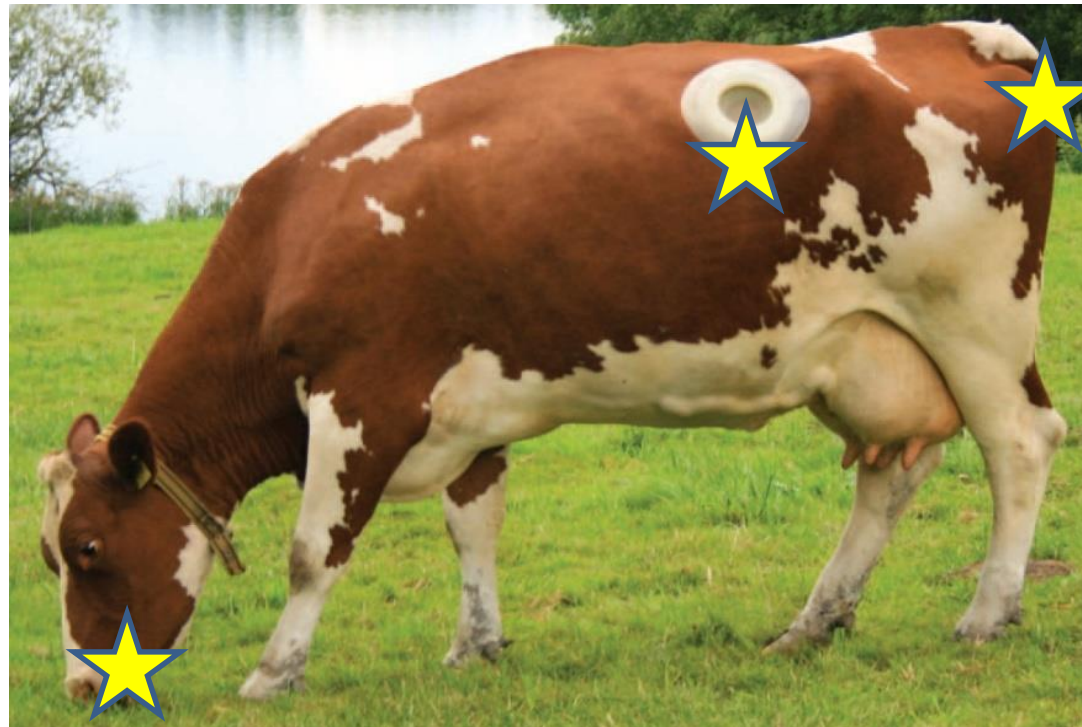
Rainer Roehe
SRUC

KEGG ID/Diet	Description	^b Estimate	^c VIP
K06001	Tryptophan synthase beta chain [EC:4.2.1.20]	0.13310	1.233
Diet conc	Concentrate based diet	-0.14695	1.204
Diet mixed	Mixed forage-concentrate diet	0.14695	1.204
K02118	V/A-type H ⁺ -transporting ATPase subunit B [EC:3.6.3.14]	0.07984	1.151
K02117	V/A-type H ⁺ -transporting ATPase subunit A [EC:3.6.3.14]	0.08564	1.133
K00638	Chloramphenicol O-acetyltransferase [EC:2.3.1.28]	0.06182	1.082
K00200	Formylmethanofuran dehydrogenase subunit A [EC:1.2.99.5]	0.06042	1.070
K03531	Fell division protein FtsZ	0.07526	1.065
K00201	Formylmethanofuran dehydrogenase subunit B [EC:1.2.99.5]	0.04859	1.049
K00399	Methyl-coenzyme M reductase alpha subunit [EC:2.8.4.1]	0.03843	1.021
K00123	Formate dehydrogenase, alpha subunit [EC:1.2.1.2]	0.03417	1.013
K03388	Heterodisulfide reductase subunit A [EC:1.8.98.1]	0.02734	0.997
K14126	F ₄₂₀ -non-reducing hydrogenase subunit A [EC:1.12.99.-]	0.00384	0.933
K01079	Phosphoserine phosphatase [EC:3.1.3.3]	0.03424	0.932
K00401	Methyl-coenzyme M reductase beta subunit [EC:2.8.4.1]	0.00484	0.909
K00527	Ribonucleoside-triphosphate reductase [EC:1.17.4.2]	0.03449	0.898
K02337	DNA polymerase III subunit alpha [EC:2.7.7.7]	-0.05338	0.886
K02837	Peptide chain release factor RF-3	0.00883	0.852
K01893	Asparaginyl-tRNA synthetase [EC:6.1.1.22]	-0.06673	0.812
K00925	Acetate kinase [EC:2.7.2.1]	-0.00903	0.812
K00656	Formate C-acetyltransferase [EC:2.3.1.54]	-0.00834	0.787
K02948	Small subunit ribosomal protein S11	-0.00400	0.734

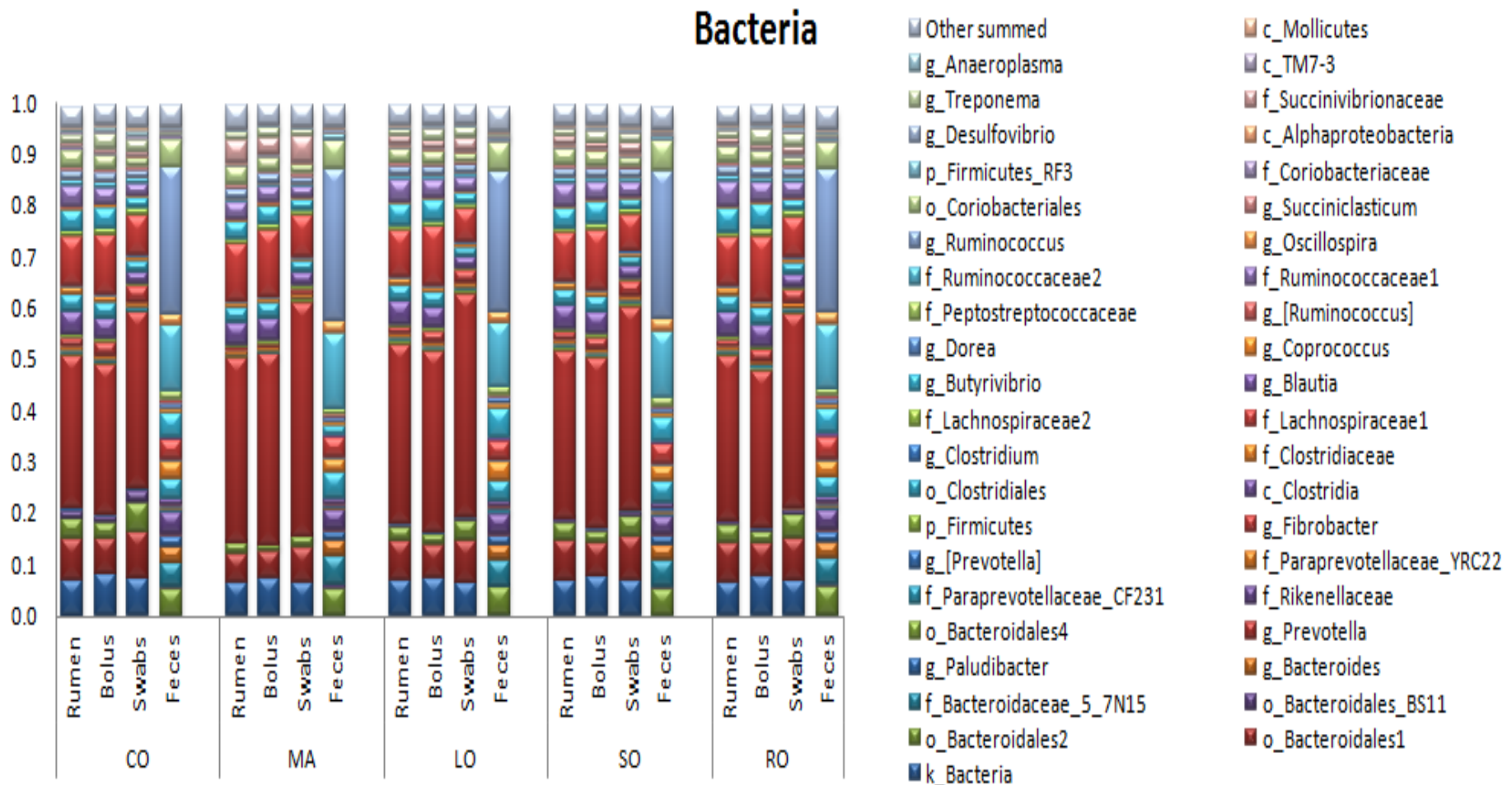
“The genes identified in Table 3 to be important explained the variation in methane emissions by 88%.”

Tools, resources, legacy

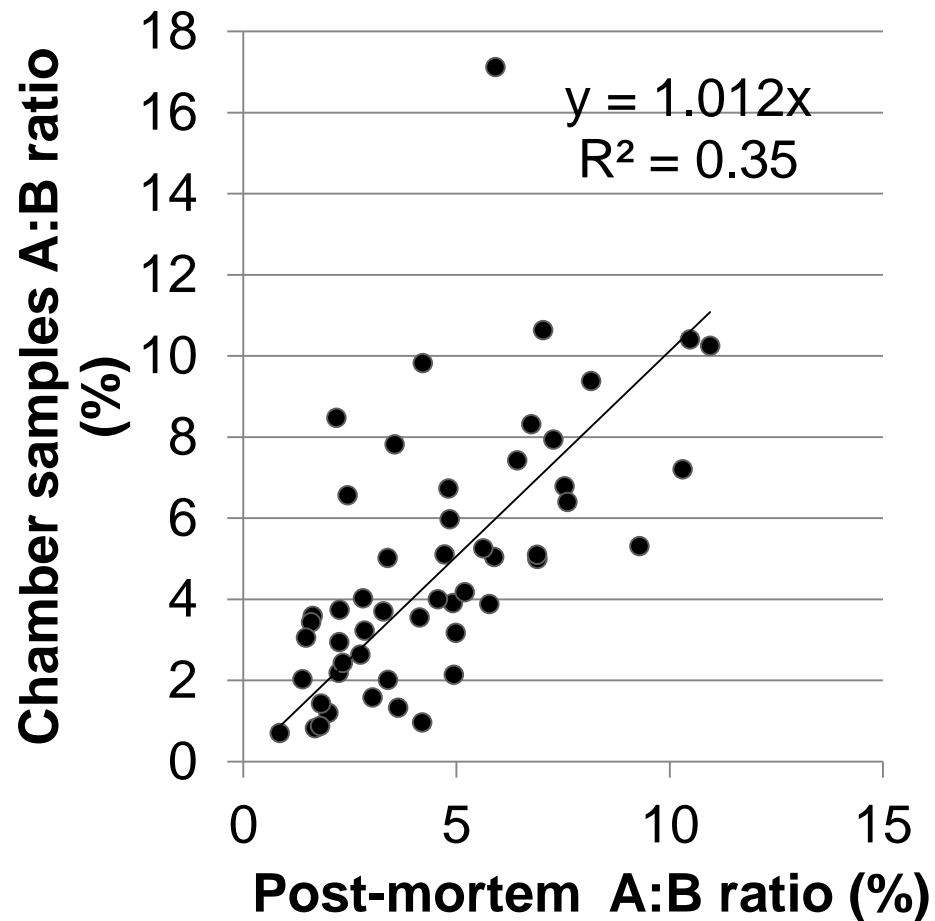
- **Proxies** Buccal-ruminal-faecal microbiomes



Proxies for rumen sampling – mouth (swab and bolus) and faeces



Proxies for rumen sampling – *post-mortem* samples



Conclusions on methane microbiome research



- Low-methane phenotype has low archaea and high Succinovibrionaceae abundance
- Differences in abundance of 22 microbial genes explained the variation in methane emissions by 88%
- *Post-mortem* sampling of digesta is a useful proxy
- Oral samples also a useful proxy

Acknowledgements

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Snelling, Graham Horgan

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