

Phylogenomic Analyses to Detect Prokaryote to Eukaryote Horizontal Gene Transfer

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Overview

- The approach used - detection of 'recent' HGT based upon phylogenetic trees
- The computer programmes used and some of their features
- Some results from analysis of the genome of *Entamoeba histolytica*
- Future directions and challenges

Why study HGT?

- What is the role of HGT, outside of endosymbiosis, in the evolution of eukaryotes?
- How much HGT has occurred? What types of genes? Where from?
- How has HGT affected the evolution of parasitic eukaryotes? Does HGT provide drug targets?

How have we chosen to look for HGT?

- A semi-automated phylogenomics approach based upon a published computer programme called PyPhy
- Use trees to detect HGT in a two-stage process:
 - Simple parsimony or p-distance trees from edited alignments
 - Bayesian trees with more sophisticated phylogenetic models as a second stage screen
 - Distance-based bootstrapping to gain a measure of confidence in groups on trees

A phylogenomic approach to microbial evolution

Thomas Sicheritz-Pontén and Siv G. E. Andersson*

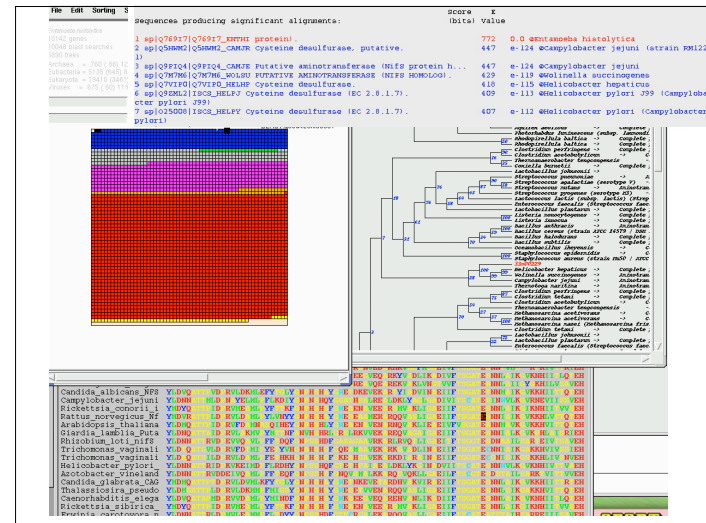
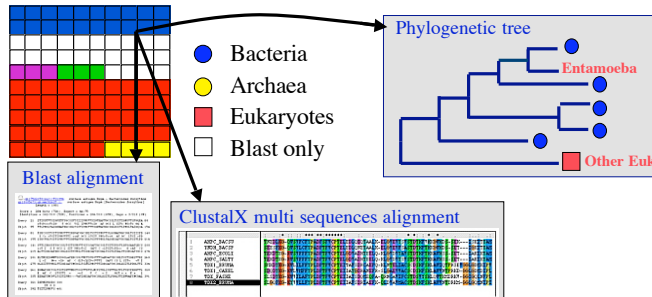
Department of Molecular Evolution, Evolutionary Biology Center, Uppsala University, 752 36 Uppsala, Sweden

Received August 3, 2000; Revised and Accepted November 6, 2000

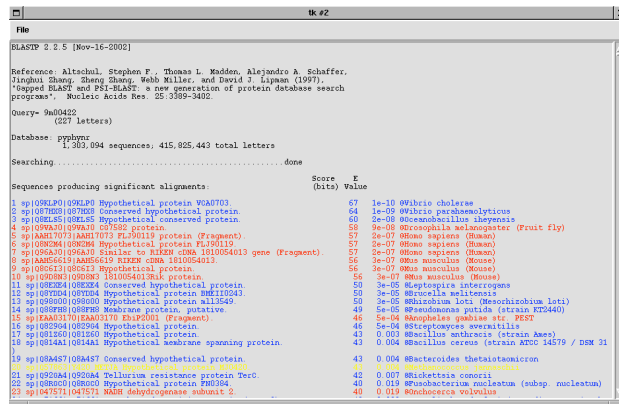
PyPhy - the Primary Screen

- The database
 - Translated (Tr)EMBL
 - Swissprot entries (for annotation)
- BlastP searches using query genome to recover homologues
 - user defined cut-off values (e.g. at least 40% similarity over >70<200% of the length of query sequence)
- Align sequences using ClustalX and trim the alignment using GBLOCKS
- Construct bootstrapped P-distance or parsimony trees
- Display results as a grid with coloured squares indicating neighbor relationships

PyPhy graphical interface



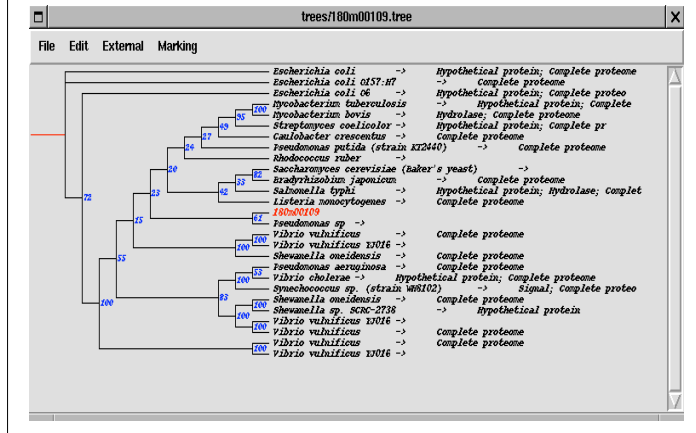
BlastP search



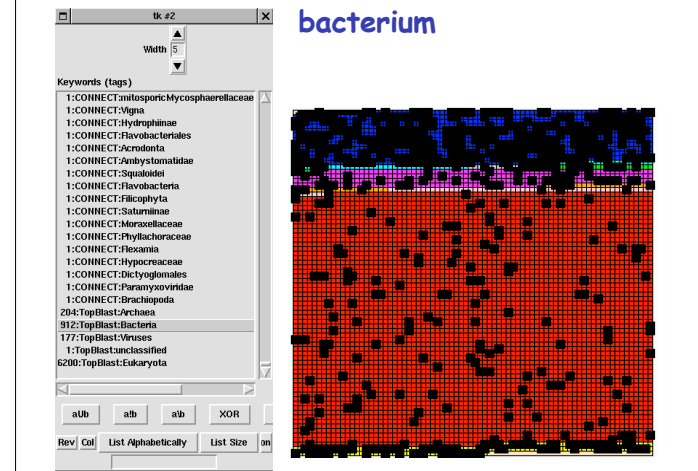
G-blocked Alignment



Bootstrapped P-distance trees



Proteins where the top BlastP hit is a bacterium



The screenshot shows a software window with a menu bar (File, Edit, Sorting, Searching, Blast tools, TNG, KEGG, Display) and a list of protein domains on the left. The central area displays a 3D model of ATP synthase embedded in a lipid bilayer membrane. Labels include 'Stator', 'Inner mitochondrial membrane', 'F1F0 ATP synthase (E. coli)', and 'ATP SYNTHESIS'. A red arrow indicates the direction of proton flow (H+). Below the diagram are several alignment tables for different subunits.

Y-type ATPase (Bacterial)

both	alpha	gamma	delta	epsilon	c	a	b
------	-------	-------	-------	---------	---	---	---

Y-type ATPase (Eukaryotic)

both	alpha	gamma	ORP	delta	epsilon	c	a
b	#	25	f	8			
e	f	b	i	k	i		

Y-type ATPase (Fungal)

A	B	C	D	E	F	I	K
1	AQ79	S4D	81	104			

The analysis of 100 genes supports the grouping of three highly divergent amoebae: *Dictyostelium*, *Entamoeba*, and *Mastigamoeba*

Eric Baptiste*, Henner Brinkmann†, Jennifer A. Lee‡, Dorothy V. Moore§, Christoph W. Sensen¶, Paul Gordon¶, Laure Durufle*, Terry Gaasterland*, Philippe Lopez*, Miklós Müller*, and Herve Philippe†

The phylogenetic tree shows the relationships between various organisms. The three amoebae (*Dictyostelium discoidium*, *Entamoeba histolytica*, and *Mastigamoeba balamuthi*) are highlighted in a red box and grouped under the label 'Conosa'. Other groups include Archaea, Kinetoplastida, Alveolata, Plantae, and Opisthokonta.

(*Entamoeba* + *Dictyostelium*) connections

The screenshot shows a software window with a menu bar (File, Edit, Sorting, Searching, Blast tools, TNG) and a list of keywords (tags) on the left. The central area displays a heatmap of connections between genes from *Entamoeba histolytica* (4183 genes) and *Dictyostelium discoideum* (441 tagged genes). The heatmap shows a dense network of connections between the two species.

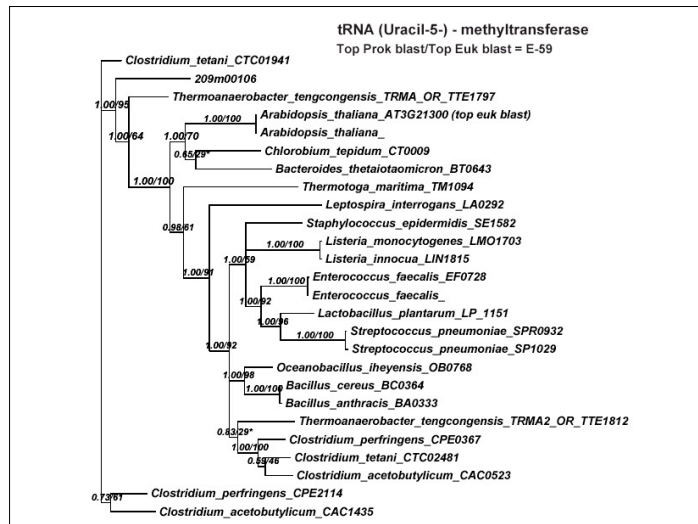
The screenshot shows a software window with a menu bar (File, Edit, Sorting, Searching, Blast tools, TNG, KEGG, Display) and a list of genes on the left. The central area displays a phylogenetic tree and a heatmap of connections between genes. The heatmap shows a dense network of connections between genes from various species, including *Entamoeba histolytica* and *Dictyostelium discoideum*.

PyPhy - the secondary screen I

- Search EST databases and hits added to the alignments
- Feed *G*-blocked alignment into MRBAYES (Huelsenbeck & Ronquist 2001)
 - WAG matrix, gamma correction for site rate variation and a proportion (pinvar) of invariant sites.
- Make Bayesian consensus trees with posterior probabilities as support values

PyPhy - the secondary screen II

- Bootstrapping to provide an additional indication of support for relationships
- Each data set is bootstrapped (100 replicates) and used to make distance matrices under the same evolutionary model as in the Bayesian analysis, using custom (P4) software
- Trees are made from the distance matrices using FastME (Desper & Gascuel 2004) and a bootstrap consensus tree made using P4
- Transfer BS support values onto the Bayesian consensus tree



letters to nature

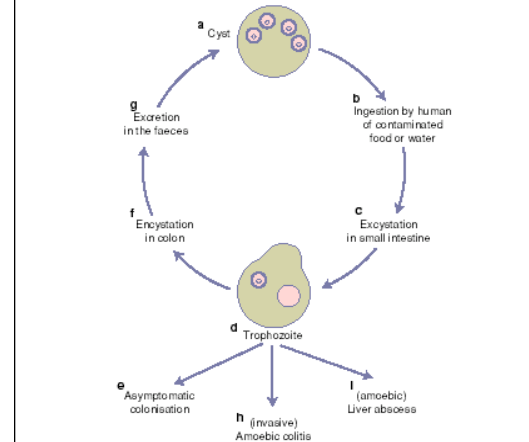
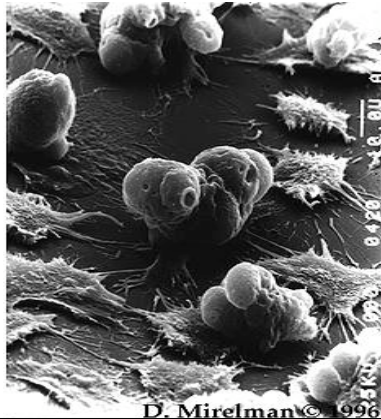
NATURE [VOL 433] 24 FEBRUARY 2005

The genome of the protist parasite *Entamoeba histolytica*

Brendan Loftus¹, Iain Anderson¹, Rob Davies², U. Cecilia M. Alsmark³, John Samuelson⁴, Paolo Amedeo¹, Paola Roncaglia¹, Matt Berriman², Robert P. Hirt³, Barbara J. Mann⁵, Tomo Nozaki⁶, Bernard Suh¹, Mihai Pop¹, Michael Duchene⁷, John Ackers⁸, Egbert Tannich⁹, Matthias Leippe¹⁰, Margit Hofer⁷, Iris Bruchhaus⁹, Ute Willhoft⁹, Alok Bhattacharya¹¹, Tracey Chillingworth¹, Carol Churcher², Zahra Hance², Barbara Harris², David Harris², Kay Jagels², Sharon Moule², Karen Mungall², Doug Ormond², Rob Squares², Sally Whitehead², Michael A. Quail², Ester Rabinowitsch², Halina Norbertczak², Claire Price², Zheng Wang¹, Nancy Guillén¹², Carol Gilchrist⁵, Suzanne E. Stroup⁹, Sudha Bhattacharya¹¹, Anuradha Lohia¹³, Peter G. Foster¹⁴, Thomas Sicheritz-Ponten¹⁵, Christian Weber¹², Upinder Singh¹⁶, Chandrama Mukherjee¹³, Najib M. El-Sayed¹, William A. Petri Jr⁵, C. Graham Clark², T. Martin Embley³, Bart Barrell², Claire M. Fraser¹ & Neil Hall^{2*}

E. histolytica trophozoites in situ

(D. Mirelman 1996)



Life cycle of *Entamoeba histolytica* and the clinical manifestations of infection in humans

Expert. Reviews in Molecular Medicine ©1999 Cambridge University Press

The *E. histolytica* genome

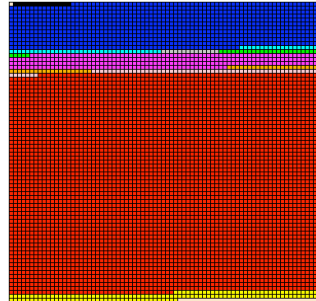
- Sequenced by TIGR and the Sanger institute (PIs Brendan Loftus and Neil Hall)
- ~ 23.7 Mbp
- 9938 predicted genes
- 1/3rd of genes have no detectable homologue in public data bases
- Large gene families, duplications are common

Aim of our HGT screen

- To identify the strongest candidate 'recent' HGT from prokaryotes to *Entamoeba*
 - The tip of the HGT iceberg?
 - Help to validate HGT as a plausible explanation for topological incongruence deeper in tree
- These cases should be the easiest to defend - paralogy or poor tree-building is unlikely to be an equally plausible explanation to HGT
- This screen is not seeking to detect more 'ancient' transfers, for example - at the base of major groups or from the alpha-proteobacterial mitochondrial endosymbiont

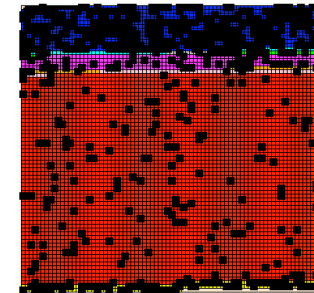
Primary screen output for *E. histolytica*

- 9938 genes
- 5740 trees
- 819 bacterial and 129 archaeal connections in the trees



Superimposed top blast hit = prokaryote

- 912 genes have a bacteria and 204 an archaea as the top BLAST hit, 107 of these genes show eukaryotic connections in the primary tree.



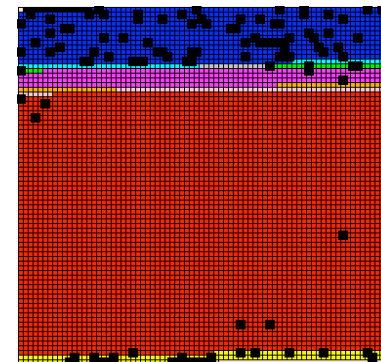
Criteria for HGT 2^o analysis

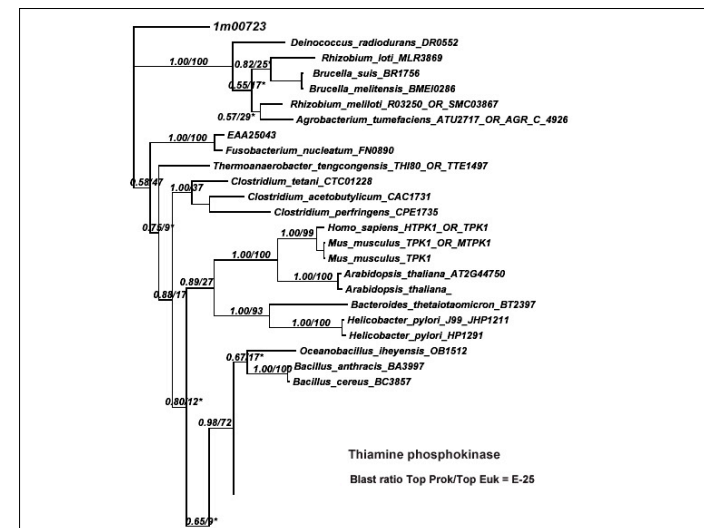
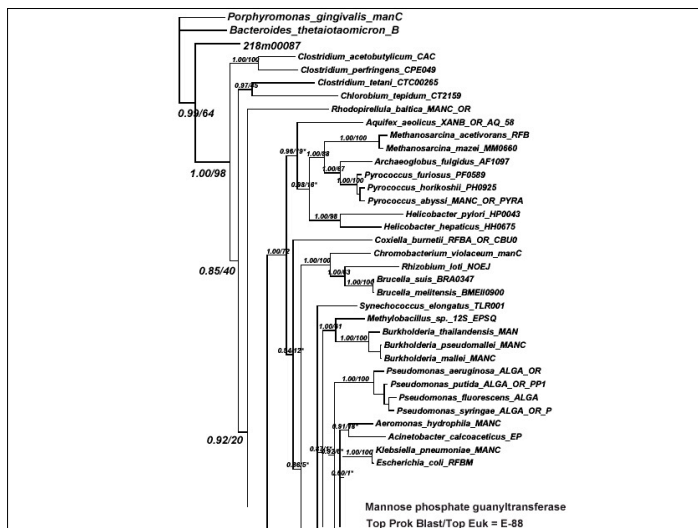
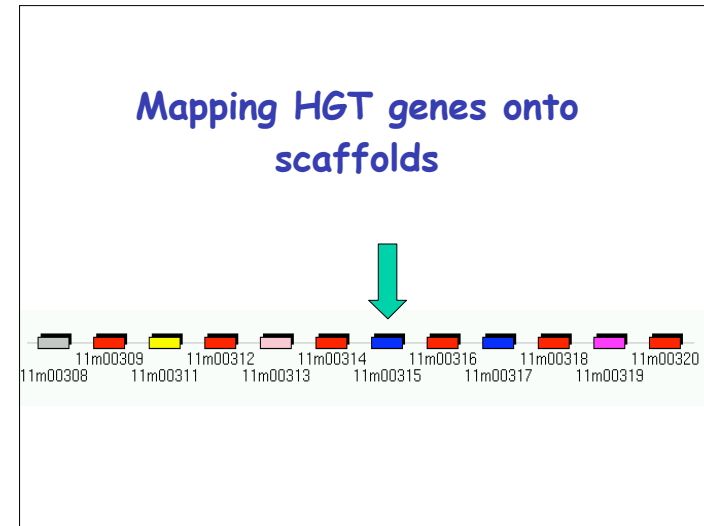
(vary in degree of ad hoc-ness)

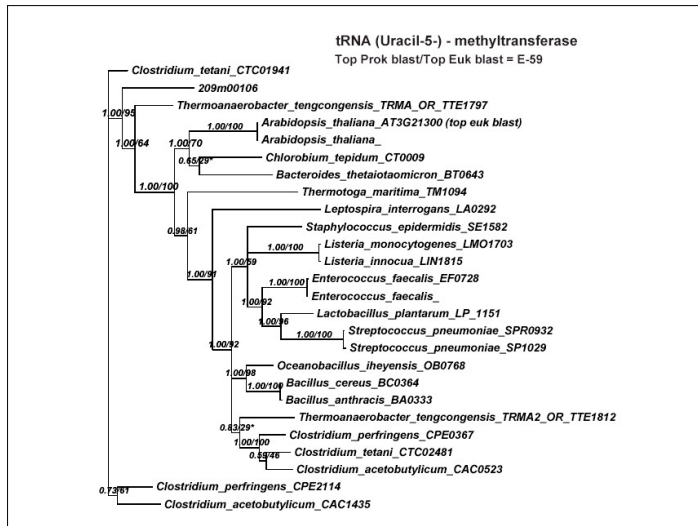
- Strongly supported tree placing *Entamoeba* within a conventional bacterial group
- No other eukaryotic sequence in tree
 - sampling is very poor, surprises occur!
- Two or more strongly supported nodes separating *Entamoeba* from any other eukaryotic sequence
- Eukaryotes never appear together in bootstrap partitions
- High ratio of prokaryote to eukaryote BlastP score

Results of the 2nd generation analysis

- 96 Bayesian trees are most simply explained by prokaryote to eukaryote HGT

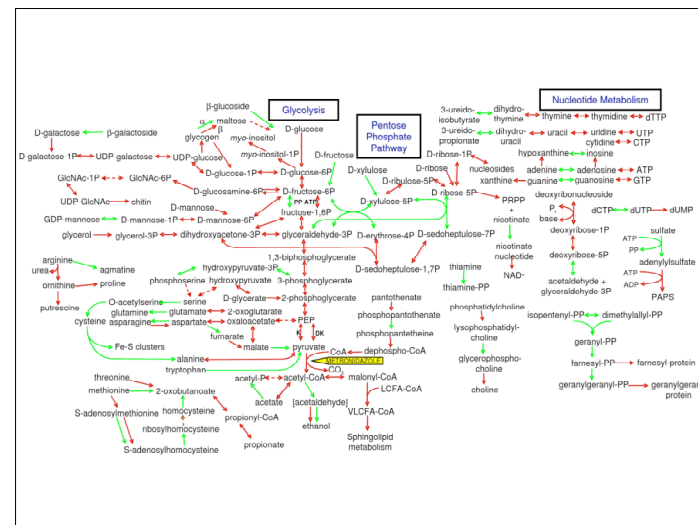
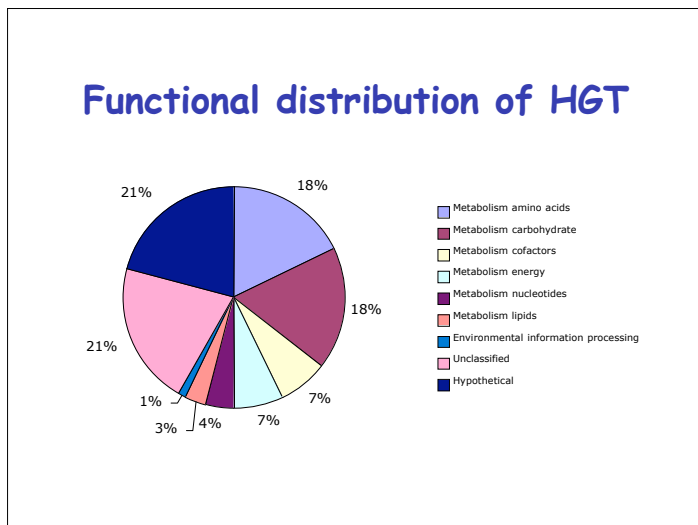






Transferred genes are involved in metabolism

121m00124	Tartrate dehydrogenase	METABOLISM
133m00129	Fructokinase	METABOLISM
400m00033	Arginine decarboxylase	METABOLISM
78m00151	Lysophospholipase L2	METABOLISM
9m00390	HesA/MoeB/ThiF family protein	METABOLISM
24m00307	NADH dehydrogenase, similar to nitrite reductase	ENERGY METABOLISM
61m00186	5-nitroimidazole antibiotic resistance protein	CELL RESCUE, DEFENCE AND VIRULENCE
30m00272	ABC transporter	TRANSPORT
289m00068	Conserved hypothetical protein	UNKNOWN FUNCTION



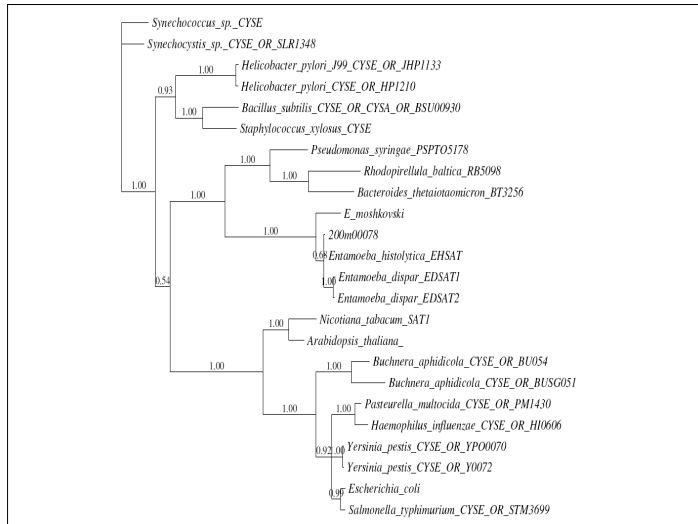
We see a broad collection of donors

Name	NN MrBayes	Best Prokaryote blast hit
100m00122	DT Deinococcus	Clostridium perfringens
189m00102	Archeal like	Pyrobaculum aerophilum
10m00331	Proteo /Xanthobact	Methanobacterium thermoautotrophicum
119m00142	Proteo /Vibrio	Bacillus subtilis
11m00315	Bact/Chlorob	Bacteroides thetaiotaomicron
209m00106	Firmicutes	Clostridium perfringens
126m00107	Bacteria like	Pseudomonas aeruginosa
130m00124	Firmicutes	Lactococcus lactis (subsp. lactis) (Streptococcus lactis)
133m00129	Bacteria like	Bacteroides thetaiotaomicron
133m00136	Bact/Chlorob	Methanosarcina mazei (Methanosarcina frisia)

Summary BlastP Statistics

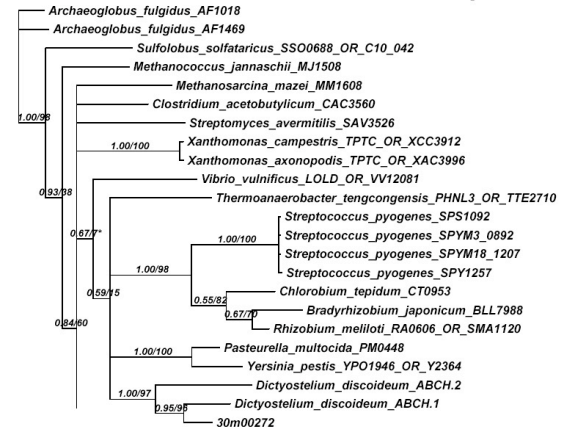
(provided as supplemental info to Loftus et al 2005)

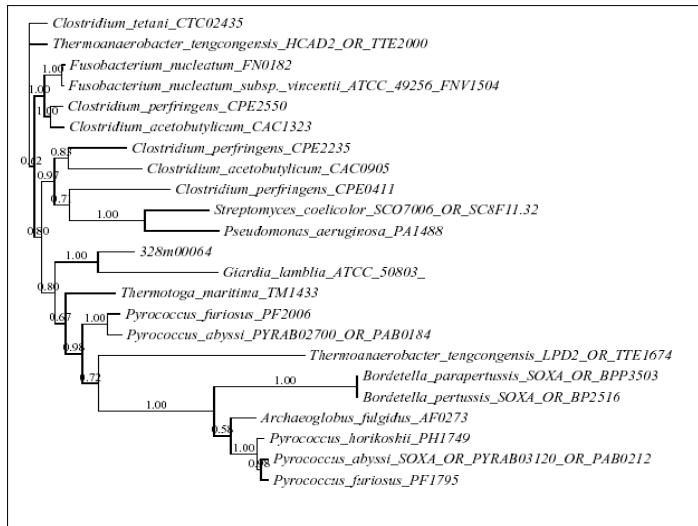
Acc. No.	Length	Top Prokaryotic blast hit	PL	ID level	%ID	Top Eukaryotic hit	EL	ID level	%ID	P E score	E E score	Ratio (P/E)
EAL47929	330	Clostridium acetobutylicum	284	77.27%	23	none	none	1.00E-19	none	0	0	0
EAL47464	604	Bacteroides thetaiotaomicron	482	79.80%	38	Phormyces sp. E2	855	110.40%	27	1.00E-86	9.00E-31	1.11E-56
EAL47236	269	Methanostaculum thermoaerophilum	265	101.26%	39	Arabidopsis thaliana	286	80.27%	21	1.00E-47	0.011	8.00E-46
EAL47026	164	Bacillus subtilis	131	80.13%	30	Homo sapiens	170	50.61%	28	1.00E-10	0.005	0.00E-08
EAL47149	343	Bacteroides thetaiotaomicron	350	102.34%	42	Phytophthora sp.	361	116.53%	32	4.00E-83	2.00E-45	2.00E-38
EAL46979	370	Yersinia pestis	355	96.04%	46	Arabidopsis thaliana	351	94.90%	34	1.00E-83	8.00E-49	0.25E-35
EAL46854	192	Pseudomonas aeruginosa	184	96.16%	41	Caenorhabditis elegans	212	54.19%	34	4.00E-39	3.00E-17	1.33E-19
EAL46757	85	Lactococcus lactis	81	95.29%	31	Musa sapientum	332	117.4%	22	1.00E-09	1.4	2.14E-10
EAL46701	264	Bacteroides thetaiotaomicron	255	100.26%	46	Arabidopsis thaliana	325	78.26%	28	13.00E-52	8.00E-14	3.75E-49
EAL46676	218	Methanobacterium thermoautotrophicum	218	100.00%	37	Oryza sativa	472	26.19%	22	15.00E-31	0.7	1.00E-31
EAL46650	419	Chryseobacterium thersites	362	100.45%	30	Schistosoma japonicum	478	49.11%	41	1.00E-35	1.00E-19	2.00E-16
EAL46599	219	Bacteroides thetaiotaomicron	229	104.16%	31	Arabidopsis thaliana	312	49.16%	29	1.00E-20	4.00E-12	2.00E-09
EAL46592	140	Archaeoglobus fulgidus	174	124.29%	49	Giardia lamblia	204	44.9%	30	15.00E-28	0.4	1.47E-26
EAL46527	656	Bacteroides thetaiotaomicron	669	101.98%	53	Schistosoma japonicum	883	134.60%	31	0	0.00E-47	0.00E-00
EAL46421	205	Clostridium acetobutylicum	219	106.83%	49	Arabidopsis thaliana	230	83.19%	53	1.00E-34	8.00E-12	1.25E-22
EAL46359	218	Clostridium perfringens	224	102.75%	65	Giardia lamblia	258	101.21%	46	2.00E-73	2.00E-43	1.00E-30
EAL46311	248	Streptococcus meningitis	258	104.03%	36	Plasmodium yoelii	407	33.17%	24	1.00E-31	0.1	4.29E-31
EAL46313	118	Prochlorococcus marisnigri	153	129.11%	42	Homo sapiens	212	187.3%	22	1.00E-21	0.85	1.12E-21
EAL46110	157	Bacteroides thetaiotaomicron	155	98.73%	49	Arabidopsis thaliana	446	27.15%	19	1.00E-34	0.89	1.09E-34
EAL46116	661	Bacillus halodurans	666	100.60%	48	Cryptosporidium parvum	679	101.65%	50	0	1.00E-172	0.00E-00
EAL46026	176	Bacteroides thetaiotaomicron	174	98.77%	50	Arabidopsis thaliana	147	30.54%	46	1.00E-40	0.00E-07	3.33E-34
EAL45981	429	Bacteroides thetaiotaomicron	427	100.00%	52	Phormyces sp. E2	484	114.84%	28	1.00E-148	0.00E-37	1.25E-109
EAL45834	259	Anabaena sp. (strain PCC 1120)	272	104.98%	27	Caenorhabditis elegans	446	49.20%	19	2.00E-19	0.005	1.00E-12
EAL45807	300	Streptomyces coelicolor	346	115.33%	32	Dicystostelium discoideum	442	110.98%	30	1.00E-39	1.00E-35	0.00E-01
EAL45618	159	Bacteroides thetaiotaomicron	147	92.14%	46	none	0	none	0	2.00E-28	none	0
EAL45586	460	Clostridium tetani	461	100.22%	47	Dicystostelium discoideum	503	181.45%	39	1.00E-118	1.00E-84	3.33E-33
EAL45565	234	Pyrobaculum aerophilum	281	120.29%	22	Arabidopsis thaliana	291	62.22%	27	1.00E-24	1.00E-14	0.00E-10
EAL45474	264	Prochlorococcus marisnigri	264	100.00%	54	Arabidopsis thaliana	284	88.64%	42	18.00E-26	0.011	1.24E-27



HGT at the base of the amoebozoans?

ABC transporter



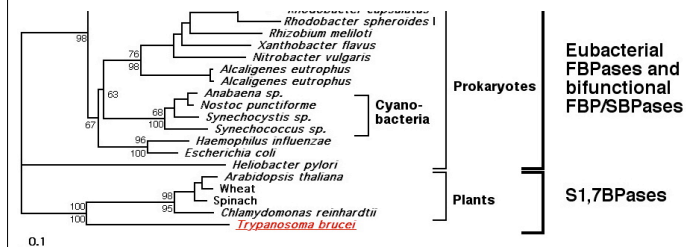


Plant-like traits associated with metabolism of *Trypanosoma* parasites

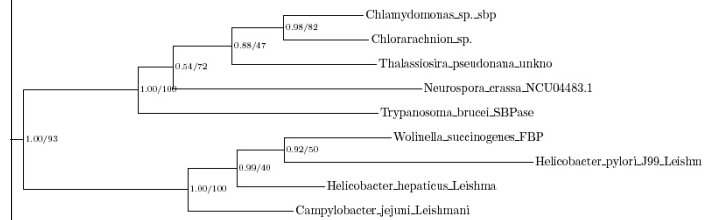
Véronique Hannaert*, Emma Saavedra*, Francis Duffieux*, Jean-Pierre Szikora*, Daniel J. Rigden⁵, Paul A. M. Michels*, and Fred R. Opperdoes*[¶]

- *Trypanosoma brucei* contains a complete open-reading frame encoding a homologue of sedoheptulose-1,7-bisphosphatase (SBPase).
- This enzyme is typical for the Calvin cycle of photosynthetic organisms and only encountered in the chloroplasts of green algae and plants
- This was taken as evidence by that *Trypanosoma* once had a plastid

http://www.icp.ucl.ac.be/~opperd/Supplementary/sbpase_nbj_tree.html



Fungi contain SBPase too!



The people who do the work



**Robert Hirt and
Thomas Sicheritz
Ponten**

Cessie Alsmark

Peter Foster

**Collaborators:
Brendan Loftus and Neil Hall (TIGR)
Matt Berriman (Sanger)**

Useful URLs

- **Pyphy** <http://www.cbs.dtu.dk/staff/thomas/pyphy/>
- **P4** <http://www.nhm.ac.uk/zoology/external/p4.htm>
- **ClustalX**
<http://www.hgmp.mrc.ac.uk/Registered/Option/clustalx.html>
- **Gblocks** <http://molevol.ibmb.csic.es/Gblocks/Gblocks.html>
- **MrBayes** <http://morphbank.ebc.uu.se/mrbayes/>
- **FastME**
<http://www.lirmm.fr/~w3ifa/MAAS/FastME/FastME.html>