EVOLUTION OF MITOCHONDRIAL IMPORT MACHINERY IN DICTYOSTELIUM DISCOIDEUM

Marcin Jąkalski, M.Sc.
Institute of Bioinformatics, University of Muenster

Outline

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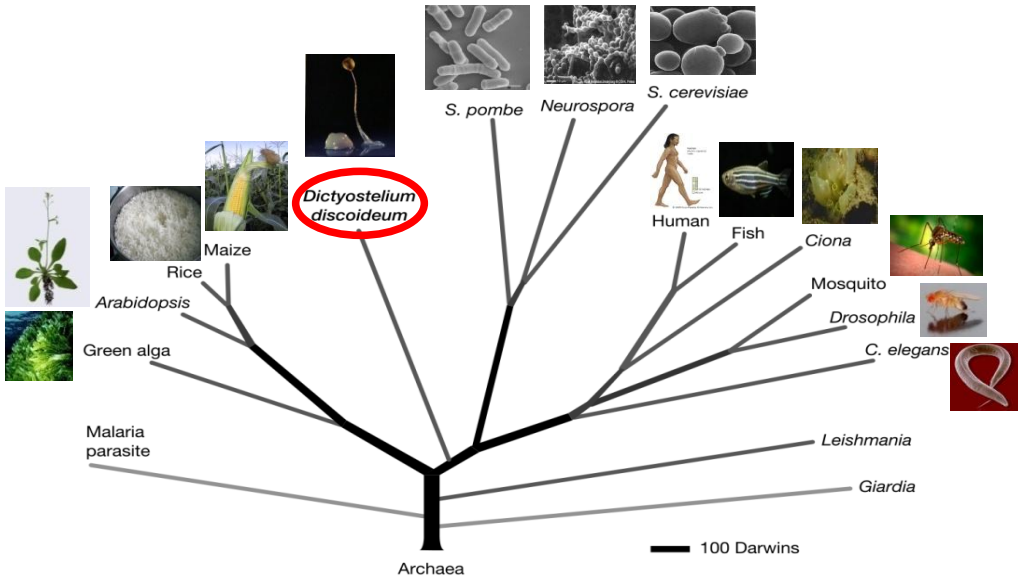
- **Background**
  - Eukaryotic tree of life
  - *Dictyostelium discoideum* as model organism
  - Mitochondrial evolution and import machinery
- **Methodology**
- **Results**
  - mitochondrial import machinery in *Dictyostelium*
  - phylogenetic relationships of eukaryotic mitochondrial β-barrles
- **Conclusions**
- **Acknowledgements**

Objectives

- identification of mitochondrial outer membrane import machinery in *Dictyostelium discoideum* (and *Acathamoeba castellanii*)
- verification of the current classification of *D. discoideum* and other *Amoebozoa* on the eukaryotic phylogenetic tree



History of living organisms classification

<table>
<tr><th>Linnaeus</th><th>Haeckel</th><th>Chatton</th><th>Copeland</th><th>Whittaker</th><th>Woese et al.</th><th>Woese et al.</th><th>Cavalier-Smith</th></tr>
<tr><td>1735</td><td>1866</td><td>1925</td><td>1938</td><td>1969</td><td>1977</td><td>1990</td><td>1998</td></tr>
<tr><td>2 kingdoms</td><td>3 kingdoms</td><td>2 empires</td><td>4 kingdoms</td><td>5 kingdoms</td><td>6 kingdoms</td><td>3 domains</td><td>6 kingdoms</td></tr>
<tr><td rowspan="2">(not treated)</td><td rowspan="2">Protista</td><td rowspan="2">Prokaryota</td><td rowspan="2">Monera</td><td rowspan="2">Monera</td><td>Eubacteria</td><td>Bacteria</td><td rowspan="2">Bacteria</td></tr>
<tr><td>Archaebacteria</td><td>Archae</td></tr>
<tr><td rowspan="4">Vegetabilia</td><td rowspan="4">Plantae</td><td rowspan="5">Eukaryota</td><td rowspan="3">Protista</td><td rowspan="2">Protista</td><td rowspan="2">Protista</td><td rowspan="5">Eukarya</td><td>Protista</td></tr>
<tr><td>Chromista</td></tr>
<tr><td>Fungi</td><td>Fungi</td><td>Fungi</td></tr>
<tr><td>Plantae</td><td>Plantae</td><td>Plantae</td><td>Plantae</td></tr>
<tr><td>Animalia</td><td>Animalia</td><td>Animalia</td><td>Animalia</td><td>Animalia</td><td>Animalia</td></tr>
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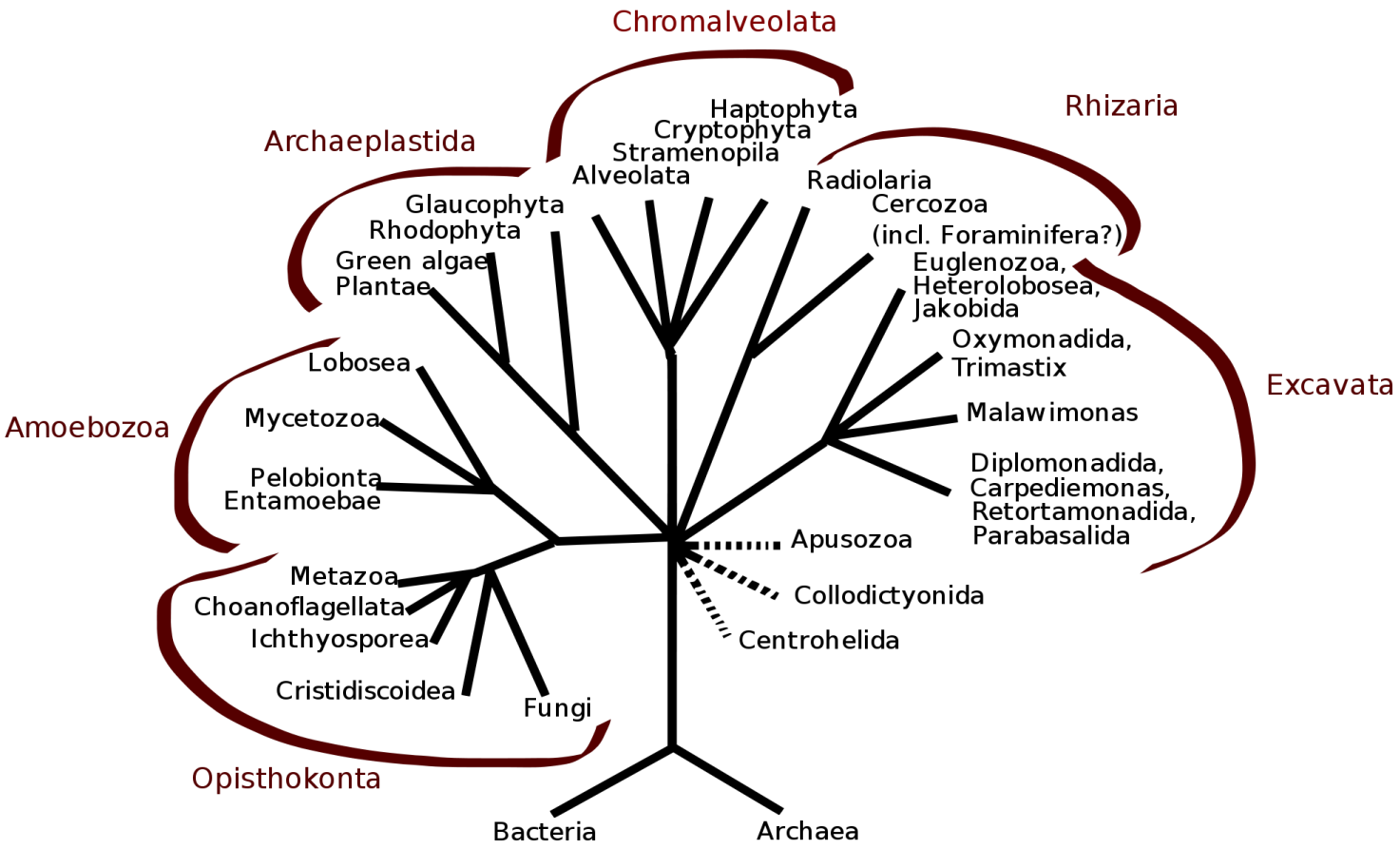
Six eukaryotic supergroups



(Adl et al. 2005)

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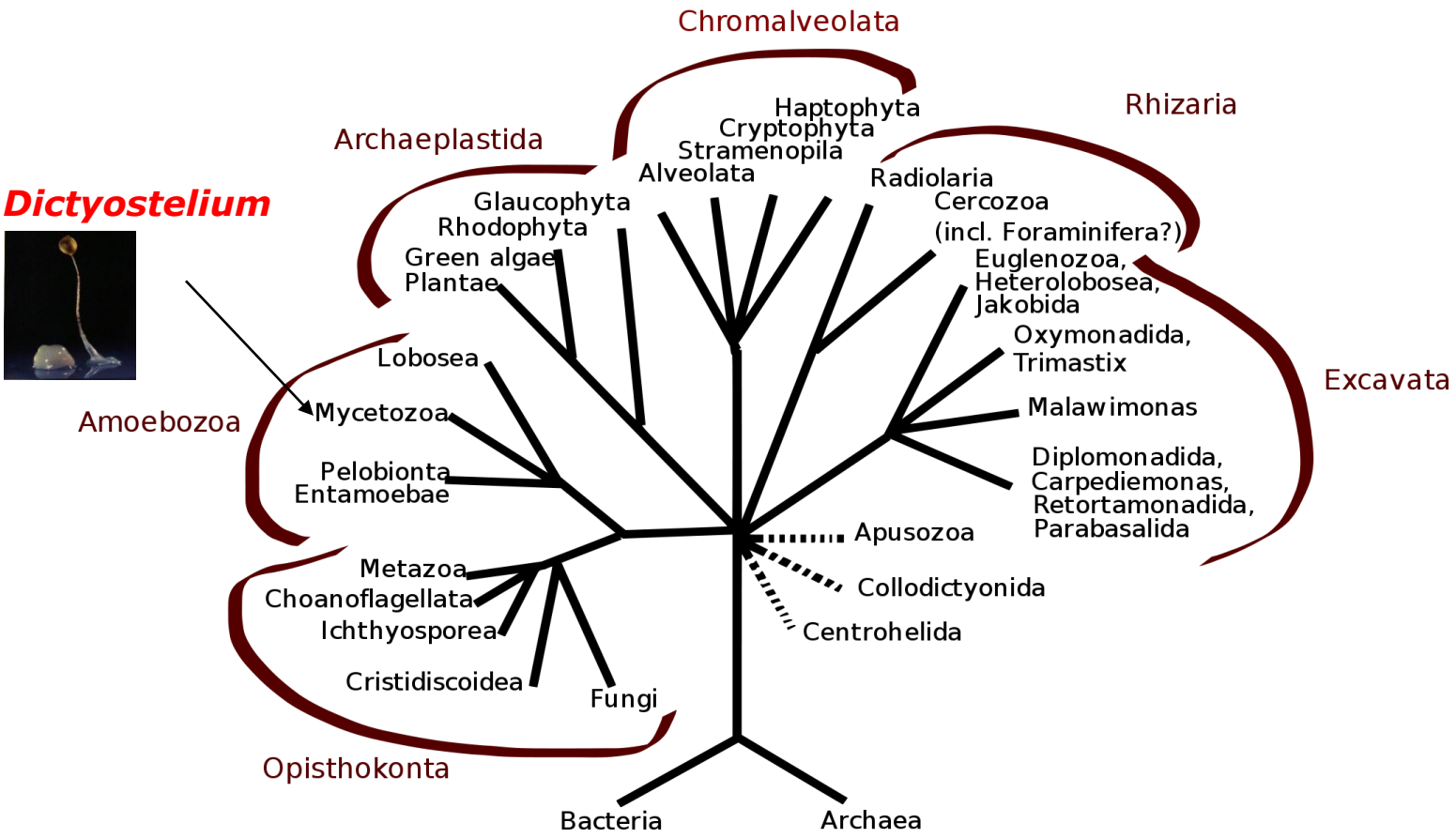
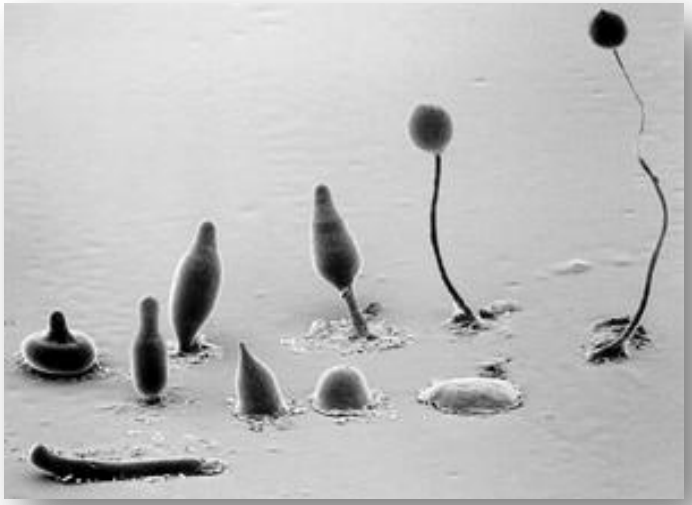


(Adl et al. 2005)

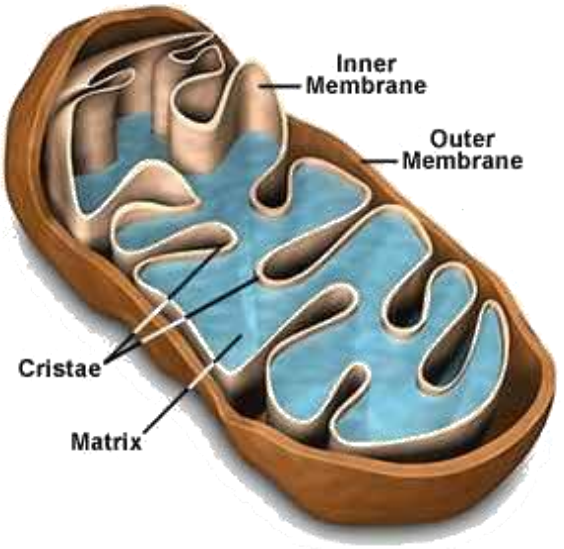
Dictyostelium discoideum

- model organism in studies of mitochondria, signal transduction, cytoskeletal function, endocytosis morphogenesis and cell division
- unique lifestyle (uni- and multicellular stages)
- genome - 6 chromosomes, 34 Mb haploid, with 77% of A+T, codes 12,500 proteins

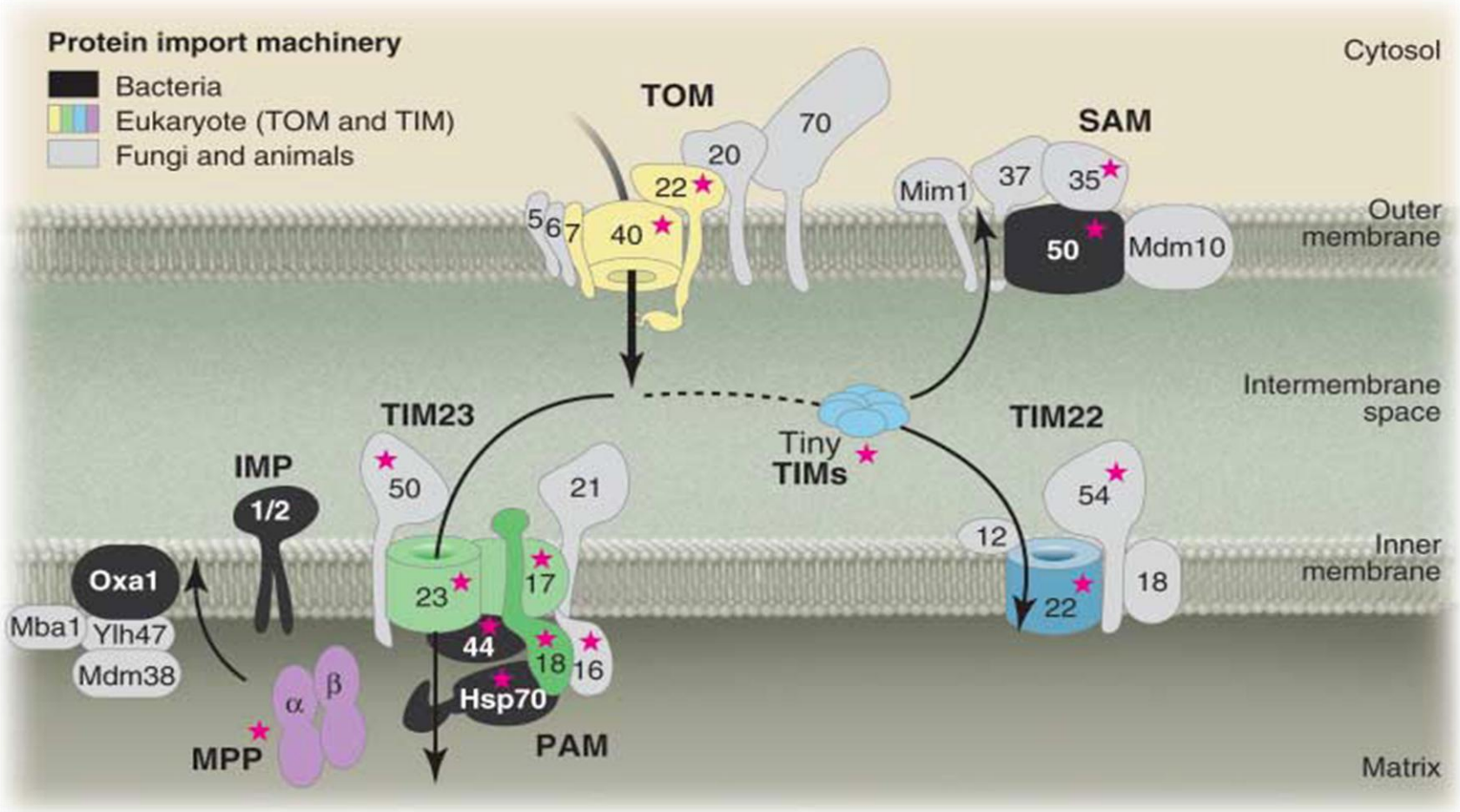


Endosymbiotic theory

- Lynn Margulis (1967, 1981) – *Alphaproteobacteria*
  - similar size
  - 2 membranes
  - small circular DNA
  - own ribosomes
  - proteins homologous to bacterial proteins



Mitochondrial import machinery

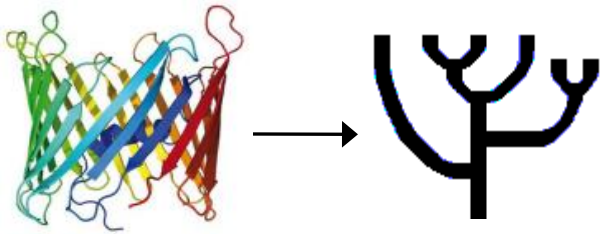


Dolezal et al. 2006

Methodology

- Identification of mitochondrial outer membrane import machinery in *Dictyostelium*
  - similarity search (BLAST)
  - HMM search (HMMER)
  - validation of candidates (PSIPRED, CDD)

- Analysis of phylogenetic relationships of eukaryotic mitochondrial β-barrels (outer membrane channels)
  - dataset collection
  - msa
  - trees construction (NJ, ML)

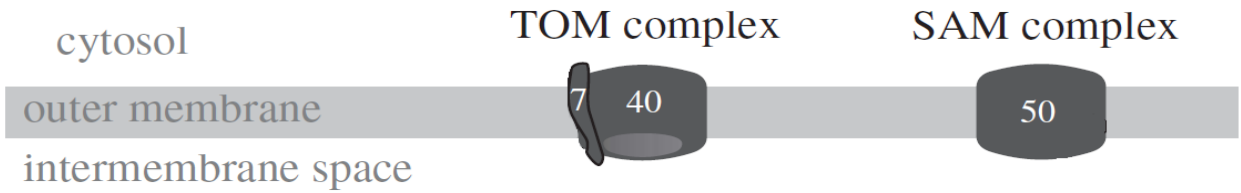


Results / Mitochondrial import machinery in *D. discoideum*

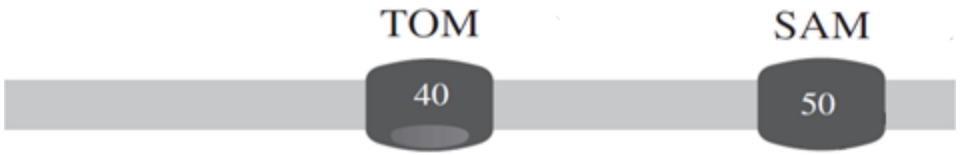
- **VDAC, Tom40, Tom7**
- **Sam50**
  - 396aa, Bacterial surface Antigen domain
  - similar to mammalian Samm50
- **Mdm12**
  - 202aa, 1 TMH
  - similar to yeast Mdm12
- **Mmm1**
  - 359aa, DUF2404 domain, 2 TMH
  - similar to yeast Mmm1
- ***Metaxin, Tom70***

Results / Reductive evolution of mitochondrial import machinery

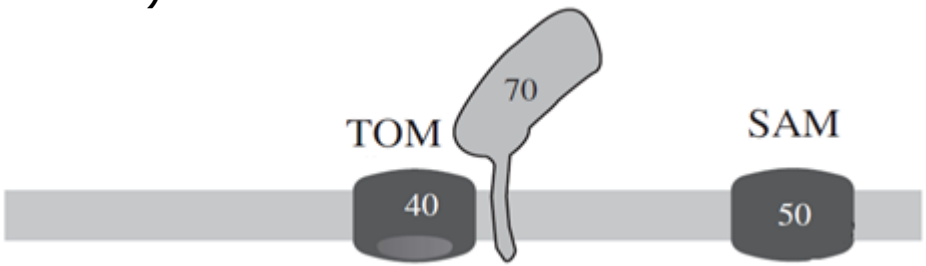
- ***Dictyostelium discoideum*** - Amoebozoa, Mycetozoa, Dictyosteliida



- *Entamoeba histolytica* - Amoebozoa, Archamoebae, Entamoebidae (Tovar et al. 1999, Dolezal et al. 2010)



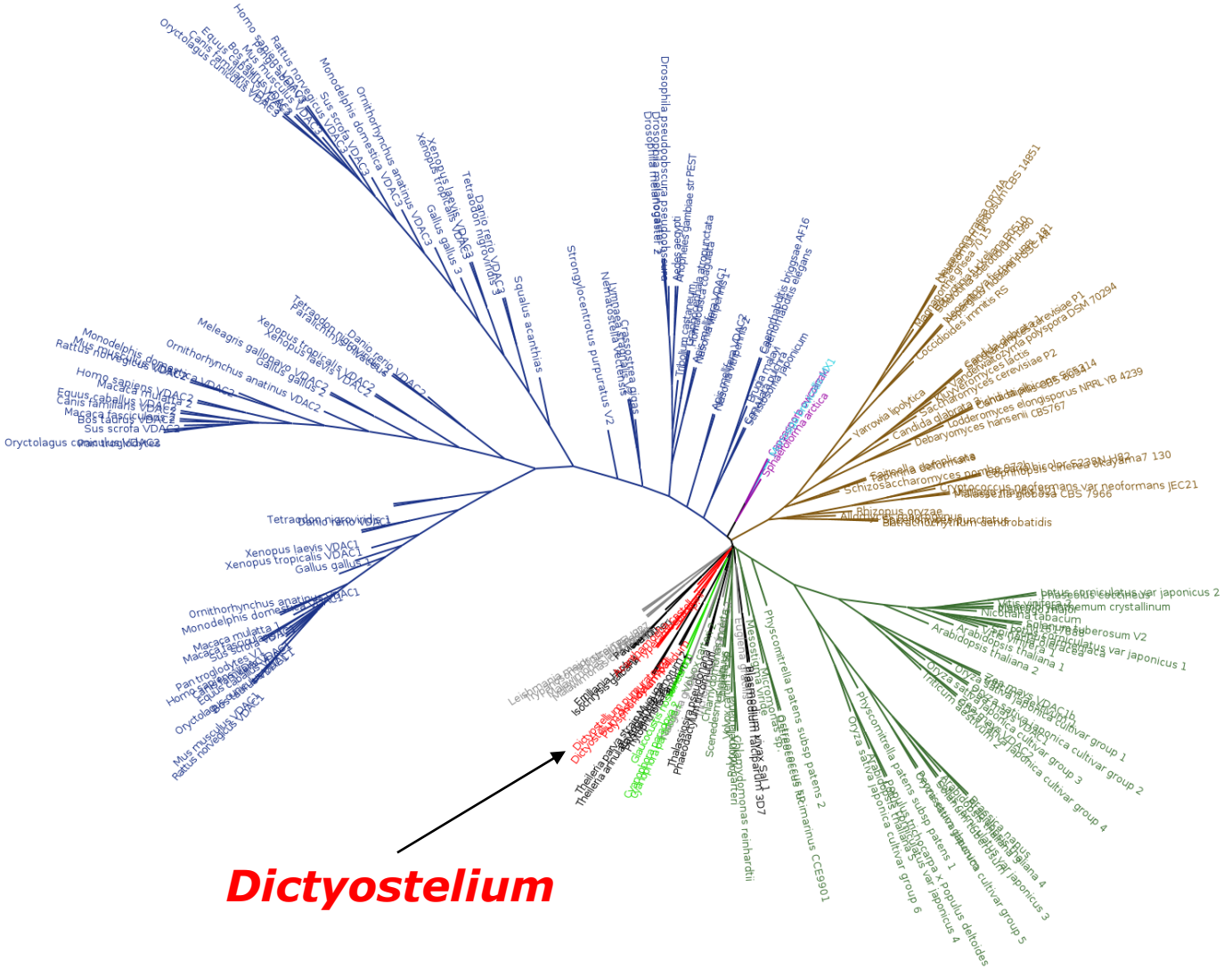
- *Encephalizoon cuniculi* - Opisthokonta, Fungi, Microsporidia (Waller et al. 2009)



Results / Dataset for phylogeny

Super-groups	Rank	No of species	Sam50	Tom40	VDAC
Amoebozoa	Eumycetozoa	4	2	3	4
	Acanthamoebidae	1	1	1	1
	Entamoebida	2	2	2	-
		7	5	6	5
Ophisthokonta	Metazoa	44	28	43	76
	Choanomonada (Choanoflagellata)	2	1	1	1
	Fungi	41	30	34	31
	Mesomycetozoa	2	-	1	2
		89	59	79	110
Archeplastida	Chloroplastida	30	12	16	48
	Glaucophyta	2	-	-	3
		32	12	16	51
Chromalveolata	Haptophyta	3	1	2	3
	Stramenopiles	4	5	4	4
	Alveolata	12	1	12	4
	Cryptophyceae	1	-	1	-
		20	7	19	11
Excavata	Malawimonas	2	-	-	2
	Parabasalia	1	-	1	1
	Preaxostyla	1	-	1	-
	Heterolobosea	1	1	1	1
	Euglenozoa	3	-	-	3
		8	1	3	7
Rhizaria	Cercozoa	1	-	1	-
		1	0	1	0
	TOTAL	157	84	124	184

Results / VDAC ML tree



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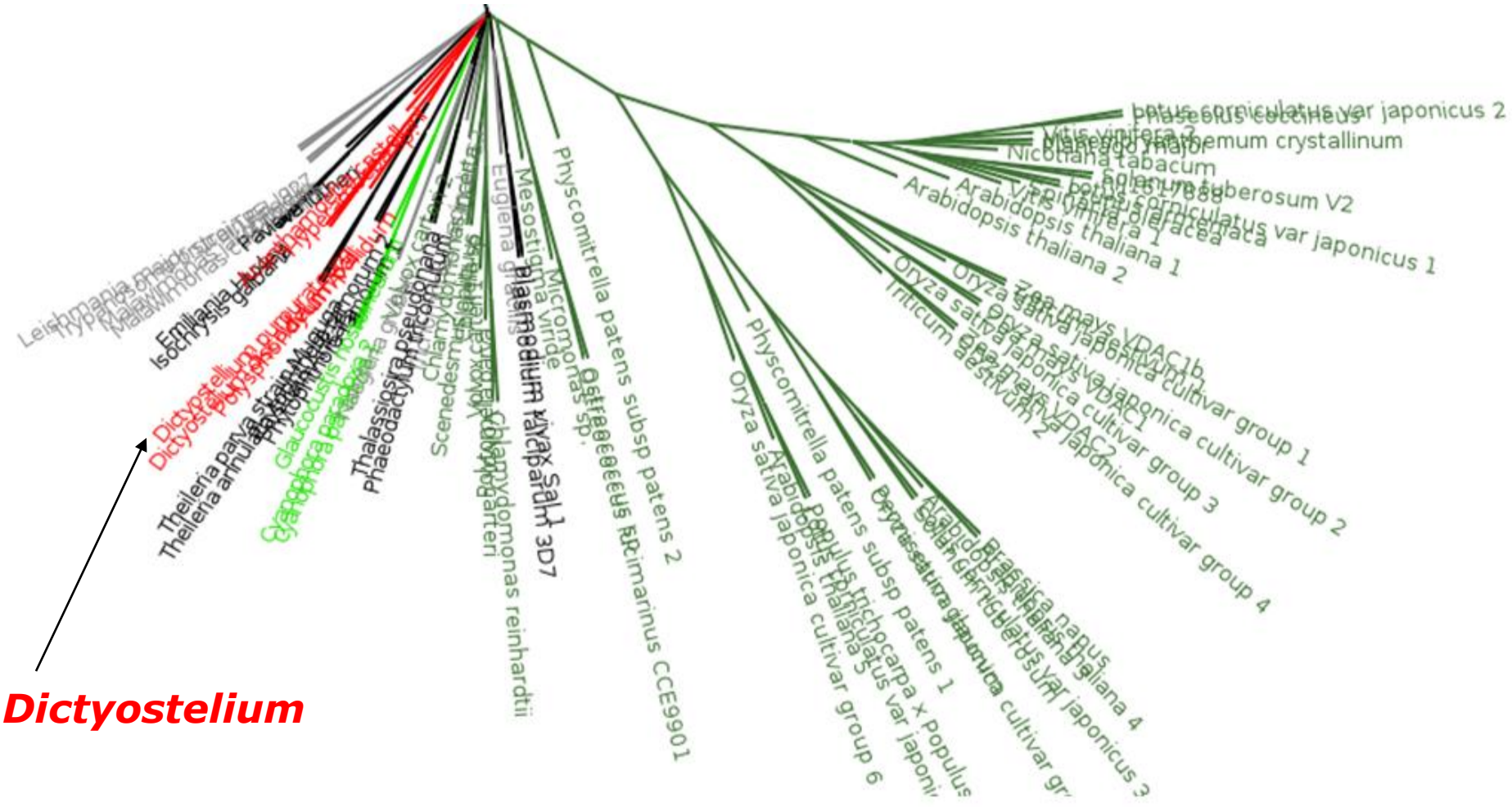
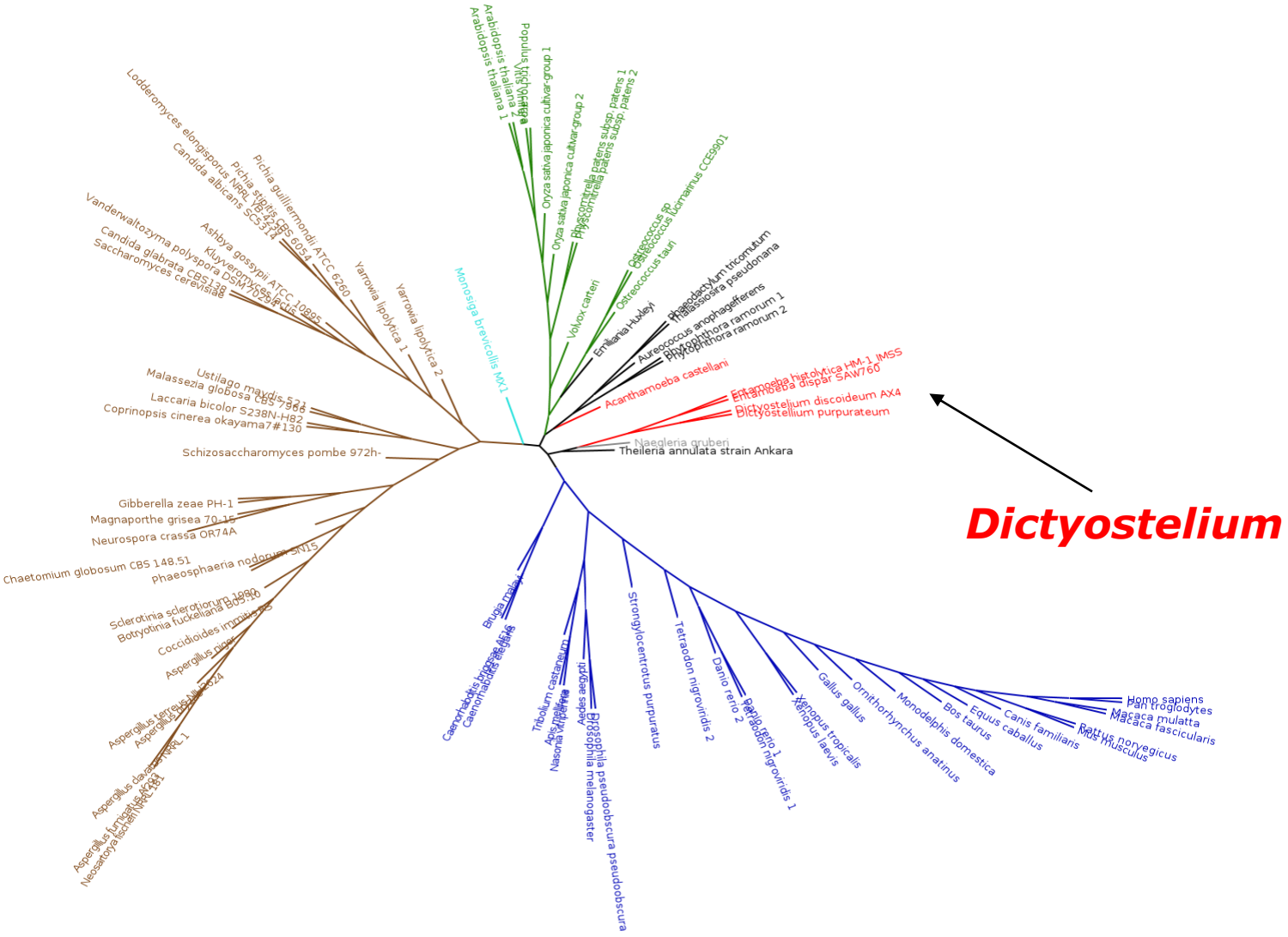
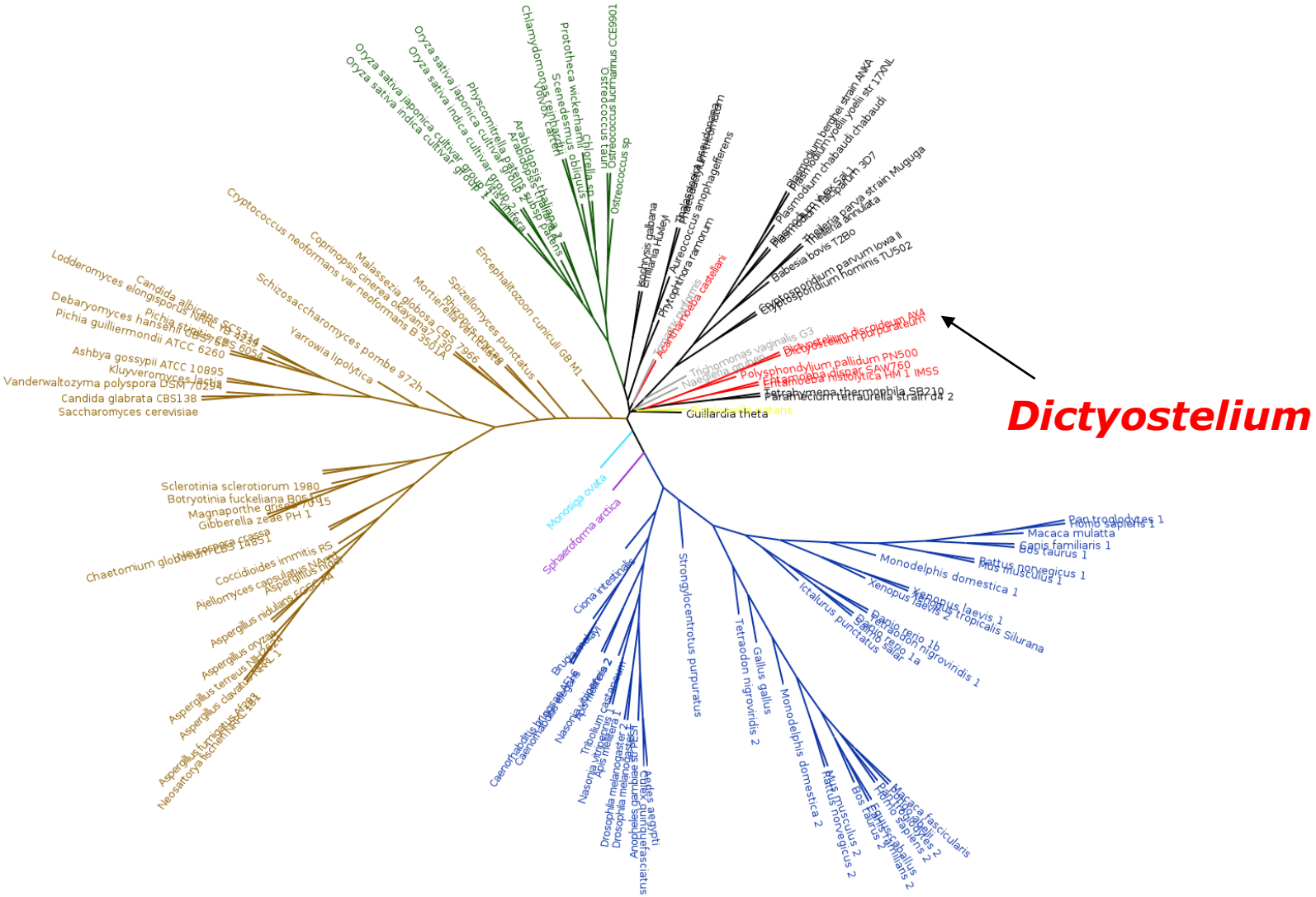


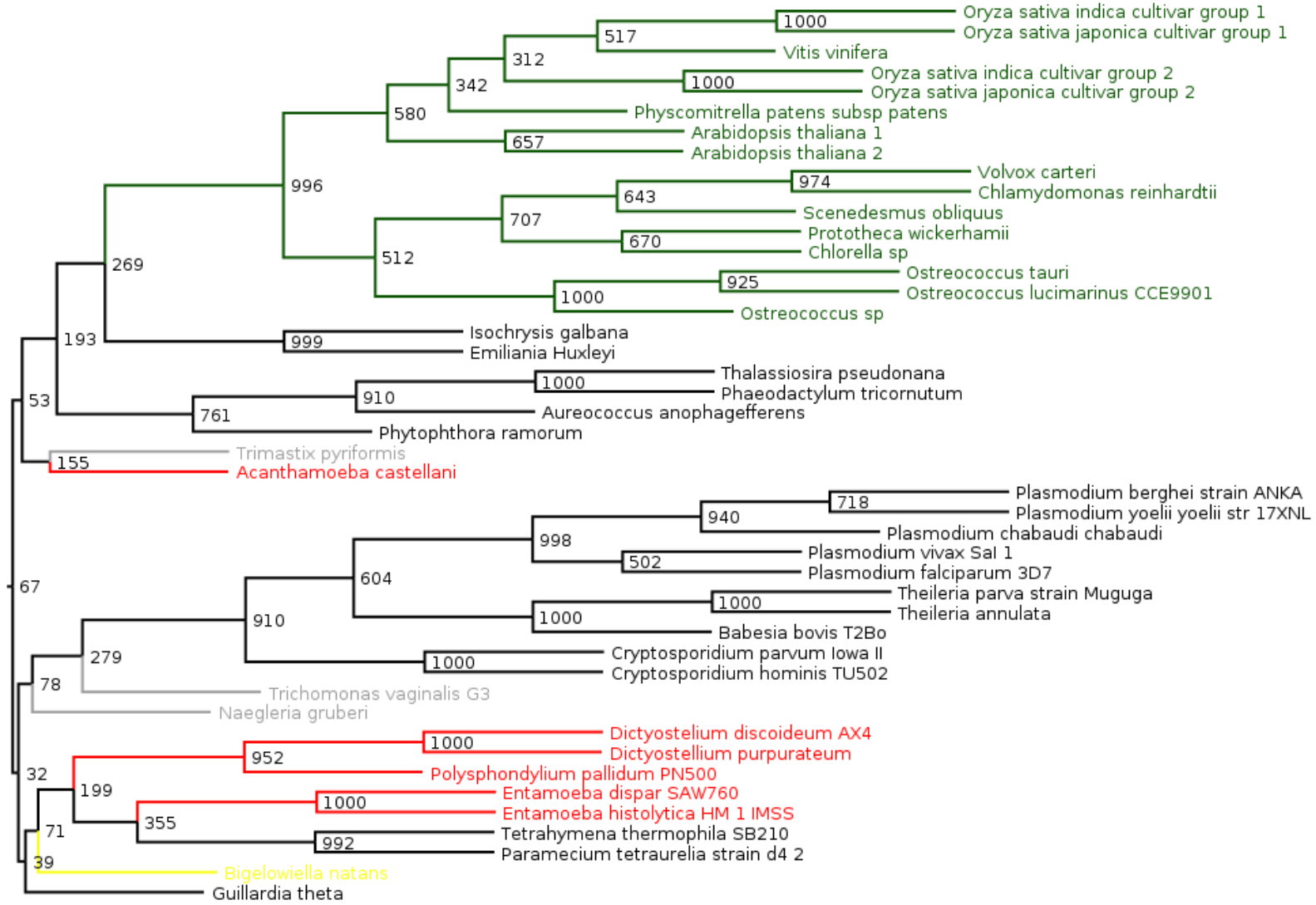
Results / Sam50 NJ tree



Results / Tom40 NJ tree



Results / Quality of trees



Conclusions

- A number of mitochondrial outer membrane import machinery subunits are missing in *D. discoideum*
  - secondary gene loss (?)
  - less hydrophobic preproteins for import (?)
  - conserved function and structure but not the sequence (?)
  - lineage specific counterparts (?)
- β-barrel proteins of mitochondrial outer membrane present high evolutionary rate
  - sequences are too diverged to obtain satisfying quality of alignment and therefore build well supported phylogenetic trees
- The position of *D. discoideum* on the eukaryotic tree cannot be verified with a high level of confidence with the usage of mitochondrial β-barrel outer membrane proteins

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