

# Supplementary Material for “Transcriptome and evolutionary analysis of *Pseudotrichomonas keilini*, a free-living anaerobic eukaryote”

## Supplementary Methods

### *Strain isolation and culture*

*Pseudotrichomonas keilini* strain TOLEDOT was isolated from salt marsh sediment at Toledo, Castile-La Mancha, Spain, coordinates 39°58'42"N, 3°39'20"W. It was maintained as a mono-eukaryotic, polyxenic culture in Dobell-Laidlaw biphasic (Dobell & Laidlaw, 1926) medium at room temperature and subcultured every two weeks. Living and protargol-stained cells of *P. keilini* were examined under a microscope 1200 BX51 (Olympus) equipped with an Olympus DP71 camera, using DIC optics for living cells. Protargol-stained preparations were prepared as follows: moist films spread on coverslips were prepared from pelleted cultures obtained by centrifugation at 500 g for 8 minutes. The films were fixed in Bouin-Hollande's fluid for approximately 15 hours, washed with 70% ethanol, and stained with 1% protargol (Bayer, I. G. Farbenindustrie) following (Nie, 1950) protocol. Micrographs of protargol-stained cells exhibiting the morphological characteristics of *P. keilini* are given in Supplementary Figure 1.

### *Transcriptome sequencing and assembly*

For transcriptome sequencing, RNA was extracted from 8 mL *P. keilini* culture using Trizol reagent (Invitrogen) and cDNA was synthesised using the SmartSeq2 protocol (Picelli et al. 2014) using poly-dT primers for first strand synthesis to enrich for eukaryotic transcripts and a template-switching oligo to enable sequencing of the 5' end of each transcript. Libraries were built at the ASU genomics core using KAPA Biosystem's LTP library preparation kit (KK8232). Library fragment size was analysed by TapeStation (Agilent), and quantified by qPCR (KAPA Library Quantification Kit, KK4835) on Thermo Fisher Scientific's Quantstudio 5 before sequencing on an Illumina MiSeq V2 using 2x250 paired end chemistry. Additional transcriptome sequencing from another 8 mL *P. keilini* culture was carried out at Génome Québec using the NEB stranded mRNA library protocol (New England Biolabs) for cDNA synthesis and library preparation before sequencing 50 million reads on the NovaSeq6000 platform using 2x100 chemistry. Transcript reads were assembled using Trinity RNA-Seq v2.8.4 (Haas et al., 2013). Predicted proteins that were 100% identical over the overlapping length were clustered using CD-HIT 4.8.1 (Li and Godzik, 2006). Reads have been deposited in the NCBI Short Read Archive under the accession PRJNA884676.

### *Gene finding and annotation*

Protein prediction from transcripts was done using TransDecoder version 5.5.0 (Haas et al., 2013). Using default parameters, we obtained a total of 83,266 proteins from the total set of assembled transcripts. As we were unable to obtain a pure culture of *P. keilini*, we applied a

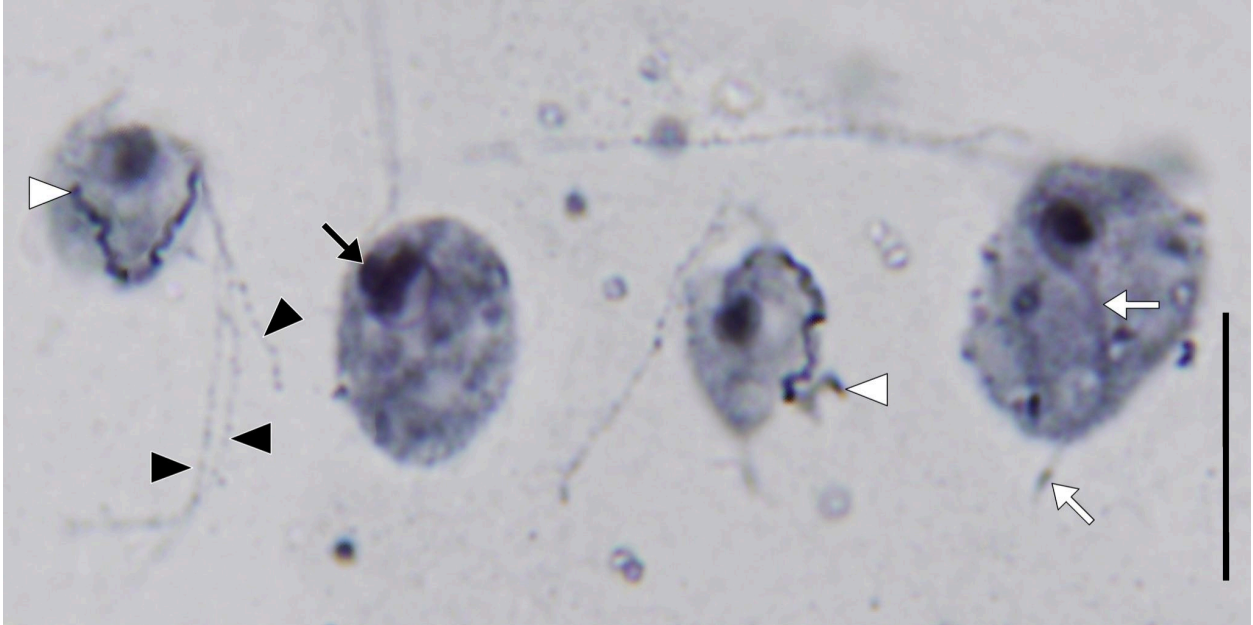
conservative BLAST-based filter to these proteins to distinguish bona fide *P. keilini* proteins from prokaryotic contaminants of the assembly. To do so, we searched the predicted proteins against a custom database containing protein sets from 38 published genomes of excavates, along with a representative sampling of 73 other eukaryotes, 148 bacteria and 146 archaea (see Supplementary Table S1). Proteins for which either (i) the best hit was from a parabasalid relative of *P. keilini*, or (ii) alternatively where the first three database hits were eukaryotic in a Diamond BLAST search were retained as putative *P. keilini* proteins. We assigned functions to the filtered *P. keilini* protein set using eggNOG-mapper v2, which uses precomputed protein families from the eggNOG database (Huerta-Cepas et al., 2016, 2017, 2019) to perform functional annotation. For proteins which could not be annotated with eggNOG, we used blastKOALA to predict functions from the KEGG database (Kanehisa et al., 2016, 2017; Kanehisa & Goto, 2000).

### *Phylogenetics*

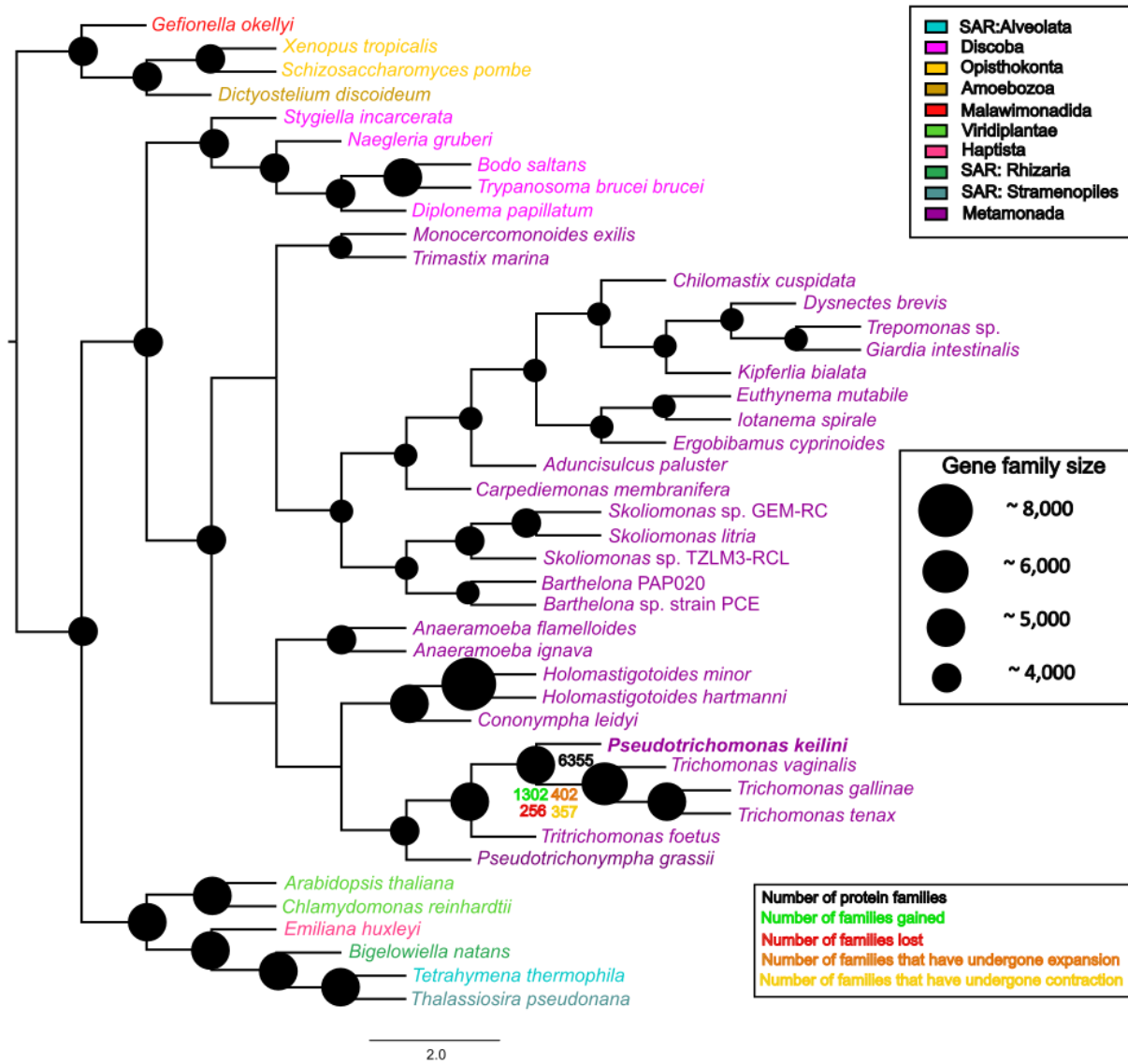
The species tree was inferred using SpeciesRax (Morel et al., 2022), based on 13,346 gene family trees clustered from 43 eukaryotic genomes using Broccoli v1.2 (Derelle et al., 2020). The branch lengths represent substitutions per site. The individual gene family trees were inferred using IQ-TREE2 (Minh et al., 2020), with 1000 rapid bootstraps, and the best-fitting model in each case (including LG+C20 and LG+C60 as potential site-heterogeneous options), was chosen by the Bayesian Information Criterion. The phylogenetic birth-death model was fit in Count following the procedure suggested in the user manual (Csűös, 2010); the final model included three categories to model variation in branch-specific lengths and duplication rates across the species tree.

### *Hydrogenosome detection*

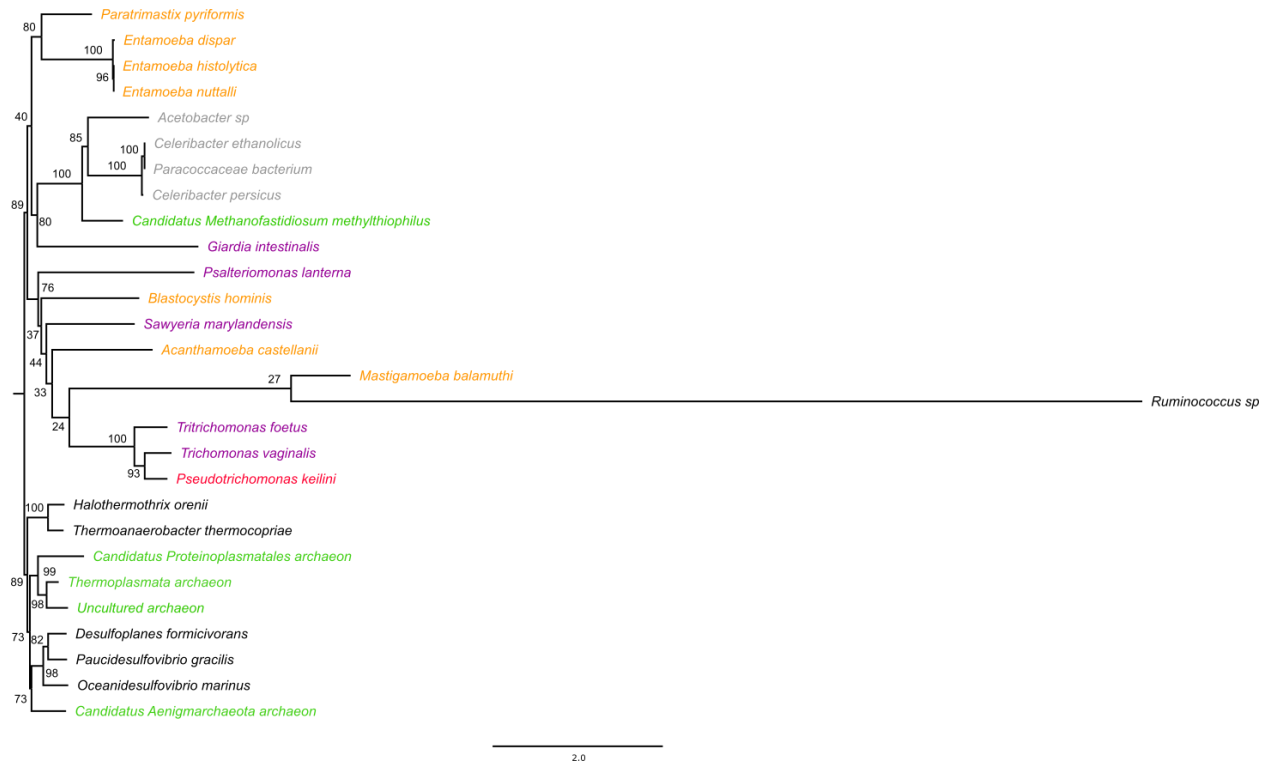
We used the supplementary table in (Stairs et al., 2015) which included key enzymes of each of the five classes of mitochondria and its related organelles classified by Muller et al. (2012). Using Diamond (Buchfink et al., 2015), we did a BLAST search for the sequences against the database of *P. keilini* filtered proteins to identify key enzymes. Other protein sequences were added for these enzymes by doing a BLAST search against the NCBI nr database and selecting the top five hits from eukaryotes, alphaproteobacteria, and other bacteria. The retrieved sequences were then aligned using MAFFT v7.390 (Kato & Standley, 2013). After checking the alignment, and selecting the most complete sequences, we then ran BMGE-1.12 (Crisuolo & Gribaldo, 2010) which trims the multiple sequence alignment to remove poorly aligned regions. The trimmed alignments were used to construct maximum-likelihood trees with IQ-TREE-v1.6.10 using the best model selected by ModelFinder (Kalyaanamoorthy et al., 2017; Nguyen et al., 2015). Finally, we used HMMER 3.2.1 (<http://hmmer.org/>) to search the filtered *P. keilini* transcriptome for homologues of mitochondrial carrier family transport proteins, with an E-value threshold of  $< 10^{-5}$ . We used the PF00153 HMM profile for the MCF mitochondrial carrier protein which contains 125,808 sequences from 1,117 species including *Trichomonas vaginalis*. HMMER profiles are probabilistic profile hidden Markov models (Durbin et al., 1998; Eddy, 1998; Krogh et al., 1994) which are used to identify distant protein homologs.



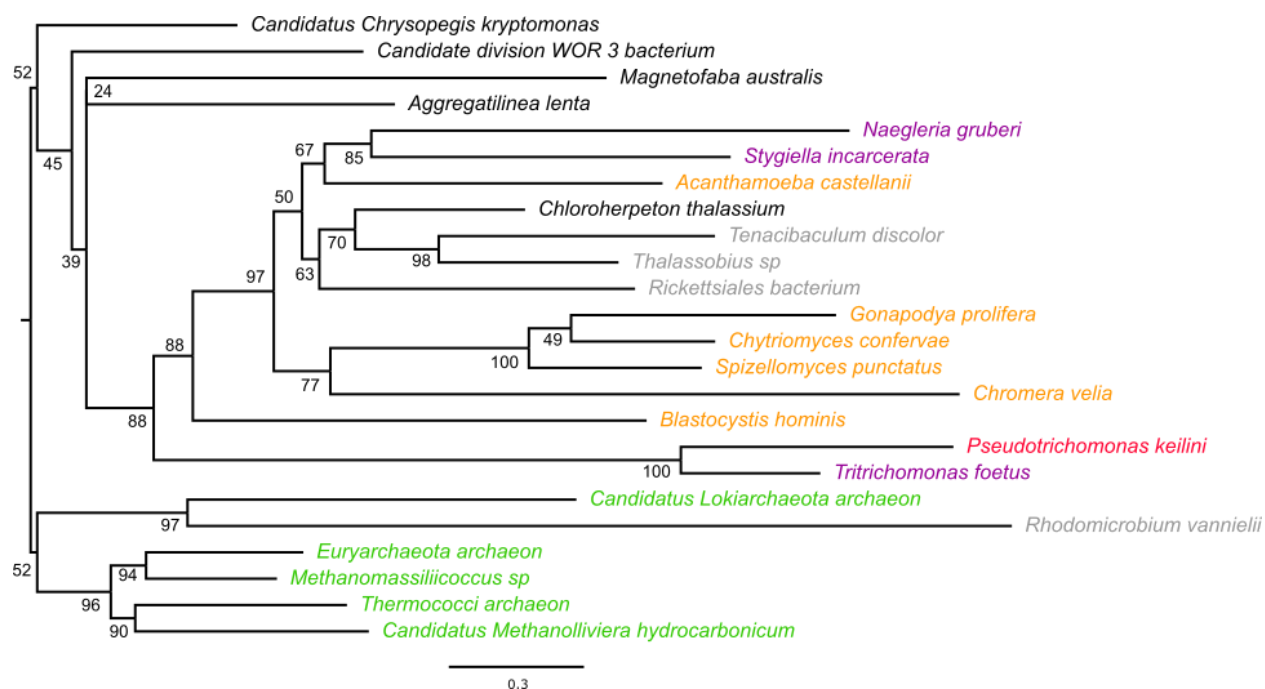
**Figure S1. Morphology of *Pseudotrichomonas keilini*.** Protargol-stained cells exhibit the diagnostic characters of this species: three anterior flagella (black arrowheads), a well developed undulating membrane that does not continue as a free flagellum (white arrowheads), a large parabasal body (this differentiates the organism from *Lacusteria*; black arrow), and a normally developed axostyle (white arrows), scale 10  $\mu$ m.



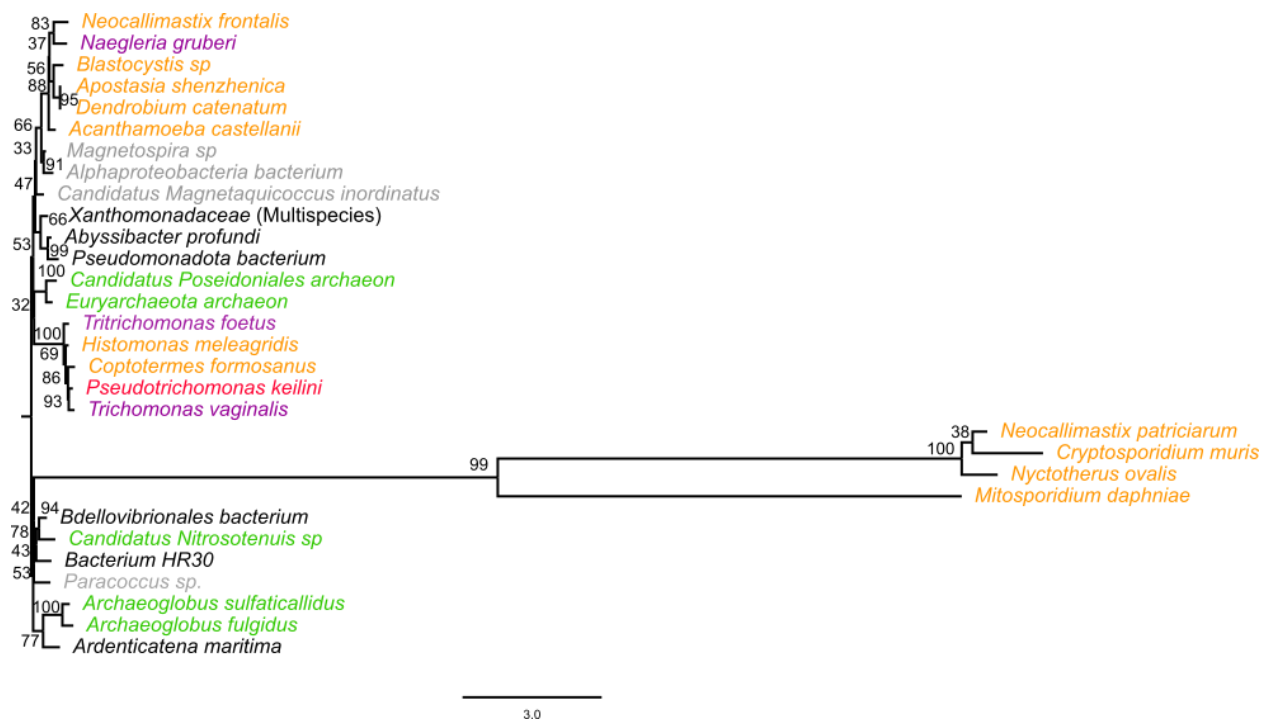
**Figure S2: Gene family evolution in parabasalids and metamonads.** We used a phylogenetic birth-death model implemented in Count ((Csűös, 2010) 2010) to map gene family evolution onto a species tree manually edited to reflect the consensus view of deep eukaryotic relationships. Numbers, and the diameter of circles, indicate gene family repertoire size at ancestral nodes, while family gains, losses, expansions and contractions are plotted for the *Trichomonas* lineage after its divergence from *P. keilini*.



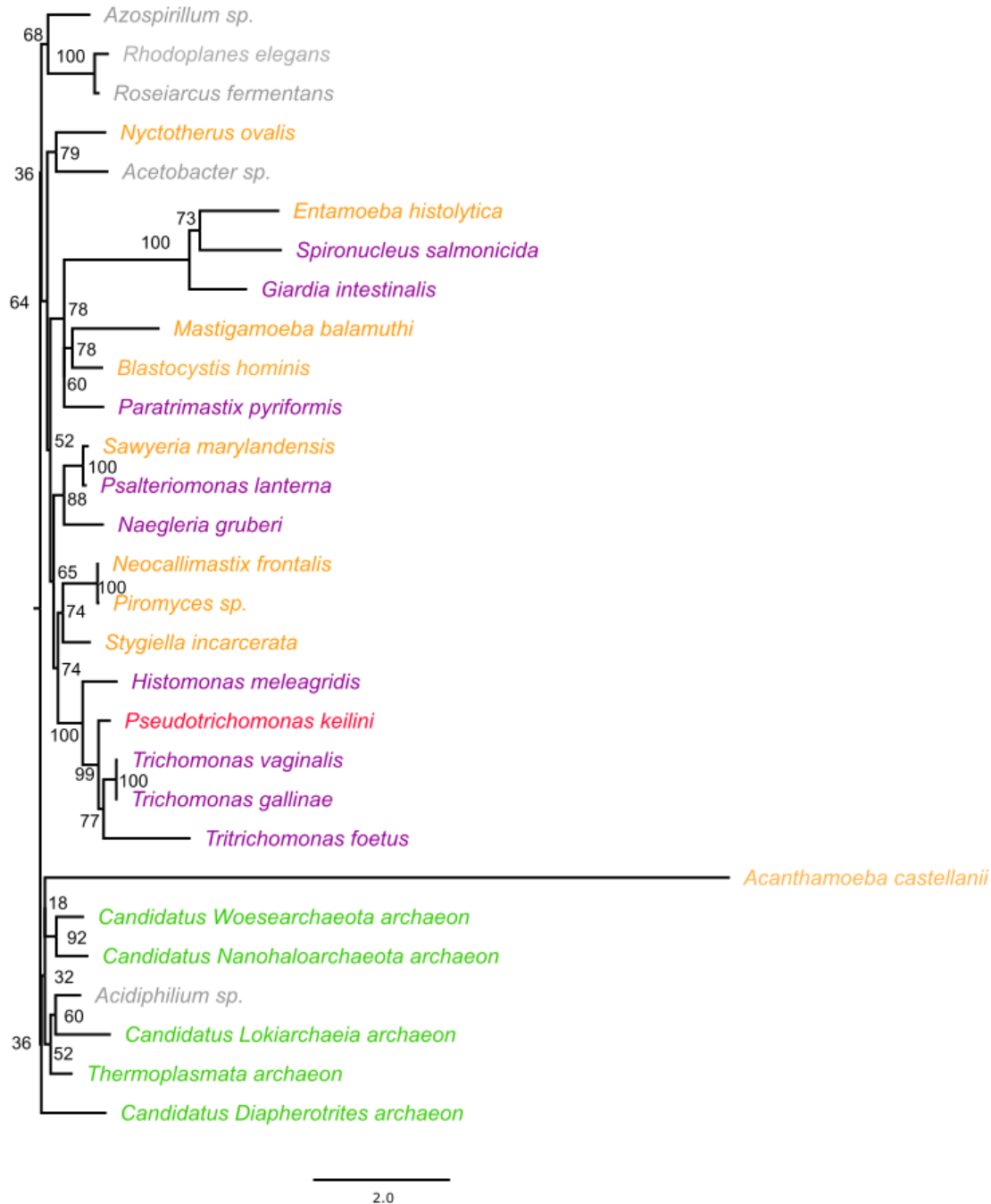
**Figure S3: Phylogeny of Pyruvate:ferredoxin oxidoreductase (PFO).** PFO is a key hydrogenosomal enzyme that catalyzes the interconversion of pyruvate to Acetyl-CoA. Most sampled excavate sequences including *Psalteriomonas lanterna* and *Sawyeria marylandensis* form a clade with the parabasalids *P. keilini*, *Trichomonas vaginalis*, and *Tritrichomonas foetus*. The maximum likelihood gene tree was inferred using IQ-TREE. Branch supports are ultrafast bootstrap values, and branch lengths are proportional to the expected number of substitutions per site, as indicated by the scale bar.



**Figure S4: Phylogenetic analysis of Acetyl:succinate CoA-transferase subunit b (ASCT1b)** which is a bidirectional enzyme that acts on the conversion of succinate to acetyl-CoA and vice versa. In the tree, *P. keilini* groups with *Tritrichomonas foetus* and they both form part of a larger clade which includes other eukaryotes, some of which are excavates. The maximum likelihood gene tree was inferred using IQ-TREE. Branch supports are ultrafast bootstrap values, and branch lengths are proportional to the expected number of substitutions per site, as indicated by the scale bar.

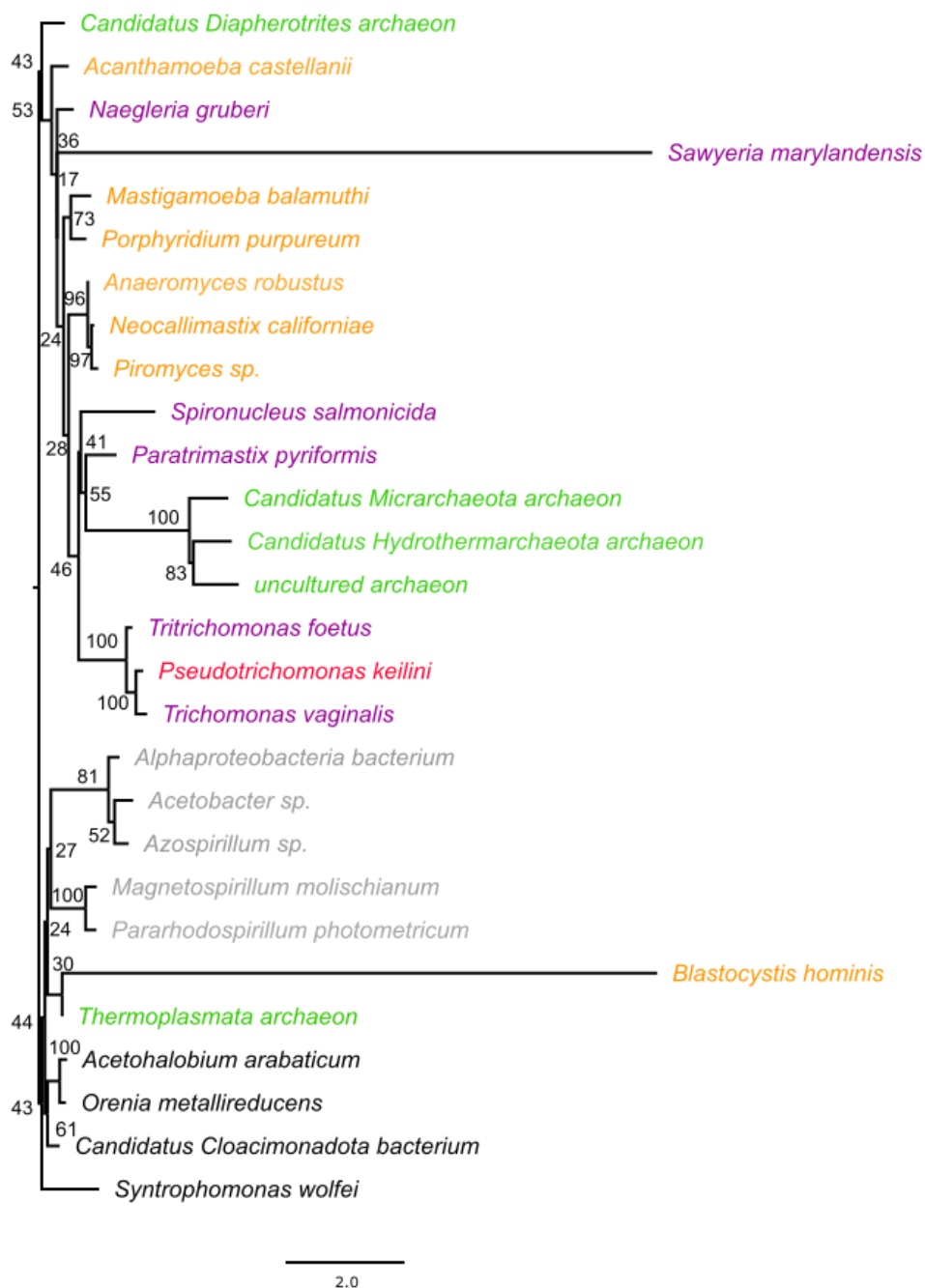


**Figure S5: Phylogenetic analysis of Succinyl coenzyme A synthetase (SCS)** which is a Krebs cycle enzyme that catalyzes the interconversion of succinyl-CoA to succinate. *Tritrichomonas foetus*, *Trichomonas vaginalis*, and *P. keilini*, but not the eukaryotes as a whole, form a clade. The maximum likelihood gene tree was inferred using IQ-TREE. Branch supports are ultrafast bootstrap values, and branch lengths are proportional to the expected number of substitutions per site, as indicated by the scale bar.

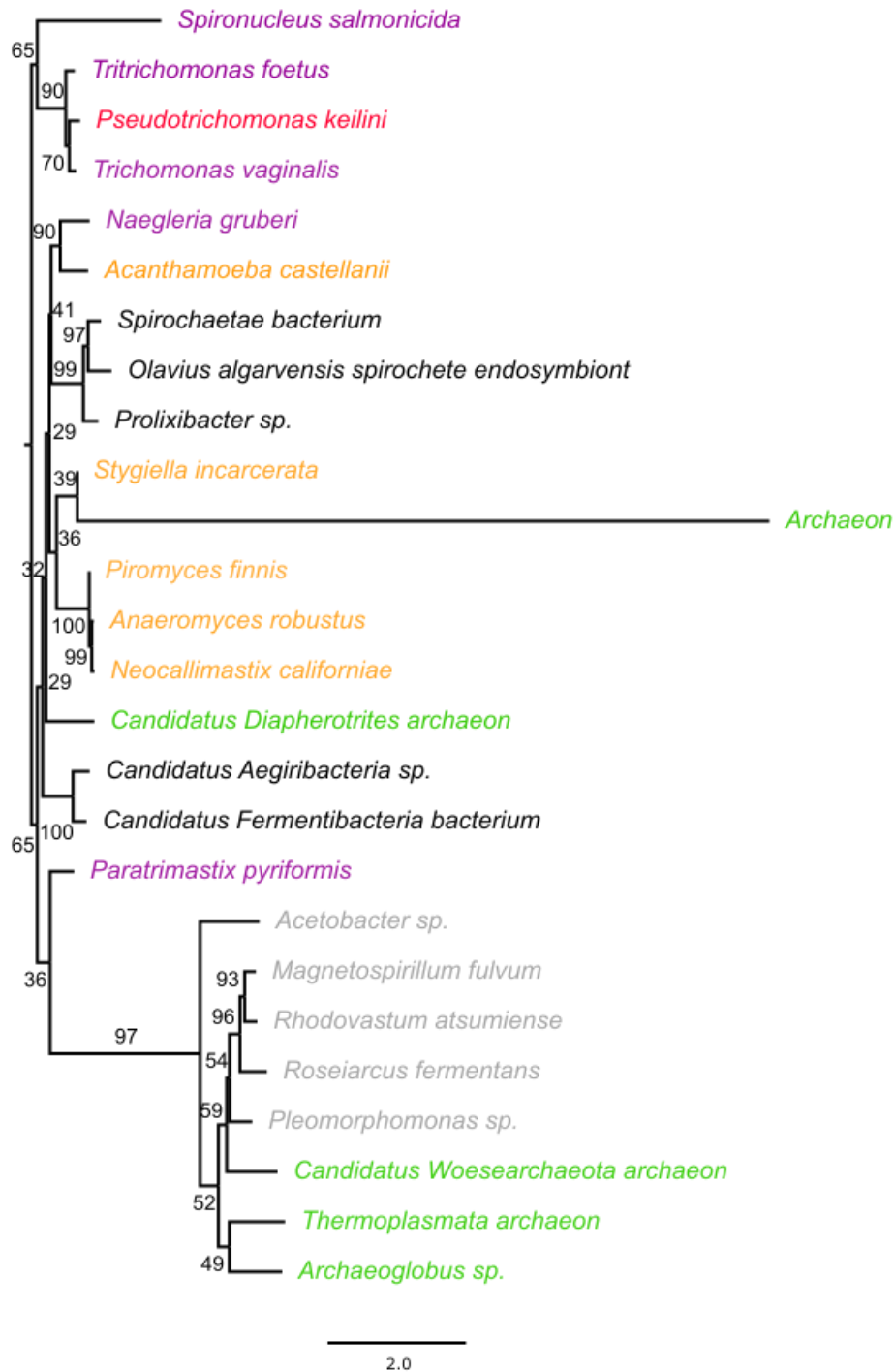


**Figure S6: Phylogeny of Fe-Fe hydrogenase (hydA) enzyme** which is responsible for the production of molecular hydrogen and one of the key hydrogenosomal enzymes. Another sequence from the excavate *Histomonas meleagridis* groups with the parabasalian clade of *P. keilini*, *Trichomonas vaginalis*, and *Tritrichomonas foetus*, *Trichomonas sp* and *Trichomonas gallinae*. The maximum likelihood gene tree was inferred using IQ-TREE. Branch supports are ultrafast bootstrap values, and branch lengths are proportional to the expected number of substitutions per site, as indicated by the scale bar.



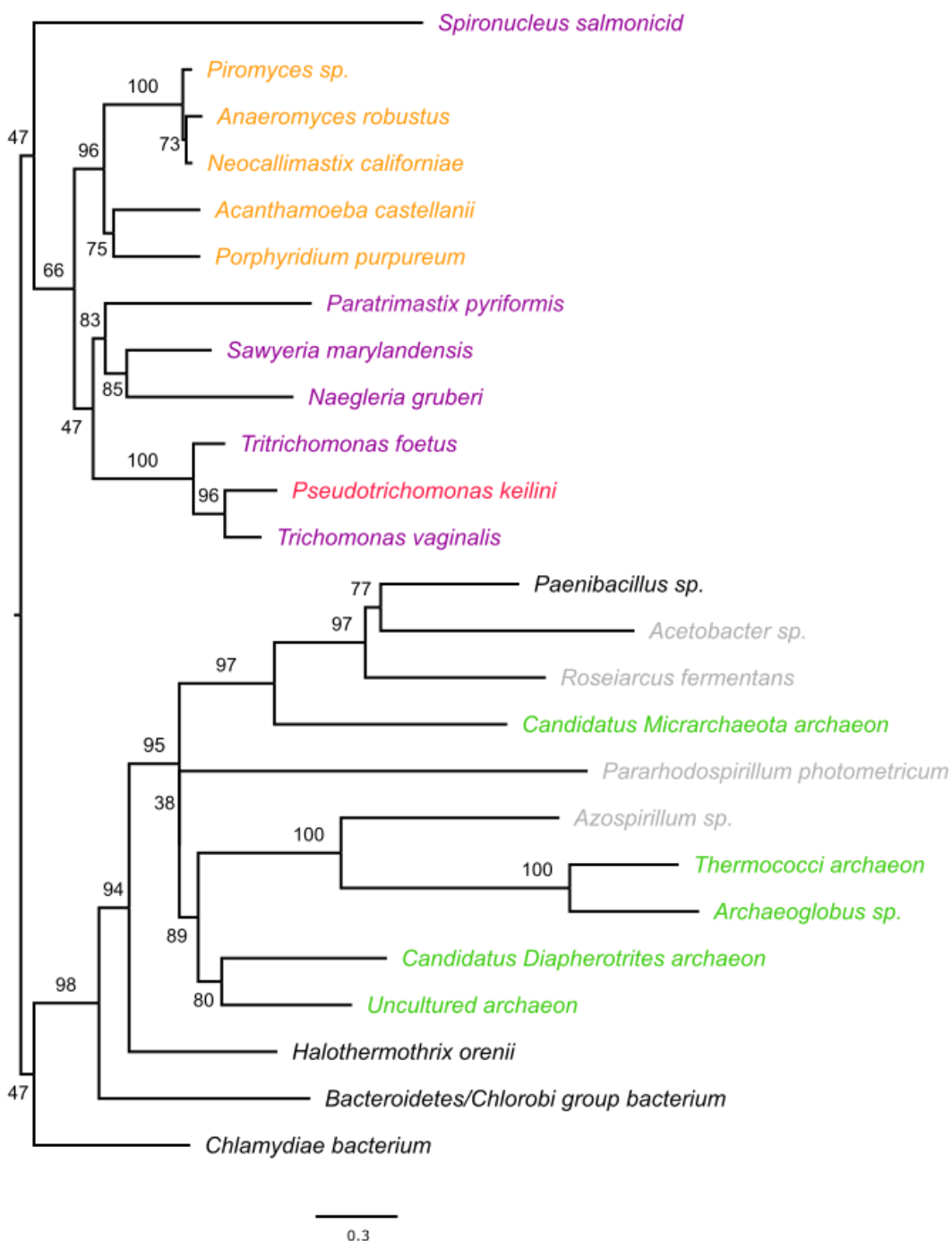


**Figure S7: Phylogeny of radical SAM domain containing protein (hydE),** one of the three maturase enzymes required for the synthesis of a mature Fe-Fe-hydrogenase(hydA). In this tree, the three parabasalids of *P. keilini*, *Trichomonas vaginalis*, and *Tritrichomonas foetus* group together. The maximum likelihood gene tree was inferred using IQ-TREE. Branch supports are ultrafast bootstrap values, and branch lengths are proportional to the expected number of substitutions per site, as indicated by the scale bar.



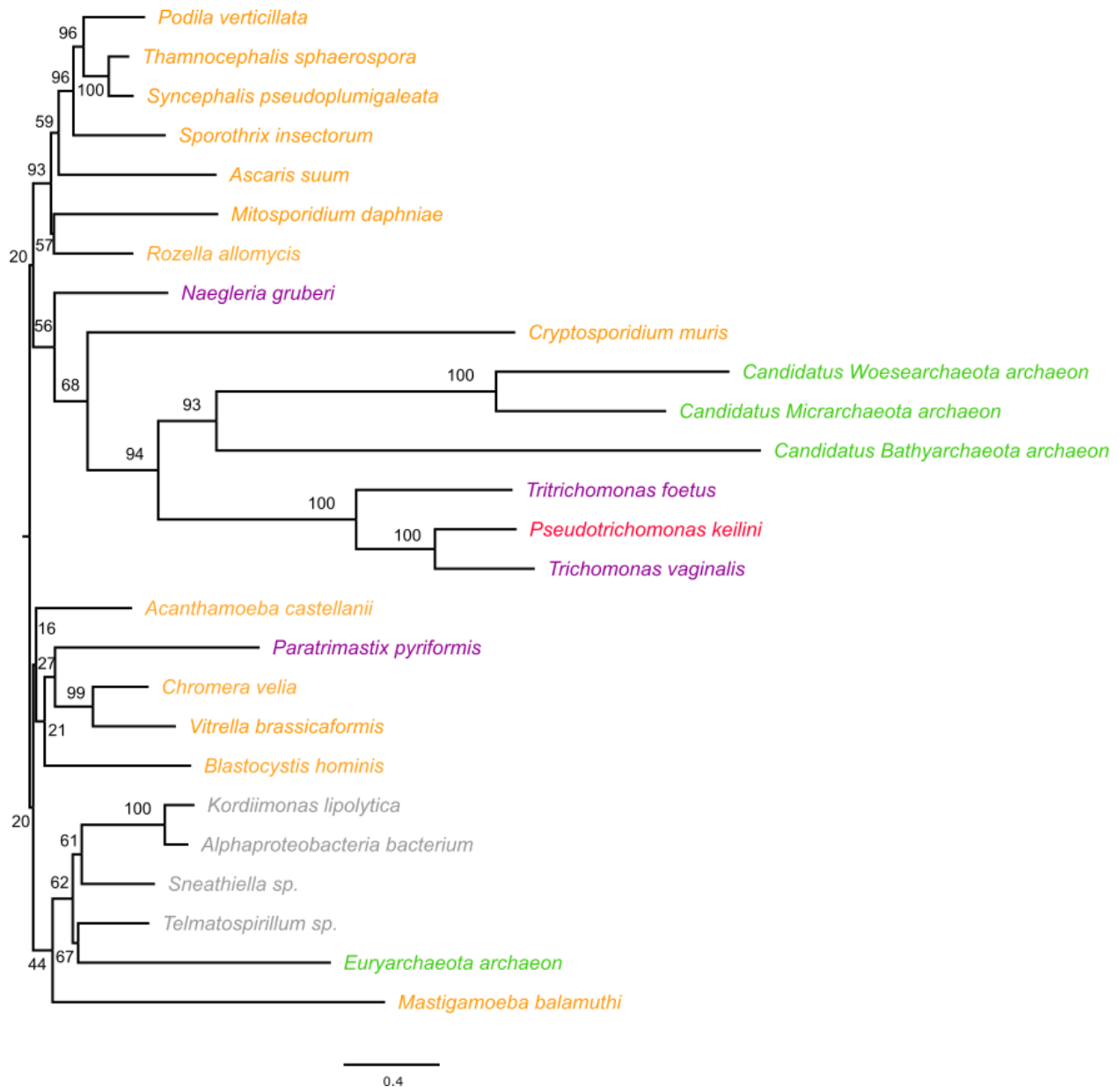
**Figure S8: Phylogeny of small GTP-binding protein (hydF)**, another one of the three maturase enzymes required for the synthesis of a mature Fe-Fe-hydrogenase(hydA). In this tree, the three parabasalids of *P. keilini*, *Trichomonas vaginalis*, and *Tritrichomonas foetus* group together. The maximum likelihood gene tree was inferred using IQ-TREE. Branch supports are

ultrafast bootstrap values, and branch lengths are proportional to the expected number of substitutions per site, as indicated by the scale bar.



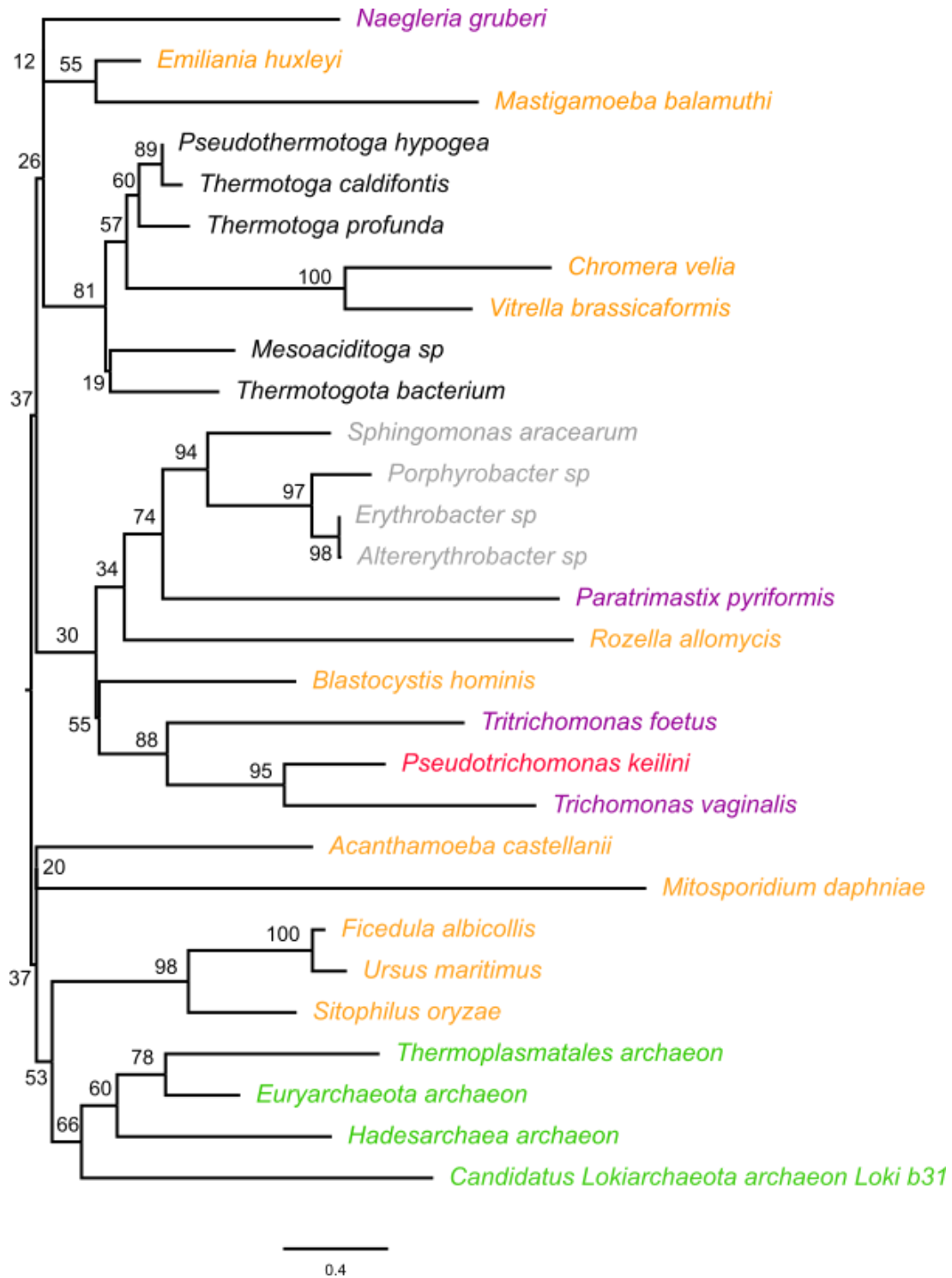
**Figure S9: Phylogeny of FeFe-hydrogenase assembly protein (hydG),** the third maturase enzyme required for the synthesis of a mature Fe-Fe-hydrogenase (hydA). A clade of the three parabasalids *P. keilini*, *Trichomonas vaginalis*, and *Tritrichomonas foetus* can be seen which is

part of a bigger clade of eukaryotes including excavates. The maximum likelihood gene tree was inferred using IQ-TREE. Branch supports are ultrafast bootstrap values, and branch lengths are proportional to the expected number of substitutions per site, as indicated by the scale bar.

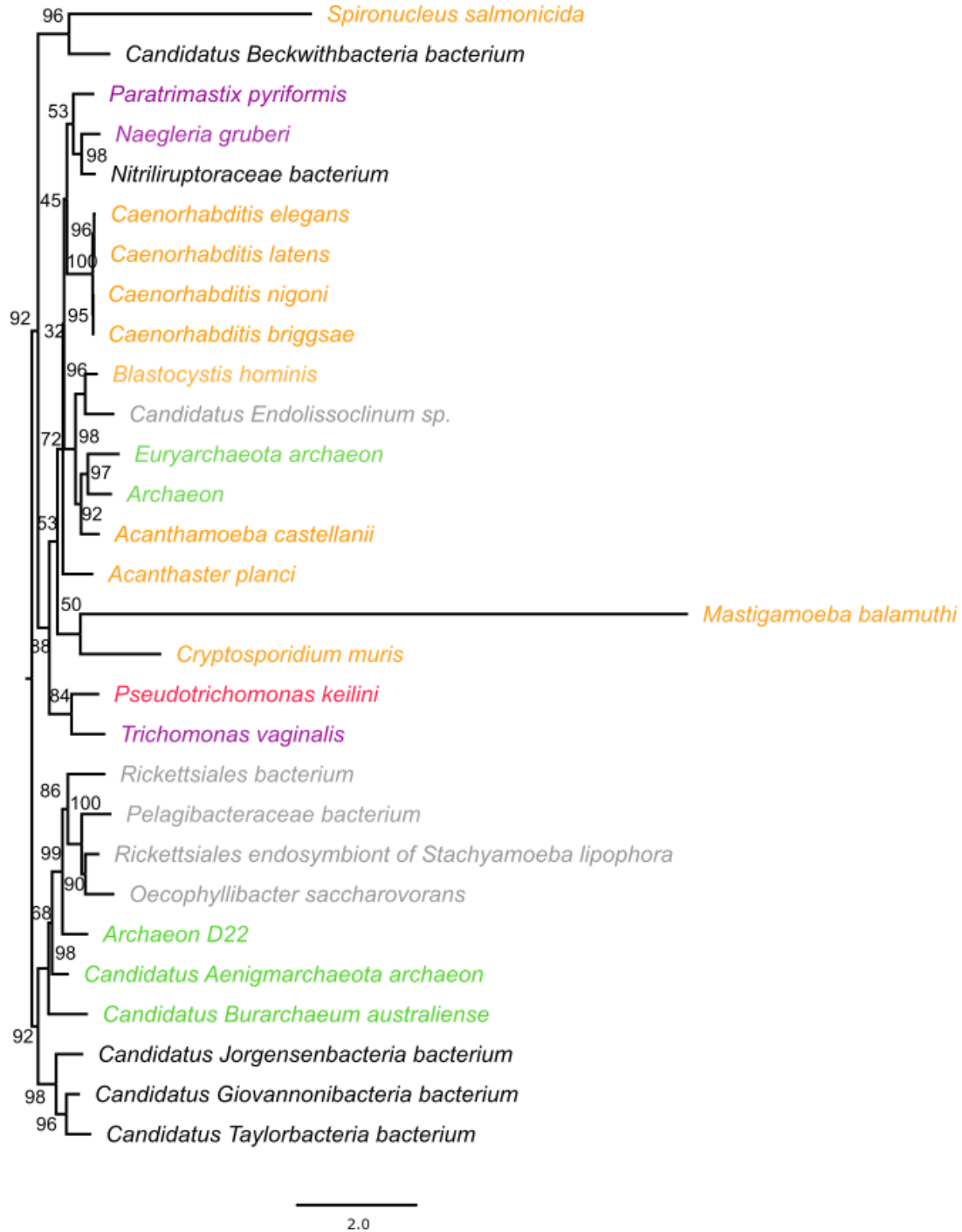


**Figure S10: Phylogeny of L-protein (GCSL),** which is part of the glycine cleavage system. The tree topology shows a clade of parabasalids consisting of *P. keilini*, *Trichomonas vaginalis*, and *Tritrichomonas foetus*, while other eukaryotic sequences form a separate clade. The

maximum likelihood gene tree was inferred using IQ-TREE. Branch supports are ultrafast bootstrap values, and branch lengths are proportional to the expected number of substitutions per site, as indicated by the scale bar.

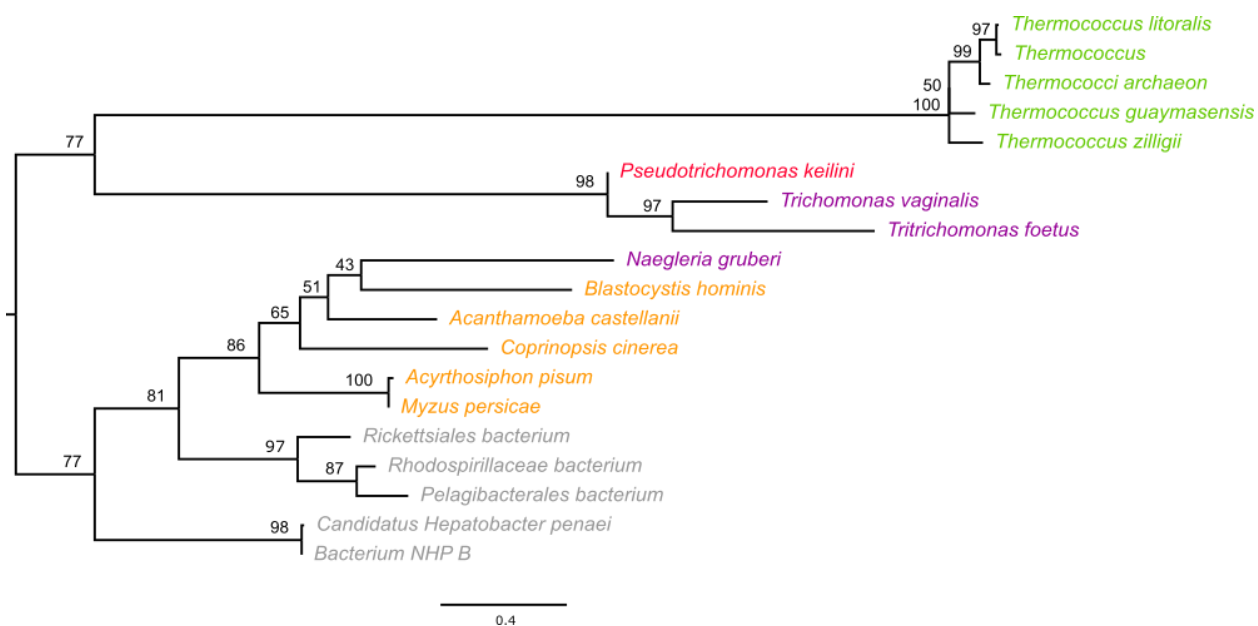


**Figure S11: Phylogeny of H-protein (GCSH)**, the second enzyme detected in *P. keilini*'s glycine cleavage system. The tree contains a clade of parabasalids including *P. keilini*, *Trichomonas vaginalis*, and *Tritrichomonas foetus* that is part of a larger clade comprising other eukaryotic and some alphaproteobacterial sequences, which could indicate an alphaproteobacterial origin of the enzyme. The maximum likelihood gene tree was inferred using IQ-TREE. Branch supports are ultrafast bootstrap values, and branch lengths are proportional to the expected number of substitutions per site, as indicated by the scale bar.



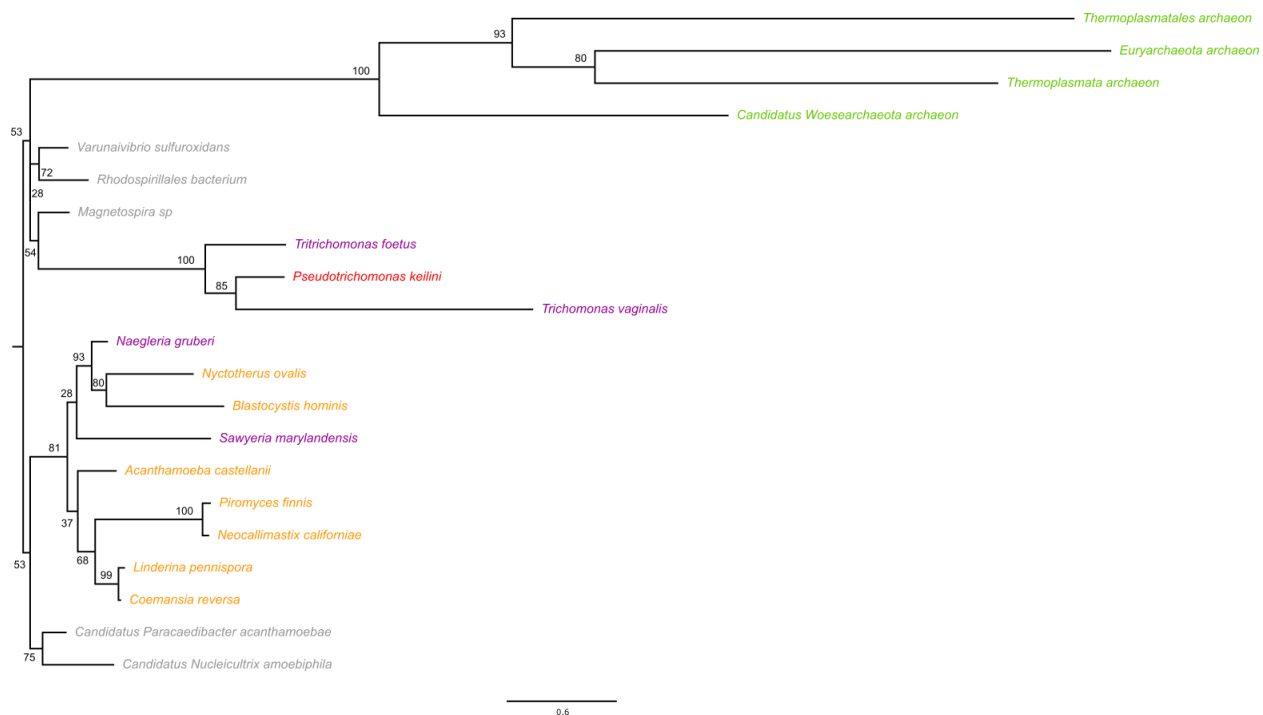
**Figure S12: Phylogenetic analysis of Serine hydroxymethyltransferase (SHMT) enzyme** which also plays a role in the glycine system. The tree shows that neither eukaryotes nor excavates are monophyletic. However, *P. keilini* and *Trichomonas vaginalis* are grouping together. The maximum likelihood gene tree was inferred using IQ-TREE. Branch supports are

ultrafast bootstrap values, and branch lengths are proportional to the expected number of substitutions per site, as indicated by the scale bar.



**Figure S13: Phylogeny of 24-kDa NADH-quinone oxidoreductase subunit E (NUOE)**, one of the components of Complex I of the electron transport chain. In this tree, the *P. keilini* sequence is grouping with the other parabasalians of *Trichomonas vaginalis* and *Tritrichomonas foetus*, and not with alphaproteobacteria. The maximum likelihood gene tree was inferred using IQ-TREE. Branch supports are ultrafast bootstrap values, and branch lengths are proportional to the expected number of substitutions per site, as indicated by the scale bar.





**Figure S14: Phylogenetic tree of 51-kDa NADH-quinone oxidoreductase subunit F (NUOF)**, another component of Complex I of the electron transport chain. The tree topology shows that the parabasal group of *P. keilini*, *Trichomonas vaginalis*, and *Tritrichomonas foetus* are grouping together within a larger clade of alphaproteobacterial sequences. This could indicate an endosymbiotic origin of the enzyme. The maximum likelihood gene tree was inferred using IQ-TREE. Branch supports are ultrafast bootstrap values, and branch lengths are proportional to the expected number of substitutions per site, as indicated by the scale bar.

**Supplementary Table S1: List of species used in the contaminant-filtering step.**

Species name	Domain	Taxonomy
<i>Cyanophora paradoxa</i>	Eukaryotes	Archaeplastida
<i>Chondrus crispus</i>		
<i>Galdieria sulphuraria</i>		
<i>Cyanidioschyzon merolae</i>		
<i>Porphyra umbilicalis</i>		
<i>Chlamydomonas reinhardtii</i>		
<i>Volvox carteri</i>		
<i>Tetraselmis sp</i>		
<i>Monoraphidium neglectum</i>		
<i>Bathycoccus prasinus</i>		
<i>Ostreococcus tauri</i>		
<i>Micromonas pusilla</i>		
<i>Auxenochlorella protothecoides</i>		
<i>Oryza sativa</i>		
<i>Arabidopsis thaliana</i>		
<i>Physcomitrella patens</i>		
<i>Selaginella moellendorffii</i>		
<i>Klebsormidium nitens</i>		
<i>Carpediemonas membranifera</i>	Eukaryotes	Excavates
<i>Dysnectes brevis</i>		
<i>Ergobibamus cyprinoides</i>		
<i>Kipferlia bialata</i>		
<i>Trepomonas sp</i>		
<i>Aduncisulcus paluster</i>		
<i>Chilomastix cuspidata</i>		
<i>Trimastix marina</i>		
<i>Naegleria gruberi</i>		
<i>Stygiella incarcerata</i>		
<i>Bodo saltans</i>		
<i>Diplonema papillatum</i>		
<i>Euglena gracilis</i>		
<i>Giardia lamblia ATCC50803</i>		
<i>Leishmania braziliensis</i>		
<i>Leishmania donovani species complex</i>		
<i>Leishmania infantum JPCM5</i>		
<i>Leishmania major Friedlin</i>		

<i>Leishmania mexicana</i> MHOM GT 2001 U1103		
<i>Leishmania panamensis</i>		
<i>Leptomonas pyrrocoris</i>		
<i>Leptomonas seymouri</i>		
<i>Phytomonas</i> sp isolate EM1		
<i>Trypanosoma brucei brucei</i>		
<i>Trypanosoma brucei gambiense</i>		
<i>Trypanosoma congolense</i> IL3000		
<i>Trypanosoma cruzi</i> CLBrener		
<i>Trypanosoma cruzi</i> Dm28c		
<i>Trypanosoma cruzi</i> ID25		
<i>Trypanosoma cruzi marinkellei</i>		
<i>Trypanosoma equiperdum</i>		
<i>Trypanosoma grayi</i>		
<i>Trypanosoma rangeli</i> SC58		
<i>Trypanosoma theileri</i>		
<i>Trypanosoma vivax</i> Y486		
<i>Trichomonas vaginalis</i> G3		
<i>Tritrichomonas foetus</i>		
<i>Monocercomonoides</i>		
<i>Ectocarpus siliculosus</i>		
<i>Thalassiosira pseudonana</i>		
<i>Fragilariopsis cylindrus</i>		
<i>Nannochloropsis gaditana</i>		
<i>Thraustotheca clavata</i>		
<i>Phaeodactylum tricornutum</i>		
<i>Thalassiosira oceanica</i>		
<i>Aureococcus anophagefferens</i>		
<i>Leptocylindrus danicus</i>		
<i>Odontella aurita</i>		
<i>Nitzschia</i> RCC80		
<i>Bolidomonas pacifica</i>		
<i>Fistulifera solaris</i>		
<i>Tetrahymena thermophila</i>		
<i>Paramecium tetraurelia</i>		
<i>Stylonychia lemnae</i>		
<i>Protoceratium reticulatum</i>		
<i>Noctiluca scintillans</i>		
<i>Togula jolla</i>		

SAR

<i>Polarella glacialis</i>		
<i>Amphidinium carterae</i>		
<i>Oxytricha trifallax</i>		
<i>Stentor coeruleus</i>		
<i>Bigelowiella natans</i>		
<i>Reticulomyxa filosa</i>		
<i>Elphidium margaritaceum</i>		
<i>Cryptophyceae sp</i>		<b>Cryptista</b>
<i>Guillardia theta</i>		
<i>Goniomonas avonlea</i>		
<i>Emiliana huxleyi</i>		<b>Haptista</b>
<i>Chrysochromulina sp</i>		
<i>Raphidiophrys heterophryoidea</i>		
<i>Acanthocystis sp</i>		
<i>Choanocystis sp</i>		
<i>Raineriophrys erinaceoides</i>		
<i>Allomyces macrogynus</i>		<b>Obazoa</b>
<i>Schizosaccharomyces pombe</i>		
<i>Spizellomyces punctatus</i>		
<i>Lobosporangium transversale</i>		
<i>Parvularia atlantis</i>		
<i>Fonticula alba</i>		
<i>Amphimedon queenslandica</i>		
<i>Limulus polyphemus</i>		
<i>Xenopus tropicalis</i>		
<i>Nematostella vectensis</i>		
<i>Lottia gigantea</i>		
<i>Monosiga brevicollis</i>		
<i>Salpingoeca rosetta</i>		
<i>Gefionella okellyi</i>		<b>Malawimonadidae</b>
<i>Ancoracysta twisti</i>		<b>Janouskovec</b>
<i>Ancyromonas sigmoides</i>		<b>Eukaryota incertae sedis; Ancoracysta</b>

<i>Fabomonas tropica</i>		<b>Ancyromonadida; Planomonadidae; Fabomonas</b>
<i>Nutomonas longa</i>		<b>Ancyromonadida; Nutomonas</b>
<i>Diphylleia rotans</i>		<b>CRuMs; Collodictyonidae; Diphylleia</b>
<i>Rigifila ramosa</i>		<b>CRuMs; Rigifilida; Rigifila</b>
<i>Candidatus Berkelbacteria bacterium</i> GWA2_46_7		<b>CPR Berkelbacteria</b>
<i>Candidatus Berkelbacteria bacterium</i> GWA2_35_9		<b>CPR Berkelbacteria</b>
<i>Candidatus Berkelbacteria bacterium</i> GWA2_38_9		<b>CPR Berkelbacteria</b>
<i>Candidatus Berkelbacteria bacterium</i> GWAE1_39_12		<b>CPR Berkelbacteria</b>
<i>Candidatus Berkelbacteria bacterium</i> CG1_02_42_45		<b>CPR Berkelbacteria</b>
<i>Candidatus Berkelbacteria bacterium</i> CG2_30_39_44		<b>CPR Berkelbacteria</b>
<i>Candidatus Berkelbacteria bacterium</i> CG2_30_43_20		<b>CPR Berkelbacteria</b>
<i>Candidatus Berkelbacteria bacterium</i> RIFCSPLOWO2_01_FULL_50_28		<b>CPR Berkelbacteria</b>
<i>Candidatus Berkelbacteria bacterium</i> RIFOXYA2_FULL_43_10		<b>CPR Berkelbacteria</b>
<i>Candidatus Candidate division WS6</i> <i>bacterium</i> GW2011_GWA2_37_6	<b>Bacteria</b>	<b>CPR Dojkabacteria</b>
<i>Candidate division WS6 bacterium</i> GW2011_GWF1_35_23		<b>CPR Dojkabacteria</b>
<i>Candidate division Kazan bacterium</i> GW2011_GWA1_50_15		<b>CPR Kazan</b>
<i>Candidate division Kazan bacterium</i> GW2011_GWA1_44_22		<b>CPR Kazan</b>
<i>Candidatus Amesbacteria bacterium</i> RIFOXYB1_FULL_44_23		<b>CPR Microgenomates</b>
<i>Candidatus Beckwithbacteria bacterium</i> GW2011_GWC1_49_16		<b>CPR Microgenomates</b>
<i>Candidatus Collierbacteria bacterium</i> RIFOXYB1_FULL_49_13		<b>CPR Microgenomates</b>
<i>Candidatus Curtissbacteria bacterium</i> RBG_16_39_7		<b>CPR Microgenomates</b>
<i>Candidatus Daviesbacteria bacterium</i> RIFCSPHIGHO2_12_FULL_37_16		<b>CPR Microgenomates</b>

<i>Candidatus Gottesmanbacteria bacterium</i> GW2011_GWA1_43_11		<b>CPR Microgenomates</b>
<i>Microgenomates group bacterium</i> GW2011_GWA2_46_16		<b>CPR Microgenomates</b>
<i>Candidatus Woykebacteria bacterium</i> GWB1_45_5		<b>CPR Microgenomates</b>
<i>Candidatus Levybacteria bacterium</i> RIFCSPHIGHO2_02_FULL_37_10		<b>CPR Microgenomates</b>
<i>Candidatus Pacebacteria bacterium</i> CG1_02_43_31		<b>CPR Microgenomates</b>
<i>Candidatus Woykebacteria bacterium</i> RBG_13_40_15		<b>CPR Microgenomates</b>
<i>Candidatus Woykebacteria bacterium</i> RBG_13_40_7b		<b>CPR Microgenomates</b>
<i>Candidatus Roizmanbacteria bacterium</i> RIFCSPLOWO2_01_FULL_45_11		<b>CPR Microgenomates</b>
<i>Candidatus Shapirobacteria bacterium</i> GW2011_GWF2_37_20		<b>CPR Microgenomates</b>
<i>Candidatus Woesebacteria bacterium</i> GW2011_GWD2_40_19		<b>CPR Microgenomates</b>
<i>Candidatus Woesebacteria bacterium</i> RIFCSPHIGHO2_01_FULL_41_10		<b>CPR Microgenomates</b>
<i>Candidatus Falkowbacteria bacterium</i> GW2011_GWE2_38_254		<b>CPR Parcubacteria</b>
<i>Candidatus Giovannonibacteria bacterium</i> RIFCSPHIGHO2_02_43_16		<b>CPR Parcubacteria</b>
<i>Candidatus Jorgensenbacteria bacterium</i> GWA1_54_12		<b>CPR Parcubacteria</b>
<i>Candidatus Jorgensenbacteria bacterium</i> RIFCSPHIGHO2_02_FULL_45_20		<b>CPR Parcubacteria</b>
<i>Candidatus Kaiserbacteria bacterium</i> RIFCSPHIGHO2_01_FULL_56_24		<b>CPR Parcubacteria</b>
<i>Parcubacteria bacterium</i> SCGC AAA011-A09		<b>CPR Parcubacteria</b>
<i>Candidatus Magasanikbacteria bacterium</i> GW2011_GWA2_45_39		<b>CPR Parcubacteria</b>
<i>Candidatus Magasanikbacteria bacterium</i> GW2011_GWA2_46_17		<b>CPR Parcubacteria</b>
<i>Candidatus Moranbacteria bacterium</i> RIFCSPHIGHO2_01_FULL_55_24		<b>CPR Parcubacteria</b>
<i>Candidatus Moranbacteria bacterium</i> GW2011_GWC2_37_8		<b>CPR Parcubacteria</b>
<i>Candidatus Nomurabacteria bacterium</i> CG1_02_31_12		<b>CPR Parcubacteria</b>
<i>Candidatus Nomurabacteria bacterium</i> RIFCSPLOWO2_01_FULL_36_10b		<b>CPR Parcubacteria</b>

<i>Candidatus Uhrbacteria bacterium</i> RIFCSPLOWO2_02_FULL_49_11		<b>CPR Parcubacteria</b>
<i>Candidatus Uhrbacteria bacterium</i> RIFCSPLOWO2_02_FULL_54_37		<b>CPR Parcubacteria</b>
<i>Candidatus Uhrbacteria bacterium</i> RIFOXYC2_FULL_47_19		<b>CPR Parcubacteria</b>
<i>Candidatus Wolfbacteria bacterium</i> RIFOXYB1_FULL_54_12		<b>CPR Parcubacteria</b>
<i>Candidatus Yanofskybacteria bacterium</i> RIFCSPHIGHO2_01_FULL_44_17		<b>CPR Parcubacteria</b>
<i>Candidatus Peregrinibacteria bacterium</i> CG1_02_41_10		<b>CPR Peregrinibacteria</b>
<i>Candidatus Peregrinibacteria bacterium</i> CG1_02_54_53		<b>CPR Peregrinibacteria</b>
<i>Candidatus Saccharibacteria bacterium</i> CG2_30_41_52		<b>CPR Saccharibacteria</b>
<i>Candidatus Saccharibacteria bacterium</i> RIFCSPHIGHO2_12_FULL_49_19		<b>CPR Saccharibacteria</b>
<i>Candidate division WWE3 bacterium</i> RIFCSPLOWO2_01_FULL_42_11		<b>CPR WWE3</b>
<i>Chloroherpeton thalassium</i> ATCC 35110		<b>FBC Chlorobi</b>
<i>Gemmatimonadetes bacterium</i> RIFCSPLOWO2_12_FULL_68_9		<b>FBC Gemmatimonadetes</b>
<i>Ignavibacteria bacterium</i> GWA2_55_25		<b>FBC Ignavibacteria</b>
<i>Candidate division Zixibacteria bacterium</i> SM23_73_2		<b>FBC Zixibacteria</b>
<i>Thermithiobacillus tepidarius</i> DSM 3134		<b>Proteobacteria</b> <b>Acidithiobacillia</b>
<i>Kordiimonas gwangyangensis</i> DSM 19435		<b>Proteobacteria</b> <b>Alphaproteobacteria</b>
<i>Pelagibacterium halotolerans</i> B2		<b>Proteobacteria</b> <b>Alphaproteobacteria</b>
<i>Rhodobacteraceae bacterium</i> CG2_30_10_405		<b>Proteobacteria</b> <b>Alphaproteobacteria</b>
<i>Granulibacter bethesdensis</i> CGDNIH1		<b>Proteobacteria</b> <b>Alphaproteobacteria</b>
<i>Tistrella mobilis</i> KA081020 65		<b>Proteobacteria</b> <b>Alphaproteobacteria</b>
<i>Anaplasma marginale</i>		<b>Proteobacteria</b> <b>Alphaproteobacteria</b>
<i>Citromicrobium</i> sp. JLT1363		<b>Proteobacteria</b> <b>Alphaproteobacteria</b>
<i>Bordetella bronchiseptica</i> RB50		<b>Proteobacteria</b> <b>BetaProteobacteria</b>

<i>Comamonas testosteroni</i> TK102		<b>Proteobacteria</b> <b>BetaProteobacteria</b>
<i>Gallionella capsiferiformans</i> ES 2		<b>Proteobacteria</b> <b>BetaProteobacteria</b>
<i>Methylovorus</i> sp. SIP3 4		<b>Proteobacteria</b> <b>BetaProteobacteria</b>
<i>Deefgea rivuli</i> DSM 18356		<b>Proteobacteria</b> <b>BetaProteobacteria</b>
<i>Bacteriovorax marinus</i> SJ		<b>Proteobacteria</b> <b>Deltaproteobacteria</b>
<i>Desulfotignum phosphitoxidans</i> FiPS 3		<b>Proteobacteria</b> <b>Deltaproteobacteria</b>
<i>Myxococcus xanthus</i> DK 1622		<b>Proteobacteria</b> <b>Deltaproteobacteria</b>
<i>Syntrophus aciditrophicus</i> SB		<b>Proteobacteria</b> <b>Deltaproteobacteria</b>
<i>Lebetimonas</i> sp. JS032		<b>Proteobacteria</b> <b>Epsilonproteobacteria</b>
<i>Anaerobiospirillum succiniciproducens</i> DSM 6400		<b>Proteobacteria</b> <b>Gammaproteobacteria</b>
<i>Haliea rubra</i> CM41 15a DSM		<b>Proteobacteria</b> <b>Gammaproteobacteria</b>
<i>Arhodomonas aquaeolei</i> DSM 8974		<b>Proteobacteria</b> <b>Gammaproteobacteria</b>
<i>Arsenophonus nasoniae</i> DSM 15247		<b>Proteobacteria</b> <b>Gammaproteobacteria</b>
<i>Legionella micdadei</i> ATCC 33218		<b>Proteobacteria</b> <b>Gammaproteobacteria</b>
<i>Methyloglobulus morosus</i> KoM1		<b>Proteobacteria</b> <b>Gammaproteobacteria</b>
<i>Saccharospirillum impatiens</i> DSM 12546		<b>Proteobacteria</b> <b>Gammaproteobacteria</b>
<i>Alkanindiges illinoisensis</i> DSM 15370		<b>Proteobacteria</b> <b>Gammaproteobacteria</b>
<i>Photobacterium profundum</i> SS9		<b>Proteobacteria</b> <b>Gammaproteobacteria</b>
<i>Frateuria terrea</i> CGMCC 1.7053		<b>Proteobacteria</b> <b>Gammaproteobacteria</b>
<i>Mariprofundus ferrooxydans</i> M34		<b>Proteobacteria</b> <b>Zetaproteobacteria</b>
<i>RIFCSPLOWO2 2 FULL 45 22</i>		<b>PVC Chlamydiae</b>
<i>Chlamydophila abortus</i> S263		<b>PVC Chlamydiae</b>
<i>Lentisphaerae bacterium</i> GWF2_52_8		<b>PVC Lentisphaerae</b>
<i>Candidatus Omnitrophica bacterium</i> CG1_02_46_14		<b>PVC Omnitrophica</b>



<i>Planctomycetes bacterium</i> GWB2_41_19		<b>PVC Planctomycetes</b>
<i>Phycisphaerae bacterium</i> SM1_79		<b>PVC Plantomycetes</b>
<i>Arcanobacterium haemolyticum</i> DSM 20595		<b>Terrabacteria Actinobacteria</b>
<i>Corynebacterium argentoratense</i> DSM 44202		<b>Terrabacteria Actinobacteria</b>
<i>Gulosibacter molinativorax</i> DSM 13485		<b>Terrabacteria Actinobacteria</b>
<i>Aestuariimicrobium kwangyangense</i> DSM 21549		<b>Terrabacteria Actinobacteria</b>
<i>Bifidobacterium animalis animalis</i> ATCC 25527		<b>Terrabacteria Actinobacteria</b>
<i>Eggerthella</i> sp. YY7918		<b>Terrabacteria Actinobacteria</b>
<i>Rhodoluna laticola</i> MWH Ta8		<b>Terrabacteria Actinobacteria</b>
<i>Enorma massiliensis</i> phI		<b>Terrabacteria Actinobacteria</b>
<i>Anaerolinea thermophila</i> UNI-1		<b>Terrabacteria Chloroflexi</b>
<i>Dehalococcoidia bacterium</i> DG_22		<b>Terrabacteria Chloroflexi</b>
<i>Chloroflexi bacterium</i> RBG_13_50_10		<b>Terrabacteria Chloroflexi</b>
<i>Aphanizomenon flos aquae</i> NIES 81		<b>Terrabacteria Cyanobacteria</b>
<i>Crinalium epipsammum</i> PCC 9333		<b>Terrabacteria Cyanobacteria</b>
<i>Oscillatoria</i> sp. PCC 7112		<b>Terrabacteria Cyanobacteria</b>
<i>Stanieria cyanosphaera</i> PCC 7437		<b>Terrabacteria Cyanobacteria</b>
<i>Bacillus anthracis</i> 52 G		<b>Terrabacteria Firmicutes</b>
<i>Marinococcus halotolerans</i> DSM 16375		<b>Terrabacteria Firmicutes</b>
<i>Fructobacillus fructosus</i> KCTC 3544		<b>Terrabacteria Firmicutes</b>
<i>Streptococcus mutans</i> GS 5		<b>Terrabacteria Firmicutes</b>
<i>Youngiibacter fragile</i> 232.1		<b>Terrabacteria Firmicutes</b>
<i>Filifactor alocis</i> ATCC 35896		<b>Terrabacteria Firmicutes</b>
<i>Orenia marismortui</i> DSM 5156		<b>Terrabacteria Firmicutes</b>
<i>Veillonella parvula</i> DSM 2008		<b>Terrabacteria Firmicutes</b>
<i>Melainabacteria bacterium</i> MEL_A1		<b>Terrabacteria Melainabacteria</b>
<i>Candidatus Melainabacteria bacterium</i> RIFCSPLOWO2_12_FULL_35_11		<b>Terrabacteria Melainabacteria</b>
<i>Mesoplasma florum</i> W37		<b>Terrabacteria Tenericutes</b>
<i>Thermosynechococcus elongatus</i> BP1		<b>Terrabacteria Cyanobacteria</b>

<i>Deinococcus geothermalis</i> DSM 11300		<b>Terrabacteria</b> <b>Deinococcus-Thermus</b>
<i>Acidobacteria bacterium</i> RBG_16_68_9		<b>Acidobacteria</b>
<i>Acidobacteria bacterium</i> RIFCSPLOWO2_02_FULLL_68_18		<b>Acidobacteria</b>
<i>Acidobacterium</i> sp. MP5ACTX8		<b>Acidobacteria</b>
<i>Holophaga foetida</i> TMBS4 DSM 6591		<b>Acidobacteria</b>
<i>Candidatus Aminicenantes bacterium</i> RBG_16_66_30		<b>Aminicenantes</b>
<i>Hydrogenobacter thermophilus</i> TK 6		<b>Aquificae</b>
<i>Thermovibrio ammonificans</i> HB 1		<b>Aquificae</b>
<i>Armatimonadetes bacterium</i> 13_1_40CM_64_14		<b>Armatimonadetes</b>
<i>Bacteroides fragilis</i> NCTC 9343		<b>Bacteroidetes</b>
<i>Marinilabilia salmonicolor</i> JCM 21150		<b>Bacteroidetes</b>
<i>Cyclobacterium marinum</i> DSM 745		<b>Bacteroidetes</b>
<i>Indibacter alkaliphilus</i> LW1		<b>Bacteroidetes</b>
<i>Epilithonimonas tenax</i> DSM 16811		<b>Bacteroidetes</b>
<i>Galbibacter</i> sp. ck I2 15		<b>Bacteroidetes</b>
<i>Gracilimonas tropica</i> DSM 19535		<b>Bacteroidetes</b>
<i>Arcticibacter svalbardensis</i> MN12 7		<b>Bacteroidetes</b>
<i>Chrysiogenes arsenatis</i> DSM 11915		<b>Chrysiogenetes</b>
<i>Denitrovibrio acetiphilus</i> DSM 12809		<b>Deferribacteres</b>
<i>Fusobacterium nucleatum nucleatum</i> ATCC 25586		<b>Fusobacteria</b>
<i>Candidatus Gracilibacteria bacterium</i> CG1_02_38_174		<b>Gracilibacteria</b>
<i>Candidatus Vecturithrix granuli</i>		<b>Modulibacteria</b>
<i>Nitrospinae bacterium</i> RIFCSPLOWO2_12_FULLL_47_7		<b>Nitrospinae</b>
<i>Nitrospirae bacterium</i> GWC2_57_9		<b>Nitrospirae</b>
<i>Nitrospira defluvii</i>		<b>Nitrospirae</b>
<i>Brachyspira murdochii</i> DSM 12563		<b>Spirochaetes</b>
<i>Leptospira biflexa</i> serovar Patoc strain 'Patoc 1 (Paris)'		<b>Spirochaetes</b>
<i>Thermanaerovibrio acidaminovorans</i> DSM 6589		<b>Synergistetes</b>
<i>Thermodesulfatator indicus</i> CIR29812 DSM 15286		<b>Thermodesulfobacteria</b>
<i>Kosmotoga olearia</i> TBF 19.5.1		<b>Thermotogae</b>
<i>Candidatus Wirthbacteria bacterium</i> CG2 30_54_11		<b>Wirthbacteria</b>

<i>Heimdallarchaeota</i> LC2	<b>Archaea</b>	<b>Asgard group Candidatus Heimdallarchaeota</b>
<i>Heimdallarchaeota</i> LC3		<b>Asgard group Candidatus Heimdallarchaeota</b>
<i>Lokiarchaeum mirabilis</i>		<b>Asgard group Candidatus Lokiarchaeota</b>
<i>Odinarchaeota</i> LCB4		<b>Asgard group Candidatus Odinarchaeota</b>
<i>Thorarchaeota</i> AB25		<b>Asgard group Candidatus Thorarchaeota</b>
<i>Candidatus Aenigmarchaeota</i> CG_4_10_14_3_um_filter_37_21		<b>DPANN group Candidatus Aenigmarchaeota</b>
<i>Candidatus Aenigmarchaeota</i> archaeon CG01_land_8_20_14_3_00_37_9		<b>DPANN group Candidatus Aenigmarchaeota</b>
<i>Candidatus Diapherotrites</i> archaeon CG11_big_fil_rev_8_21_14_0_20_37_9		<b>DPANN group Candidatus Diapherotrite</b>
archaeon GW2011_AR10 ( <i>Diapherotrites</i> archaeon AR10?)		<b>DPANN group Candidatus Diapherotrite</b>
<i>Candidatus Huberarchaea</i> <i>crystalense</i>		<b>DPANN group Candidatus Huberarchaea</b>
<i>Candidatus Micrarchaeum acidiphilum</i> ARMAN-2		<b>DPANN group Candidatus Micrarchaeota</b>
<i>Candidatus Micrarchaeota</i> archaeon A_DKE		<b>DPANN group Candidatus Micrarchaeota</b>
<i>Candidatus Micrarchaeota</i> archaeon <i>Mia14</i>		<b>DPANN group Candidatus Micrarchaeota</b>
<i>Candidatus Nanosalina</i>		<b>DPANN group Candidatus Nanohaloarchaeota</b>
<i>Candidatus Nanosalinarum</i> (sp. J07AB56)		<b>DPANN group Candidatus Nanohaloarchaeota</b>
<i>Candidatus Nanopetramus</i> SG9		<b>DPANN group Candidatus Nanohaloarchaeota</b>
archaeon GW2011_AR13		<b>DPANN group Candidatus Pacearchaeota</b>
archaeon GW2011_AR1		<b>DPANN group Candidatus Pacearchaeota</b>
archaeon GW2011_AR6		<b>DPANN group Candidatus Pacearchaeota</b>
<i>Candidatus Parvarchaeum acidiphilum</i> ARMAN-4		<b>DPANN group Candidatus Parvarchaeota</b>
<i>Candidatus Parvarchaeum acidophilus</i> ARMAN-5		<b>DPANN group Candidatus Parvarchaeota</b>
archaeon GW2011_AR15		<b>DPANN group Candidatus Woesearchaeota</b>
archaeon GW2011_AR20		<b>DPANN group Candidatus Woesearchaeota</b>

<i>archaeon GW2011_AR4</i>		<b>DPANN group Candidatus Woesearchaeota</b>
<i>Nanoarchaeum equitans</i>		<b>DPANN group Nanoarchaeota</b>
<i>Candidatus Nanobsidianus stetteri</i>		<b>DPANN group Nanoarchaeota</b>
<i>Candidatus Nanopusillus acidilobi</i>		<b>DPANN group Nanoarchaeota</b>
<i>Candidatus Altiarchaeum CG_4_9_14_0_8_um_filter_32_206</i>		<b>DPANN group Candidatus Altiarchaeales</b>
<i>CG_SM1 (Alt 1) - Candidatus Altiarchaeum hamiconexum</i>		<b>DPANN group Candidatus Altiarchaeales</b>
<i>WOR_SM1_SCG (Alt 2)</i>		<b>DPANN group Candidatus Altiarchaeales</b>
<i>MSI_SMI (Alt 1) - Candidatus Altiarchaeum hamiconexum</i>		<b>DPANN group Candidatus Altiarchaeales</b>
<i>Candidatus Altiarchaeales ex4484_2</i>		<b>DPANN group Candidatus Altiarchaeales</b>
<i>Aciduliprofundum boonei (T469)</i>		<b>Euryarchaeota Aciduliprofundum boonei</b>
<i>Archaeoglobus fulgidus (DSM 4304)</i>		<b>Euryarchaeota Archaeoglobi</b>
<i>Archaeoglobus sulfaticallidus</i>		<b>Euryarchaeota Archaeoglobi</b>
<i>Hadesarchaea archaeon YNP_N21</i>		<b>Euryarchaeota Hadesarchaea</b>
<i>Hadesarchaea YNP_45</i>		<b>Euryarchaeota Hadesarchaea</b>
<i>Natronomonas pharaonis (DSM 2160)</i>		<b>Euryarchaeota Halobacteria</b>
<i>Haloarcula marismortui</i>		<b>Euryarchaeota Halobacteria</b>
<i>Halobacterium salinarum NRC-1</i>		<b>Euryarchaeota Halobacteria</b>
<i>Halomicrobium mukohataei</i>		<b>Euryarchaeota Halobacteria</b>
<i>Haloferax volcanii</i>		<b>Euryarchaeota Halobacteria</b>
<i>Natronococcus amylolyticus</i>		<b>Euryarchaeota Halobacteria</b>
<i>Natronobacterium gregoryi (SP2)</i>		<b>Euryarchaeota Halobacteria</b>
<i>Methanobacterium bryantii</i>		<b>Euryarchaeota Methanobacteria</b>
<i>Methanobacterium formicicum</i>		<b>Euryarchaeota Methanobacteria</b>

<i>Methanobacterium</i> sp. 42_16		<b>Euryarchaeota</b> <b>Methanobacteria</b>
<i>Methanobacterium subterraneum</i>		<b>Euryarchaeota</b> <b>Methanobacteria</b>
<i>Methanothermobacter marburgensis</i>		<b>Euryarchaeota</b> <b>Methanobacteria</b>
<i>Methanothermobacter thermoautotrophicus</i>		<b>Euryarchaeota</b> <b>Methanobacteria</b>
<i>Methanobrevibacter arboriphilus</i>		<b>Euryarchaeota</b> <b>Methanobacteria</b>
<i>Methanobrevibacter cuticularis</i>		<b>Euryarchaeota</b> <b>Methanobacteria</b>
<i>Methanobrevibacter filiformis</i>		<b>Euryarchaeota</b> <b>Methanobacteria</b>
<i>Methanobrevibacter oralis</i> JMR01		<b>Euryarchaeota</b> <b>Methanobacteria</b>
<i>Methanobrevibacter millerae</i>		<b>Euryarchaeota</b> <b>Methanobacteria</b>
<i>Methanobrevibacter ruminantium</i>		<b>Euryarchaeota</b> <b>Methanobacteria</b>
<i>Methanobrevibacter smithii</i>		<b>Euryarchaeota</b> <b>Methanobacteria</b>
<i>Methanosphaera cuniculi</i>		<b>Euryarchaeota</b> <b>Methanobacteria</b>
<i>Methanosphaera stadtmannae</i>		<b>Euryarchaeota</b> <b>Methanobacteria</b>
<i>Methanosphaera</i> sp. WGK6		<b>Euryarchaeota</b> <b>Methanobacteria</b>
<i>Methanothermus fervidus</i>		<b>Euryarchaeota</b> <b>Methanobacteria</b>
<i>Methanocaldococcus jannaschii</i>		<b>Euryarchaeota</b> <b>Methanococci</b>
<i>Methanotorrus formicicus</i>		<b>Euryarchaeota</b> <b>Methanococci</b>
<i>Methanococcus maripaludis</i>		<b>Euryarchaeota</b> <b>Methanococci</b>
<i>Methanothermococcus thermolithotrophicus</i>		<b>Euryarchaeota</b> <b>Methanococci</b>
<i>Methanocella arvoryzae</i> (Rice Cluster I)		<b>Euryarchaeota</b> <b>Methanomicrobia</b>
<i>Methanocella paludicola</i> (SANA E)		<b>Euryarchaeota</b> <b>Methanomicrobia</b>
<i>Methanocorpusculum parvum</i>		<b>Euryarchaeota</b> <b>Methanomicrobia</b>
<i>Methanocorpusculum labreanum</i>		<b>Euryarchaeota</b> <b>Methanomicrobia</b>

<i>Methanoculleus bourgensis</i>		<b>Euryarchaeota</b> <b>Methanomicrobia</b>
<i>Methanospirillum hungatei</i>		<b>Euryarchaeota</b> <b>Methanomicrobia</b>
<i>Candidatus Methanoperedens nitroreducens</i> (ANME-2d)		<b>Euryarchaeota</b> <b>Methanomicrobia</b>
<i>Methanotherix soehngenii</i>		<b>Euryarchaeota</b> <b>Methanomicrobia</b>
<i>Methanotherix thermoacetophila</i> (PT)		<b>Euryarchaeota</b> <b>Methanomicrobia</b>
<i>Methanococcoides methylutens</i>		<b>Euryarchaeota</b> <b>Methanomicrobia</b>
<i>Methanohalophilus mahii</i>		<b>Euryarchaeota</b> <b>Methanomicrobia</b>
<i>Methanosarcina acetivorans</i>		<b>Euryarchaeota</b> <b>Methanomicrobia</b>
<i>Methanosarcina barkeri str. Fusaro</i>		<b>Euryarchaeota</b> <b>Methanomicrobia</b>
<i>Methanosarcina barkeri</i> CM1		<b>Euryarchaeota</b> <b>Methanomicrobia</b>
<i>Methanosarcina mazei</i>		<b>Euryarchaeota</b> <b>Methanomicrobia</b>
<i>Methanosarcina spelaei</i>		<b>Euryarchaeota</b> <b>Methanomicrobia</b>
<i>Methermicoccus shengliensis</i>		<b>Euryarchaeota</b> <b>Methanomicrobia</b>
<i>Methanosarcinales archaeon ex4484_138</i> (GoM-ArcI)		<b>Euryarchaeota</b> <b>Methanomicrobia</b>
<i>Methanosarcinales archaeon ex4572_44</i> (GoM-ArcI)		<b>Euryarchaeota</b> <b>Methanomicrobia</b>
<i>Candidatus Syntrophoarchaeum caldarius</i> (ANME-2 - GoM-Arch87-2)		<b>Euryarchaeota</b> <b>Methanomicrobia</b>
ANME-2 cluster archaeon HR1		<b>Euryarchaeota</b> <b>Methanomicrobia</b>
ANME-1 cluster archaeon ex4572_4		<b>Euryarchaeota</b> <b>Methanomicrobia</b>
Arc I group archaeon U1Isi0528_Bin055		<b>Euryarchaeota</b> <b>Methanomicrobia</b>
<i>Methanonatronarchaeum thermophilum</i>		<b>Euryarchaeota</b> <b>Methanonatronarchaeia</b>
<i>Methanopyrus kandleri</i> AV19		<b>Euryarchaeota</b> <b>Methanopyri</b>
<i>Methanopyrus KOL6</i>		<b>Euryarchaeota</b> <b>Methanopyri</b>
<i>Theionarchaea archaeon DG-70-1</i>		<b>Euryarchaeota</b> <b>Theionarchaea</b>

<i>Theionarchaea archaeon DG-70</i>		<b>Euryarchaeota Theionarchaea</b>
<i>Pyrococcus furiosus</i>		<b>Euryarchaeota Thermococci</b>
<i>Pyrococcus abyssi</i>		<b>Euryarchaeota Thermococci</b>
<i>Pyrococcus horikoshii</i>		<b>Euryarchaeota Thermococci</b>
<i>Thermococcus kodakarensis</i>		<b>Euryarchaeota Thermococci</b>
<i>Methanomassiliicoccus luminyensis</i>		<b>Euryarchaeota Thermoplasmata</b>
<i>Candidatus Methanomassiliicoccus intestinalis</i>		<b>Euryarchaeota Thermoplasmata</b>
<i>Candidatus Methanoplasma termitum</i>		<b>Euryarchaeota Thermoplasmata</b>
<i>Acidiplasma aeolicum</i>		<b>Euryarchaeota Thermoplasmata</b>
<i>Ferroplasma acidarmanus fer1</i>		<b>Euryarchaeota Thermoplasmata</b>
<i>Picrophilus torridus</i>		<b>Euryarchaeota Thermoplasmata</b>
<i>Thermoplasma volcanium</i>		<b>Euryarchaeota Thermoplasmata</b>
<i>Thermoplasmatales archaeon SCGC AB-539-N05</i>		<b>Euryarchaeota Thermoplasmata</b>
<i>Thermoplasmatales archaeon B_DKE</i>		<b>Euryarchaeota Thermoplasmata</b>
<i>Cuniculiplasma sp. C_DKE</i>		<b>Euryarchaeota Thermoplasmata</b>
<i>uncultured marine group II euryarchaeote</i>		<b>Euryarchaeota unclassified</b>
<i>Marine group III euryarchaeote CG-Epi2</i>		<b>Euryarchaeota unclassified</b>
<i>MSBL1 archaeon SCGC-AAA259E19</i>		<b>Euryarchaeota unclassified</b>
<i>MCG-1</i>		<b>TACK group Candidatus Bathyarchaeota</b>
<i>MCG-6</i>		<b>TACK group Candidatus Bathyarchaeota</b>
<i>MCG-15</i>		<b>TACK group Candidatus Bathyarchaeota</b>
<i>Candidatus Bathyarchaeota archaeon BA2</i>		<b>TACK group Candidatus Bathyarchaeota</b>
<i>Geothermarchaeota (ex4572_27)</i>		<b>TACK group Candidatus Geothermarchaeota</b>

<i>Candidatus Korarchaeum cryptofilum</i>		<b>TACK group Candidatus Korarchaeota</b>
<i>Methanomethylicus mesodigestum</i> V2		<b>TACK group Candidatus Verstraetearchaeota</b>
<i>Methanosuratus petracarbonis</i> V4		<b>TACK group Candidatus Verstraetearchaeota</b>
<i>Acidilobus saccharovorans</i>		<b>TACK group Crenarchaeota</b>
<i>Aeropyrum camini</i>		<b>TACK group Crenarchaeota</b>
<i>Aeropyrum pernix</i> (K1)		<b>TACK group Crenarchaeota</b>
<i>Hyperthermus butylicus</i>		<b>TACK group Crenarchaeota</b>
<i>Ignisphaera aggregans</i> (DSM 17230)		<b>TACK group Crenarchaeota</b>
<i>Ignicoccus hospitalis</i>		<b>TACK group Crenarchaeota</b>
<i>Ignicoccus islandicus</i> (DSM 13165)		<b>TACK group Crenarchaeota</b>
<i>Staphylothermus marinus</i>		<b>TACK group Crenarchaeota</b>
<i>Fervidicoccus fontis</i>		<b>TACK group Crenarchaeota</b>
<i>Sulfolobus solfataricus</i>		<b>TACK group Crenarchaeota</b>
<i>Caldivirga maquilingensis</i>		<b>TACK group Crenarchaeota</b>
<i>Pyrobaculum aerophilum</i> (str. IM2)		<b>TACK group Crenarchaeota</b>
<i>Thermofilum pendens</i>		<b>TACK group Crenarchaeota</b>
<i>Candidatus Caldiarchaeum subterraneum</i>		<b>TACK group Aigarchaeota</b>
<i>Cenarchaeum symbiosum</i> A		<b>TACK group Thaumarchaeota</b>
<i>Nitrosopumilus koreensis</i>		<b>TACK group Thaumarchaeota</b>
<i>Nitrosopumilus maritimus</i> SCM1		<b>TACK group Thaumarchaeota</b>
<i>Nitrosopumilus limnia</i>		<b>TACK group Thaumarchaeota</b>
<i>Candidatus Nitrosomarinus catalina</i>		<b>TACK group Thaumarchaeota</b>
<i>Candidatus Nitrososphaera gargensis</i>		<b>TACK group Thaumarchaeota</b>
<i>Candidatus Nitrocosmicus oleophilus</i>		<b>TACK group Thaumarchaeota</b>



<i>Candidatus Nitrosocaldus islandicus</i>		<b>TACK group Thaumarchaeota</b>
<i>Marine group I thaumarchaeote SCGC RSA3</i>		<b>TACK group Thaumarchaeota</b>
<i>Thaumarchaeota archaeon SCGC AB-539-E09</i>		<b>TACK group Thaumarchaeota</b>
<i>Thaumarchaeota archaeon BS4 (Candidatus Nitrosocaldus cavascurensis)</i>		<b>TACK group Thaumarchaeota</b>
<i>Thaumarchaeota Fn1</i>		<b>TACK group Thaumarchaeota</b>
<i>Candidatus Marsarchaeota G2 archaeon BE_D</i>		<b>unclassified</b>
<i>Candidatus Marsarchaeota G1 archaeon OSP_C</i>		<b>unclassified</b>

**Table S2: BUSCO analysis for *P. keilini* and selected other parabasalids and metamonads.**

Phylum	Species	Data type	Complete	Fragmented	Missing	Source
Anaeramoebae	<i>Anaeramoeba flamelloides</i>	genome	47	26	27	(Stairs et al., 2021)
Anaeramoebae	<i>Anaeramoeba ignava</i>	genome	61	15	24	(Stairs et al., 2021)
Fornicata	<i>Dysnectes brevis</i>	transcriptome	42	14	44	PRJNA315708
Fornicata	<i>Giardia intestinalis (2020)</i>	genome	24	6	70	(Stairs et al., 2021)
Fornicata	<i>Kipferlia bialata</i>	genome	39	11	50	(Tanifuji et al., 2018), PRJDB5223
Fornicata	<i>Trepomonas sp.</i>	transcriptome	20	6	74	(Stairs et al., 2021)
Parabasalia	<i>Cononympha_leidyi (3 single cells)</i>	transcriptome	22	10	68	(Nishimura et al., 2020), PRJDB8546
Parabasalia	<i>Dientamoeba fragilis</i>	transcriptome	49	13	38	(Barratt et al., 2015), PRJNA284312
Parabasalia	<i>Histomonas meleagridis</i>	transcriptome	49	8	43	PRJEB19109

Parabasalialia	<i>Holomastigotoides hartmanni</i> (3 single cells)	transcriptome	19	13	68	(Nishimura et al., 2020), PRJDB8546
Parabasalialia	<i>Holomastigotoides minor</i> (3 single cells)	transcriptome	20	12	68	(Nishimura et al., 2020), PRJDB8546
Parabasalialia	<i>Pentatrichomonas hominis</i>	transcriptome	50	8	42	(Handrich et al., 2019), PRJNA340083
Parabasalialia	<i>Pseudotrichomonas keilini</i>	transcriptome	47	11	42	this study
Parabasalialia	<i>Pseudotrichonympha grassi</i> (3 single cells)	transcriptome	36	8	56	(Nishimura et al., 2020), PRJDB8546
Parabasalialia	<i>Trichomitus batrachorum</i>	transcriptome	47	10	43	(Handrich et al., 2019), PRJNA340083
Parabasalialia	<i>Trichomonas gallinae</i>	transcriptome	43	9	48	(Handrich et al., 2019), PRJNA340083
Parabasalialia	<i>Trichomonas tenax</i>	transcriptome	48	8	44	(Handrich et al., 2019), PRJNA340083
Parabasalialia	<i>Trichomonas vaginalis</i>	transcriptome	53	6	41	(Stairs et al., 2021), PRJNA938429
Parabasalialia	<i>Tritrichomonas foetus</i>	transcriptome	49	6	45	(Morin-Adeline et al., 2014), SRX540117
Preaxostyla	<i>Monocercomonoides exilis</i>	genome	55	8	37	(Stairs et al., 2021)

**Table S3: List of proteins on the hydrogenosome surface.**

<b>Protein Accession Number</b>	<b>gene_ID</b>	<b>P_keilini protein header</b>	<b>function</b>
XP_001323739.1	TVAG_005 910	Pkeilini_DN667_c0_g2_i1.p1	50S-ribosomal-protein-L2,-putative
XP_001323765.1	TVAG_006 170	Pkeilini_DN989_c0_g1_i1.p1	60S-ribosomal-protein-L19,-putative
XP_001323773.1	TVAG_006 250	Pkeilini_DN915_c0_g1_i1.p1	30S-ribosomal-protein-S8,-putative
XP_001311950.1	TVAG_008 680	Pkeilini_DN1020_c0_g1_i1.p1	tubulin-epsilon-chain,-putative
XP_001582527.1	TVAG_013 060	Pkeilini_DN4044_c0_g1_i1.p1	60S-ribosomal-protein-L3,-putative
XP_001582635.1	TVAG_014 160	Pkeilini_DN645_c0_g1_i1.p2	60S-ribosomal-protein-L12,-putative
XP_001329886.1	TVAG_015 800	Pkeilini_DN460_c0_g1_i2.p1	60S-ribosomal-protein-L23,-putative
XP_001317481.1	TVAG_020 040	Pkeilini_DN3914_c0_g3_i1.p1	ribosomal-protein-S9,-putative
XP_001314734.1	TVAG_020 480	Pkeilini_DN508_c0_g2_i1.p1	40S-ribosomal-protein-S18,-putative
XP_001300897.1	TVAG_033 590	Pkeilini_DN670_c0_g1_i1.p1	40S-ribosomal-protein-S6,-putative
XP_001326922.1	TVAG_038 050	Pkeilini_DN414_c0_g1_i2.p1	50S-ribosomal-protein-L24p,-putative
XP_001315155.1	TVAG_040 820	Pkeilini_DN16934_c0_g1_i1.p2	40S-ribosomal-protein-S17,-putative
XP_001301101.1	TVAG_041 350	Pkeilini_DN3310_c1_g1_i1.p1	30S-40S-ribosomal-protein,-putative
XP_001312328.1	TVAG_043 060	Pkeilini_DN163620_c0_g1_i1.p1	fructose-bisphosphate-aldolase,-putative
XP_001315627.1	TVAG_043 500	Pkeilini_DN242_c0_g2_i1.p1	enolase,-putative
XP_001314691.1	TVAG_044 510	Pkeilini_DN842_c0_g2_i4.p1	heat-shock-protein-70-(HSP70)-4,-putative
XP_001330678.1	TVAG_044 560	Pkeilini_DN374_c0_g1_i1.p1	50S-ribosomal-protein-L5p,-putative
XP_001323300.1	TVAG_045 010	Pkeilini_DN206053_c0_g1_i1.p1	glucokinase,-putative

XP_001308576.1	TVAG_045 340	Pkeilini_DN420_c0_g1_i1.p1	polyadenylate-binding-protein,-putative
XP_001298952.1	TVAG_047 460	Pkeilini_DN207414_c0_g1_i1.p1	40S-ribosomal-protein-S3a,-putative
XP_001321104.1	TVAG_051 160	Pkeilini_DN182683_c0_g1_i1.p1	60S-acidic-ribosomal-protein-P0,-putative
XP_001306738.1	TVAG_054 130	Pkeilini_DN92336_c3_g1_i1.p1	60S-ribosomal-protein-L7,-putative
XP_001330619.1	TVAG_054 500	Pkeilini_DN198864_c0_g1_i1.p1	50S-ribosomal-protein-L6p,-putative
XP_001308434.1	TVAG_054 830	Pkeilini_DN184234_c0_g1_i1.p1	phosphoglucomutase,-putative
XP_001323508.1	TVAG_061 890	Pkeilini_DN161037_c0_g1_i1.p1	60S-ribosomal-protein-L18a,-putative
XP_001323512.1	TVAG_061 930	Pkeilini_DN218798_c0_g1_i1.p1	glucose-6-phosphate-isomerase,-putative
XP_001320140.1	TVAG_064 640	Pkeilini_DN170420_c1_g1_i4.p1	ribosomal-protein-L5,-putative
XP_001318569.1	TVAG_066 030	Pkeilini_DN1889_c0_g1_i1.p1	40S-ribosomal-protein-S8,-putative
XP_001328958.1	TVAG_067 400	Pkeilini_DN828_c0_g1_i2.p1	elongation-factor-1-alpha,-putative
XP_001584514.1	TVAG_071 920	Pkeilini_DN103_c0_g1_i1.p1	40S-ribosomal-protein-S23,-putative
XP_001321243.1	TVAG_073 860	Pkeilini_DN2499_c0_g3_i2.p1	phosphoenolpyruvate-protein-phosphotransferase,-putative
XP_001324843.1	TVAG_074 480	Pkeilini_DN283_c0_g1_i2.p1	60S-ribosomal-protein-L10a,-putative
XP_001324856.1	TVAG_074 610	Pkeilini_DN1754_c0_g1_i2.p1	60S-ribosomal-protein-L10,-putative
XP_001320891.1	TVAG_079 260	Pkeilini_DN9_c0_g1_i2.p1	phosphofructokinase,-putative
XP_001579459.1	TVAG_083 260	Pkeilini_DN570_c0_g4_i1.p1	60S-ribosomal-protein-L17,-putative
XP_001318159.1	TVAG_087 140	Pkeilini_DN173700_c0_g1_i1.p1	arp2/3,-putative
XP_001304619.1	TVAG_092 750	Pkeilini_DN690_c0_g1_i1.p1	glucokinase
XP_001303733.1	TVAG_094 720	Pkeilini_DN605_c0_g1_i1.p2	60S-ribosomal-protein-L34,-putative

XP_001321933.1	TVAG_098 450	Pkeilini_DN218705_c0_g1_i1.p 1	40S-ribosomal-protein-S4,-putative
XP_001303193.1	TVAG_099 490	Pkeilini_DN184416_c0_g1_i1.p 1	glucose-kinase,-putative
XP_001580437.1	TVAG_101 690	Pkeilini_DN165121_c0_g1_i1.p 1	60S-ribosomal-protein-L24,-putative
XP_001324008.1	TVAG_106 800	Pkeilini_DN1152_c0_g1_i1.p1	30S-ribosomal-protein-S3,-putative
XP_001581388.1	TVAG_110 140	Pkeilini_DN167_c0_g3_i5.p2	ubiquitin,-putative
XP_001300910.1	TVAG_112 230	Pkeilini_DN79301_c0_g2_i1.p1	60S-ribosomal-protein-L13,-putative
XP_001579013.1	TVAG_113 710	Pkeilini_DN54853_c0_g1_i1.p2	phosphoglycerate-mutase,-putative
XP_001324949.1	TVAG_117 480	Pkeilini_DN330_c0_g1_i3.p1	30S-ribosomal-protein-S11,-putative
XP_001276845.1	TVAG_119 330	Pkeilini_DN19_c0_g6_i1.p1	60S-ribosomal-protein-L21,-putative
XP_001276908.1	TVAG_119 970	Pkeilini_DN208907_c0_g1_i1.p 1	conserved-hypothetical-protein
XP_001276929.1	TVAG_120 180	Pkeilini_DN9217_c0_g1_i1.p1	40S-ribosomal-protein-S10,-putative
XP_001277020.1	TVAG_121 100	Pkeilini_DN426_c0_g1_i2.p1	60S-ribosomal-protein-L18,-putative
XP_001322974.1	TVAG_121 550	Pkeilini_DN1040_c0_g1_i1.p1	60S-ribosomal-protein-L27a,-putative
XP_001324688.1	TVAG_128 790	Pkeilini_DN68345_c0_g1_i2.p1	60S-ribosomal-protein-L4,-putative
XP_001324775.1	TVAG_139 320	Pkeilini_DN43315_c0_g1_i1.p1	heat-shock-protein,-putative
XP_001320113.1	TVAG_142 440	Pkeilini_DN3544_c0_g1_i2.p1	40S-ribosomal-protein-S2,-putative
XP_001579934.1	TVAG_146 910	Pkeilini_DN183802_c0_g1_i1.p 1	glyceraldehyde-3-phosphate-dehydrog enase,-putative
XP_001309550.1	TVAG_148 950	Pkeilini_DN639_c0_g1_i1.p1	50S-ribosomal-protein-L15e,-putative
XP_001309564.1	TVAG_149 090	Pkeilini_DN976_c0_g2_i4.p1	actin,-putative
XP_001303635.1	TVAG_152 720	Pkeilini_DN330_c0_g1_i3.p1	40S-ribosomal-protein-S14,-putative

XP_001317545.1	TVAG_153 560	Pkeilini_DN358_c0_g1_i4.p1	heat-shock-protein,-putative
XP_001313895.1	TVAG_154 680	Pkeilini_DN5492_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001310739.1	TVAG_157 940	Pkeilini_DN5492_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001580742.1	TVAG_178 000	Pkeilini_DN52_c0_g1_i2.p1	60S-ribosomal-protein-L23a,-putative
XP_001583987.1	TVAG_182 370	Pkeilini_DN69_c0_g3_i2.p1	chaperonin-containing-t-complex-protein-1,-gamma-subunit,-tcpg,-putative
XP_001584002.1	TVAG_182 520	Pkeilini_DN79301_c0_g2_i1.p1	60S-ribosomal-protein-L13,-putative
XP_001580136.1	TVAG_190 450	Pkeilini_DN1019_c0_g1_i1.p1	kakapo,-putative
XP_001320814.1	TVAG_191 140	Pkeilini_DN528_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001581272.1	TVAG_192 620	Pkeilini_DN206419_c0_g1_i1.p1	actin-depolymerizing-factor,-putative
XP_001308024.1	TVAG_204 360	Pkeilini_DN168_c0_g2_i1.p1	malate-dehydrogenase,-putative
XP_001308025.1	TVAG_204 370	Pkeilini_DN206053_c0_g1_i1.p1	glucokinase,-putative
XP_001303801.1	TVAG_205 910	Pkeilini_DN184234_c0_g1_i1.p1	phosphoglucomutase,-putative
XP_001319786.1	TVAG_212 020	Pkeilini_DN5548_c1_g1_i1.p1	transketolase,-putative
XP_001325073.1	TVAG_222 040	Pkeilini_DN5492_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001310373.1	TVAG_226 870	Pkeilini_DN5492_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001303253.1	TVAG_234 160	Pkeilini_DN219244_c0_g1_i1.p1	arp2/3-complex-20-kD-subunit,-putative
XP_001581543.1	TVAG_239 310	Pkeilini_DN1019_c0_g2_i2.p1	bollus-pemphigoid-antigen,-putative
XP_001320867.1	TVAG_240 050	Pkeilini_DN516_c0_g1_i1.p1	40S-ribosomal-protein-sa,-putative
XP_001323701.1	TVAG_248 450	Pkeilini_DN1700_c0_g1_i1.p1	peptidyl-tRNA-hydrolase,-putative
XP_001579239.1	TVAG_253 650	Pkeilini_DN168_c0_g2_i1.p1	malate-dehydrogenase,-putative

XP_001323043.1	TVAG_258 220	Pkeilini_DN7684_c0_g1_i1.p1	glycosyltransferase,-putative
XP_001317828.1	TVAG_263 740	Pkeilini_DN242_c0_g1_i2.p1	enolase,-putative
XP_001313821.1	TVAG_265 950	Pkeilini_DN56722_c0_g1_i1.p1	60S-ribosomal-protein-L32,-putative
XP_001579758.1	TVAG_268 050	Pkeilini_DN220_c0_g1_i1.p1	phosphoglycerate-kinase,-putative
XP_001330352.1	TVAG_272 970	Pkeilini_DN362_c0_g1_i1.p1	40S-ribosomal-protein-S24,-putative
XP_001321781.1	TVAG_276 310	Pkeilini_DN217279_c1_g1_i1.p1	starch-branching-enzyme-II,-putative
XP_001321791.1	TVAG_276 410	Pkeilini_DN3469_c0_g2_i1.p1	translation-elongation-factor,-putative
XP_001304599.1	TVAG_277 390	Pkeilini_DN160592_c0_g1_i1.p2	peptidyl-prolyl-cis-trans-isomerase-A,-putative
XP_001582074.1	TVAG_282 580	Pkeilini_DN52882_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001582118.1	TVAG_283 020	Pkeilini_DN587_c0_g1_i1.p1	initiation-factor-5A,-putative
XP_001330357.1	TVAG_292 580	Pkeilini_DN16084_c3_g1_i1.p1	immunophilin,-putative
XP_001307577.1	TVAG_293 770	Pkeilini_DN160642_c0_g1_i1.p1	phosphofructokinase,-putative
XP_001315498.1	TVAG_299 380	Pkeilini_DN330_c0_g1_i3.p1	30S-ribosomal-protein-S11,-putative
XP_001311055.1	TVAG_300 000	Pkeilini_DN163620_c0_g1_i1.p1	fructose-bisphosphate-aldolase,-putative
XP_001304306.1	TVAG_319 220	Pkeilini_DN362_c0_g2_i1.p1	40S-ribosomal-protein-S24,-putative
XP_001322282.1	TVAG_329 460	Pkeilini_DN242_c0_g2_i1.p1	enolase,-putative
XP_001317525.1	TVAG_336 940	Pkeilini_DN690_c0_g1_i1.p1	glucokinase,-putative
XP_001319342.1	TVAG_342 830	Pkeilini_DN330_c0_g1_i3.p1	40S-ribosomal-protein-S14/30S-ribosomal-protein-S11,-putative
XP_001328722.1	TVAG_348 090	Pkeilini_DN207414_c0_g1_i1.p1	40S-ribosomal-protein-S3a,-putative
XP_001328746.1	TVAG_348 330	Pkeilini_DN921_c0_g2_i1.p1	glycogen-phosphorylase,-putative

XP_001326306.1	TVAG_351 310	Pkeilini_DN351_c0_g1_i1.p1	plastin,-putative
XP_001308720.1	TVAG_354 020	Pkeilini_DN160502_c0_g1_i1.p1	contractin,-putative
XP_001307153.1	TVAG_360 700	Pkeilini_DN163620_c0_g1_i1.p1	fructose-bisphosphate-aldolase,-putative
XP_001307814.1	TVAG_370 550	Pkeilini_DN670_c0_g1_i1.p1	40S-ribosomal-protein-S6,-putative
XP_001305702.1	TVAG_373 720	Pkeilini_DN2724_c0_g1_i10.p2	pyruvate-kinase,-putative
XP_001306345.1	TVAG_376 130	Pkeilini_DN16874_c0_g1_i1.p3	gelsolin,-putative
XP_001304373.1	TVAG_380 910	Pkeilini_DN600_c0_g3_i1.p1	DEAD-box-ATP-dependent-RNA-helicase,-putative
XP_001295243.1	TVAG_381 030	Pkeilini_DN382_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001302740.1	TVAG_381 690	Pkeilini_DN208349_c0_g1_i1.p1	NAD-dependent-epimerase/dehydratase,-putative
XP_001314248.1	TVAG_383 940	Pkeilini_DN220_c0_g1_i1.p1	phosphoglycerate-kinase,-putative
XP_001581778.1	TVAG_391 760	Pkeilini_DN450_c0_g1_i1.p2	phosphofructokinase,-putative
XP_001327217.1	TVAG_397 250	Pkeilini_DN690_c0_g1_i1.p1	glucokinase,-putative
XP_001328502.1	TVAG_423 320	Pkeilini_DN79301_c0_g2_i1.p1	60S-ribosomal-protein-L13,-putative
XP_001325139.1	TVAG_430 830	Pkeilini_DN9_c0_g1_i2.p1	phosphofructokinase,-putative
XP_001302863.1	TVAG_435 000	Pkeilini_DN16084_c3_g1_i1.p1	conserved-hypothetical-protein
XP_001313948.1	TVAG_442 070	Pkeilini_DN184416_c0_g1_i1.p1	glucose-kinase,-putative
XP_001579592.1	TVAG_462 920	Pkeilini_DN160642_c0_g1_i1.p1	phosphofructokinase,-putative
XP_001325501.1	TVAG_464 120	Pkeilini_DN330_c0_g1_i1.p1	30S-ribosomal-protein-S11,-putative
XP_001325506.1	TVAG_464 170	Pkeilini_DN242_c0_g2_i1.p1	enolase,-putative
XP_001322192.1	TVAG_482 430	Pkeilini_DN426_c0_g1_i2.p1	60S-ribosomal-protein-L18,-putative



XP_001322532.1	TVAG_491 670	Pkeilini_DN207881_c0_g1_i1.p 1	malic-enzyme,-putative
XP_001305512.1	TVAG_496 160	Pkeilini_DN211320_c0_g1_i1.p 1	phosphofructokinase,-putative

**Table S4: List of putative membrane proteins, enzymes and hypothetical proteins identified in the hydrogenosome**

Protein Accession Number	gene_ID	P_keilini protein header	function
XP_001584373.1	TVAG_070500	Pkeilini_DN58243_c4_g1_i1.p1	Rab7g-protein,-putative
XP_001584268.1	TVAG_185900	Pkeilini_DN211195_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001584214.1	TVAG_185340	Pkeilini_DN171252_c0_g1_i1.p1	Rab32,-putative
XP_001584210.1	TVAG_185300	Pkeilini_DN25505_c0_g1_i1.p1	Rab11,-putative
XP_001584188.1	TVAG_185080	Pkeilini_DN175067_c0_g1_i1.p1	ran,-putative
XP_001584134.1	TVAG_183850	Pkeilini_DN5301_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001584128.1	TVAG_183790	Pkeilini_DN568_c0_g1_i1.p1	malic-enzyme,-putative
XP_001584099.1	TVAG_183500	Pkeilini_DN541_c0_g1_i1.p1	succinate-thiokinase- $\zeta$ -subunit
XP_001584080.1	TVAG_183300	Pkeilini_DN194471_c0_g1_i1.p1	aminotransferase-class-V,-putative
XP_001584012.1	TVAG_182620	Pkeilini_DN13155_c3_g1_i1.p1	nitrate,-fromate,-iron-dehydrogenase,-putative
XP_001583906.1	TVAG_076670	Pkeilini_DN1671_c1_g1_i1.p1	small-GTPase-RAB,-putative
XP_001583890.1	TVAG_076510	Pkeilini_DN175_c0_g1_i1.p1	2-amino-3-ketobutyrate-coenzyme-A-ligase,-putative
XP_001583862.1	TVAG_076230	Pkeilini_DN584_c0_g1_i1.p1	nucleotide-binding-protein,-putative
XP_001583785.1	TVAG_075460	Pkeilini_DN206_c3_g1_i1.p1	Rab5b,-putative

XP_001583584.1	TVAG_036230	Pkeilini_DN26_c4_g1_i1.p1	RAB,-putative
XP_001583562.1	TVAG_036010	Pkeilini_DN160973_c0_g1_i1.p1	A-type-flavoprotein
XP_001583303.1	TVAG_377960	Pkeilini_DN26_c4_g1_i1.p1	Rab8,-putative
XP_001583118.1	TVAG_093060	Pkeilini_DN877_c0_g2_i1.p1	Rabx30-protein,-putative
XP_001583042.1	TVAG_456910	Pkeilini_DN941_c2_g5_i1.p1	Rabx32-protein,-putative
XP_001583028.1	TVAG_456770	Pkeilini_DN419_c0_g1_i1.p2	iron-sulfur-cluster-assembly-protein,-putative
XP_001582848.1	TVAG_249220	Pkeilini_DN953_c0_g3_i1.p1	Rab2,-putative
XP_001582728.1	TVAG_237680	Pkeilini_DN208767_c1_g1_i1.p1	ADP,ATP-carrier-protein,-putative
XP_001582674.1	TVAG_237140	Pkeilini_DN842_c0_g4_i1.p1	heat-shock-protein,-putative
XP_001582391.1	TVAG_198430	Pkeilini_DN195283_c0_g1_i1.p1	14-3-3-protein-sigma,-gamma,-zeta,-beta/alpha,-putative
XP_001582383.1	TVAG_198350	Pkeilini_DN6701_c0_g1_i1.p2	hypothetical-protein
XP_001582360.1	TVAG_198110	Pkeilini_DN688_c0_g3_i1.p1	pyruvate-flavodoxin-oxidoreductase,-putative
XP_001582336.1	TVAG_167250	Pkeilini_DN392_c0_g1_i1.p1	chaperonin,-putative
XP_001582335.1	TVAG_167240	Pkeilini_DN16677_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001582155.1	TVAG_283380	Pkeilini_DN2717_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001581795.1	TVAG_391930	Pkeilini_DN184428_c0_g1_i1.p1	RAB,-putative
XP_001581497.1	TVAG_238830	Pkeilini_DN568_c0_g1_i1.p1	malic-enzyme,-putative
XP_001581254.1	TVAG_192440	Pkeilini_DN941_c3_g1_i1.p1	RAC,-putative
XP_001580948.1	TVAG_402160	Pkeilini_DN21606_c0_g1_i1.p1	guanine-nucleotide-exchange-factor,-putative
XP_001580911.1	TVAG_130330	Pkeilini_DN22962_c0_g1_i1.p1	conserved-hypothetical-protein

XP_001580780.1	TVAG_178380	Pkeilini_DN160711_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001580752.1	TVAG_178100	Pkeilini_DN194385_c0_g1_i1.p1	hypothetical-protein
XP_001580745.1	TVAG_178030	Pkeilini_DN160251_c0_g1_i1.p1	plasma-membrane-calcium-transporting-ATPase,-putative
XP_001580733.1	TVAG_177910	Pkeilini_DN1508_c0_g1_i1.p1	hypothetical-protein
XP_001580703.1	TVAG_433130	Pkeilini_DN285_c1_g1_i1.p1	heat-shock-protein,-putative
XP_001580655.1	TVAG_432650	Pkeilini_DN198613_c0_g1_i1.p1	nitrogen-fixation-protein-nifu,-putative
XP_001580601.1	TVAG_228780	Pkeilini_DN501_c0_g1_i1.p1	alcohol-dehydrogenase,-putative
XP_001580529.1	TVAG_136740	Pkeilini_DN184428_c0_g1_i1.p1	RAB,-putative
XP_001580481.1	TVAG_136260	Pkeilini_DN26_c5_g1_i1.p1	GTP-binding-protein-ypt10,-putative
XP_001580416.1	TVAG_101480	Pkeilini_DN12181_c0_g1_i1.p1	RAB,-putative
XP_001580394.1	TVAG_101260	Pkeilini_DN13976_c0_g1_i1.p1	Rab32,-putative
XP_001580191.1	TVAG_214300	Pkeilini_DN15514_c0_g1_i1.p2	conserved-hypothetical-protein
XP_001580148.1	TVAG_190580	Pkeilini_DN58662_c0_g1_i1.p1	Clan-MG,-family-M24,-aminopeptidase-P-like-metallopeptidase
XP_001580142.1	TVAG_190510	Pkeilini_DN4576_c0_g1_i1.p1	GTP-binding-protein-rit,-putative
XP_001580044.1	TVAG_247370	Pkeilini_DN184336_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001579755.1	TVAG_268020	Pkeilini_DN194634_c1_g1_i1.p1	aspartate-aminotransferase,-putative
XP_001579748.1	TVAG_267950	Pkeilini_DN207024_c0_g1_i1.p1	lysyl-tRNA-synthetase,-putative
XP_001579740.1	TVAG_267870	Pkeilini_DN269_c0_g1_i2.p1	malic-enzyme,-putative
XP_001579545.1	TVAG_462450	Pkeilini_DN676_c0_g2_i1.p1	RAB,-putative
XP_001579538.1	TVAG_462370	Pkeilini_DN14322_c0_g1_i3.p1	Rabx19-protein,-putative

XP_001579477.1	TVAG_083440	Pkeilini_DN112439_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001579475.1	TVAG_083420	Pkeilini_DN147688_c0_g2_i1.p1	conserved-hypothetical-protein
XP_001579216.1	TVAG_122960	Pkeilini_DN52162_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001579154.1	TVAG_122340	Pkeilini_DN1085_c1_g2_i1.p1	RAB,-putative
XP_001579147.1	TVAG_122270	Pkeilini_DN3020_c0_g2_i1.p1	RAB,-putative
XP_001579030.1	TVAG_113880	Pkeilini_DN13377_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001579029.1	TVAG_113870	Pkeilini_DN182694_c0_g1_i1.p1	Acetyl-CoA-hydrolase,-putative
XP_001578953.1	TVAG_225930	Pkeilini_DN28069_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001276882.1	TVAG_119710	Pkeilini_DN22730_c0_g1_i1.p1	Clan-ME,-family-M16,-insulinase-like-metallopeptidase
XP_001330794.1	TVAG_320200	Pkeilini_DN3462_c0_g2_i1.p1	Rab5b,-putative
XP_001330618.1	TVAG_054490	Pkeilini_DN201059_c0_g1_i1.p1	Tryptophanase,-putative
XP_001330504.1	TVAG_383530	Pkeilini_DN177099_c0_g1_i1.p1	RAB,-putative
XP_001330829.1	TVAG_217400	Pkeilini_DN211716_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001330450.1	TVAG_047890	Pkeilini_DN626_c0_g1_i1.p1	succinate-thiokinase-a-subunit
XP_001314415.1	TVAG_090060	Pkeilini_DN941_c2_g4_i1.p2	GTP-binding-protein-Rab2,-putative
XP_001330332.1	TVAG_272760	Pkeilini_DN218827_c0_g1_i1.p1	glutathione-reductase,-putative
XP_001330320.1	TVAG_217870	Pkeilini_DN19306_c0_g1_i1.p2	nucleotide-binding-protein,-putative
XP_001330242.1	TVAG_325080	Pkeilini_DN183480_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001330232.1	TVAG_324980	Pkeilini_DN8882_c0_g2_i2.p2	ATP-synthase,-putative
XP_001330176.1	TVAG_395550	Pkeilini_DN182694_c0_g1_i1.p1	Acetyl-CoA-hydrolase,-putative

XP_001329730.1	TVAG_454230	Pkeilini_DN184377_c0_g1_i1.p1	Rab15,-13,-10,-1,-35,-5,-and,-putative
XP_001329309.1	TVAG_297650	Pkeilini_DN601_c0_g1_i1.p1	grpe-protein,-putative
XP_001329276.1	TVAG_297320	Pkeilini_DN26_c4_g1_i1.p1	small-GTPase-rabi,-putative
XP_001329126.1	TVAG_150540	Pkeilini_DN58243_c4_g1_i1.p1	RAB-2,4,14,-putative
XP_001329022.1	TVAG_447580	Pkeilini_DN69533_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001328863.1	TVAG_434770	Pkeilini_DN1107_c0_g1_i1.p1	Rab5b,-putative
XP_001328770.1	TVAG_348580	Pkeilini_DN1522_c0_g1_i1.p1	Rabx26-protein,-putative
XP_001328523.1	TVAG_423530	Pkeilini_DN4238_c0_g1_i1.p2	conserved-hypothetical-protein
XP_001328415.1	TVAG_340860	Pkeilini_DN12251_c0_g1_i1.p2	Rab7g-protein,-putative
XP_001328285.1	TVAG_278280	Pkeilini_DN26_c1_g1_i1.p2	Rabx22-protein,-putative
XP_001328194.1	TVAG_262210	Pkeilini_DN172140_c0_g1_i1.p1	tricarboxylate-transport-protein,-putative
XP_001328129.1	TVAG_165340	Pkeilini_DN626_c0_g1_i1.p1	succinate-thiokinase-a-subunit
XP_001328031.1	TVAG_159810	Pkeilini_DN953_c0_g5_i1.p2	small-GTPase-RAB,-putative
XP_001328023.1	TVAG_159730	Pkeilini_DN206_c0_g2_i2.p1	Rab78,-putative
XP_001327882.1	TVAG_405730	Pkeilini_DN7495_c0_g1_i1.p1	Rabx37-protein,-putative
XP_001327427.1	TVAG_201980	Pkeilini_DN7689_c0_g1_i1.p1	Rab15,-13,-10,-1,-35,-5,-and,-putative
XP_001327371.1	TVAG_392650	Pkeilini_DN781_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001327338.1	TVAG_392320	Pkeilini_DN199807_c0_g1_i1.p1	groes-chaperonin,-putative
XP_001327242.1	TVAG_019190	Pkeilini_DN75143_c0_g2_i1.p1	chaperone-protein-DNAj,-putative
XP_001327227.1	TVAG_397350	Pkeilini_DN160922_c0_g1_i1.p1	Rab9,-putative

XP_001326968.1	TVAG_038530	Pkeilini_DN196128_c0_g1_i1.p1	ornithine-carbamoyltransferase,-putative
XP_001326958.1	TVAG_038420	Pkeilini_DN5113_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001326942.1	TVAG_038250	Pkeilini_DN900_c0_g1_i1.p2	Rab12,-putative
XP_001326936.1	TVAG_038190	Pkeilini_DN8719_c0_g1_i1.p1	small-GTPase-rabi,-putative
XP_001326908.1	TVAG_461020	Pkeilini_DN3412_c0_g1_i1.p1	ABC-transporter,-putative
XP_001326833.1	TVAG_393370	Pkeilini_DN196009_c0_g1_i1.p1	Rab23,-putative
XP_001326755.1	TVAG_388650	Pkeilini_DN30752_c0_g1_i1.p1	serine-palmitoyltransferase-I,-putative
XP_001326629.1	TVAG_255980	Pkeilini_DN9830_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001326421.1	TVAG_373310	Pkeilini_DN85431_c0_g1_i1.p1	RAB,-putative
XP_001326329.1	TVAG_351540	Pkeilini_DN25815_c0_g1_i1.p1	2,4-dienoyl-CoA-reductase-[NADPH],-putative
XP_001326325.1	TVAG_351500	Pkeilini_DN196009_c0_g1_i1.p1	Rab7,-putative
XP_001326307.1	TVAG_351320	Pkeilini_DN17_c0_g2_i1.p1	purine-nucleoside-phosphorylase,-putative
XP_001326088.1	TVAG_044270	Pkeilini_DN494_c1_g1_i1.p1	RAB,-putative
XP_001326029.1	TVAG_468220	Pkeilini_DN13197_c0_g1_i1.p2	conserved-hypothetical-protein
XP_001325929.1	TVAG_371800	Pkeilini_DN80221_c1_g1_i1.p1	conserved-hypothetical-protein
XP_001325705.1	TVAG_206500	Pkeilini_DN3171_c0_g1_i1.p1	Hydroxylamine-reductase,-putative
XP_001325649.1	TVAG_424580	Pkeilini_DN175264_c0_g1_i1.p1	mevalonate-kinase,-putative
XP_001325272.1	TVAG_212310	Pkeilini_DN12195_c0_g1_i1.p1	Rabx21-protein,-putative
XP_001325171.1	TVAG_404940	Pkeilini_DN2224_c0_g1_i1.p1	RAB,-putative
XP_001324855.1	TVAG_074600	Pkeilini_DN195601_c0_g1_i1.p1	tyrosine-aminotransferase,-putative

XP_001324836.1	TVAG_074410	Pkeilini_DN1405_c0_g2_i1.p1	Rabx41-protein,-putative
XP_001324773.1	TVAG_139300	Pkeilini_DN111_c0_g1_i1.p1	phosphoenolpyruvate-carboxykinase,-putative
XP_001324770.1	TVAG_139270	Pkeilini_DN26_c1_g1_i1.p2	Rab10,-putative
XP_001324695.1	TVAG_128860	Pkeilini_DN211282_c0_g1_i1.p1	RAB-18,-putative
XP_001324545.1	TVAG_161280	Pkeilini_DN494_c0_g3_i2.p1	Rab32,-putative
XP_001324510.1	TVAG_160930	Pkeilini_DN15873_c0_g1_i1.p1	Periplasmic-[Fe]-hydrogenase,-putative
XP_001324271.1	TVAG_038950	Pkeilini_DN941_c0_g4_i1.p1	Rabx3-protein,-putative
XP_001324262.1	TVAG_038850	Pkeilini_DN184336_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001324197.1	TVAG_271570	Pkeilini_DN50689_c0_g1_i1.p1	equilibrative-nucleoside-transporter,-putative
XP_001324090.1	TVAG_362470	Pkeilini_DN7689_c0_g1_i1.p1	Rab17,-putative
XP_001323999.1	TVAG_106710	Pkeilini_DN726_c0_g3_i1.p1	conserved-hypothetical-protein
XP_001323967.1	TVAG_106390	Pkeilini_DN1297_c0_g1_i1.p1	RAB-36-and,-putative
XP_001323815.1	TVAG_081640	Pkeilini_DN46067_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001323810.1	TVAG_081590	Pkeilini_DN34893_c0_g2_i1.p1	Rab5b,-putative
XP_001323776.1	TVAG_006280	Pkeilini_DN9662_c1_g1_i1.p1	RAB,-putative
XP_001323774.1	TVAG_006260	Pkeilini_DN12195_c0_g3_i1.p1	GTP-binding-protein-ypt10,-putative
XP_001323750.1	TVAG_006020	Pkeilini_DN172145_c0_g1_i1.p1	vacuolar-ATP-synthase-subunit-ac39,-putative
XP_001323626.1	TVAG_379850	Pkeilini_DN1175_c2_g1_i1.p1	Rab32,-putative
XP_001323600.1	TVAG_379590	Pkeilini_DN3503_c0_g1_i1.p2	GTPase_rho,-putative
XP_001323596.1	TVAG_379550	Pkeilini_DN195601_c0_g1_i1.p1	tyrosine-aminotransferase,-putative

XP_001323527.1	TVAG_343980	Pkeilini_DN164738_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001323468.1	TVAG_127520	Pkeilini_DN407_c0_g1_i1.p1	N-ethylmaleimide-reductase,-putative
XP_001323391.1	TVAG_498620	Pkeilini_DN403_c0_g1_i1.p1	ankyrin-repeat-containing-protein,-putative
XP_001323367.1	TVAG_498380	Pkeilini_DN66760_c1_g1_i1.p1	Rab20,-putative
XP_001323255.1	TVAG_410350	Pkeilini_DN1857_c0_g1_i1.p2	conserved-hypothetical-protein
XP_001323219.1	TVAG_446990	Pkeilini_DN187988_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001323218.1	TVAG_446980	Pkeilini_DN187988_c0_g1_i1.p1	Rab9,-putative
XP_001323182.1	TVAG_446610	Pkeilini_DN2285_c0_g2_i1.p1	small-GTPase-rabd,-putative
XP_001322993.1	TVAG_121740	Pkeilini_DN171252_c0_g1_i1.p1	Rab5,-putative
XP_001322920.1	TVAG_364210	Pkeilini_DN14322_c0_g1_i1.p1	Rabx37-protein,-putative
XP_001322907.1	TVAG_157610	Pkeilini_DN953_c0_g5_i1.p2	rho4,-putative
XP_001322830.1	TVAG_282070	Pkeilini_DN497_c1_g2_i1.p1	Rab8,-putative
XP_001322821.1	TVAG_281980	Pkeilini_DN197200_c0_g1_i1.p1	centrosomal-protein-of-135-kDa,-putative
XP_001322722.1	TVAG_484100	Pkeilini_DN953_c0_g4_i2.p1	Rabx26-protein,-putative
XP_001322593.1	TVAG_109540	Pkeilini_DN41445_c0_g1_i1.p1	serine-hydroxymethyltransferase,-putative
XP_001322449.1	TVAG_118780	Pkeilini_DN108422_c0_g1_i1.p1	calmodulin,-putative
XP_001322271.1	TVAG_329350	Pkeilini_DN7422_c1_g1_i1.p2	Rab5,-putative
XP_001322074.1	TVAG_259320	Pkeilini_DN676_c0_g1_i2.p1	Rabx18-protein,-putative
XP_001322061.1	TVAG_259190	Pkeilini_DN541_c0_g2_i1.p1	succinate-thiokinase- $\gamma$ -subunit
XP_001321827.1	TVAG_056480	Pkeilini_DN941_c2_g5_i1.p1	ran,-putative



XP_001321627.1	TVAG_420260	Pkeilini_DN183133_c0_g1_i1.p1	ATP-synthase-beta-subunit,-putative
XP_001321621.1	TVAG_420200	Pkeilini_DN608_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001321561.1	TVAG_395100	Pkeilini_DN1052_c0_g1_i1.p1	Rab17,-putative
XP_001321469.1	TVAG_133030	Pkeilini_DN257_c0_g1_i1.p2	NADH-ubiquinone-oxidoreductase-flavoprotein,-putative
XP_001321321.1	TVAG_230580	Pkeilini_DN688_c0_g3_i1.p1	pyruvate-flavodoxin-oxidoreductase,-putative
XP_001321292.1	TVAG_180430	Pkeilini_DN953_c0_g2_i1.p1	RAB-36-and,-putative
XP_001321289.1	TVAG_180400	Pkeilini_DN173495_c0_g1_i1.p1	Phospholipase-C-precursor,-putative
XP_001320926.1	TVAG_079630	Pkeilini_DN124701_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001320922.1	TVAG_079570	Pkeilini_DN211774_c0_g1_i1.p1	Rabx19-protein,-putative
XP_001320829.1	TVAG_239660	Pkeilini_DN176_c0_g1_i1.p1	cysteine-desulfurylase,-putative
XP_001320436.1	TVAG_308190	Pkeilini_DN174_c0_g1_i1.p1	ral,-putative
XP_001320125.1	TVAG_064490	Pkeilini_DN235_c0_g1_i1.p1	rubrerythrin,-putative
XP_001319879.1	TVAG_430220	Pkeilini_DN3256_c0_g1_i1.p1	RAB,-putative
XP_001319653.1	TVAG_419720	Pkeilini_DN171937_c0_g1_i1.p1	aspartate-aminotransferase,-putative
XP_001319283.1	TVAG_311860	Pkeilini_DN65569_c0_g2_i1.p1	conserved-hypothetical-protein
XP_001319140.1	TVAG_057110	Pkeilini_DN182581_c0_g1_i1.p1	Clan-SC,-family-S33,-methylesterase-like-serine-peptidase
XP_001319122.1	TVAG_056930	Pkeilini_DN14322_c0_g1_i1.p1	RAB-36-and,-putative
XP_001319084.1	TVAG_340390	Pkeilini_DN842_c0_g4_i1.p1	heat-shock-protein-70-(HSP70)-4,-putative
XP_001319083.1	TVAG_340380	Pkeilini_DN91169_c0_g1_i2.p1	conserved-hypothetical-protein
XP_001319074.1	TVAG_340290	Pkeilini_DN269_c0_g1_i2.p1	malic-enzyme,-putative

XP_001318961.1	TVAG_310250	Pkeilini_DN111_c0_g1_i1.p1	phosphoenolpyruvate-carboxykinase,-putative
XP_001318941.1	TVAG_310050	Pkeilini_DN13155_c3_g1_i1.p1	nitrate,-fromate,-iron-dehydrogenase,-putative
XP_001318893.1	TVAG_211200	Pkeilini_DN953_c0_g8_i1.p1	Rabx21-protein,-putative
XP_001318848.1	TVAG_056190	Pkeilini_DN725_c1_g1_i1.p1	Clan-MH,-family-M20,-peptidase-T-like-metallopeptidase
XP_001318715.1	TVAG_257310	Pkeilini_DN941_c2_g3_i1.p1	Rab21,-putative
XP_001318541.1	TVAG_065750	Pkeilini_DN104585_c0_g1_i1.p1	sucrose-transport-protein,-putative
XP_001318394.1	TVAG_098820	Pkeilini_DN195601_c0_g1_i1.p1	tyrosine-aminotransferase,-putative
XP_001318376.1	TVAG_068130	Pkeilini_DN269_c0_g1_i2.p1	malic-enzyme,-putative
XP_001317955.1	TVAG_100550	Pkeilini_DN144216_c0_g1_i1.p1	glycine-cleavage-system-H-protein,-putative
XP_001317694.1	TVAG_286490	Pkeilini_DN3462_c4_g1_i1.p1	RAB,-putative
XP_001317473.1	TVAG_019960	Pkeilini_DN317_c0_g2_i3.p1	conserved-hypothetical-protein
XP_001317292.1	TVAG_191660	Pkeilini_DN184795_c0_g1_i1.p1	groes-chaperonin,-putative
XP_001317232.1	TVAG_416520	Pkeilini_DN1175_c2_g1_i1.p1	RAB-18,-putative
XP_001317174.1	TVAG_040030	Pkeilini_DN1216_c0_g1_i1.p1	Iron-sulfur-flavoprotein
XP_001317169.1	TVAG_039980	Pkeilini_DN108_c0_g2_i1.p3	superoxide-dismutase,-putative
XP_001317041.1	TVAG_226310	Pkeilini_DN474_c2_g1_i1.p1	conserved-hypothetical-protein
XP_001316822.1	TVAG_233350	Pkeilini_DN50982_c0_g1_i1.p1	Clan-ME,-family-M16,-insulinase-like-metallopeptidase
XP_001316713.1	TVAG_453070	Pkeilini_DN258_c0_g1_i1.p1	Rab2,-putative
XP_001316606.1	TVAG_350580	Pkeilini_DN941_c0_g1_i1.p1	Rab21,-putative
XP_001316281.1	TVAG_203620	Pkeilini_DN392_c0_g1_i1.p1	rubisco-subunit-binding-protein-alpha-subunit,-ruba,-putative

XP_001316041.1	TVAG_454570	Pkeilini_DN1322_c0_g1_i1.p1	Embryonic-protein-DC-8,-putative
XP_001315530.1	TVAG_193770	Pkeilini_DN953_c0_g5_i1.p2	RAB,-putative
XP_001315422.1	TVAG_049830	Pkeilini_DN266_c0_g1_i1.p1	disulfide-oxidoreductase,-putative
XP_001315408.1	TVAG_049690	Pkeilini_DN217534_c0_g1_i1.p1	thiamin-pyrophosphokinase,-putative
XP_001315345.1	TVAG_328940	Pkeilini_DN160822_c0_g1_i1.p1	alcohol-dehydrogenase,-putative
XP_001315025.1	TVAG_008100	Pkeilini_DN1297_c0_g1_i1.p1	Rabx38-protein,-putative
XP_001314995.1	TVAG_370000	Pkeilini_DN12195_c0_g1_i1.p1	Rabx30-protein,-putative
XP_001314846.1	TVAG_260830	Pkeilini_DN5872_c0_g2_i1.p1	conserved-hypothetical-protein
XP_001314811.1	TVAG_254890	Pkeilini_DN688_c0_g3_i1.p1	pyruvate-flavodoxin-oxidoreductase,-putative
XP_001314747.1	TVAG_020610	Pkeilini_DN1975_c0_g1_i1.p2	RAB-18,-putative
XP_001314705.1	TVAG_055550	Pkeilini_DN3462_c0_g1_i1.p1	Rab8,-putative
XP_001314029.1	TVAG_412220	Pkeilini_DN269_c0_g1_i2.p1	malic-enzyme,-putative
XP_001313967.1	TVAG_442270	Pkeilini_DN4169_c0_g1_i1.p1	GTP-binding-protein-yptv3,-putative
XP_001313958.1	TVAG_442170	Pkeilini_DN38635_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001313802.1	TVAG_265760	Pkeilini_DN407_c0_g1_i1.p1	N-ethylmaleimide-reductase,-putative
XP_001313682.1	TVAG_096630	Pkeilini_DN217534_c0_g1_i1.p1	thiamin-pyrophosphokinase,-putative
XP_001313584.1	TVAG_208470	Pkeilini_DN160426_c0_g1_i1.p1	threonyl-tRNA-synthetase,-putative
XP_001313551.1	TVAG_181000	Pkeilini_DN206_c0_g5_i1.p1	RAB-19,-41-and,-putative
XP_001313356.1	TVAG_114310	Pkeilini_DN4486_c0_g1_i1.p1	peroxiredoxins,-prx-1,-prx-2,-prx-3,-putative
XP_001313153.1	TVAG_257780	Pkeilini_DN272_c2_g3_i1.p1	biotin-synthase,-putative

XP_001312991.1	TVAG_196220	Pkeilini_DN207877_c0_g1_i1.p1	protein-brittle-1,-chloroplast-precursor,-putative
XP_001312927.1	TVAG_029020	Pkeilini_DN1080_c0_g1_i1.p1	small-GTPase-rabh,-putative
XP_001312785.1	TVAG_424920	Pkeilini_DN66760_c1_g1_i1.p1	RAB-3-and,-putative
XP_001312753.1	TVAG_088050	Pkeilini_DN392_c0_g1_i1.p1	chaperonin,-putative
XP_001312620.1	TVAG_468600	Pkeilini_DN182200_c0_g1_i1.p1	WD-repeat-protein,-putative
XP_001312469.1	TVAG_241150	Pkeilini_DN26_c1_g1_i1.p2	Rabx24-protein,-putative
XP_001312254.1	TVAG_152430	Pkeilini_DN159673_c0_g1_i1.p1	lysyl-tRNA-synthetase,-putative
XP_001312198.1	TVAG_112840	Pkeilini_DN187988_c0_g1_i1.p1	Rabx15-protein,-putative
XP_001312168.1	TVAG_296220	Pkeilini_DN160340_c0_g1_i1.p1	NADH-dehydrogenase-24-kDa-subunit,-putative
XP_001312142.1	TVAG_236570	Pkeilini_DN4000_c0_g1_i1.p1	Rab32,-putative
XP_001312073.1	TVAG_115470	Pkeilini_DN195996_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001311960.1	TVAG_008790	Pkeilini_DN71584_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001311873.1	TVAG_321030	Pkeilini_DN195918_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001311871.1	TVAG_321010	Pkeilini_DN49090_c0_g2_i1.p1	AMP-dependent-ligase/synthetase,-putative
XP_001311861.1	TVAG_242960	Pkeilini_DN688_c0_g3_i1.p1	pyruvate-flavodoxin-oxidoreductase,-putative
XP_001311772.1	TVAG_409800	Pkeilini_DN207636_c0_g1_i1.p1	RAB,-putative
XP_001311734.1	TVAG_479760	Pkeilini_DN47132_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001311148.1	TVAG_263350	Pkeilini_DN3153_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001311109.1	TVAG_047800	Pkeilini_DN2125_c0_g1_i1.p1	Rab11,-putative
XP_001310420.1	TVAG_335500	Pkeilini_DN17664_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001310180.1	TVAG_037570	Pkeilini_DN13155_c0_g4_i1.p1	NADH-ubiquinone-oxidoreductase,-putative

XP_001310176.1	TVAG_037530	Pkeilini_DN8689_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001309934.1	TVAG_440200	Pkeilini_DN164467_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001309798.1	TVAG_390750	Pkeilini_DN26408_c0_g3_i1.p2	RAB-18,-putative
XP_001309776.1	TVAG_470110	Pkeilini_DN197003_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001309717.1	TVAG_216900	Pkeilini_DN953_c0_g9_i1.p1	ras,-putative
XP_001309656.1	TVAG_075320	Pkeilini_DN3946_c0_g1_i1.p2	vacuolar-proton-ATPase,-putative
XP_001309521.1	TVAG_018050	Pkeilini_DN217915_c0_g1_i1.p2	RAB-18,-putative
XP_001309408.1	TVAG_277590	Pkeilini_DN160251_c0_g1_i1.p1	cation-transporting-ATPase,-putative
XP_001309398.1	TVAG_024790	Pkeilini_DN494_c0_g1_i1.p2	septum-promoting-GTP-binding-protein,-putative
XP_001309295.1	TVAG_337970	Pkeilini_DN106_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001309218.1	TVAG_264120	Pkeilini_DN34504_c0_g3_i1.p1	pecanex,-putative
XP_001309182.1	TVAG_205390	Pkeilini_DN350_c0_g1_i2.p1	GTPase-mss1/trme,-putative
XP_001308879.1	TVAG_077910	Pkeilini_DN4238_c0_g1_i1.p2	conserved-hypothetical-protein
XP_001308636.1	TVAG_256470	Pkeilini_DN217534_c0_g1_i1.p1	thiamin-pyrophosphokinase,-putative
XP_001308553.1	TVAG_047210	Pkeilini_DN2207_c0_g1_i1.p1	2-hydroxyacid-dehydrogenase,-putative
XP_001308528.1	TVAG_459470	Pkeilini_DN1975_c0_g1_i1.p2	Rab9,-putative
XP_001308237.1	TVAG_032090	Pkeilini_DN622_c0_g1_i1.p1	Co-chaperone-protein-HscB-Hsc20,-mitochondrial-precursor,-putative
XP_001308201.1	TVAG_051830	Pkeilini_DN3462_c4_g1_i1.p1	RAC,-putative
XP_001308200.1	TVAG_051820	Pkeilini_DN172140_c0_g1_i1.p1	tricarboxylate-transport-protein,-putative
XP_001308167.1	TVAG_336320	Pkeilini_DN3171_c0_g2_i1.p1	Hydroxylamine-reductase,-putative

XP_001307912.1	TVAG_048600	Pkeilini_DN1721_c0_g1_i1.p1	small-GTPase-rabi,-putative
XP_001307775.1	TVAG_049140	Pkeilini_DN108_c0_g2_i1.p3	superoxide-dismutase-[fe],-putative
XP_001307690.1	TVAG_346230	Pkeilini_DN41178_c0_g1_i1.p2	conserved-hypothetical-protein
XP_001307488.1	TVAG_300910	Pkeilini_DN199171_c0_g1_i1.p1	Rabx18-protein,-putative
XP_001307405.1	TVAG_458060	Pkeilini_DN157931_c0_g2_i1.p1	conserved-hypothetical-protein
XP_001307320.1	TVAG_194760	Pkeilini_DN64940_c0_g1_i1.p1	guanine-nucleotide-exchange-factor,-putative
XP_001307251.1	TVAG_466790	Pkeilini_DN206198_c0_g1_i1.p1	pyruvate-flavodoxin-oxidoreductase,-putative
XP_001307088.1	TVAG_105770	Pkeilini_DN206198_c0_g1_i1.p1	pyruvate-flavodoxin-oxidoreductase,-putative
XP_001307064.1	TVAG_080400	Pkeilini_DN953_c0_g10_i1.p1	Rabx31-protein,-putative
XP_001306984.1	TVAG_151010	Pkeilini_DN2353_c0_g1_i3.p3	Rabx19-protein,-putative
XP_001306776.1	TVAG_224980	Pkeilini_DN160002_c0_g1_i1.p1	Clan-MH,-family-M20,-peptidase-T-like-metallopeptidase
XP_001306669.1	TVAG_455090	Pkeilini_DN4238_c0_g1_i1.p2	conserved-hypothetical-protein
XP_001306447.1	TVAG_132350	Pkeilini_DN830_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001306356.1	TVAG_104710	Pkeilini_DN1085_c0_g1_i1.p1	Rabx3-protein,-putative
XP_001306230.1	TVAG_277050	Pkeilini_DN27394_c0_g1_i1.p1	Citrate-lyase-beta-chain,-putative
XP_001305871.1	TVAG_489800	Pkeilini_DN222480_c0_g1_i1.p1	NADH-dehydrogenase-51-kDa-subunit,-putative
XP_001305709.1	TVAG_361590	Pkeilini_DN13155_c0_g4_i1.p1	NADH-ubiquinone-oxidoreductase,-putative
XP_001305704.1	TVAG_361540	Pkeilini_DN419_c0_g1_i1.p2	iron-sulfur-assembly-protein,-putative
XP_001305570.1	TVAG_349870	Pkeilini_DN26_c0_g1_i2.p1	Rabx15-protein,-putative
XP_001305426.1	TVAG_344520	Pkeilini_DN914_c0_g1_i1.p1	conserved-hypothetical-protein

XP_001305403.1	TVAG_331490	Pkeilini_DN9662_c1_g1_i1.p1	Rabx18-protein,-putative
XP_001305368.1	TVAG_327470	Pkeilini_DN771_c0_g1_i1.p1	alcohol-dehydrogenase,-putative
XP_001305213.1	TVAG_262750	Pkeilini_DN160677_c0_g1_i1.p1	vacuolar-ATP-synthase-subunit-H,-putative
XP_001305174.1	TVAG_164890	Pkeilini_DN182694_c0_g1_i1.p1	Acetyl-CoA-hydrolase,-putative
XP_001305114.1	TVAG_386080	Pkeilini_DN170837_c0_g1_i1.p1	Clan-MG,-family-M24,-aminopeptidase-P-like-metallopeptidase
XP_001305092.1	TVAG_383350	Pkeilini_DN196009_c0_g1_i1.p1	RAB-2,4,14,-putative
XP_001304655.1	TVAG_384490	Pkeilini_DN429_c0_g1_i1.p1	Rab9,-putative
XP_001304618.1	TVAG_092740	Pkeilini_DN219560_c0_g1_i1.p1	small-GTPase-rabi,-putative
XP_001304246.1	TVAG_065320	Pkeilini_DN2353_c0_g1_i3.p3	Rab21,-putative
XP_001304133.1	TVAG_220970	Pkeilini_DN16958_c0_g1_i1.p2	RAB,-putative
XP_001304067.1	TVAG_147840	Pkeilini_DN210433_c0_g1_i1.p1	Rab21,-putative
XP_001304062.1	TVAG_147790	Pkeilini_DN6516_c0_g2_i1.p1	cysteine/methionine-metabolism-pyridoxal-5-phosphate-enzymes,-putative
XP_001303981.1	TVAG_144730	Pkeilini_DN541_c0_g1_i1.p1	succinate-thiokinase- $\zeta$ -subunit
XP_001303783.1	TVAG_445730	Pkeilini_DN199807_c0_g1_i1.p1	groes-chaperonin,-putative
XP_001303674.1	TVAG_092170	Pkeilini_DN1127_c0_g1_i1.p1	preprotein-translocase-secy-subunit,-putative
XP_001303658.1	TVAG_134510	Pkeilini_DN219911_c0_g1_i1.p1	RAB-18,-putative
XP_001303641.1	TVAG_328110	Pkeilini_DN26_c0_g1_i2.p1	RAB-18,-putative
XP_001303632.1	TVAG_152690	Pkeilini_DN407_c0_g1_i1.p1	N-ethylmaleimide-reductase,-putative
XP_001303268.1	TVAG_385350	Pkeilini_DN183936_c0_g1_i1.p1	thioredoxin,-putative
XP_001303252.1	TVAG_234150	Pkeilini_DN109943_c0_g1_i1.p1	conserved-hypothetical-protein

XP_001303146.1	TVAG_415960	Pkeilini_DN3256_c0_g1_i1.p1	Rab9,-putative
XP_001303059.1	TVAG_088220	Pkeilini_DN195601_c0_g1_i1.p1	aspartate-aminotransferase,-putative
XP_001302997.1	TVAG_371280	Pkeilini_DN12181_c0_g1_i1.p1	Rab2,-putative
XP_001302917.1	TVAG_124590	Pkeilini_DN4394_c0_g1_i1.p1	Rab6,-putative
XP_001302913.1	TVAG_124540	Pkeilini_DN158973_c0_g1_i1.p1	GTPase_rho,-putative
XP_001302832.1	TVAG_320300	Pkeilini_DN184375_c0_g1_i1.p1	GTP-binding-protein-Rab2,-putative
XP_001302560.1	TVAG_386000	Pkeilini_DN159371_c0_g1_i1.p1	Receptor-expression-enhancing-protein,-putative
XP_001302496.1	TVAG_356810	Pkeilini_DN144134_c0_g2_i1.p1	NimA-like-protein
XP_001302364.1	TVAG_327760	Pkeilini_DN1216_c0_g1_i1.p1	Iron-sulfur-flavoprotein
XP_001302013.1	TVAG_261280	Pkeilini_DN2117_c0_g1_i1.p1	GTP-binding-protein-ypt11,-putative
XP_001301760.1	TVAG_025980	Pkeilini_DN11926_c1_g1_i2.p1	glutamate-dehydrogenase,-putative
XP_001301244.1	TVAG_158270	Pkeilini_DN222101_c0_g1_i1.p1	RAB,-putative
XP_001301100.1	TVAG_041340	Pkeilini_DN199807_c0_g1_i1.p1	groes-chaperonin,-putative
XP_001301044.1	TVAG_422690	Pkeilini_DN1052_c0_g1_i1.p1	Rab9,-putative
XP_001301038.1	TVAG_422630	Pkeilini_DN622_c0_g1_i1.p1	Co-chaperone-protein-HscB-Hsc20,-mitochondrial-precursor,-putative
XP_001300804.1	TVAG_293370	Pkeilini_DN207058_c0_g1_i1.p1	nucleoside-diphosphate-kinase,-putative
XP_001300601.1	TVAG_001130	Pkeilini_DN53254_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001300482.1	TVAG_318670	Pkeilini_DN626_c0_g1_i1.p1	succinate-thiokinase-a-subunit
XP_001300294.1	TVAG_015270	Pkeilini_DN6278_c0_g1_i2.p2	small-GTPase-rabh,-putative
XP_001300248.1	TVAG_169740	Pkeilini_DN219911_c0_g1_i1.p1	RAB-19,-41-and,-putative



XP_001299687. 1	TVAG_440690	Pkeilini_DN26_c4_g1_i1.p1	small-GTPase-rabh,-putative
XP_001299604. 1	TVAG_527180	Pkeilini_DN327_c2_g1_i1.p1	RAB,-putative
XP_001299513. 1	TVAG_177600	Pkeilini_DN183618_c0_g1_i1.p1	glycine-cleavage-system-H-protein ,-putative
XP_001299483. 1	TVAG_450220	Pkeilini_DN96108_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001299482. 1	TVAG_399860	Pkeilini_DN71456_c0_g1_i1.p1	Ferredoxin-2
XP_001299372. 1	TVAG_499340	Pkeilini_DN497_c1_g1_i1.p1	hypothetical-protein
XP_001299219. 1	TVAG_528800	Pkeilini_DN3462_c4_g1_i1.p1	RAB-2,4,14,-putative
XP_001299204. 1	TVAG_450060	Pkeilini_DN13377_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001298987. 1	TVAG_126970	Pkeilini_DN207052_c0_g1_i1.p1	RAB-GDP-dissociation-inhibitor,-p utative
XP_001298262. 1	TVAG_022530	Pkeilini_DN30686_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001297863. 1	TVAG_377380	Pkeilini_DN1554_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001297704. 1	TVAG_085320	Pkeilini_DN2659_c0_g1_i1.p1	small-GTPase-rabi,-putative
XP_001297366. 1	TVAG_530140	Pkeilini_DN160991_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001297292. 1	TVAG_504530	Pkeilini_DN211774_c0_g1_i1.p1	Rabx26-protein,-putative
XP_001296818. 1	TVAG_060820	Pkeilini_DN1085_c1_g1_i1.p1	RAB,-putative
XP_001296212. 1	TVAG_082020	Pkeilini_DN159740_c0_g1_i1.p1	conserved-hypothetical-protein
XP_001294582. 1	TVAG_547520	Pkeilini_DN218014_c0_g1_i1.p1	threonine-synthase,-putative
XP_001294517. 1	TVAG_416100	Pkeilini_DN568_c0_g1_i1.p1	malic-enzyme,-putative

**Table S5: List of annotated genes gained in the trichomonas lineage after the split from their common ancestor with *P. keilini***

Gene family	Annotation	<i>T. vaginalis</i> gene ID	KEGG_ko
OG_792	Acetyltransf_1	TvagiEBg036290	ko:K20793
OG_5491	Acetyltransf_1,Pkinase,TPR_8	TvagiEBg053982	ko:K04345
OG_24374	biological adhesion	TvagiEBg038277	NA
OG_24315	biological adhesion	TvagiEBg036641	ko:K10402
OG_6750	biological adhesion	TvagiEBg023227	NA
OG_7081	biological adhesion	TvagiEBg023531	NA
OG_7139	biological adhesion	TvagiEBg055182	ko:K14000
OG_7120	biological adhesion	TvagiEBg043549	NA
OG_16843	biological adhesion	TvagiEBg047580	NA
OG_16996	biological adhesion	TvagiEBg036104	ko:K14000
OG_17311	biological adhesion	TvagiEBg041328	ko:K15152
OG_170	cAMP-dependent protein kinase activity	TvagiEBg039290	ko:K04345,ko:K10409,ko:K19584
OG_1811	Chromo,DUF4208,Helicase_C,PHD,SNF2_N	TvagiEBg028330	ko:K11367
OG_2044	DNA polymerase type B, organellar and viral	TvagiEBg047836	NA
OG_3706	twin BRCT domain	TvagiEBg028318	ko:K10728

OG_2184	endoplasmic reticulum-plasma membrane tethering	TvagiEBg028132	ko:K12486,ko:K19938
OG_3706	twin BRCT domain	TvagiEBg028318	ko:K10728
OG_1726	spectrin binding	TvagiEBg042842,TvagiEBg055069	ko:K15502,ko:K15503
OG_6482	Glycine rich protein	TvagiEBg030396	NA
OG_6775	GTPase activity	TvagiEBg034972	NA
OG_6405	GTPase activity	TvagiEBg041930	NA
OG_2058	islet amyloid polypeptide processing	TvagiEBg048541,TvagiEBg048542	ko:K01341,ko:K01360,ko:K08673
OG_1775	Exhibits S-adenosyl-L-methionine-dependent methyltransferase activity	TvagiEBg025243	NA
OG_2007	MyD88-dependent toll-like receptor signaling pathway	TvagiEBg044672,TvagiEBg044653	ko:K18809
OG_5666	Myb-like DNA-binding domain	TvagiEBg022475,TvagiEBg041712,TvagiEBg055335,TvagiEBg046332	ko:K09422
OG_16767	nerve growth factor signaling pathway	TvagiEBg056182	ko:K15503,ko:K21440
OG_6132	nuclear import signal receptor activity	TvagiEBg055732	NA
OG_24385	PDZ domain binding	TvagiEBg057545	ko:K16072,ko:K19878,ko:K20478
OG_1974	Phage tail repeat like	TvagiEBg019118	NA

OG_24226	spectrin binding	TvagiEBg046228	NA
OG_5955	protein kinase activity	TvagiEBg050562,TvagiEBg050583	ko:K04345
OG_5921	protein serine/threonine kinase activity	TvagiEBg025205,TvagiEBg054198,TvagiEBg046872	NA
OG_1756	protein serine/threonine kinase activity	TvagiEBg023811,TvagiEBg041668	ko:K02216
OG_1026	protein serine/threonine kinase activity	TvagiEBg028031,TvagiEBg049667	ko:K08813,ko:K16312
OG_4577	protein serine/threonine kinase activity	TvagiEBg038832,TvagiEBg048970	ko:K04428,ko:K17533,ko:K08798
OG_6690	protein serine/threonine kinase activity	TvagiEBg056404	ko:K08857,ko:K20879
OG_24246	protein ubiquitination	TvagiEBg000145	NA
OG_24247	protein ubiquitination	TvagiEBg033567,TvagiEBg035052	ko:K20129,ko:K15502,ko:K15503
OG_1735	protein ubiquitination	TvagiEBg021138,TvagiEBg024708,TvagiEBg012479,TvagiEBg001122,TvagiEBg001731,TvagiEBg006733,TvagiEBg000512,TvagiEBg008998,TvagiEBg002326	ko:K10325,ko:K12591
OG_6128	protein ubiquitination	TvagiEBg049297,TvagiEBg043655	NA
OG_16927	protein ubiquitination	TvagiEBg039035	ko:K15502,ko:K15503,ko:K20032,ko:K21440
OG_2368	protein serine/threonine kinase activity	TvagiEBg032765,TvagiEBg047551,TvagiEBg016996,TvagiEBg058648,TvagiEBg059205,TvagiEBg052716	ko:K04345,ko:K07376,ko:K13302,ko:K13303
OG_7079	regulation of centriole replication	TvagiEBg021289	ko:K06631,ko:K07298,ko:K08269,ko:K08850,ko:K13412,ko:K21358

OG_6445	regulation of choline O-acetyltransferase activity	TvagiEBg049095,TvagiEBg050875	ko:K01404,ko:K08654,ko:K12813
OG_4922	Reprolysin_4	TvagiEBg044791,TvagiEBg044795,TvagiEBg050876	NA
OG_19677	Reversible hydration of carbon dioxide	TvagiEBg041073,TvagiEBg054911	ko:K01673
OG_5952	Ribonuclease H protein	TvagiEBg028675,TvagiEBg029501,TvagiEBg028461,TvagiEBg038876,TvagiEBg037831,TvagiEBg030542,TvagiEBg040960,TvagiEBg032543,TvagiEBg027125	ko:K12879
OG_18737	Ribosomal L29e protein family rpl29	TvagiEBg037733,TvagiEBg034570.TvagiEBg047046	ko:K02905
OG_1071	Right handed beta helix region	TvagiEBg032352,TvagiEBg045961,TvagiEBg045962,TvagiEBg020968,TvagiEBg028656,TvagiEBg002344,TvagiEBg017538,TvagiEBg051902,TvagiEBg052658,TvagiEBg055958	NA
OG_19828	RNA polymerase I core binding	TvagiEBg026015	ko:K11294
OG_24227	RNA polymerase II transcription regulator recruiting activity	TvagiEBg000087,TvagiEBg054872,TvagiEBg058883	NA
OG_6053	RNA binding	TvagiEBg023316	ko:K13154
OG_19443	sensory perception of sound	TvagiEBg028625,TvagiEBg051265	ko:K05747
OG_1990	serine-type endopeptidase activity	TvagiEBg052350,TvagiEBg052355,TvagiEBg052562,TvagiEBg052386,TvagiEBg052378	NA
OG_24486	spectrin binding	TvagiEBg054770	NA

OG_1846	spectrin binding	TvagiEBg037450,TvagiEBg037444,TvagiEBg037452,TvagiEBg030906,TvagiEBg037447,TvagiEBg059166,TvagiEBg032170,TvagiEBg028616,TvagiEBg027013	ko:K15502,ko:K15503
OG_124	spectrin binding,protein ubiquitination	TvagiEBg036895,TvagiEBg039181,TvagiEBg056857,TvagiEBg058173,TvagiEBg051128,TvagiEBg025287,TvagiEBg039180,TvagiEBg025285,TvagiEBg025286,TvagiEBg046793,TvagiEBg045177,TvagiEBg055607,TvagiEBg059132,TvagiEBg049319	ko:K15502,ko:K15503,ko:K20032,ko:K21440
OG_1726	spectrin binding	TvagiEBg042842,TvagiEBg055069	ko:K15502,ko:K15503
OG_6626	spectrin binding	TvagiEBg031656	NA
OG_7116	spectrin binding	TvagiEBg041913	NA
OG_17052	spectrin binding	TvagiEBg045359	ko:K10380
OG_2826	Sulfatase	TvagiEBg043468,TvagiEBg056542,TvagiEBg038218,TvagiEBg049604,TvagiEBg020034,TvagiEBg037070,TvagiEBg040806,TvagiEBg056543,TvagiEBg039040,TvagiEBg044578,TvagiEBg019289,TvagiEBg044686,TvagiEBg051277,TvagiEBg050423,TvagiEBg040807,TvagiEBg049450,TvagiEBg015897,TvagiEBg021826,TvagiEBg019340	NA
OG_5283	thiosulfate sulfurtransferase activity	TvagiEBg056003	ko:K01011
OG_701	thiomorpholine-carboxylate dehydrogenase activity	TvagiEBg027824,TvagiEBg042806	ko:K01750,ko:K18258

OG_218	trehalase (brush-border membrane glycoprotein)	TvagiEBg044074,TvagiEBg044075	ko:K01194
OG_2106	unfolded protein binding, ATP binding	TvagiEBg052412,TvagiEBg036462,TvagiEBg045708,TvagiEBg056047,TvagiEBg055070,TvagiEBg023823,TvagiEBg038251,TvagiEBg052522,TvagiEBg022564,TvagiEBg052488,TvagiEBg052411	ko:K04043

## *P. keilini* hydrogenosomal preprotein import system is similar to other hydrogenosomes

Import into the hydrogenosome requires transport across two plasma membranes, in canonical mitochondrial organisms the inner and outer membranes have evolutionarily unrelated translocase complexes, which contain a core translocase and accessory components which assist in preprotein import. The Translocase of the Outer Membrane (TOM) complex has an essential conserved translocase, TOM40, and orthologs have been functionally characterised as the preprotein translocases in isolated hydrogenosomes (Makki et al., 2019). Characteristic of these proteins is a beta-barrel fold of the pfam hmm family [Porin\\_3](#), the number of paralogs of this protein varies from lineage to lineage in excavates with kinetoplastids such as *T. brucei* having two copies of a highly diverged protein termed ATOM (Pusnik et al., 2011), and *T. vaginalis* as many as six (Rada et al., 2011). We identified two partial sequences in *P. keilini* (Pfam Porin\_3, E-value  $1.1 \times 10^{-6}$ ,  $2.3 \times 10^{-5}$ ). Whilst neither sequence is complete, both have beta barrel topology similar to translocases identified in *T. vaginalis* by PRED\_TMBB (Bagos et al., 2004). In most eukaryotes the TOM complex has accessory proteins which assist in preprotein import and binding, these subunits seem to have independently emerged in different eukaryotic lineages though are assumedly functionally similar. Several accessory proteins have been identified to the *T. brucei* TOM complex (Schneider, 2018), though appear absent in the *T. vaginalis* (Makki et al., 2019). No strong homologs to the accessory proteins from the yeast system are present in *P. keilini* nor ATOM11, 12, 14, 46, 69 of the *T. brucei* TOM complex.

Preproteins destined for insertion into the outer membrane are handled subsequent to Tom40 import by another bacterially evolved beta barrel protein SAM50 which is the core translocase of the SAM complex (Kozjak et al., 2003). We did not detect SAM50 in *P. keilini*.

In contrast to the ancestrally bacterial beta barrel translocases of the outer membrane, import through and insertion into the inner membrane is facilitated by proteins of eukaryotic innovation termed Translocase of the Inner Membrane (TIM) complexes. In many eukaryotes

the inner membrane translocases have functionally diverged to do slightly different tasks, in yeast three related proteins Tim17, 22, 23 form the core translocases to two different complexes the Tim22 complex mediating the insertion of membrane proteins and Tim23 for lumen destined proteins. In *P. keilini* two proteins were identified with homology to the inner membrane TIM 17 family (Pfam Tim17, E-values  $3.3 \times 10^{-8}$ ,  $4.6 \times 10^{-8}$ ), this compares to the 4 identified in *T. vaginalis* where it is not yet clear whether they form functionally discrete complexes (Rada et al., 2011). Like the outer membrane, the translocase complexes of the inner membrane have accessory subunits which assist in preprotein import, the most significant of these the Presequence translocase-Associated Motor (PAM) which involves both matrix and membrane associated proteins. The membrane associating components, Tim44 Pam16 (Pfam Pam16, E-value  $8.4 \times 10^{-5}$ ), and Pam18 were all found in *P. keilini* as well as the matrix proteins Mge1 and mtHSP70. The PAM motor has also been characterised in *T. vaginalis* (Rada et al., 2011) and is similarly complete. Some imported preproteins undergo signal sequence cleavage with a specific Mitochondrial Processing Protease (MPP). In many eukaryotes the MPP is composed of evolutionarily related  $\alpha/\beta$  subunits, but other organisms including *T. vaginalis* have undergone streamlining to a single subunit (Šmíd et al., 2008). In our dataset we find only a single MPP type protease, suggesting that the reductive evolution of this complex occurred before the speciation of *P. keilini*.

In summary the *P. keilini* hydrogenosome retains the core translocases of the inner and outer membranes, and closely resembles the architecture of the *Trichomonas* hydrogenosome, with complete PAM and a single MPP, but without other accessory proteins. The copy number of the core translocases is lower than that of *T. vaginalis*. Particularly interesting in this respect are the two Tim17 family proteins, which would suggest that the scope for functional diversity of the TIM complexes is limited to those two proteins and could be another example of a reductive evolution in the preprotein import system.

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