Identifying protists in NYC subway samples

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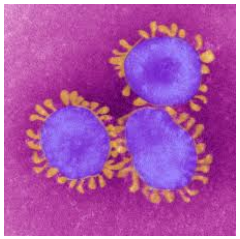
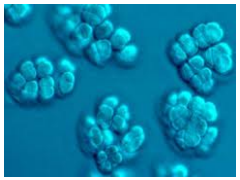
An NYU Grand Challenge Project: Mapping the New York City "MetaGenome"

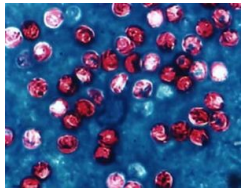
coronavirus



Methanosarcina

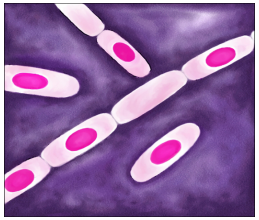


Cryptosporidium

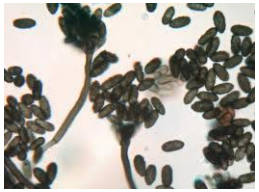


Lactobacillus

Bacillus cereus



Stachybotrys



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NYU LANGONE MEDICAL CENTER

CENTER FOR GENOMICS AND SYSTEMS BIOLOGY
NEW YORK UNIVERSITY

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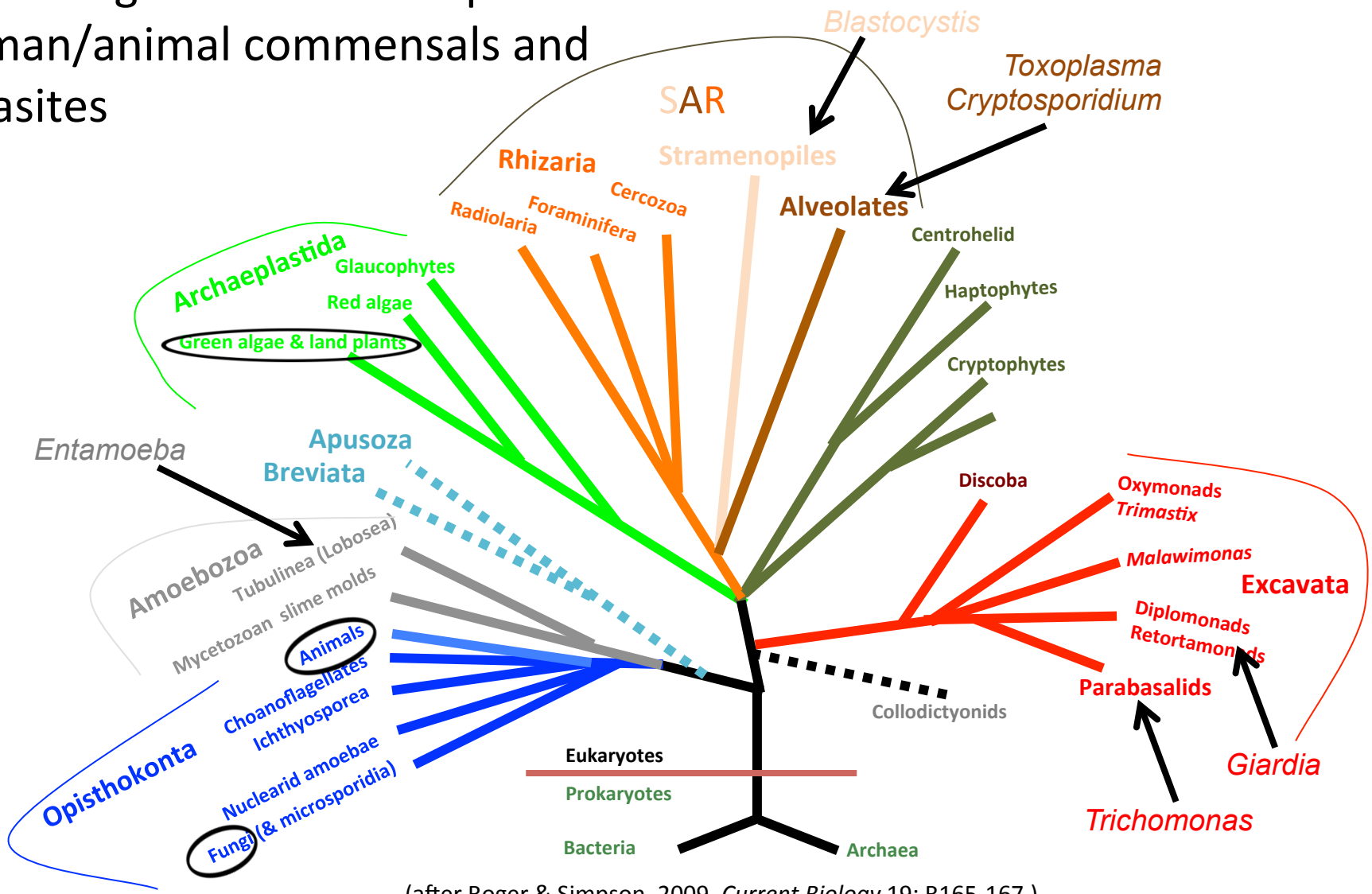
Protists are found through out the eukaryotic tree of life

- Single-celled eukaryotes
- Free-living environmental protists
- Human/animal commensals and parasites

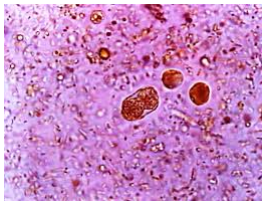


(after Roger & Simpson, 2009. *Current Biology* 19: R165-167.)

Many protists are zoonotic

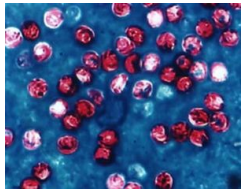
- Transmissible between humans, domestic animals and wildlife
- 16-20% of emerging zoonoses
- Many are digestive and reproductive parasites
- Cause global poor health and economic losses

Blastocystis

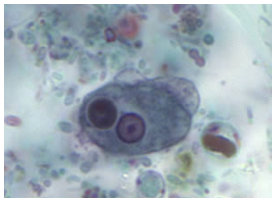


Giardia intestinalis

Cryptosporidium

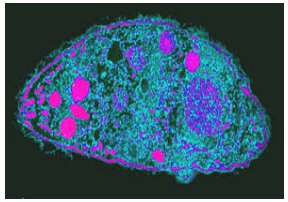


Entamoeba histolytica



Trichomonads

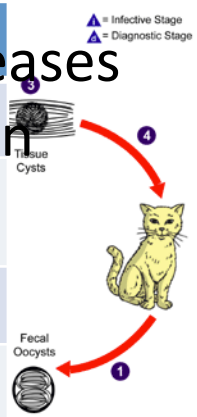
Toxoplasma gondii



Tritrichomonas foetus

- Despite this, emerging parasitic zoonoses are neglected diseases
- Few studies have investigated their emergence and spread in urban environments

Animal	Disease	Incidence	Consequences
Cats	Diahrrea	~1/3 of cats worldwide	Non-fatal
Cattle	Vaginosis, endometritis	Unknown but worldwide	Spontaneous abortion, infertility, herd loss
Pigs	Commensal	68-98% domestic pigs	NA
Humans	Pulmonary infections	Unknown	Exacerbate symptoms, prolong illness



CDC
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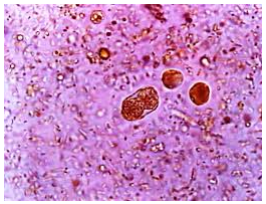
http://www.dpd.cdc.gov/dpdx

Project goals

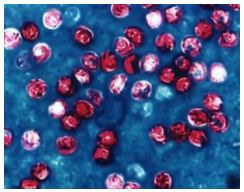
- Investigate population dynamics and genetic diversity of zoonotic protists present in New York City
  - Use NGS methods to identify, track, and characterize zoonotic protist communities in NYC
  - Associate their presence with local wildlife and domestic animal populations.

Blastocystis

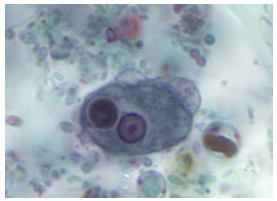


Giardia intestinalis

Cryptosporidium

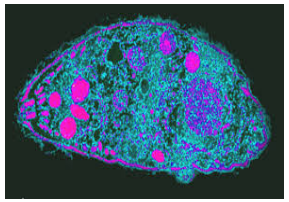


Entamoeba histolytica



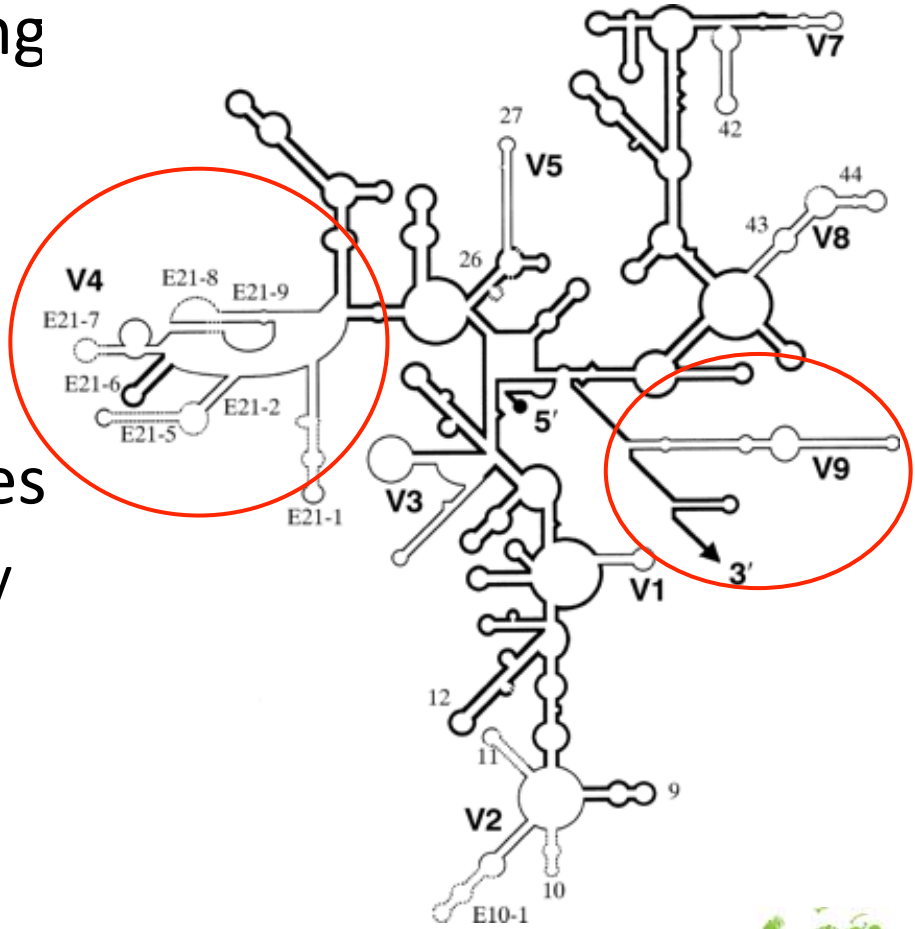
Trichomonads

Toxoplasma gondii



How to identify protists in environmental samples?

- 18S rRNA amplicon sequencing
  - Small subunit of rRNA
  - Present in all eukaryotes
  - Has 9 variable regions
- V9 and V4 are good candidates
  - Illumina sequencing technology
  - V9
    - Vertebrate blocking primer
    - Three domain primers
  - V4
    - Largest variable region
    - Eukaryote specific



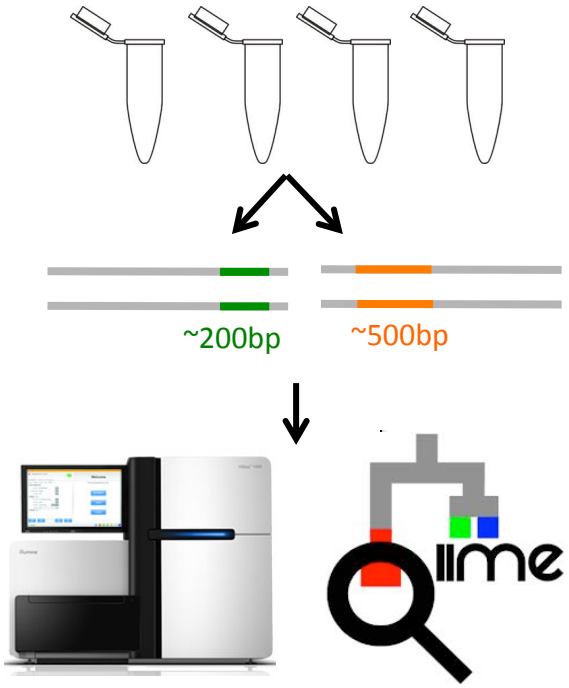
Ueda-Nishimura and Mikata 2000

earth microbiome project

18S protocol testing and data analysis

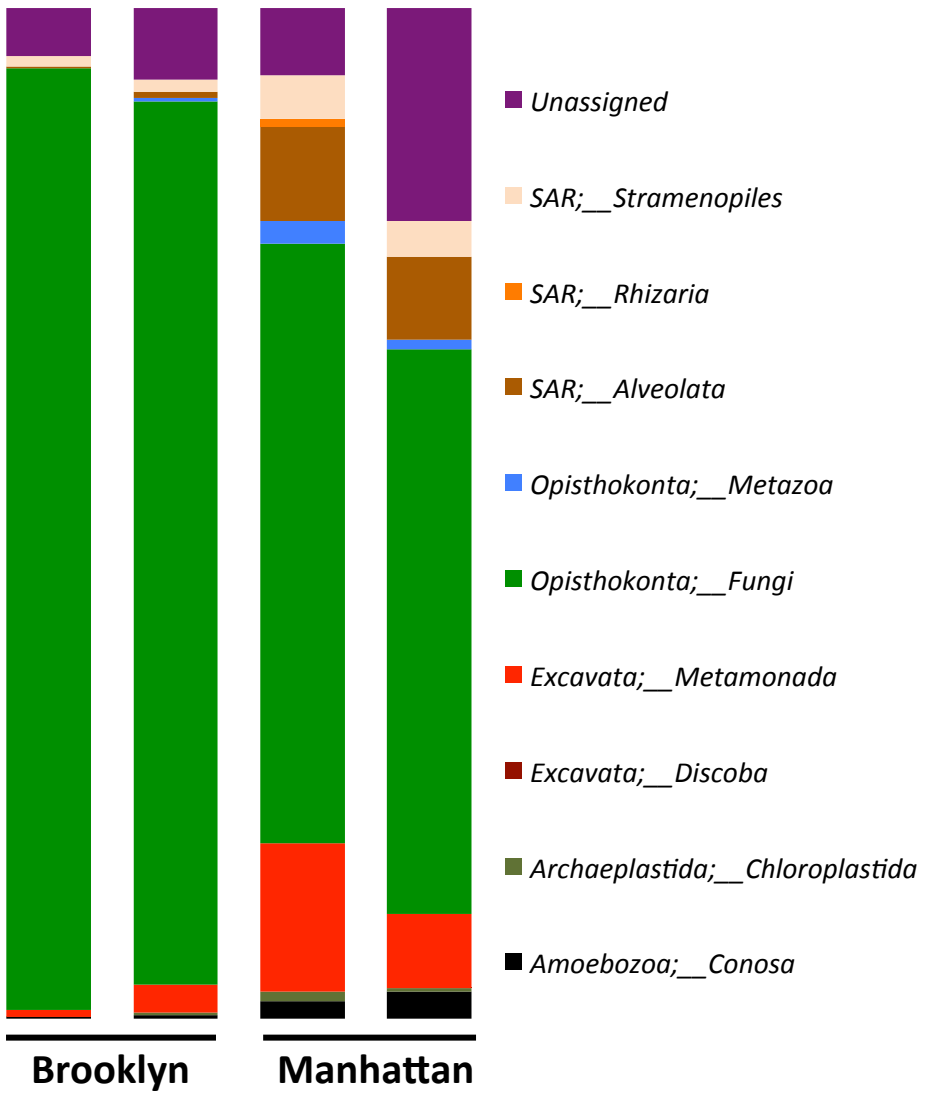
- DNA from four subway swabs
  - July 2013
- Amplicon PCR
  - V9 and V4 regions
- 18S paired-end sequencing
  - Illumina Miseq
- Compare results
  - QIIME based pipeline
  - *de novo* OTUs at 98% identity
  - Curated SILVA reference database

Borough	Collection site
Brooklyn	Bedford Ave Turnstile
Brooklyn	Bedford Ave wooden bench
Manhattan	8th Ave wooden bench
Manhattan	6th Ave turnstile



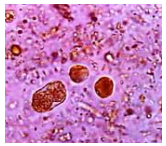
Majority of eukaryotes identified were fungi

- 9 major clades, 33 genera
- Dominated by fungi
  - 50-90% of each sample
- Less metazoan DNA than might expected
  - Mostly insects and mice
- Manhattan samples have higher diversity
- Numerous protists detected as well

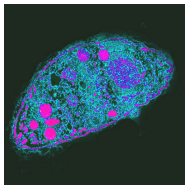


Several parasitic protists are present

- Zoonotic taxa of interest
  - *Blastocystis*
    - Gut parasite



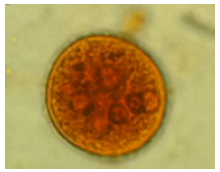
  - *Toxoplasma gondii*
    - Toxoplasmosis



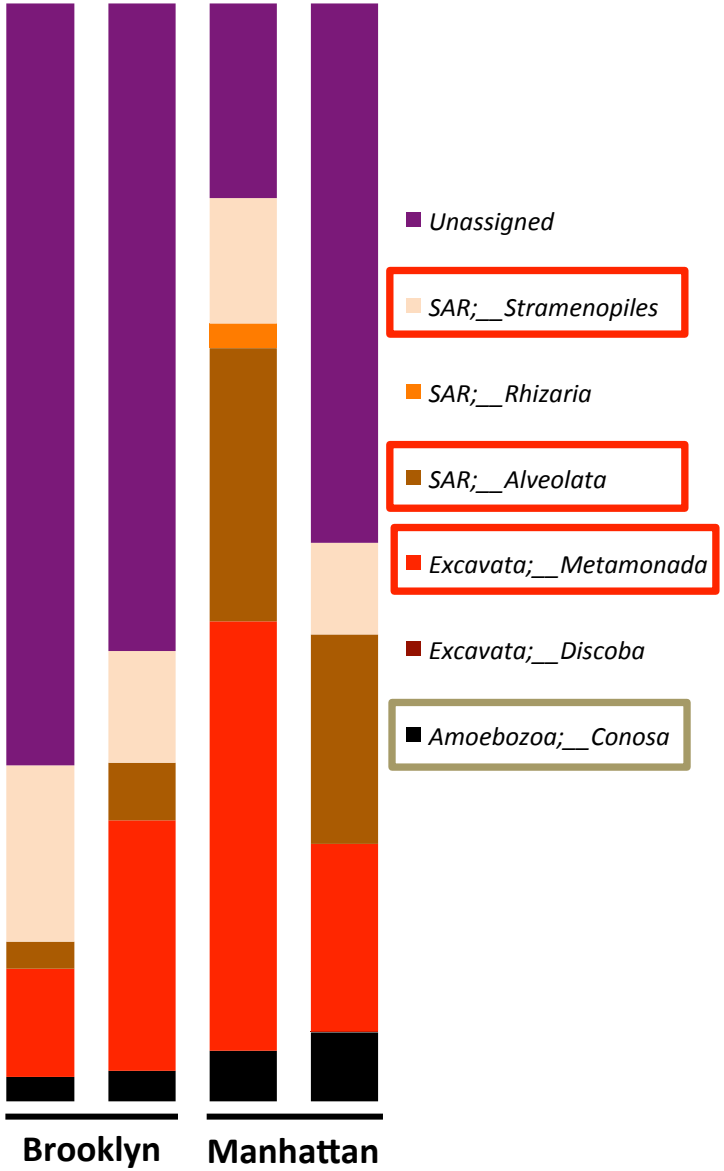
  - *Trichomonas vaginalis*
    - Most common human non-viral STD

  - *Tritrichomonas foetus*
    - Parasite of cats & dogs & humans

- Human/animal commensals
  - *Entamoeba dispar*
    - Commensal gut taxa



- Many unassigned taxa



Summary and future work

- Protists can be identified in environmental samples with the 18S rRNA gene
  - Diverse communities
  - Including many taxa of interest

- Lots of unidentified sequences
  - Need better databases for identification

EUK REF

- Collect and sequence other NYC environments:
  - Sewage
  - Soil
  - ATMs

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