

Feb 3rd, 9:00 AM - 10:00 AM

Digging Out the Devils: Molecular Examination of Amoeba-like Cells from Cranial Tissue of the Endangered Rio Grande Silvery Minnow

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Hamel, Brandon, "Digging Out the Devils: Molecular Examination of Amoeba-like Cells from Cranial Tissue of the Endangered Rio Grande Silvery Minnow" (2018). *Undergraduate Research Symposium*. 8.
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Molecular Examination of Amoeba-like Cells from Cranial Tissue of the Endangered Rio Grande Silvery Minnow



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The Plight of the Rio Grande Silvery Minnow (*Hybognathus amarus*)



- Fish species endemic to southwestern United States
- Critically Endangered
 - Human alteration of Rio Grande
- Now occupies ~5% of its original range.
- Aquaculture efforts started in 2000 through U.S. Fish and Wildlife Service



Trouble in Paradise



- Unusual symptoms in the summer of 2012
 - Spinning behavior
 - Chronic low-level mortalities
- Viability of restocking efforts questioned
- Investigation undertaken by VIMS at the College of William and Mary
 - Water and habitat quality
 - Pathogenic microbe screens
 - Gross necropsies of affected specimens
- All came back negative, leaving the cause a mystery

The Plot Thickens



- A second investigation in 2013 discovered an unusual cell in the connective tissue of the cranial floor
 - Amoeba-like cell
 - Possible infectious organism
 - Identification a priority

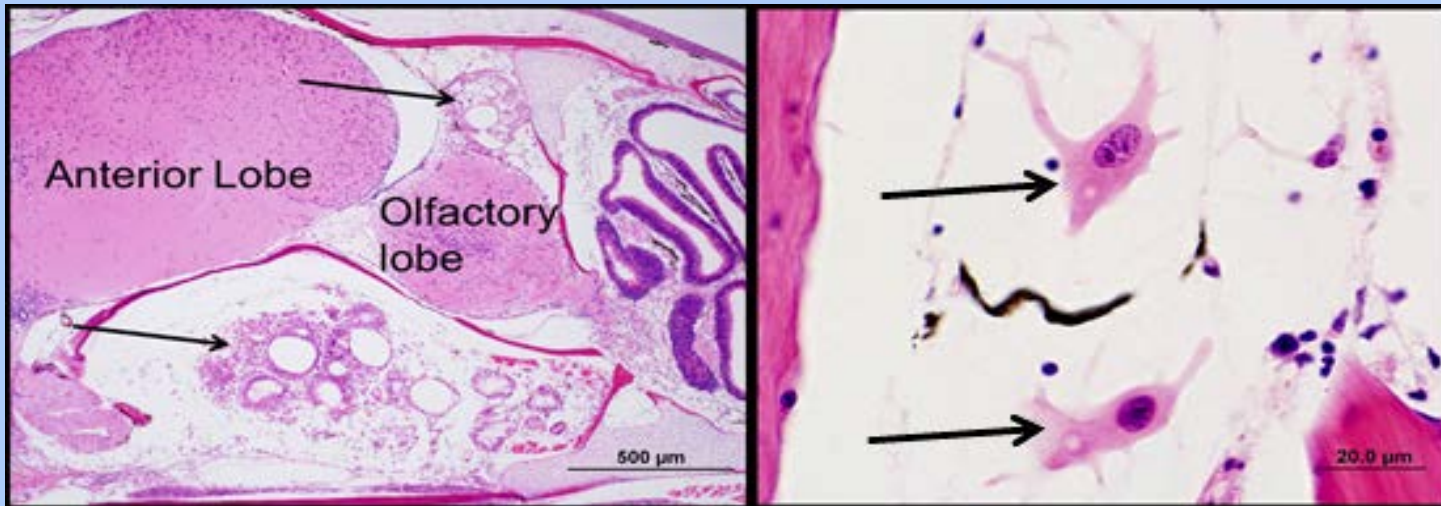


Photo Credit:
Dr. Wolfgang K.
Vogelbein, The
Virginia Institute
of Marine
Science, College
of William and
Mary

Amoebic Infectious Agents – The Suspects



- Pathogenic Amoebae
 - *Acanthamoeba*, *Balmuthia*, *Neoparamoeba* spp.
 - ✦ Amoebic Gill Disease
- Parasites with Amoeboid Life Stages
 - Myxozoans
 - ✦ *Myxobolus cerebralis* – causative agent of whirling disease
 - Infection causing whirling behavior similar to observed symptoms
 - ✦ *Henneguya*, *Kudoa*, *Buddenbrockia*, *Tetrascapuloides* spp.
 - Many infect gills or other organs, but new or related species a possibility

Preliminary Investigation



- PCR – Polymerase Chain Reaction
 - Molecular technique used to amplify and visualize a target DNA sequence in a sample
 - General, nonspecific primers designed to amplify a wide range of myxozoan and amoebic species
 - Testing performed on digested samples from affected fish
- No amplification of DNA targets
- Identity of amoebic organism still a mystery

Going Deeper With Illumina Sequencing

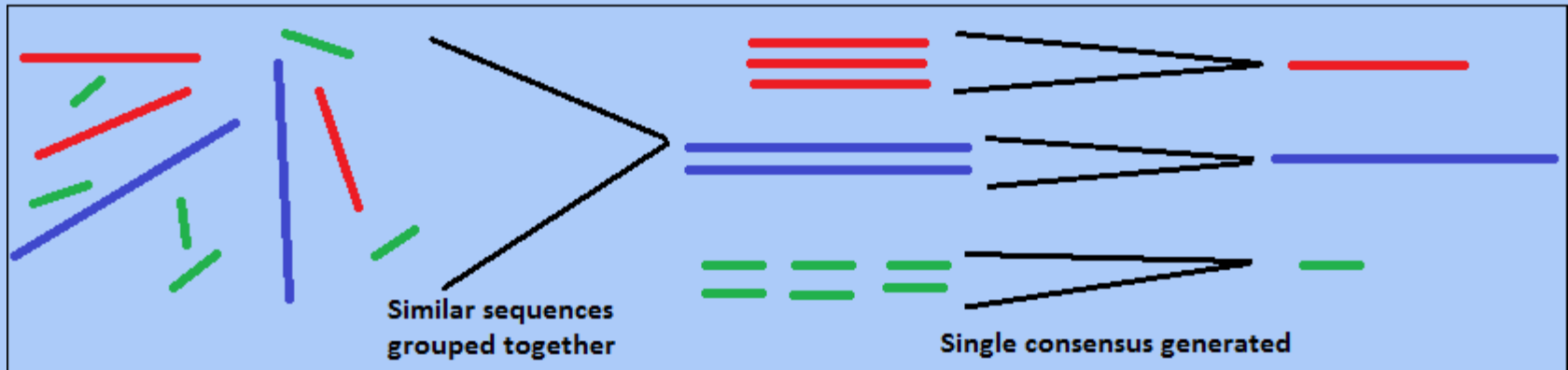


- Taking a DNA ‘snapshot’
- Generic Eukaryotic primer sets would amplify all 18S sequences present in sample
- Sequences would be analyzed using Illumina MiSeq platform
 - Uses NGS to collect up to 8 Gb of sequencing data
 - ✦ For reference, older sequencing methods collect up to 10 Kb/day
 - Allows sequencing of multiple loci at once
 - ✦ Two loci examined

Sifting for Gold



- ~450,000 sequences retrieved from each of the two eukaryotic 18S loci amplified
 - A program known as cdhit was used to cluster similar sequences together
 - Reduced number of different sequences to ~1000 per loci

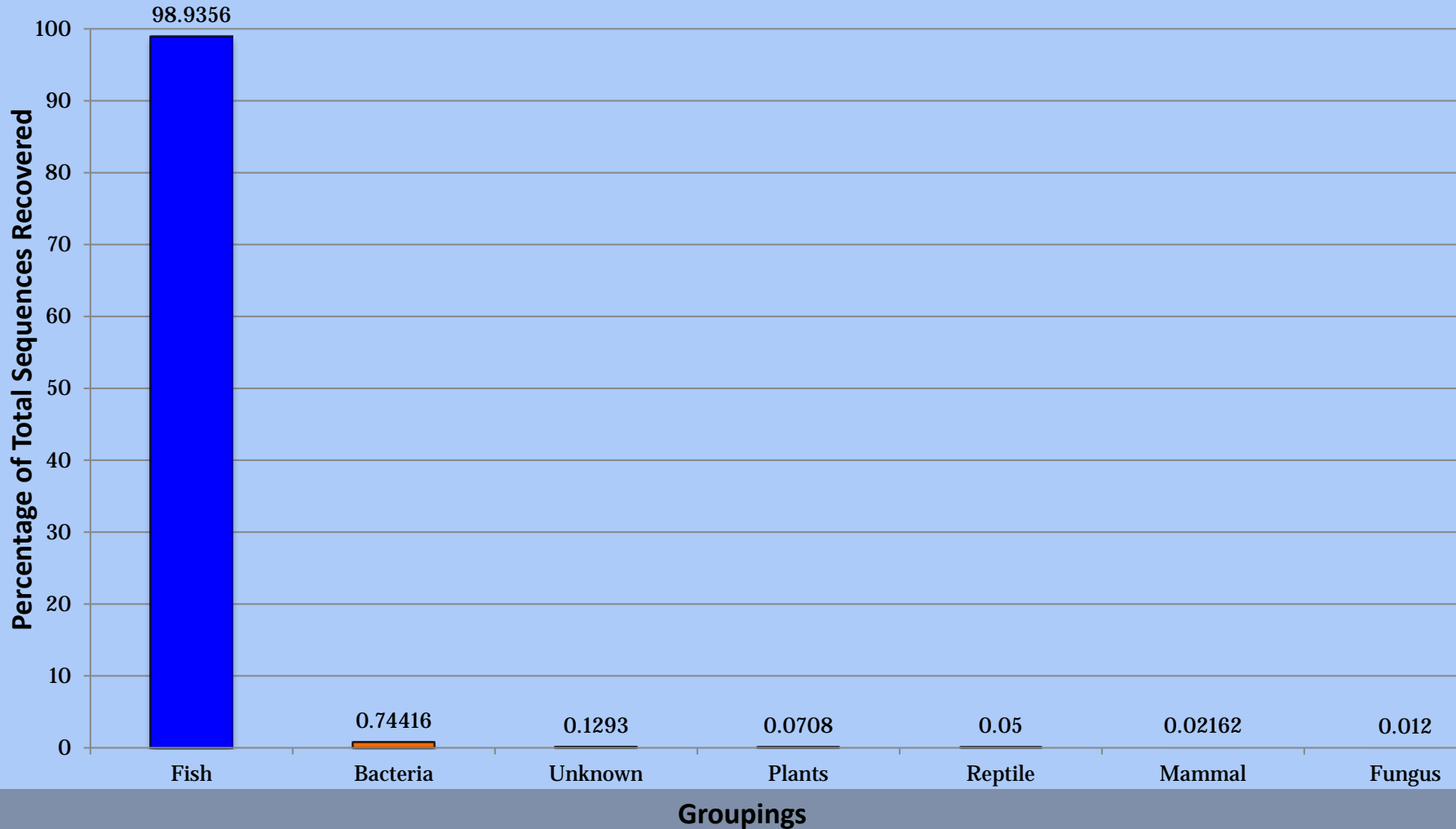


- Clusters representing more than 0.01% of the total number of sequences were analyzed using BLAST in Geneious[®] software

Results - 1st Locus (EUK 1136)



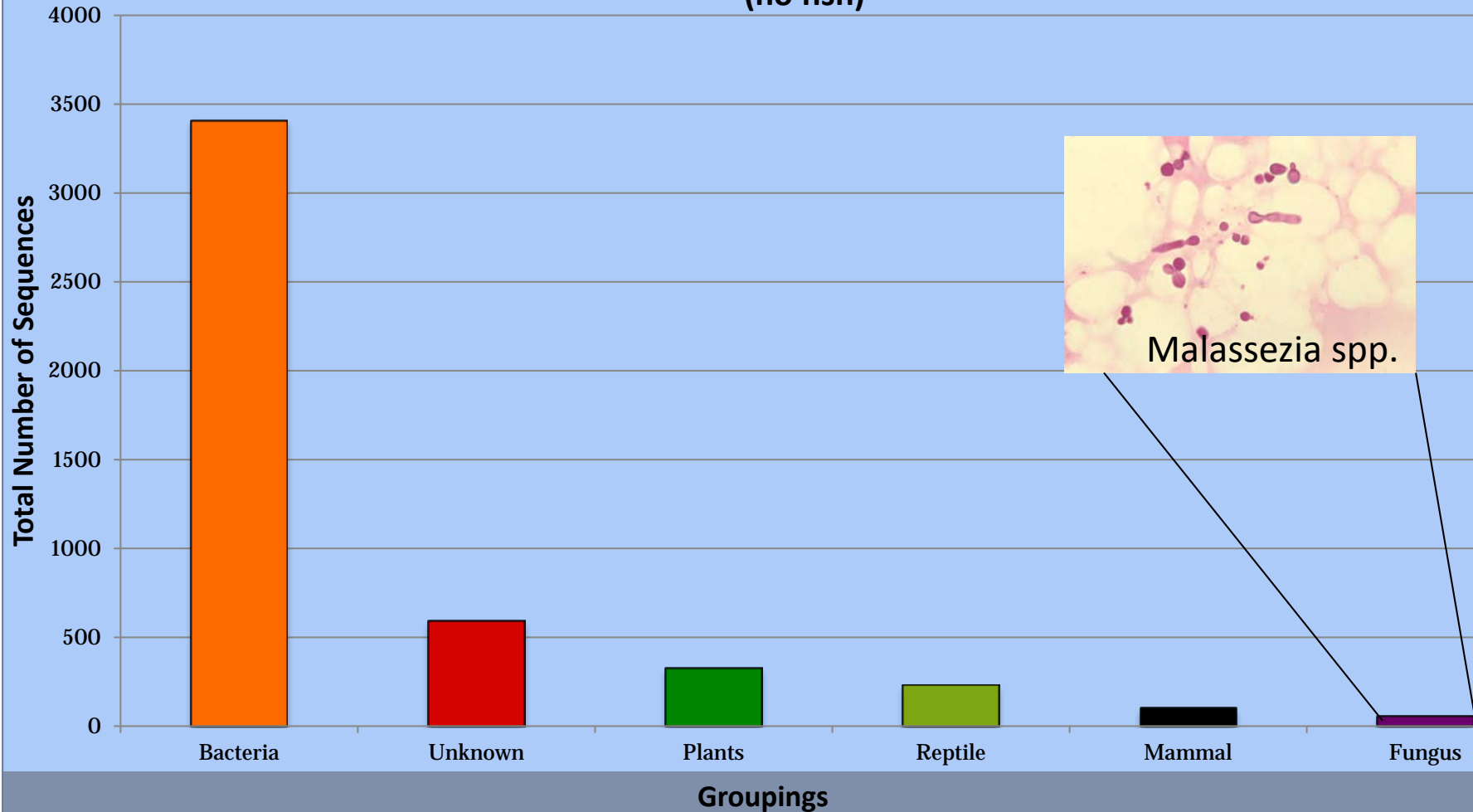
Distribution of BLAST Results from Illumina Sequencing of EUK 1136 Locus



Results – 1st Locus (EUK 1136)



**BLAST Results from Illumina Sequencing of EUK F_R Locus with Background Removed
(no fish)**



Results – 2nd Locus (EUK F_R)



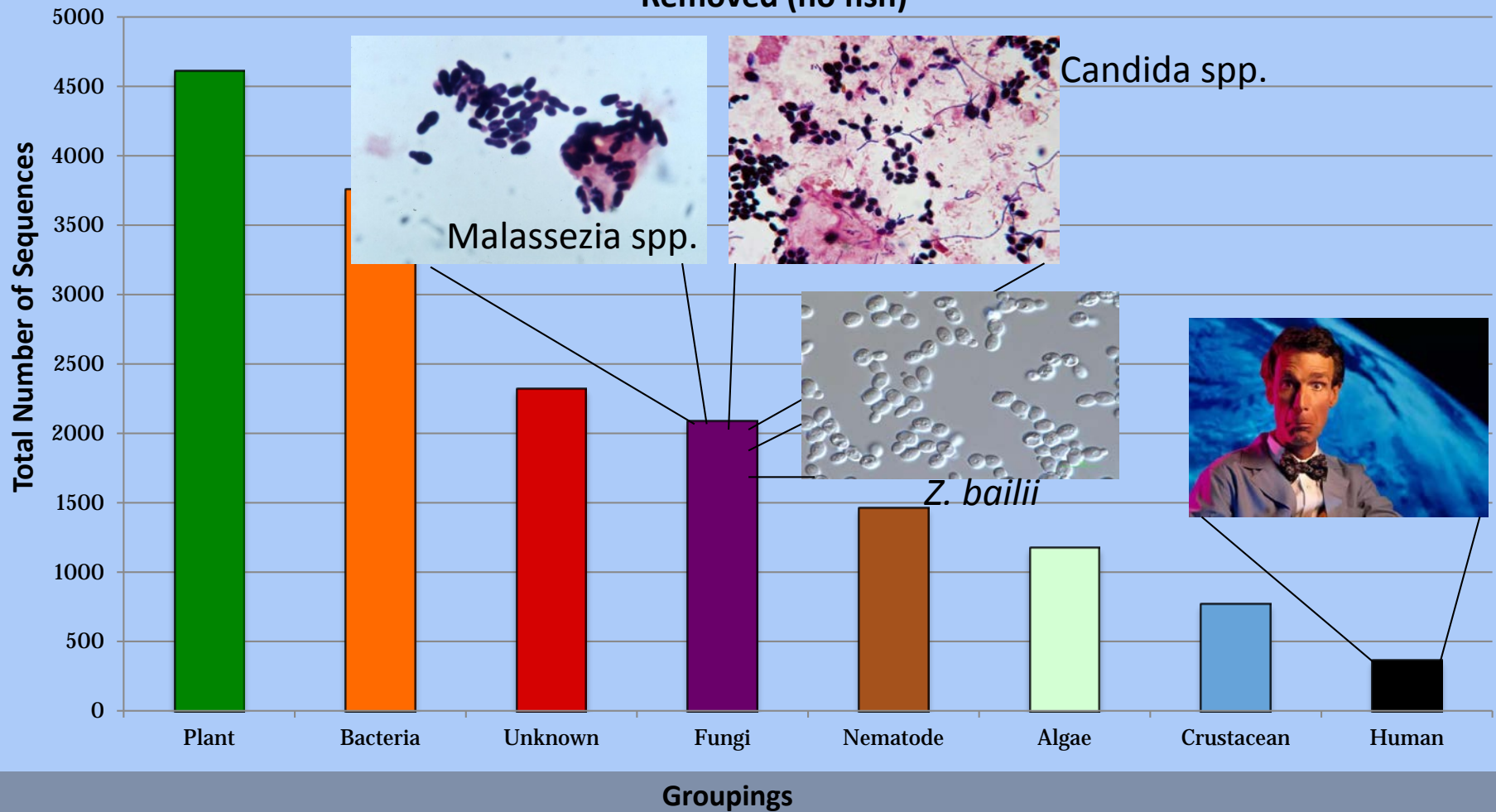
Distribution of BLAST Results from Illumina Sequencing of EUK F_R Locus



Results - 2nd Locus (EUK F_R)



BLAST Results from Illumina Sequencing of EUK F_R Locus with Background Removed (no fish)



Discussion/Conclusion



- No Amoeba or related species detected from either locus
 - Unknown Sequences?
- Possible alternatives
 - Pathological host cell
 - Primer sets not specific for organism
 - Detection threshold of Illumina not tested
- Results of this study unable to provide identification for mysterious cell.

References



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Questions?