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Heterotrophic Protists as Useful Models for Studying Microbial Food Webs in a Model Soil Ecosystem and the Universality of Complex Unicellular Life

Andrew Robert Thompson

A dissertation submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

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ABSTRACT

Heterotrophic Protists as Useful Models for Studying Microbial Food Webs in a Model Soil Ecosystem and the Universality of Complex Unicellular Life

Andrew Robert Thompson
Department of Biology, BYU
Doctor of Philosophy

Heterotrophic protists, consisting largely of the Cercozoa, Amoebozoa, Ciliophora, Discoba and some Stramenopiles, are a poorly characterized component of life on Earth. They play an important ecological role in soil communities and provide key insights into the nature of one of life's most enigmatic evolutionary transitions: the development of the complex unicell. Soil ecosystems are crucial to the functioning of global biogeochemical cycles (e.g. carbon and nitrogen) but are at risk of drastic change from anthropogenic climate change. Heterotrophic protists are the primary regulators of bacterial diversity in soils and as such play integral roles in biogeochemical cycling, nutrient mobilization, and trophic cascades in food webs under stress. Understanding the nature of these changes requires examining the rates, diversity, and resiliency of interactions that occur between soil organisms. However, soils are the most taxonomically diverse ecosystems on Earth and disentangling the complexities of dynamic and varied biotic interactions in them requires a unique model system. The McMurdo Dry Valleys of Antarctica, one of the harshest terrestrial environments on Earth, serve as a model soil ecosystem owing to their highly reduced biological diversity. Exploring the functioning of heterotrophic protists in these valleys provides a way to test the applicability of this model system to other soil food webs. However, very little is known about their taxonomic diversity, which is a strong predictor of function. Therefore, I reviewed the Antarctic literature to compile a checklist of all known terrestrial heterotrophic protists in Antarctica. I found significant geographical, methodological, and taxonomic biases and outlined how to address these in future research programs. I also conducted a molecular survey of whole soil communities using 18 shotgun metagenomes representing major landscape features of the McMurdo Dry Valleys. The results revealed the dominance of Cercozoa and point to an Antarctic heterotrophic protist soil community that is taxonomically diverse and reflects the structure and composition of communities at lower latitudes. To investigate whether biotic interactions or abiotic factors were a larger driver for Antarctic heterotrophic protists, I conducted variation partitioning using environmental data (e.g. moisture, pH and electrical conductivity). Biotic variables were more significant and accounted for more of the variation than environmental variables. Taken together, it is clear that heterotrophic protists play key ecological roles in this ecosystem. Deeper insights into the ecology of these organisms in the McMurdo Dry Valleys also have implications for the search for complex unicellular life in our universe. I discuss the theoretical underpinnings of searching for these forms of life outside of Earth, conclude that they are likely to occur, and postulate how future missions could practically search for complex unicells.

Keywords: heterotrophic soil protists, McMurdo Dry Valleys, shotgun metagenomics, network analysis, life on Mars, key evolutionary innovations, universal complex unicellular life

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I would like to thank the MCM LTER for allowing me the opportunity to participate in their science initiatives and to be part of the team. Working in the Antarctic was an eye-opening experience from both a personal and professional perspective. Seeing how engaging and effective interdisciplinary science is has impressed upon me the importance of collaboration and maintaining a big picture perspective even when tackling specialized problems.

I would like to thank Taylor Thompson, my wife, who has been a huge support. I would not have and could not have completed this achievement without her. She has encouraged me constantly to keep pushing forward, to always do the best that I can. She has also been a steady, grounding force, helping me to see the value of taking breaks to rest and recharge, and being upbeat and positive when I was feeling down and overwhelmed. She has done all of this while

sacrificing so many of her own personal goals and ambitions, and I hope to be able to one day return the favor.

I would also like to thank Asher, my son, for being a relatively easygoing kid this last year and always making time for his Dad when he needed to smile.

I am grateful to my parents and siblings who have been extremely supportive of this endeavor and have continually been cheering me on. I would also like to thank my wife's family for their support and encouragement.

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TABLE OF CONTENTS

TITLE PAGE i

ABSTRACT ii

ACKNOWLEDGEMENTS iii

TABLE OF CONTENTS v

LIST OF TABLES ix

LIST OF FIGURES x

Chapter 1: Checklist of terrestrial protists from continental and peninsular Antarctica, including the South Shetland Islands 1

Abstract 2

Introduction 3

Methods 4

Results 5

Discussion 36

Acknowledgements 39

References 40

Chapter 2: Towards characterizing a microbial ecosystem: A review of the taxonomic and functional diversity of heterotrophic soil protists (protozoa) in Antarctic terrestrial ecosystems..59

Abstract

Introduction 61

Taxonomic diversity of HSPs in continental Antarctica 64

HSP diversity across habitats 70
Drivers of community structure 72
Abiotic factors 72
Biotic drivers: co-occurrence and trophic interactions 77
Methodological biases and challenges 79
Insights and future studies 82
Conclusions 84
Acknowledgements 85
References 86
Chapter 3: Shotgun metagenomes reveal the diversity of heterotrophic soil protists in a model polar ecosystem 138
Abstract 139
Introduction 140
Methods 142
Sampling methods and locations 142
Environmental parameters 143
DNA extraction and sequence generation 144
Taxonomic assignment of reads 145
Submission to MG-RAST 147
Results 147
Heterotrophic protists 149
Other eukaryotes 150
Environmental drivers 151

Discussion ... 152
MDV diversity trends ... 152
Drivers of eukaryotic diversity in MDV soils ... 154
Shotgun metagenomics in MDV soils present unique benefits and challenges ... 155
Conclusions ... 155
Acknowledgements ... 156
References ... 157
Supplementals ... 175
Sequencing and post-processing ... 175
Chapter 4: Contributions of Heterotrophic protists to a soil food web in a model polar desert ecosystem: Evidence that biotic factors matter ... 179
Abstract ... 180
Introduction ... 181
Methods ... 182
Shotgun metagenome sequencing and SSU identification ... 182
Statistical analyses ... 184
Results ... 186
Biotic vs. abiotic drivers ... 186
General composition of co-occurrence types ... 188
Trends in network analyses ... 188
Discussion ... 190
Conclusions ... 194

Acknowledgements 195
References 196
Chapter 5: Searching for complex unicellular life in the Universe: Are key evolutionary innovations repeatable? 219
Abstract 211
Introduction 221
The universality of complex unicellular life 222
The rarity of eukaryogenesis 223
Eukaryogenesis via predation of parasitism 223
Universal ecological niche space 224
Functional eukaryotes are identifiable 225
Testing for functional eukaryote analogs 227
Complex unicellular life on Mars 228
The initial evolution of eukaryote-analogs on Mars: Time and oxygen 228
Evolutionary limits in extreme environments 230
Understanding the limits of eukaryotes on Earth 231
Testing for non-independent lineages of life on Mars 233
Conclusions 234
Acknowledgements 235
References 236

LIST OF TABLES

Table 1.1: Taxonomic summary of terrestrial protozoa in continental Antarctica. 58

Supp. Table 2.1: Comprehensive list of studies on protozoan biodiversity in continental Antarctica. 109

Supp. Table 2.2: Presence/Absence for Antarctic heterotrophic protists by region and habitat. 113

Table 3.1: Site, sequencing and processing statistics 166

Table 3.2: Taxonomic breakdown of normalized SSU reads.. 167

Table 3.3: Taxonomic assignment table (genus).. 168

Supp. Table 3.1: Averages for environmental variables for all sites... 177

Supp. Table 3.2: PERMANOVA output table.. 178

Supp. Table 4.1: Partial redundancy analysis results by biotic subdivision and statistical component for each HSP response group.. 211

Supp. Table 4.2: Taxonomic reference for OTUs in network diagrams.............................. 216

LIST OF FIGURES

Figure 2.1: Alpha diversity and study count by biodiversity region.. 104

Figure 2.2: Study location and methodological type.. 105

Figure 2.3: Overall study target bias by morphological group.. 106

Figure 2.4: Ratio of shared to unique taxa by region.. 107

Figure 2.5: Taxa by habitat and by region. 108

Figure 3.1: Map of study area 171

Figure 3.2: Species richness by site and group. 172

Figure 3.3: Relative abundance of normalized reads by site. 173

Figure 3.4: Site community composition comparisons.. 174

Supp. Figure 3.1: Rarefaction curves for entire eukaryotic communities. 176

Figure 4.1: Variation Partitioning with All Biota as biotic component and environmental variables as abiotic component 204

Figure 4.2: Proportion of association types among OTUs of all groups given by the pairwise analysis of co-occurrence.. 205

Figure 4.3: Network of pairwise co-occurrence associations for Cercozoa 206

Figure 4.4: Network of pairwise co-occurrence associations for Ciliophora 207

Figure 4.5: Network of pairwise co-occurrence associations for Amoebozoa 208

Figure 4.6: Network of pairwise co-occurrence associations for Discoba and other heterotrophic protists 209

Figure 4.7: Proportion of bacterial phyla associated with HSP response groups 210

Figure 5.1: Common targets in the search for life against evolutionary time............................ 244

Figure 5.2: Schematic for isolating extraterrestrial phagotrophic unicells. 245

Chapter 1

Checklist of terrestrial protists from continental and peninsular Antarctica, including the South Shetland Islands

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Abstract

Heterotrophic soil protists encompass lineages that are both evolutionarily ancient and highly diverse, providing an untapped wealth of scientific insight. Yet, the diversity of free-living heterotrophic soil protists is still largely unknown. In an effort to contribute to our understanding of protist diversity, we present a checklist of these protists from Antarctica. As a pole, Antarctica is especially susceptible to rising temperatures caused by anthropogenic climate change. No comprehensive evaluation of heterotrophic soil protist diversity exists for this continent. Establishing a baseline for future conservation efforts of Antarctic protists is therefore important. Our literature search found 236 taxa identified to species and an additional 303 taxa identified to higher taxonomic levels in 54 studies spanning over 100 years of research. Isolated by distance, climate, and the circumpolar vortex, Antarctica is the most extreme continent on Earth: it is not unreasonable to think that it may host physiologically and evolutionarily unique species of protists, yet currently most species discovered in Antarctica are considered cosmopolitan. Additional sampling of the more extreme, intra-continental zones will likely result in the discovery of more novel and unique taxa.

Introduction

The global diversity of free-living protists is not known although estimates range from <30,000 (Mora et al. 2011) to over 1 million species (Adl et al. 2007, Cotterill et al. 2008, Larsen et al. 2017), with many in between (Appeltans et al. 2012, Pawlowski et al. 2012, de Vargas et al. 2015). Improved understanding of protistan diversity of soils in ice-free regions around Antarctica (approximately 0.5% of the continent (Burton-Johnson et al. 2016)) can help to refine these estimates. As the most extreme and isolated continent on Earth, Antarctic soils are home to many phylogenetically and physiologically unique species. Unfortunately, these soil communities are on the verge of experiencing major shifts in the face of climate change (Amesbury et al. 2017), and some of the more specialized species may risk extinction (Frenot et al. 2005, Hughes et al. 2015), including heterotrophic soil protists (HSPs) that play key roles in nutrient cycling and community structure. Conservation of these at-risk, scientifically intriguing species is therefore a high priority (Chown and Convey 2007), yet a checklist of HSP species for Antarctica does not exist. Here, we present the known diversity of Antarctic HSPs in order to establish a baseline for conservation efforts and a framework for future protist biodiversity research in Antarctica's ice-free regions.

As a group, HSPs possess a high degree of morphological, physiological, evolutionary and ecological diversity (Doolittle et al. 1996, Couteaux and Darbyshire 1998, Geisen et al. 2018). They play unique and essential roles in soil ecosystems, including promoting prey diversity and mobilizing nutrients to higher trophic levels (Corliss 2004, Clarholm 2005, Anderson 2012, Rønn et al. 2012, Wilkinson et al. 2012). In Antarctica, these protists have been studied for over 100 years (Richters 1908, Sudzuki 1979, Czechowski et al. 2016). The most recent review of this diversity listed 50 zooflagellates, 15 gymnamoebae, 60 testate amoeba and

75 ciliates, or 200 total species, yet the studies were heavily biased towards the Antarctic Peninsula, South Orkney Islands and other maritime Antarctic Islands (Smith 1996). Generally, the northern peninsula is warmer and wetter relative to continental sites and hosts large swaths of moss beds and input from seabirds and other marine mammals. Coastal continental sites (i.e. East Antarctica, Dronning Maud Land, Enderby Land) are colder and dryer but still experience moisture, chemical and biological input from the sea. Intra-continental sites (i.e. Transantarctic Mountains, Ellsworth Land and Mountains, South & North Victoria Land, Prince Charles Mountains) host ice free regions that are among the driest and coldest on Earth and are often used as analogs for other planets (e.g. Mars) (Doran et al. 2010, Heldmann et al. 2013). Overall, Antarctica's environment is extreme, and even higher productivity sites (e.g. Northern Antarctic Peninsula) are still limited in their biodiversity. In the coldest and driest sites, terrestrial life is limited to low diversity microbial communities in sandy, mineral soils (Priscu 2013). Assessing HSP diversity in these latter regions is especially important to the conservation of these highly unique ecosystems.

Methods

This checklist focuses on continental and peninsular Antarctica but includes the South Shetland Islands and Elephant Island due to their proximity to the northern tip of the peninsula. We reviewed all studies we could find on HSPs in these regions since the beginning of formal research in Antarctica, with the earliest dated at 1907 (Richters 1907), and the most recent in 2018 (Park et al. 2018). Searches were performed using variations on the keywords "Antarctica", "terrestrial", "moss" and "soil" coupled with "protist", "protozoa", "ciliate", "ciliophora",

“testate”, “amoeba”, “flagellate”, “cercozoa”, “excavata”, “euglenozoa”, “mycetozoa” and “slime mould” in Web of Science, SCOPUS and Google scholar, and by following citation chains in all articles found. (Ing B and Smith 1983, Putzke et al. 2004)We include a brief taxonomic history for each entry since taxonomic identifications have changed for many species over the last 100 years and many of the records reviewed are ecological and at times reflect erroneous designations. To ensure accuracy, an additional search was performed using the same databases to verify the most recent accepted taxonomic position and list pertinent nomenclatural changes.

Results

In our review of 54 studies on HSP diversity in Antarctica, we recovered a total of 539 taxa (Table 1). Of this total, 236 were identified to species: 95 Ciliophora, 84 Amoebozoa (including 7 species of slime mold (Horak 1966, Ing B and Smith 1983, Arambarri and Spinedi 1989, Putzke et al. 2004)), 39 Cercozoa, 13 Excavata, 3 Stramenopiles, 1 Apicomplexan (*Colpodella edax*) and 1 *incertae sedis* (*Polypseudopodius bacterioides*). An additional 303 taxa not identified to species were recorded, 194 of which were identified as far as genus. The 109 remaining include the records from those studies that did not identify past the morphological phylum level (i.e. ciliate, flagellate, testate amoeba)(Steele et al. 1994) as well as unclassified OTUs from molecular studies (Fell et al. 2006, Czechowski et al. 2016, Obbels et al. 2016).

SAR: Stramenopile (Chrysophyceae)

Oikomonas mutabilis Kent, 1880

Oikomonas termo (Müller, 1773)

Monas termo Müller, 1773 (*orig.*)

Heterochromulina termo (Ehrenberg) (*syn. no year*)

Oikomonas termo (Müller, 1773) Kent, 1880 (*reclass.*)

Oikomonas termo Ehrenberg, 1838 (*error in authorship*)

Oicomonas termo Ehrenberg, 1838 (*error in authorship, misspelling*)

SAR: Stramenopile (Dictyochophyceae)

Actinomonas mirabilis Kent, 1880

SAR: Alveolata (Apicomplexa)

Colpodella edax (Klebs, 1892)

Bodo edax Klebs, 1892 (*orig.*)

Heteromita angusta Dujardin, 1841 (*syn.*)

Bodo caudatus Stein, 1878 *sensu* Hänel, 1979 (*syn. in part*) see *Parabodo caudatus*

Spiromonas angusta (Dujardin, 1841) Kent, 1881 (*syn.*)

Bodo celer Klebs, 1892 (*syn. no year*)

Colpodella angusta (Dujardin, 1841) Simpson and Patterson, 1996 (*syn.*)

Colpodella edax (Klebs, 1892) Simpson and Patterson, 1996 (*reclass.*)

SAR: Alveolata (Ciliophora)

Acineria uncinata Tucolesco, 1962

Acineria uncinata Dujardin, 1841 (*error in authorship*)

Acuholosticha paranotabilis (Foissner, Agatha and Berger 2002)

Uroleptus paranotabilis Foissner, Agatha and Berger 2002 (*orig.*)

Cuadiholosticha paranotabilis (Foissner, Agatha and Berger, 2002) Berger, 2006 (*reclass.*)

Acuholosticha paranotabilis (Foissner, Agatha and Berger 2002) Li et al., 2017 (*reclass.*)

Anteholosticha rectangular Jung, Park and Kim, 2016

Anteholosticha sigmoidea (Foissner, 1982)

Holosticha sigmoidea Foissner, 1982 (*orig.*)

Anteholostigma sigmoidea (Foissner, 1982) Berger, 2003 (*reclass.*)

Blepharisma hyalinum Perty, 1849

Blepharisma hyalinum Perty, 1852 (*error in year*)

Bryophyllum loxophylliforme Kahl, 1931

Bryophyllum tegularum Kahl, 1931

Adumbratosticha tetracirrata (Buitkamp and Wilbert, 1974)

Holosticha tetracirrata Buitkamp and Wilbert, 1974 (*orig.*)

Caudiholosticha tetracirrata (Buitkamp and Wilbert, 1974) Berger, 2003 (*reclass.*)

Adumbratosticha tetracirrata (Buitkamp and Wilbert, 1974) Li et al., 2017 (*reclass.*)

Cinetochilum margaritaceum (Ehrenberg, 1831)

Cyclidium margaritaceum Ehrenberg, 1831 (*orig.*)

Cinetochilum margaritaceum (Ehrenberg, 1831) Perty, 1852 (*reclass.*)

Cinetochilum margarcliclum (Ehrenberg) (*misspelling*)

Codonella cratera (Leidy, 1877)

Difflugia crater Leidy, 1877 (*orig.*)

Codonella lac ustris Entz, 1885 (*syn.*)

Codonella cratera (Leidy, 1877) Imhof, 1885 (*reclass.*)

Colpoda californica Kahl, 1931

Colpoda cucullus (Müller, 1773)

Kolpoda cucullus Müller, 1773 (*orig.*)

Colpoda cucullus (Müller, 1773) Gmelin, 1790 (*reclass.*)

Colpoda cuculla (Müller, 1773): Hada, 1967 (*misspelling*)

Colpoda ecaudata (Liebmann, 1936)

Cyclidium ecaudatum Liebmann, 1936 (*orig.*)

Balantiophorus minutus Schewiakoff *sensu* Watson, 1945 (*syn*.)

Colpoda ecaudata (Liebmann, 1936) Foissner, Blatterer, Berger and Kohmann, 1991 (*reclass* .)

Colpoda inflata (Stokes, 1884)

Tillina inflata Stokes, 1884 (*orig.*)

Colpoda rouxi Kahl, 1926 (*syn*.)

Colpoda inflata (Stokes, 1884) Kahl, 1931 (*reclass*.)

Colpoda inflata (Stokes, 1885) Kahl, 1931 (*reclass*., *error in year*)

Colpoda maupasi Enriques, 1908

Colpoda fastigata Kahl, 1931 (*syn*.)

Colpoda matritensis Ocariz, Rico and Munoz, 1965 (*syn*.)

Colpoda steinii Maupas, 1883

Colpoda steini Maupas, 1883: Sudzuki, 1979 (*misspelling*)

Tillina saprophila Stokes, 1884 (*syn*.)

Colpoda saprophila (Stokes, 1884) (*syn*.)

Colpoda duodenaria Taylor and Furgason, 1938 (*syn*.)

Colpoda steni (*misspelling*)

Colpoda dragescoi Chardez, 1981 (*syn*.)

Cyclidium glaucoma Müller, 1786

Cyclidium muscicola Kahl, 1931

Cyrtohymena candens (Kahl, 1932)

Steinia candens Kahl, 1932 (*orig*.)

Steinia simplex Dragesco, 1966 (*syn*.)

Cyrtohymena candens (Kahl, 1932) Foissner, 1989 (*reclass.*)

Cyrtohymena citrina (Berger and Foissner, 1987)

Steinia citrina Foissner, 1985 (*nomen nudum*)

Steinia citrina Berger and Foissner, 1987 (*orig.*)

Cyrtohymena citrina (Berger and Foissner, 1987) Foissner, 1989 (*reclass.*)

Cyrtolophosis acuta Kahl, 1926

Cyrtolophosis mucicola Stokes, 1885

Dichilum cuneiforme Schewiakoff, 1889

> *Dichilium cuneiforme* Schewiakoff (*misspelling*)
>
> *Dichilum cunciforme* (*misspelling*)
>
> *Dichilum cuneiforme* Schewiakoff, 1892 (*error in year*)

Drepanomonas revoluta Penard, 1922

> *Drepanomonas borzai* Lepsi, 1948 (*syn.*)

Drepanomonas sphagni Kahl, 1931

Enchelys polynucleata (Foissner, 1984)

> *Enchelydium polynucleatum* Foissner, 1984 (*orig.*)
>
> *Enchelys polynucleata* (Foissner, 1984) Foissner, Agatha and Berger, 2002 (*reclass.*)

Epispathidium papilliferum (Kahl, 1930)

> *Spathidium papilliferum* Kahl, 1930 (*orig.*)
>
> *Epispathidium papilliferum* (Kahl, 1930) Foissner, 1984 (*reclass.*)

Fuscheria lacustris Song and Wilbert, 1989

Fuscheria terricola Berger, Foissner and Adam, 1983

Gastronauta derouxi Blatterer and Foissner, 1992

Gonostomum affine (Stein, 1859)

> *Oxytricha affinis* Stein, 1859 (*orig.*)
>
> *Plagiotricha (Gonostomum) affinis* Stein, 1859 (*syn.*)
>
> *Stichochaeta affinis* (Stein, 1859) Gourret and Roeser, 1888 (*syn.*)
>
> *Gonostomum algicola* Gellért, 1942 (*syn.*)

Gonostomum bryonicolum Gellért, 1956 (*syn.*)

Gonostomum ciliophorum Gellért, 1956 (*syn.*)

Gonostomum spirotrichoides Gellért, 1956 (*syn.*)

Gonostomum geleii Gellért, 1957 (*syn.*)

Gastrostyla affine (Stein, 1859) Borror, 1972 (*syn.*)

Trachelostyla bryonicolum (Gellért, 1956) Borror, 1972 (*syn.*)

Trachelostyla ciliophorum (Gellért, 1956) Borror, 1972 (*syn.*)

Trachelostyla geleii (Gellért, 1957) Borror, 1972 (*syn.*)

Trachelostyla spirotrichoides (Gellért, 1956) Borror, 1972 (*syn.*)

Trachelostyla canadensis Buitkamp and Wilbert, 1974 (*syn.*)

Trachelostyla affine (Stein, 1859) Small and Lynn, 1985 (*syn.*)

Gonostomum singhii Kamra, Kumar and Sapra, 2008 (*syn.*)

Grossglockneria acuta Foissner, 1980

Halteria grandinella (Müller, 1773)

Trichoda grandinella Müller, 1773 (*orig.*)

Halteria grandinella (Müller, 1773) Dujardin, 1841 (*reclass*.)

Hemiurosomoida longa (Gelei and Szabodos, 1950)

Oxytricha longa Gelei and Szabodos, 1950 (*orig*.)

Urosomoida longa (Gelei and Szabodos, 1950) Foissner et al., 1991 (*reclass*.)

Hemiurosomoida longa (Gelei and Szabodos, 1950) Singh and Kamra, 2015 (*reclass*.)

Heterourosomoida lanceolata (Shibuya, 1930)

Oxytricha lanceolata Shibuya, 1930 (*orig*.)

Heterourosomoida lanceolata (Shibuya, 1930) Singh and Kamra, 2015 (*reclass*.)

Holosticha pullaster (Müller, 1773)

Trichoda pullaster Müller, 1773 (*orig.*)

Oxytricha pullaster (Müller, 1773) (*syn.*)

Kerona pullaster (Müller, 1773) (*syn.*)

Amphisia micans (Engelmann, 1862) (*syn.*)

Oxytricha micans Engelmann, 1862 (*syn.*)

Holosticha micans (Engelmann, 1862) (*syn.*)

Oxytricha alba Fromentel, 1876 (*syn.*)

Amphisia multiseta Sterki, 1878 (*syn.*)

Holosticha simplicis Wang and Nie, 1932 (*syn.*)

Keronopsis retrovacuolata (Tucolesco, 1952) (*syn.*)

Holosticha kessleri var. aquae-dulcis Buchar, 1957 (*syn.*)

Keronopsis litoralis Gellért and Tamas, 1958 (*syn.*)

Holosticha danubialis Kaltenbach, 1960 (*syn.*)

Holosticha retrovacuolata Tucolesco, 1962 (*syn.*)

Holosticha coronata Vuxanovici, 1963 (*syn.*)

Holosticha minima Vuxanovici, 1963 (*syn.*)

Holosticha rhomboedrica Vuxanovici, 1963 (*syn.*)

Holosticha rhomboedrica f. eliptica Vuxanovici, 1963 (*syn.*)

Holosticha rhomboedrica f. lata Vuxanovici, 1963 (*syn.*)

Holosticha rostrata Vuxanovici, 1963 (*syn.*)

Holosticha rostrata f. pitica Vuxanovici, 1963 (*syn.*)

Holosticha rostrata var. mononucleata Stiller, 1974 (*syn.*)

Pseudokeronopsis retrovacuolata (Tucoleso, 1962) Borror and Wicklow, 1983 (*syn.*)

Holosticha pullaster (Müller, 1773) Foissner, Blatterer, Berger and Kohmann, 1991 (*reclass.*)

Homalogastra setosa Kahl, 1926

Kahlilembus attenuatus (Smith, 1897)

Lembus attenuata Smith, 1897 (*orig.*)

Lembus fusiformis Kahl, 1926 (*syn.*)

Cohnilembus fusiformis Kahl 1926 (*syn.*)

Kahlilembus attenuatus (Smith, 1897) Foissner, Berger and Kohmann, 1994 (*reclass.*)

Keronopsis helluo Penard, 1922

Lamtostyla perisincirra (Hemberger, 1985)

Tachysoma perisincirra Hemberger, 1985 (*orig.*)

Lamtostyla perisincirra (Hemberger 1985) Berger and Foissner 1987 (*reclass.*)

Lamtostylides edaphoni (Berger and Foissner, 1987)

Amphisiella edaphoni Berger and Foissner, 1987 (*orig.*)

Lamtostyla edaphoni Berger and Foissner, 1987 (*syn.*)

Lamtostylides edaphoni (Berger and Foissner, 1987) Berger, 2008 (*reclass.*)

Leptopharynx costatus Mermod, 1914

Leptopharynx sphagnetorum (Levander, 1900)

Trichopelma sphagnetorum Levander, 1900 (*syn*.)

Trichoderum sphagnetorum (Levander, 1900) Strand, 1942 (*syn*.)

Leptopharynx sphagnetorum (Levander, 1900) Corliss, 1960 (*reclass.*)

Microdiaphanosoma arcuatum (Grandori and Grandori, 1934)

Diaphanosoma arcuata Grandori and Grandori, 1934 (*orig.*)

Microthorax elegans Giraud, 1863

Microthorax simulans (Kahl, 1926)

Microthorax simulans (Kahl, 1926) Kahl, 1931

Nassula tuberculata Foissner, Agatha and Berger, 2002

Nivaliella plana Foissner, 1980

Odontochlamys wisconsinensis (Kahl, 1931)

Chilodonella wisconsinensis Kahl, 1931 (*orig.*)

Odontochlamys wisconsinensis (Kahl, 1931) Petz and Foissner, 1997 (*reclass.*)

Opercularia curvicaule (Penard, 1922)

Pyxidium curvicaule Penard, 1922 (*orig.*)

Pyxidium arboricolum Biegel, 1954 (*syn.*)

Pyxidium arboricola Biegel, 1954 (*syn*)

Opercularia arboricolum Biegel, 1954 (*syn.*)

Opercularia arboricola (Biegel, 1954) Foissner, 1981 (*syn.*)

Opercularia curvicaule (Penard, 1922) Foissner, 1998 (*reclass.*)

Orthamphisiella breviseries Foissner, Agatha, and Berger, 2002

Orthamphis breviseries Foissner, Agatha, and Berger, 2002: Fell, 2006 (*misspelling*)

Oxytricha fallax Stein, 1859

Oxytricha granulifera Foissner and Adam, 1983

Oxytricha opisthomuscorum Foissner, Blatterer, Berger and Kohmann, 1991

Oxytricha setigera Stokes, 1981

Paradileptus elephantinus (Svec, 1897)

Dileptus elephantinus Svec, 1897 (*orig.*)

Pelagodileptus elephantinus Svec, 1897 (*syn.*)

Paradileptus elephantinus (Svec, 1897) Kahl, 1931 (*reclass.*)

Amphileptus moniliger Ehrenberg, 1835 (*syn.*)

Amphileptus flagellatus Rousselet, 1890 (*syn.*)

Paradileptus flagellatus (Rousselet, 1890) Wenrich, 1929 (*syn.*)

Paradileptus robustus Wenrich, 1929 (*syn.*)

Paradileptus conicus Wenrich, 1929 (*syn.*)

Paradileptus ovalis Huber-Pestalozzi, 1945 (*syn.*)

Paradileptus estensis Canella, 1951 (*syn.*)

Paradileptus minutus Dragesco, 1972 (*syn.*)

Paraenchelys terricola Foissner, 1984

Paraholosticha muscicola (Kahl, 1932)

Keronopsis muscicola Kahl, 1932 (*orig*.)

Paraholosticha muscicola (Kahl, 1932) Wenzel, 1953 (*reclass.*)

Paramecium putrinum Claparède and Lachmann, 1858

Paramecium trichium Stokes, 1885 (*syn.*)

Paroxytricha longigranulosa (Berger and Foissner, 1989)

Oxytricha longigranulosa Berger and Foissner, 1989 (*orig*.)

Paroxytricha longigranulosa (Berger and Foissner, 1989) Foissner, 2016 (*reclass*.)

Plagiocampa difficilis Foissner, 1981

Platyophrya vorax Kahl, 1926

Pleuroplitoides smithi Foissner, 1996

Pleurotricha lanceolata (Ehrenberg, 1835)

Stylonychia lanceolata Ehrenberg, 1835 (*orig.*)

Pleurotricha lanceolata (Ehrenberg, 1835) Stein, 1859 (*reclass.*)

Protospathidium fraterculum Xu and Foissner, 2005

Protospathidium serpens (Kahl, 1930) Foissner, 1981 (*syn. in part*)

Protospathidium terricola Foissner, 1998

Pseudochilodonopsis mutabilis Foissner, 1981

Pseudocohnilembus pusillus (Quennerstadt, 1869)

Lembus pusillus Quennerstadt, 1869

Pseudocohnilembus pusillus (Quennerstadt, 1869) Foissner and Wilbert, 1981 (*reclass.*)

Pseudocyrtolophosis alpestris Foissner, 1980

Pseudoholophrya terricola Berger, Foissner, and Adam, 1984

Pseudonotohymena antarctica Park, Jung, Min and Kim 2016

Pseudoplatyophrya nana (Kahl, 1926)

Platyophrya nana Kahl, 1926 (*orig.*)

Pseudoplatyophrya nana (Kahl, 1926) Foissner, 1980 (*reclass.*)

Pseudoplatyophrya saltans Foissner, 1988

Rigidohymena quadrinucleata (Dragesco and Njiné, 1971)

Steinia quadrinucleata Dragesco and Njiné, 1971 (*orig.*)

Cyrtohymena quadrinucleata (Dragesco and Njiné, 1971) Foissner, 1989 (*syn.*)

Rigidohymena quadrinucleata (Dragesco and Njiné, 1971) Berger, 2011 (*reclass.*)

Rurikoplites alpinus (Kahl, 1932)

Dileptus alpinus Kahl, 1932 (*orig.*)

Rurikoplites alpinus (Kahl, 1932) Vd'ačný and Rajter, 2015 (*reclass.*)

Sathrophilus muscorum (Kahl, 1931)

Saprophilus muscorum Kahl, 1931 (*orig.*)

Sathrophilus muscorum (Kahl, 1931) Corliss, 1960 (*reclass.*)

Spathidium claviforme Kahl, 1930

Spathidium seppelti Foissner, 1997

Sphaerophrya terricola Foissner, 1986

Sterkiella histriomuscorum Foissner, Blatterer, Berger, and Kohmann, 1991

Oxytricha trifallax Hunter, Cartinhour, Williams and Herrick, 1989 (*nomen nudum*)

Parasterkiella thompsoni (Foissner, 1996)

Sterkiella thompsoni Foissner, 1996 (*orig.*)

Parasterkiella thompsoni (Foissner, 1996) Küppers et al., 2011 (*reclass.*)

Tachysoma pellionellum (Müller, 1773)

Oxytricha pellionella Stein, 1859 (*syn.*)

Tachysoma agilis Stokes, 1887 (*syn.*)

Tachysoma pellionellum (Müller, 1773) Borror, 1972 (*reclass.*)

Tetrahymena rostrata Kahl, 1926

Trochilia minuta (Roux, 1899)

Dysteropsis minuta Roux, 1899 (*orig.*)

Trochilia minuta (Roux, 1901) (*error in year*)

Trochilia minuta (Kahl, 1931) (*error in authorship*)

Trochilia minuta (Roux, 1901) Kahl, 1931 (*reclass.*)

Uroleptus (*Caudiholosticha*) *antarctica* Park, Min and Kim 2018

Uronema nigricans (Müller, 1786)

Cyclidium nigricans Müller, 1786 (*orig.*)

Cryptochilium nigricans (Müller, 1773) Maupas, 1883 (*syn.*)

Uronema nigricans (Müller, 1786) Florentin, 1901 (*reclass*.)

Uronema parduczi Foissner, 1971 (*syn*.)

Urosomoida antarctica Foissner, 1996

Urosomoida granulifera Foissner, 1996

Urotricha agilis (Stokes, 1886)

Balanitozoon agilis Stokes, 1886 (*orig*.)

Urotricha agilis (Stokes, 1886) Kahl, 1930 (*reclass.*)

Vorticella astyliformis Foissner, 1981

Vorticella companula Ehrenberg, 1831

Vorticella aperta Fromental, 1874 (*syn.*)

Vorticella infusionum Dujardin, 1841

Vorticella microstoma Ehrenberg, 1830

Vorticella striata Dujardin, 1841

SAR: Rhizaria (Cercozoa)

Allantion tachyploon Sandon, 1924

Assulina muscorum Greeff, 1888

Asculina muscora Greeff: Hada, 1967 (*misspelling both species and genus*)

Assulina seminulum Leidy, 1879 (*syn., in part*)

Assulina minor Penard, 1890 (*syn.*)

Assulina seminulum (Ehrenberg, 1848)

Difflugia seminulum Ehrenberg, 1848 (*orig.*)

Asculina seminulum (Ehrenberg) (*misspelling*)

Difflugia Assulina seminulum Ehrenberg, 1871 (*syn.*)

Difflugia semen Ehrenberg, 1871 (*syn.*)

Euglypha brunnea Leidy, 1874 (*syn.*)

Euglypha seminulum Ehrenberg, 1845 (*syn., error in year)*

Euglypha seminulum Leidy, 1878 (*syn.*)

Assulina seminulum (Ehrenberg, 1848) Leidy, 1879 (*reclass.*)

Biomyxa vagans Leidy, 1879

Cavernomonas stercoris Vickerman, 2009 *in* Bass et al., 2009

Cercomonas agilis (Moroff, 1904)

Dimastigamoeba agilis Moroff, 1904 (*orig.*)

Cercobodo agilis (Moroff, 1904) Lemmermann, 1914 (*reclass.*)

Cercobodo agilis Martin (*error in authorship*)

Cercomonas agilis (Moroff, 1904) Mylnikov and Karpov, 2004 (*reclass.*)

Cercomonas longicauda Dujardin, 1841

Dimorpha longicauda (Dujardin, 1841) Klebs, 1892(*syn.*)

Cercobodo longicauda (Dujardin, 1841) Lemmerman, 1913 (*syn.*)

Cercomonas longicauda Stein (*error in authorship*)

Cercomonas plasmodialis (Mylnikov, 1985)

Cercobodo plasmodialis Mylnikov, 1985 (*orig.*)

Cercomonas plasmodialis (Mylnikov, 1985) Mylnikov, 1992 (*reclass.*)

Cercomonas vibrans (Sandon, 1927)

Cercobodo vibrans (Sandon, 1927) (*orig.*)

Cercomonas vibrans (Sandon, 1927) Mylnikov and Karpov, 2004 (*reclass.*)

Clathrulina elegans Cienkowski, 1867

Podosphaera haeckeliana Archer, 1869 (*syn.*)

Elaster greeffi Grimm, 1872 (*syn.*)

Clathrulina cienkowskyi Mereshkowsky, 1877 (*syn.*)

Clathrulina cienkowskyi ssp. *ovalis* von Daday, 1885 (*syn.*)

Clathrulina stuhlmanni Schaudinn, 1897 (*syn.*)

Clathrulina cienkowskii Mereshkowsky, 1877: Penard, 1913 (*misspelling*)

Clathrulina ovalis (von Daday, 1885) Deflandre, 1926 (*syn*.)

Corythion aerophila (Decloitre, 1850)

Trinema enchelys aerophila Decloitre, 1950 (*orig.*)

Corythion constricta (Certes, 1889)

Trinema constricta Certes, 1889 (*orig.*)

Corythion constricta (Certes, 1889) Jung, 1942 (*reclass.*)

Corythion dubium Tاránek, 1881

Arcella constricta Ehrenberg, 1841 (*syn., in part*)

Arcella disphaera Ehrenberg, 1841 (*syn., in part*)

Trinema acinus Leidy, 1879 (*syn., in part*)

Trinema constricta Certes, 1889 (*syn*.)

Euglypha bryophila Brown, 1911

Euglypha α Vejdovsky, 1882 (*syn*.)

Euglypha cristata Penard, 1890 (*syn., in part*)

Euglypha ciliata (Ehrenberg, 1848)

Difflugia ciliata Ehrenberg, 1848 (*orig.*)

Euglypha setigera Perty, 1852 (*syn. in part*)

Difflugia pilosa Ehrenberg, 1871 (*syn*.)

Difflugia ciliata Ehrenberg, 1871 (*syn*., *error in year*)

Euglypha ciliata (Ehrenberg, 1848) Leidy, 1878 (*reclass.*)

Euglypha ciliata f. glabra Wailes, 1915

Euglypha compressa Carter, 1864

Euglypha ampullacea Hertwig and Lesser, 1874 (*syn*.)

Euglypha ciliata Leidy, 1879 (*syn*., *in part*)

Euglypha α Vejdovsky, 1882 (*syn*., *in part*)

Euglypha compressa f. glabra Cash, 1915

Euglypha cristata Leidy, 1874

Euglypha denticulata Brown, 1912

Euglypha laevis (Ehrenberg, 1845)

Difflugia laevis Ehrenberg, 1845 (*orig.*)

Euglypha laevis (Ehrenberg, 1845) Perty, 1849 (*reclass.*)

Euglypha alveolata Leidy, 1879 (*syn., in part*)

Euglypha γ Vejdovsky, 1882 (*syn.*)

Euglypha rotunda Wailes and Penard, 1911

Euglypha rotunda Wailes (*error in authorship*)

Euglypha strigosa (Ehrenberg, 1871)

Difflugia strigosa Ehrenberg, 1871 (*orig.*)

Difflugia Setigerella strigosa Ehrenberg, 1871 (*syn.*)

Euglypha strigosa (Ehrenberg, 1871) Leidy, 1878 (*reclass.*)

Euglypha ciliata var. *strigosa* Leidy, 1879 (*syn., in part*)

Euglypha heterospina Penard, 1890 (*syn.*)

Euglypha strigosa f. glabra Wailes, 1898

Euglypha tuberculata Dujardin, 1841

Difflugia areolata Ehrenberg, 1841 (*syn.*)

Euglypha alveolata Dujardin, 1841 (*syn., in part*)

Euglypha tuberculosa Dujardin, 1841 (*syn.*)

Difflugia alveolata Pritchard, 1861 (*syn.*)

Euglypha pusilla Entz, 1877 (*syn.*)

Euglypha β Vejdovsky, 1882 (*syn.*)

Lecythium hyalinum Hertwig and Lesser, 1874

Paracercomonas crassicauda (Dujardin, 1836)

Cercomonas crassicauda Dujardin, 1836 (*orig.*)

Paracercomonas crassicauda (Dujardin, 1836) Bass and Cavalier-Smith, 2009 *in* Bass et al., 2009 (*reclass.*)

Cercomonas crassicauda Alexeieff (*error in authorship*)

Cercomonas crasicauda Lemmermann (*error in authorship*)

Pseudodifflugia gracilis Schlumberger, 1845

Pleurophrys sphaerica Claparède and Lachmann, 1858 (*syn*.)

Pleurophrys angulata Mereschkovsky, 1879 (*syn*.)

Pseudodifflugia gracilis var. *terricola* Bonnet and Thomas, 1960

Sainouron mikroteron Sandon, 1924

Spongomonas uvella Stein, 1878

Trachelocorythion pulchellum (Penard, 1890)

Corythion pulchellum Penard, 1890 (*orig.*)

Chorythion pulchellum Awerintzew, 1907 (*syn*.)

Trachelocorythion pulchellum (Penard, 1890) Bonnet, 1979 (*reclass.*)

Trinema contraria Decloitre, 1961

Trinema complanatum Penard, 1890

Trinema acinus Leidy, 1879 (*syn., in part*)

Trinema enchelys (Ehrenberg, 1838)

Difflugia enchelys Ehrenberg, 1838 (*orig., in part*)

Trinema acinus Dujardin, 1841 (*syn.*)

Arcella enchelys Ehrenberg, 1844 (*syn.*)

Arcela enchelys Ehrenberg, 1854 (*misspelling*, *error in year*)

Euglypha pleurostoma Carter, 1857 (*syn.*)

Euglypha enchelys Wallich, 1864 (*syn.*)

Trinema (*Difflugia*) *enchelli* Crevier, 1870 (*syn.*)

Trinema enchelys (Ehrenberg, 1838) Leidy, 1878 (*reclass.*)

Trinema enchelys (Ehrenberg, 1938) Leidy, 1878 (*error in year*)

Trinema enchelys (Ehrenberg, 1838) Leidy, 1879 (*error in year*)

Trinema enchelys Leidy (*error in authorship*)

Trinema lineare Penard, 1890

Difflugia enchelys Ehrenberg, 1838 (*orig., in part*)

Arcella hyalina Ehrenberg, 1841 (*syn.*)

Arcella enchelys Ehrenberg, 1847 (*syn.*)

Arcella enchelys Ehrenberg, 1854 (*error in year*)

Arcella enchelys alpha Ehrenberg, 1854 (*syn.*)

Trinema acinus Leidy, 1879 (*syn., in part*)

Trinema enchelys f. beta Awerintzew, 1906 (*syn.*)

Trinema lineare var. *truncatum* Chardez, 1964

Valkanovia elegans Schönborn, 1964

Excavata

Astasia inflata Dujardin, 1841

Bodo angustus (Dujardin, 1841)

Bodo angusta Dujardin, 1841 (*orig.*)

Bodo angustus (Dujardin, 1841) Bütschli 1883

Bodo globosus Stein, 1878

Bodo globose Stein, 1878 (*orig.*)

Bodo saltans Ehrenberg, 1831

Bodo jaculans Perty (*syn.*)

Naegleria gruberi (Schardinger, 1899)

Amoeba gruberi Schardinger, 1899 (*orig.*)

Naegleria gruberi (Schardinger, 1899) Wilson, 1916 (*reclass.*)

Naegleria neopolaris De Jonckheere, 2006

Parabodo caudatus (Dujardin, 1841)

Amphimonas caudatus Dujardin, 1841 (*orig.*)

Bodo caudatus (Dujardin, 1841) Stein, 1878 (*reclass.)*

Bodo alexeieffi Lemm. (*syn. no year*)

Bodo asiaticus Castellanii and Chalmers (*syn. no year*)

Bodo compressus Lemm. (*syn. no year*)

Bodo cruzi Hartm. and Chagas (*syn. no year*)

Bodo josephi Belar (*syn. no year*)

Bodo mutabilis Klebs 1892 (*syn*.)

Bodo obovatus Lemm. (*syn. no year*)

Bodo putrinus (Stokes) Lemm. (*syn. no year*)

Heteronema minima Form. (*syn. no year*)

Bodo caudatus Hollande (*error in authorship*)

Bodo cudatus (*misspelling*)

Parabodo caudatus (Dujardin 1841) Vickerman *in* Moreira, López-García and Vickerman 2004

Paratrimastix pyriformis (Klebs, 1893)

Tetramitus pyriformis Klebs, 1893 (*orig.*)

Coelotrichomastix convexa Hollande, 1939 (*syn*.)

Trimastix convexa (Hollande, 1939) Grassé, 1952 (*syn*.)

Percolomonas pyriformis (Klebs, 1893) Larsen and Patterson, 1990 (*syn*.)

Trimastix pyriformis (Klebs, 1893) Bernard et al. 2000 (*reclass.*)

Paratrimastix pyriformis (Klebs, 1893) Zhang, Táborsky, Silberman, Pánek, Čepička and Simpson, 2015 (*reclass.*)

Paravahlkampfia ustiana (Page, 1974)

Vahlkampfia ustiana Page, 1974 (*orig.*)

Paravahlkampfia ustiana (Page, 1974) Brown and De Jonckheere, 1999 (*reclass*.)

Peranemopsis trichophora (Ehrenberg, 1832)

Trachelius trichophorus Ehrenberg, 1832 (*orig.*)

Peranema trichophora Ehrenberg, 1838 (*error in year*)

Peranema trichophora (Ehrenberg, 1832) Dujardin, 1841 (*reclass.*)

Peranema trichophorum (Ehrenberg 1832) Stein, 1859 (*syn.*)

Paranema trichophorum (Ehrenberg 1832) Stein, 1878 (*syn.*)

Peranemopsis trichophora (Ehrenberg 1832) Péterfi, 1986 (*reclass.*)

Peranemopsis trichophora (Ehrenberg 1832) Péterfi, 1988 (*error in year*)

Petalomonas angusta (Klebs, 1893)

Petalomonas mediocanellata var. *angusta* Klebs, 1893 (*orig.*)

Petalomonas angusta (Klebs, 1893) Lemmermann, 1910 (*reclass.*)

Petalomonas augusta (Klebs, 1893) Lemmermann, 1910 (*misspelling*)

Petalomonas mediocanellata Stein, 1878

Tetramitus rostratus Perty, 1852

Vahlkampfia limax (Vahlkampf, 1905)

Amoeba limax Vahlkampf, 1905 (*orig.*)

Amoeba proteus Dujardin, 1841 (*syn., in part*)

Vahlkampfia limax (Vahlkampf, 1905) Chatton, 1912 (*reclass.*)

Amoebozoa

Acanthamoeba castellanii (Douglas, 1930)

Acanthamoeba castellanii (Douglas, 1930) Volkonsky, 1931 (*reclass.*)

Acanthamoeba castellani (Douglas, 1930) (*misspelling*)

Acanthamoeba polyphaga (Puschkarew, 1913)

Amoeba discoides Schaeffer, 1916

Amoeba discoides Greeff (*error in authorship*)

Amoeba limicola Rhumbler, 1894

Amoeba limicola Rhumbler, 1894 (*orig.*)

Pelomyxa limicola (Rhumbler, 1894) Bovee 1951 (*syn.*)

Pelomyxa limnicola (Rhumbler, 1894) (*misspelling*)

Arcella arenaria Greeff, 1866

Arcella aureola Maggi, 1883 (*syn.*)

Arcella microstoma Penard, 1890 (*syn.*)

Arcella arenaria var. *compressa* Chardez, 1965

Arcella arenaria var. *sphagnicola* Deflandre, 1928

Arcella vulgaris Ehrenberg, 1830

Arcella vulgaris Ehr. (*abbrev. author*)

Astramoeba radiosa (Ehrenberg, 1830)

Amoeba radiosa Ehrenberg, 1830 (*orig.*)

Calomyxa metallica (Berk., 1837)

Physarum metallicum Berk., 1837 (*orig*.)

Cornuvia metallica (Berk.) Rostafinsky, 1876 (*reclass*.)

Oligonema aeneum P. Karst., 1879 (*syn*.)

Perichaena krupii Racib., 1889 (*syn*.)

Perichaena plasmodiocarpa Blytt, Förh, 1892 (*syn*.)

Margarita metallica (Berk.) Lister, 1894 (*reclass*.)

Margarita pictoviana Moore, 1902 (*syn*.)

Margarita metallica var. *intermedia* Meylan, 1910 (*syn*.)

Margarita metallica var. *plasmodiocarpa* (Blytt) R.E. Fr., 1912 (*reclass*.)

Cornuvia metallica var. *intermedia* (Meylan, 1910) Sacc. & Trotter, 1913 (*reclass*.)

Calomyxa metallica (Berk., 1837) Nieuwl., 1916 (*reclass*.)

Calomyxa metallica var. *megaspora* Yamamoto & Nannenga-Bremekamp 1990, in Nannenga-Bremekamp & Yamamoto, 1990 *(syn*.)

Centropyxis aculeata (Ehrenberg, 1832)

Arcella aculeata Ehrenberg, 1832 (*orig.*)

Difflugia aculeata Perty, 1852 (*syn.*)

Echinopyxis aculeata Claparède et Lachmann, 1859 (*syn.*)

Centropyxis aculeata (Ehrenberg, 1832) Stein, 1859 (*reclass.*)

Centropyxis aculeata Stein, 1857 (*error in authorship, error in year*)

Centropyxis aerophila Deflandre, 1929

Difflugia constricta Ehrenberg, 1838 (*syn.*, *in part*)

Arcella arctiscon Ehrenberg, 1854 (*syn.*)

Centropyxis aerophila var. *sphagnicola* Deflandre, 1929

Centropyxis cassis (Wallich, 1864)

Centropyxis cassis (Wallich, 1864) Deflandre, 1929 (*reclass.*)

Centropyxis constricta (Ehrenberg, 1838)

Difflugia constricta Ehrenberg, 1838 (*orig.*)

Arcella consricta Ehrenberg, 1841 (*syn*.)

Centropyxis constricta (Ehrenberg, 1838) Deflandre, 1929 (*reclass.*)

Centropyxis elongata (Penard, 1890)

Difflugia constricta var. *elongata* Penard, 1890 (*orig.*)

Centropyxis elongata (Penard, 1890) Thomas, 1959 (*reclass.*)

Centropyxis minuta Deflandre, 1929

Difflugia constricta Leidy, 1879 (*syn.*)

Difflugia constricta Penard, 1902 (*syn.*)

Centropyxis sylvatica (Deflandre, 1929)

Centropyxis aerophila var. *sylvatica* Deflandre, 1929 (*orig.*)

Centropyxis sylvatica (Deflandre, 1929) Bonnet and Thomas, 1955 (*reclass.*)

Cryptodifflugia compressa Penard, 1902

Cryptodifflugia sacculus (Penard, 1902)

Difflugiella sacculus Penard, 1902 (*orig*.)

Cryptodifflugia sacculus (Penard, 1902) Deflandre, 1953 (*reclass*.)

Cryptodifflugia oviformis Penard, 1890

Difflugiella oviformis Bonnet and Thomas, 1955 (*syn*.)

Cryptodifflugia operculata Page, 1966 (*syn*.)

Cyclopyxis eurystoma Deflandre, 1929

Centropyxis (*Cyclopyxis*) *eurystoma* Deflandre, 1929

Diderma antarcticolum Horak, 1966S

Diderma crustaceum (Peck, 1873)

Diderma crustaceum Peck, 1873 (*orig*.)

Chondrioderma crustaceum (Peck, 1873) Peck., 1878 [“1879”] (*syn*.)

Chondrioderma crustaceum (Peck, 1873) Berl., 1888 [Comb. Superfl., previously proposed by Peck, 1878]

Diderma niveum (Rostafinsky, 1874)

Chondrioderma niveum Rostafinsky, 1874 (*orig*.)

Chondrioderma physaroides Rostafinsky, 1874 (*syn*.)

Diderma albescens Phillips, 1877 (*syn*.)

Chondrioderma albescens (Phillips, 1877) Massee, 1892 (*reclass*.)

Diderma niveum (Rostafinsky, 1874) Sheldon 1895 (*reclass*.)

Diderma niveum (Rostafinsky, 1874) Kuntze, Revis., 1898 (*reclass*.) [Comb. Superfl., previously proposed by Sheldon, 1895]

Diderma niveum (Rostafinsky, 1874) Macbride, 1899 [Comb. Superfl., previously proposed by Sheldon, 1895] (*reclass*.)

Diderma niveum f. *pulverulentum* Meylan, 1922 (*syn*.)

Diderma niveum f. *endoleucum* Meylan, 1924 (*syn*.)

Diderma niveum var. *ferrugineum* Meylan, 1924 (*syn*.)

Diderma niveum var. *ferruginea* Meylan, 1924 (*misspelling*)

Diderma subcaeruleum Kowalski, 1968 (*syn*.)

Diderma cristatosporum Sánchez, Moreno and Illana, 2002 (*syn*.)

Diderma niveum var. *cristatosporum* (Sánchez, Moreno and Illana, 2002) Singer, Moreno, Illana and Sánchez, 2003 *in* Moreno, Singer, Illana and Sánchez, 2003 (*reclass*.)

Difflugia ampullula Playfair, 1918

Difflugia bryophila (Penard, 1902)

Difflugia piriformis var. *bryophila* Penard, 1902 (*orig.*)

Difflugia oblonga var. *longicollis* Gassowsky, 1936 (*syn.*)

Difflugia bryophila (Penard, 1902) Jung, 1942 (*reclass.*)

Difflugia longicollis (Gassowsky, 1936) Ogden and Hedley, 1980 (*syn.*)

Difflugia gassowskii Ogden, 1983 (*syn.*)

Difflugia globulosa Dujardin, 1837

Difflugia proteiformis globularis Wallich, 1864 (*syn.*)

Difflugia globularis (Wallich, 1864) Leidy, 1877 (*syn.*)

Difflugia chardezi Godeanu, 1972 (*syn.*)

Difflugia lanceolata Penard, 1890

Difflugia lucida Penard, 1890

Difflugia manicata var. *langhovdensis* Sudzuki, 1964

Difflugia mica Frenzel, 1892

Difflugia pristis Penard, 1902

Difflugia pulex Penard, 1890

Difflugia minuta var. *minor* Godeanu, 1972 (*syn.*)

Difflugia ovalisina Beyens et Chardez, 1994 (*syn.*)

Certesella certesi (Penard, 1911)

Nebela certesi Penard, 1911 (*orig.*)

Certesella certesi (Penard, 1911) Loeblich and Tappan, 1961 (*reclass*.)

Cryptodifflugia apiculata (Cash, 1904)

Difflugiella apiculata Cash, 1904 (*orig*.)

Cryptodifflugia apiculata (Cash, 1904) Page, 1966 (*reclass*.)

Diplochlamys gruberi Penard, 1909

Diplochlamys timida Penard, 1909

Diplochlamys vestita Penard, 1909

Echinamoeba silvestris Page, 1975

Pyxidicula operculata (Agardh, 1827)

Frustulia operculata Agardh, 1827 (*orig*.)

Cymbella operculata (Agardh, 1827) Agardh, 1830 (*reclass*.)

Galionella operculata (Agardh, 1827) Ehrenberg, 1834 (*reclass.*)

Pyxidicula operculata (Agardh, 1827) Ehrenberg, 1838 (*reclass.*)

Pyxidicula operculata Ehrenberg (*error in authorship*)

Heleopera petricola Leidy, 1879

Heleopera sylvatica Penard, 1890

Hyalosphenia elegans (Leidy, 1874)

Difflugia elegans Leidy, 1874 (*orig*.)

Hyalosphenia elegans (Leidy, 1874) Leidy, 1879 (*reclass.*)

Hyalosphenia turfacea Tatánek, 1881 (*syn.*)

Hyalosphenia elegans Leidy var. *major* Decloitre, 1964

Hyalosphenia minuta Cash, 1891

Hyalosphenia subflava Cash, 1909

Hyalosphenia subflava Cash and Hopkinson (*error in authorship*)

Hyalosphenia subflava Hopkinson (*error in authorship*)

Leptoderma megaspora Arambarri and Spinedi, 1989

Mayorella clavabellans Bovee, 1970

Mayorella vespertilio (Penard, 1902)

Amoeba vespertilio Penard, 1902 (*orig.*)

Mayorella vespertilio (Penard, 1902) LaPage, 1922 (*reclass*.)

Microchlamys patella (Claparède and Lachmann, 1859)

Pseudochlamys patella Claparède and Lachmann, 1859 (*orig.*)

Microchlamys patella (Claparède and Lachmann, 1859) Cockerell, 1911 (*reclass.*)

Microcorycia tessellata (Penard, 1917)

Corycia tessellata Penard, 1917 (*orig*.)

Microcorycia tessellata (Penard, 1917) Chardez, 1965 (*reclass*.)

Microcorycia bryophila Decloitre, 1974 (*syn*.)

Microcorycia flava (Greeff, 1866)

Amphizonella flava Greeff, 1866 (*orig.*)

Corycia flava (Greeff, 1866) Penard, 1902 (*syn.*)

Microcorycia flava (Greeff, 1866) Cockerell, 1911 (*reclass.*)

Microcorycia radiata (Brown, 1912)

Corycia radiata Brown, 1912 (*orig.*)

Microcorycia radiata (Brown, 1912) Hopkinson, 1919 (*reclass.*)

Nebela bohemica Taránek 1882 var. *adelia* Decloitre, 1964

Nebela collaris (Ehrenberg, 1848)

Difflugia collaris Ehrenberg, 1848 (*orig.)*

Diffluga cancellata Ehrenberg, 1848 (*syn.*)

Difflugia reticulata Ehrenberg,1848 (*syn.*)

Difflugia carpio Ehrenberg, 1854 (*syn.*)

Difflugia laxa Ehrenberg, 1871 (*syn.*)

Difflugia cellulifera Ehrenberg, 1874 (*syn.*)

Nebela numata Leidy 1874 (*syn.*)

Nebela collaris (Ehrenberg 1848) Leidy, 1879 (*reclass.*)

Nebela bohemica Taranek, 1882 (*syn.*)

Nebela sphagnophila (Steinecke) Van Oye, 1933 (*syn. no year*)

Nebela tincta var. major Deflandre 1936 (*syn.*)

Nebela tincta f. stenostoma Jung 1936 (*syn.*)

Nebela tincta (Leidy, 1879)

Hyalosphenia tincta Leidy, 1879 (*orig.*)

Euglypha bursella Veidowsky (*syn., no year*)

Nebela bursella Vejdovsky, 1882 (*syn.*)

Nebela minor Penard, 1902 (*syn.*)

Nebela tincta (Leidy, 1879) Awerintzew, 1906 (*reclass.*)

Nebela parvula Cash, 1909 (*syn.*)

Oligonema dancoii Arambarri and Spinedi, 1989

Padaungiella lageniformis (Penard, 1890)

Nebela lageniformis Penard, 1890 (*orig.*)

Nebela lageniformes Penard, 1890 (*misspelling*)

Padaungiella lageniformis (Penard, 1890) Lara and Todorov 2012 (*reclass*.)

Padaungiella wailesi (Deflandre, 1936)

Nebela wailesi Deflandre, 1936 (*orig*.)

Padaungiella wailesi (Deflandre, 1936) Lara and Todorov, 2012 (*reclass*.)

Parmulina cyathus Penard, 1902

Phalansterium solitarium Sandon, 1924

Phryganella acropodia (Hertwig and Lesser, 1874)

Difflugia acropodia Hertwig and Lesser, 1874 (*orig.*)

Phryganella acropodia (Hertwig and Lesser, 1874) Hopkinson, 1909 (*reclass.*)

Phryganella acropodia Penard (*error in authorship*)

Phryganella hemisphaerica (Penard, 1890)

Pseudodifflugia hemisphaerica Penard, 1890 (*orig.*)

Difflugia globulosa Leidy, 1879 (*syn*., *in part*)

Phryganella hemisphaerica (Penard, 1890) Penard, 1902 (*reclass*.)

Plagiopyxis callida var. *grandis* Thomas, 1958

Plagiopyxis declivis Thomas, 1955

Plagiopyxis labiata Penard, 1910

Centropyxia labiata Bartoš, 1947

Stenamoeba stenopodia (Page, 1969)

Platyamoeba stenopodia Page, 1969 (*orig*.)

Stenamoeba stenopodia (Page, 1969) Smirnov, Nassonova, Chao & Cavalier-Smith, 2007 (*reclass.*)

Saccamoeba limax (Dujardin, 1841)

Amoeba limax Dujardin, 1841 (*orig.*)

Saccamoeba limax (Penard, 1902) (*error in authorship*)

Saccamoeba stagnicola Page, 1974

Schoenbornia viscicula Schönborn, 1964

Thecamoeba striata (Penard, 1890)

Thecamoeba striata (Penard, 1890) Schaeffer, 1926 (*reclass.*)

Thecamoeba terricola (Greeff, 1866)

Amoeba terricola Greeff, 1866 (*orig.*)

Thecamoeba terricola (Greeff, 1866) Lepşi, 1960 (*reclass.*)

Thecamoeba verrucosa (Ehrenberg, 1838)

Thecamoeba verrucosa (Ehrenberg, 1838) Schaeffer, 1926 (*reclass.*)

Trichamoeba osseosaccus Schaeffer, 1926

Trichamoeba osseocuccus Schaeffer (*misspelling*)

Trichia antarctica Arambarri and Spinedi, 1989

Trichia varia (Pers., 1792)

Stemonitis varia Pers., 1792 (*orig.*)

Trichia varia (Pers., 1792) Pers., 1794 (*reclass*.)

Trichia olivacea Pers., 1796 (*syn*.)

Trichia cordata Pers., 1800 (*syn*.)

Trichia nigripes var. *cordata* (Pers., 1800) Pers., 1801 (*syn*.)

Trichia nigripes var. *cordata* (Pers., 1800) Alb. & Schwein., 1805 (*syn*.)

Trichia cylindrica Pers., 1800 (*syn.*)

Trichia nigripes var. *cylindrica* (Pers., 1800) Pers., 1801 (*syn*.)

Trichia nigripes Pers., 1801 (*syn*.)

Trichia varia var. *diluta* Pers., 1801 (*syn*.)

Trichia varia var. *subrufescens* Pers., 1801 (*syn*.)

Trichia varia var. *nigripes* (Pers., 1792) Rostafinsky, 1875 (*syn*.)

Lycoperdon luridum Hedw., 1802 (*syn*.)

Trichia varia var. sessilis Rostafinsky, 1875 (*syn*.)

Trichia aculeata Celak., 1893 (*syn*.)

Trichia varia var. *aurata* Meylan, 1908 (*syn*.)

Trichia varia var. *irregularis* Meylan, 1908 (*syn*.)

Trichia varia var. *olivacea* Brândza, 1928 (*syn*.)

Trichia synspora Kowalski & McNichols *in* Kowalski,1974 (*syn*.)

Trigonopyxis arcula (Leidy, 1879)

Difflugia arcula Leidy, 1879 (*orig.*)

Trigonopyxis arcula (Leidy, 1879) Penard, 1912 (*reclass.*)

Cystidina arcula (Leidy, 1879) Volz, 1929 (*syn.*)

Vannella contorta (Moran and Anderson 2007)

Platyamoeba contorta Moran and Anderson 2007 (*orig*.)

Vannella contorta (Moran and Anderson 2007) Smirnov, Nassonova, Chao & Cavalier-Smith, 2007 (*reclass.*)

Vannella mira (Schaeffer, 1926)

Flabellula mira Schaeffer, 1926 (*orig*.)

Vannella mira (Schaeffer, 1926) Bovee, 1965 (*reclass*.)

Vannella simplex (Wohlfarth-Bottermann, 1960)

Hyalodiscus simplex Wohlfarth-Bottermann, 1960 (*orig*.)

Vannella simplex (Wohlfarth-Bottermann, 1960) Bovee, 1965 (*reclass*.)

Vermamoeba vermiformis (Page, 1967)

Hartmannella vermiformis Page, 1967 (*orig*.)

Hartmannella vermiformes Page, 1967 (*misspelling*)

Hartmanella vermiformes Page, 1967 (misspelling)

Vermamoeba vermiformis (Page, 1967) Smirnov and Cavalier-Smith, 2011 (*reclass.*)

Incertae sedis

Polypseudopodius bacterioides Puschkarew, 1913

Incomplete records

Cochliopodium tentaculatus

Stylonychia mytilus–complex

Bodo terricolus Martin

Heteromita globosa (Stein, 1878)

Heteromita globosa (Stein, 1878) Kent, 1881 (*reclass.*)

A number of taxonomic designations for the taxa recovered have changed since the original record was published or were ambiguous. (Dillon et al. 1968) reported *Pelomyxa* (or *Amoeba*) *limnicola* (a probable misspelling), though a search of the literature failed to find this species. Bovee (1951) proposed to move *Amoeba limicola* to *Pelomyxa limicola* and the latter designation was used in several ecological papers in subsequent decades (Bovee 1965, Dillon et al. 1968); however, *A. limicola* is still considered accepted in online databases (ITIS 2018). The numerous species added to the genus *Pelomyxa* in the 19th and 20th centuries were later reduced to a single valid species (Griffin 1988, Whatley and Chapman 1990), *Pelomyxa palustris*, although no mention of *Pelomyxa limicola* was made in this move (Goodkov et al. 2004). Thus, we retain *Amoeba limicola* and its associated synonyms in this checklist. Due to the difficulty in distinguishing between some *Stylonychia* species (Haentzsch et al. 2006), Mieczan and Tarkowska-Kukuryk (2014) reported a *Stylonychia sp*. as *Stylonychia mytilus*-complex, which includes *S. lemnae, S. mytilus, S. ammermanni,* and *S. harbinensis*. We include this record due to

its ecological significance even though it is taxonomically incomplete. We placed *Euglypha bursella* Veidowsky under *Nebela bursella* Vejdovsky, 1882 as the authors are similar and no occurrence of *E. bursella* was found in database searches beyond the ecological paper we reviewed (Richters 1908). No further taxonomic information could be found than what was given for *Cochliopodium tentaculatus* from Sudzuki (1979) and *Bodo terricolus* Martin from Smith (1972) and these are included as incomplete taxonomic records. *Centropyxis aerophila* var. *sphagnicola* from Golemansky and Todorov (2004) is now treated as part of the *C. aerophila* complex (Foissner and Korganova 2000), but as this would have resulted in a loss of potentially valuable ecological information, we retain its original nomenclature in this checklist. Howe et al. (2009) split *Heteromita globosa*, a very common soil flagellate, into 5 new genera and 29 new species, rendering the original name invalid. However, as the records of *H. globosa* from the Antarctic literature predated this change and provided no taxonomic diagnoses, pictures or sequence information for their identifications of their organisms we retain *H. globosa* in our checklist to avoid confusion (Sandon and Cutler 1924, Lawley et al. 2004, Bamforth et al. 2005). *Microcorycia bryophila* from Sudzuki (1979), synonymized with *M. tessellata* in Badewitz (2004), was considered by the latter author as a suspicious record because the species was listed with a "?" in the paper's checklist. We retain it here because there are in fact two records of it in that paper (Sudzuki 1979), one of which was not considered ambiguous by Sudzuki. *Mayorella clavabellans* and *M. vespertilio* may now be considered invalid (Smirnov and Brown 2004a, Glotova et al. 2018), however we were unable to find confirmation so we retained these records in this list. Finally, Dumack et al. (2017) split the genus *Lecythium* into two but retained *Lecythium hyalinum*, reported in Smith (1972) as a valid species. As no taxonomic information was reported in the latter paper, we cannot determine whether *L. hyalinum* sensu Smith, 1972

belongs to the new genus, *Fisculla* and thus retain it as it was originally reported. Foissner et al. (2002) retroactively reassigned the *Paruroleptus notabilis* Foissner, 1982 and *Nassula picta* Greeff, 1888 reported in Foissner (1996) as *Uroleptus paranotabilis* (now *Acuholosticha paranotabilis*) and *Nassula tuberculata* respectively on the grounds that the original isolates had been misidentified. Finally, Hada (1966) reported a total of 37 protists yet due to ambiguity over the source of the moss used for analysis (freshwater or terrestrial) we did not include these species in our checklist. Sudzuki (1979) attributes some of the species from Hada's 1966 study to "Antarctic Moss", potentially implying their terrestrial origin; however, it is still not clear from this latter study whether these species were in fact terrestrial or aquatic in origin.

Discussion

The numbers presented here reflect the most comprehensive taxonomic summary of HSPs in continental and peninsular Antarctica to date. Interestingly, climate change has probably already impacted this diversity, especially that recorded in the earliest studies from the peninsular zone (Richters 1907, 1908, Penard 1911, Sandon and Cutler 1924, Smith 1972, 1974, 1978, Sudzuki 1979) which might have sampled a different community than can be found today in the same sites (Royles et al. 2016) due to invasions (Hughes et al. 2015) or warming (Nielsen and Wall 2013). How many species of terrestrial protists, if any, in Antarctica remain to be discovered is difficult to estimate. Foissner (1996) estimated an order of magnitude difference between soil ciliate diversity in the Antarctic and in Alpine and temperate zones. Chao et al. (2006) reported 644 described and 320 undescribed soil ciliate species from five continents (not including Antarctica or North America), with no less than 400 and no more than 1000 species from any

single continent. Additionally, they estimated global soil ciliate diversity at a minimum of 1900 species. Our review of the literature found 201 terrestrial ciliate taxa (89 identified to species and 112 additional records), which suggests that a significant proportion of terrestrial Antarctic ciliate species may have been found, though an unknown degree of overlap between described and undescribed species confounds this conclusion. Specific estimates for the diversity of other heterotrophic protist groups in soils are scarce, but Adl et al. (2007)predicted total richness by group (not only from soils) at approximately 17,000 Amoebozoa, 5,000 Cercozoa, 30,000 Ciliophora, and 3,000 Excavata species. Intriguingly, the relative proportion of these global estimates for each group is mirrored by that of our list of Antarctic protists – ciliates (55% of the total of these groups globally vs. 41% in antarctica), Amoebozoa (31% vs. 36%), Cercozoa (9% vs. 17%), and Excavata (5% vs. 6%). However, this pattern might only reflect the past sampling bias towards ciliates and testate amoeba (an unofficial term that includes members of the Amoebozoa and Cercozoa) and misrepresents the potential diversity of underexplored flagellate groups (e.g. other Cercozoa, Excavata). Additionally, of the 180 total genera found, 42 were not recorded with a species identification, indicating at least as many additional species not included in this checklist. Additional ciliate genera account for the majority of these genera (28), but Amoebozoa (7), Cercozoa (3), Excavata (2), an Opisthokont and a Stramenopile are also represented (data not shown). Moreover, of the 147 remaining genera, 48 were reported without an associated species identification at least once in addition to being reported elsewhere to species. Therefore, this current list greatly underestimates the total diversity of terrestrial Antarctic protists.

There seems to be a trend among early studies to declare a complete lack of endemicity among Antarctic fauna after finding that most communities were similar to those found elsewhere

(Sandon and Cutler 1924, Janetschek 1963, Sudzuki 1964, Todorov and Golemansky 1996). In fact, the majority of taxa found by morphological studies have been described as non-endemic (Todorov and Golemansky 1996, Petz 1997, Petz and Foissner 1997) and include such widespread species as *Colpoda cucullus*, *C. inflata, C. steinii*, *Centropyxis aerophila, Assulina muscorum, Euglypha rotunda*, *E. laevis* and *Heteromita globosa*. Possible explanations for this pattern could be that culturing techniques biased towards generalist, r-selected taxa that are indeed more cosmopolitan, or that examination of samples involved accidental inoculation with local species (as many of these studies were undertaken at their authors' home institutions), or that the observations reflected reality. If true, the assumption that Antarctic protists are specially adapted to such uniquely harsh environmental conditions, would be undermined.

Conversely, mounting evidence suggests that many Antarctic microbial species are not recent transplants but are instead native fauna that arrived long before the most recent glacial maxima (Chown and Convey 2007, Vyverman et al. 2010) or are demonstrably distinct from their non-Antarctic relatives (Boenigk et al. 2006). Moreover, cryptic species are common in protists (Adl et al. 2007, Venter et al. 2018) and distinguishing species in some groups (i.e. naked amoeba (Amoebozoa) and flagellates (Cercozoa, Excavata, etc.)) is notoriously difficult using morphological analysis alone (Smirnov and Brown 2004b, Venter et al. 2018). Thus far, sampling appears to be skewed towards areas that are more likely to experience invasion and host cosmopolitan taxa due to their higher latitudes and milder climate, like the peninsula and coastal Antarctic sites. Additional sampling effort of more extreme, intra-continental sites (e.g. Ellsworth Land & the Ellsworth, Transantarctic, and Prince Charles Mountains) could yield a greater number of uniquely Antarctic species. There have been species found that appear to be restricted to the Antarctic, including three of the reported slime molds: *Leptoderma megaspora*,

Oligonema dancoii, and *Trichia antarctica* (Stephenson et al. 2007). *Urosomoida antarctica* possesses numerous unique characters (Foissner 1996) while *Pseudonotohymena antarctica* *Spathidium seppelti*, and *Urosomoida granulifera* have yet to be found outside Antarctica (Petz et al. 2007, Park et al. 2017). Tyml et al. (2016) reported two strains of *Naegleria neopolaris* that matched Arctic 18S sequences exactly (from Greenland and Svalbard), a taxon apparently exclusive to the poles. Moreover, certain populations of Antarctic species otherwise indistinguishable from their more temperate counterparts exhibit different growth preferences (Bamforth et al. 2005) and body sizes (Roland et al. 2017). Whether these differences are indicative of cryptic species or are only physiological responses to the extremes of the environment remains unexplored. Thus, Antarctica appears to host both cosmopolitan and endemic species of terrestrial protists, although the relative amounts may differ by geographic region. Additional focus on assessing this diversity, as well as finding the unique and endemic species, is needed if we are to establish a baseline for Antarctic conservation and for the study of the unique ecology of these ecosystems.

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Table 1.1: Taxonomic summary of terrestrial protozoa in continental Antarctica.

Table 1 - Taxonomic Summary of Terrestrial Protozoa in Continental Antarctica			
	Taxa Identified to Species	**Taxa not identified to Species**	**All**
Ciliophora	95	113	208
Amoebozoa	84	92	176
Cercozoa	39	47	86
Excavata	13	17	30
Other	5	34	39
Total	**236**	**303**	**539**

Chapter 2

Towards characterizing a microbial ecosystem: A review of the taxonomic and functional diversity of heterotrophic soil protists (protozoa) in Antarctic terrestrial ecosystems

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Abstract

Heterotrophic soil protists (formerly soil protozoa) are an important component of soil ecosystems around the globe. More of an ecological grouping than a phylogenetic one, they comprise a large group of generally unicellular, heterotrophic eukaryotes that serve as the primary grazers of bacterial populations. As such, they are integrally connected to key biogeochemical processes carried out by bacteria, like carbon and nitrogen cycling, upon which Earth's biosphere relies. The full breadth and nature of these interactions and the roles different HSP species play in soils around the world are still largely unknown. An ideal environment in which to answer some of these fundamental questions are the ice-free regions of terrestrial Antarctica. These soils constitute some of the harshest terrestrial environments known on Earth. High salinity, low moisture, oligotrophy, and low temperatures among other factors severely constrain biotic diversity here, leaving a community that is almost entirely microbial in composition. Unfortunately, little is known about either the ecology or the diversity of heterotrophic soil protists in these Antarctic soil ecosystems. In an effort to gain a better insight into how diversity and function are linked for protists on this continent, I here present a review of the diversity of these organisms across Antarctic soils, including their regional distribution, community composition by habitat and a review of their functional ecology. Additionally, I discuss useful methods for a more complete assessment of this diversity as well as associated difficulties and pitfalls.

Introduction

Identifying links between biodiversity and function in soils is a priority for understanding both fundamental ecological principles as well as for predicting and mitigating the effects of climate change on the biosphere (Wall 2005, Wall 2007, Nielsen et al. 2011, Chakraborty et al. 2012, Potter et al. 2013). Heterotrophic soil protists (HSPs) play critical roles in regulating soil bacterial communities and influencing plant functioning, yet the extent and nature of their influence on soil community dynamics and nutrient cycling is not well understood (Wardle 2006, Corno and Jurgens 2008, Saleem et al. 2013, Geisen 2016, Geisen et al. 2017). One reason may be that soils are highly diverse ecosystems and characterizing the sheer number and variety of biotic and abiotic interactions that are present is unfeasible. Continental Antarctica is home to some of the most depauperate soils on Earth (Wall 2005). Extreme low average temperatures, limited growth periods, very little liquid water, and highly nutrient limited and saline soils severely restrict biotic diversity (Adams et al. 2006, Barret et al. 2006). The complexity of the food web is thus reduced, potentially to such a degree that answering fundamental questions about the functional roles HSPs play in soil ecosystems becomes feasible (Wall 2007). The influence that HSPs in particular have on soil processes is likely felt primarily through species-specific interactions, modulated by distribution, ecophysiology, prey preference, and life history traits of HSPs themselves and their prey, predators, and pathogens (Bell et al. 2010, Glucksman et al. 2010, Rønn et al. 2012, Saleem et al. 2013). Understanding the nature of these interactions in any system requires intimate knowledge of HSPs' taxonomic and functional diversity (Glucksman et al. 2010), yet there is currently a dearth of information in this regard (Rønn et al. 2012, Wilkinson et al. 2012, Geisen 2016). In protists, certain ecological functions (e.g. feeding preferences) can be inferred from taxonomy (Adl et al. 2018). However, relatively little work has

been done to assess taxonomic diversity in Antarctic terrestrial environments over the last century (Acuña-Rodríguez et al. 2014) and the most recent reviews are now two decades old (Smith 1996, Foissner 1998). Thompson et al. (*in review*) constructed a species checklist of HSPs in Antarctica by reviewing all relevant literature (Table S1), arriving at a total of 236 species and 303 additional taxa not identified to species (Table S2). Using this checklist and its literature reviewed as a baseline, I here focus on how this continent-wide diversity is structured at regional and local scales. I also explore how communities in mineral soils and mosses are influenced by abiotic and biotic drivers associated with the Antarctic environment and its non-protist communities. Finally, I discuss the biases in our current understanding of this diversity, the challenges associated with assessing it, and what can be done to overcome each.

Due to their intermediary size, HSPs form an essential link between soil bacteria and metazoan predators, like nematodes, which are too large to exploit many micro-soil habitats where bacteria are capable of thriving (Wilkinson et al. 2012). They can increase the productivity of bacteria by maintaining log-scale growth and enabling the growth of less abundant species (Crotty et al. 2012, Saleem et al. 2012, Saleem et al. 2013). They also prey on other groups, including fungi, other protists, and even nematodes and other micrometazoa (Bjørnlund and Rønn 2008, Rønn et al. 2012, Geisen et al. 2015, Geisen 2016, Park et al. 2017a). Fewer studies have focused on these interactions, but they are likely to be more commonplace and important links in the food web than previously thought (Geisen 2016). Recent research thus shows how crucial soil protist research is to understanding general rules about soil community stability, resilience and nutrient cycling. High level taxonomy in protists has undergone many changes since much of the literature cited for this review was published (Adl et al. 2007, Ruggiero et al. 2015, Adl et al. 2018). Traditionally, taxa were organized morphologically into ciliates, testate

amoeba, naked amoeba and flagellates; all but the first are now known to be paraphyletic. Modernly, some testate amoeba have been assigned to Cercozoa and others to Amoebozoa; naked amoeba are generally placed within the Amoebozoa, but some are now considered Excavata (e.g. vahlkampfids); flagellated protists are heavily polyphyletic and are included in the opisthokonts, excavates, cercozoans, apicomplexans and stramenopiles (Adl et al. 2007). I use the traditional names for these groups when discussing patterns of certain study biases.

Less than 0.5% of the Antarctic landmass is ice free (Burton-Johnson et al. 2016), with the single largest region (~ 0.18%) located in Victoria Land on the border of the Ross Sea (Levy 2012). These soils are poorly developed and possess some of the lowest levels of bioavailable carbon in any terrestrial ecosystem. They are also characterized by high levels of salt (due to a general lack of leaching processes), little to no precipitation (and almost always in the form of snow when it does occur), and very little moisture. During the winter, temperatures at terrestrial sites are almost constantly below the known lower threshold for biological activity (~-20C) and even during the summer, frequent freeze-thaw cycles and shaded soils can drop below temperatures where most organisms are able to remain active. I use the 16 biodiversity regions defined by Terauds and Lee (2016) as a discussion framework, but due to a paucity of records, I combine the four peninsular regions into two: Northwest and Northeast Antarctic Peninsula become North Antarctic Peninsula and Central South and South Antarctic Peninsula become South Antarctic Peninsula.

Taxonomic diversity of HSPs in continental Antarctica

The climate and growing seasons of Antarctic ice-free regions range from relatively mild and long (e.g. the peninsula) to extremely harsh and short (e.g. the Transantarctic Mountains (TM)). It is reasonable to expect therefore that regional diversity will reflect this gradient of extremes, while also sharing certain widely distributed taxa that are adapted generalists. Indeed, latitudinal studies show decreasing diversity with increasing latitude (Foissner 1996, Lawley et al. 2004). Moreover, no single region in Antarctica captures the entire diversity of the whole continent. The Northern Antarctic Peninsula (NAP), East Antarctica (EA), and South Victoria Lands (SVL) have an order of magnitude higher taxonomic richness (155, 52, and 50 species respectively) than the rest of the regions (all of which have 5 or fewer species with the exception of Adélie Land (AL), Enderby Land (EBL) and Dronning Maud Land (DML) which have 10, 15 and 12 species respectively)(Fig 1a). Of the four best investigated regions, EBL has the most additional taxa not identified to species (91) and SVL the fewest (34), while the NAP and EA had an additional 64 and 77 taxa respectively. This review focuses on taxa identified to species, but the diversity of taxa identified to only genus is included in a master species list (Table S3).

Diversity estimates are influenced by the uneven distribution of studies in each region and the asymmetric sampling effort of each of those studies (Fig 2). Experimental designs across studies varied, differing in terms of approach (methodology and target biota) and scope (sampling effort). In addition, sampling effort was frequently unavailable, making direct comparisons between regions challenging. As a proxy for sampling effort, I use the number of studies per region to evaluate trends in richness and distribution (Fig 1b). Intra-continental regions (e.g. SVL, North Victoria Land (NVL), TM, and Prince Charles Mountains (PCM)) have been the subject of fewer studies than higher latitude, coastal sites (e.g. EA and NAP), and two

of the intra-continental sites (Ellsworth Land (EL) and Ellsworth Mountains (ELM)) remain completely unstudied (Fig 1b, Fig 2). Study density is strongly skewed towards NAP and SVL (Fig 1b) and taxa counts for South Antarctic Peninsula (SAP), AL, TM, Marie Byrd Land (MBL), PCM and DML are disproportionately lower. MBL has been the focus of only one study each, and the TM, NVL, PCM, SAP and AL of only two each. NAP has received the most attention by far with 25 studies, more than twice as many as the next most studied region, SVL, with 12 studies (Fig 1b), while DML and EA have been the subject of 5 and 6 studies respectively. Possible explanations behind this geographic bias could include logistics – i.e. those regions that are the most accessible like the northern Antarctic Peninsula - and national resources allocated to science support. McMurdo station, by far the largest station in Antarctica, borders the Ross Sea region where the McMurdo Dry Valleys of SVL are located. From Figure 2 it is apparent that even the sampling distribution of SVL itself is heavily biased towards areas within easy reach of McMurdo Station. Similar local biases occur in the NAP, DML and EA, despite their relatively high study number and taxonomic count. Although SVL has been the site of more studies, EA has yielded more taxonomic records per study. This is due to the comprehensiveness or scale of the studies carried out in those respective regions.

Ciliophora account for the largest proportion of Antarctic HSPs, followed by Amoebozoa, Cercozoa, Excavata and then members of the Opisthokonta, Apicomplexa, Stramenopile and non-ciliate Alveolates (collectively referred to here as “Other”) (Fig 1a). In a latitudinal study (Lawley et al. 2004), both Cercozoa and Ciliophora were found at all latitudes sampled while Euglenozoa (Excavata) were not found south of maritime Antarctica. Numerous other studies have found Euglenozoa in EA, EBL, SVL and the NAP (Sudzuki 1979, Smith 1985, Bamforth et al. 2005) (Table S2). EA, SVL and NAP possess a similar relative

composition of Ciliophora, Amoebozoa and Cercozoa, even though sampling effort and study number are not equal between them (Fig 1) - EA and SVL have around one third the recorded species as the NAP. Amoebozoa and Ciliophora dominate these three regions in terms of relative richness (Fig 1a), and there is a strong bias towards amoebozoan testate amoeba. Well over half of Amoebozoa taxa in the NAP, EA and EBL are testates (75%, 100% and 100% respectively) while SVL Amoebozoa are slightly more diverse (only 39% testate amoeba). Cercozoan testate amoeba are similarly dominant in the records, making up 71%, 100%, 100%, and 67% of total Cercozoa in these regions, respectively. Amoebozoa are not reported from MBL, the TM, NVL or the PCM, while Ciliophora are reported from every region except AL and SAP, where the authors of those studies intentionally only investigated testate amoeba (Decloître 1960, 1964, Royles et al. 2013). These observations reflect the focus of most morphological studies on either testate amoeba (Penard 1911, 1913, Decloître 1960, 1964, Smith 1987, Todorov and Golemansky 1996) or ciliates (Ryan et al. 1989, Foissner 1996, Petz and Foissner 1996, Petz 1997, Petz and Foissner 1997, Mieczan and Tarkowska-Kukuryk 2014, Velasco-Castrillon et al. 2014) (Fig 3). Possibly due to this bias, testate amoeba diversity and distribution, especially in the NAP, are relatively well understood (Royles et al. 2016, Roland et al. 2017). The molecular studies do not bias towards testate amoeba and ciliates but do recover fewer amoebozoan sequences (3 out of 36) than other groups (Table S2). Studies using both morphological and molecular means are biased towards ciliates (Jung et al. 2015, Park et al. 2017a, Park et al. 2017b), and the single study that investigated flagellates at the exclusion of other protists (Hodgson et al. 2010) only did so because their algal primers also detected Cercozoa. Thus, it is not surprising that the known diversity of Antarctic naked Amoeba (Amoebozoa), Cercozoa, and Excavata is much lower than that of ciliate and testate amoeba. Excavates (including

euglenozoans) make up only a small part of the overall diversity and are recorded from only the NAP, SVL and EA. Cercozoa have been recorded from every region sampled, but this diversity is predominantly comprised of testate amoeba (27 out of 39 total Cercozoa) while ‘flagellates’ only account for 23%. Flagellate Cercozoa are likely to be more abundant and ubiquitous in Antarctic soils and mosses than is currently understood since they are a dominant component of these ecosystems in other more temperate climates, largely due to their small size and rapid response time to environmental changes (Foissner 1991). A Heteromitidae and *Paulinella sp.* (both Cercozoa) were found at 82°S, in the Dufek Massif in the Transantarctic Mountains, the most southern site in any study reviewed (Hodgson et al. 2010), although whether these samples came from strictly terrestrial sites is unclear. No other HSPs were found and these Cercozoa are small indicating perhaps an ecological constraint on the distribution of other HSP groups. Cercozoa have an overall lower richness than Ciliophora (Fig 1a), probably because many flagellates (Cercozoa, Excavata and other lineages) have superficially similar morphologies, which coupled with their small size (many are less than 10 microns in length) makes them easier to overlook and identify (Boenigk 2008, Venter et al. 2018). Future studies focusing on flagellate groups specifically will be necessary to better understand HSP diversity and function in these ecosystems.

An interesting pattern in Antarctic biodiversity is the striking difference between the communities of the Peninsula and the rest of the continent (Chown and Convey 2007). This division is delineated by the Gressit line and holds true for many but not all biotic groups. To see if the present data could shed light on whether this pattern holds for HSPs, I distinguished regionally unique taxa from any taxon shared with at least one other region (Fig 4). Overall, 81% of all taxa identified to species are unique to one region. This could be indicative that aeolian

dispersal in Antarctica is restricted and that these communities survived *in situ* during the last glacial maximum (Convey et al. 2009, Fraser et al. 2014). Alternatively, this could also be an artefact of regional sampling biases. Both EA and SVL have a roughly equal number of shared and unique species, while 76% of taxa from the NAP are unique and 67% of taxa from EBL are shared (data not shown). Comparing total peninsular diversity (NAP with SAP) to combined continental diversity (EBL, SVL, NVL, EA, PCM, TM, AL, MBL, and DML) revealed that 39 species were shared between the two, out of 156 and 118 total species for the peninsula and continent, respectively. The peninsula produced a combined total of 27 studies and the continent 36, suggesting that this result may not purely be a problem of sampling bias. A species accumulation curve for each region should be constructed in the future after a comprehensive multi-regional analysis to explore these patterns further. Presently, the diversity and ambiguity of sampling schemes in these studies obviate reliable construction and analysis in the present review.

Antarctic HSP diversity appears to be fairly heterogeneous and distinct from region to region. Only 19 of the 236 identified species were encountered in three or more regions (Table S3) and could potentially be considered members of most communities across the continent. Thus, the study of the autecology and function of these taxa may warrant greater emphasis than others. The most widespread taxon is *Corythion dubium* Taránek, 1881 (a cercozoan testate amoeba) which occurs in eight of the 11 regions studied, including on nunataks in MBL (Broady 1989) and probably in EBL (Hada 1966). This trend is noted by other authors (Broady et al. 1987, Royles et al. 2016) and *C. dubium* may owe its wide distribution to being strongly r-selected and other unique physiological adaptations (Smith 1985, Petz and Foissner 1997). It is absent only from the TM and the PCM, possibly because it is a moss-associated organism and

studies in both regions have only examined soil (Fig 5). *Pseudoplatyophrya nana* (Kahl, 1926) (Ciliophora) was found in five of the biodiversity regions MBL. Seven species were found in four of the biodiversity regions, *Assulina muscorum* Greeff, 1888 (Cercozoa), *Centropyxis aerophila* Deflandre, 1929 (Amoebozoa), *Colpoda cucullus* (Müller, 1773) (Ciliophora), *C. inflata* (Stokes, 1884), *C. steinii* Maupas, 1883, *Euglypha rotunda* Wailes and Penard, 1911 (Cercozoa), and *Leptopharynx costatus* Mermod, 1914 (Ciliophora), while 10 were found in three biodiversity regions and 25 were found in two regions, leaving 192 identified species that were only found in a single region – 81% of total identified species. Most of these species are considered to be globally distributed, and their distribution around the Antarctic continent may be more a result of their ability to disperse than their local dominance. Of the 19 species found in three or more regions, 9 were testate amoeba (4 Amoebozoa and 5 Cercozoa), 9 were Ciliophora, one was a cercozoan flagellate and none were naked amoeba. The species found outside of NAP, EBL, EA and SVL were *Corythion dubium*, *Assulina muscorum*, *Euglypha rotunda*, *Heteromita globosa* (Stein, 1878), *Trinema lineare* Penard, 1890, *Leptopharynx costatus*, *Pseudoplatyophrya nana* and *Paradileptus elephantinus* (Svec, 1897), *Difflugia lucida* Penard, 1890, *Colpoda inflata*, *Centropyxis aerophila*, and *Homalogastra setosa* Kahl, 1926. Considering that sampling outside of these four regions has been comparatively scarce, the recovery of these species may indicate their relative dominance in these communities. Of the 19 species found in three or more regions, 15 were recovered from both soil and moss environments while 2 were recovered from only moss, which is unexpected as moss-dwelling HSPs in Antarctica are more than twice as diverse as those recovered from soil and moss (Fig 5a). Species like those found in both soil and moss may be distributed more broadly because of their more flexible ecological requirements. Moss specialist richness is likely to be much higher only where moss communities are abundant

and diverse (i.e. NAP), but only a single species, the globally distributed flagellate *Heteromita globosa*, was found exclusively in soils in three or more regions. *H. globosa* has recently been split into 5 novel genera and 29 novel species and there are probably a variety of distinctly Antarctic *H. globosa* species, but it is not possible to retroactively identify the exact species isolated by the authors reviewed here (Sandon and Cutler 1924, Lawley et al. 2004, Bamforth et al. 2005). The *H. globosa* group of flagellates is frequently considered to be one of the most important HSPs in terms of its abundance, ubiquity, and role as a bacterial grazer and future research into its distribution, diversity, and ecology in Antarctica will be needed to fully understand the function of these ecosystems.

HSP diversity across habitats

I assigned all taxa identified to species for which habitat information was recorded (Table S3) to one of four categories (Fig 5a): 'Soil', 'Moss', 'Soil & Moss', and 'Other' which includes samples that were ornithogenic, vegetated (beyond mosses) or were unclear in their origins. Soils in continental Antarctica are generally underdeveloped mineral soils with varying levels of moisture and are rarely vegetated. Moss habitats exist on the continent in areas that are consistently moist, but generally the largest concentration of mosses is found at higher latitudes in the NAP, where they form expansive banks (Royles et al. 2016). Our categories do not effectively capture the subtle differences across the gradient of moss and mineral soil habitats, but the distinction between the two, which are often physically associated habitats, was not usually carefully separated in the studies reviewed. Future biodiversity assessments should take care to correlate their analyses with descriptive habitat types that include moisture content,

vegetation cover and sampling depth. A fifth category, “Not Reported”, encompasses those five species that did not have any associated habitat information (Table S3). The habitat with the highest HSP diversity for all regions was ‘Moss’ (116 unique species), followed by ‘Soil & Moss’ (50 species) and then ‘Soil’ (35 species). ‘Moss’ was the habitat with the highest taxonomic richness in NAP while ‘Soil & Moss’ had the most species diversity in EA, and SVL. Species richness in ‘Soil’ was greatest in SVL and entirely absent from EBL. It is clear that our understanding of HSP diversity in NAP is heavily biased towards moss species, and further investigations into mineral soil habitats in NAP, EA and EBL are needed. Some taxa assigned to ‘Moss’ may also occur in Antarctic mineral soil, inhabiting the soil directly under moss beds or soil blown over moss beds as cover. Ciliophora and Amoebozoa (mostly testate amoeba) are the most species-rich phyla in ‘Moss’, while Ciliophora diversity dominates ‘Soil’ and ‘Soil & Moss’ habitats (Fig 5b). Heterotrophic flagellates from Cercozoa and Excavata are most diverse in ‘Soils’ (3 of 7 species) and least diverse in ‘Moss’ (1 of 7) (Table S3). Antarctic mosses appear to have a low degree of endemicity (Chown and Convey 2007), which might explain the prevalence of cosmopolitan species if protist cysts and mosses disperse together (Thompson et al. *in review*). That ciliate richness is highest in all habitats is not consistent with the expected dominance in arid soil environments of Amoebozoa and Cercozoa (Geisen et al. 2014), though the simplicity of our habitat categories may be concealing environmental nuances, like moisture content. Smith (1996) observed that ciliates were only significant proportions of the soil community in guano-influenced sites (regardless of moisture content) and Bamforth et al. (2005), sampling mineral soils, noted that ciliates and testate amoeba were not common. Conversely, Petz (1997) reported on average 74% of their soil and moss samples contained ciliates. The diversity of Antarctic soil HSP communities at the local scale is low and consists of primarily

euryoecious, r-selected species. For example, Foissner (1996) found an average of 2.2 ciliates per soil sample (NAP and SVL) and Bamforth et al. (2005) (SVL) recorded only 1-6 flagellate species per soil sample. Naked amoeba in SVL soils are common, but primarily consist of *Acanthamoeba sp.* and *Vermameoba* (=*Hartmanella*) *sp*. (Bamforth et al. 2005). In Antarctic moss communities, (NAP, EA) *Corythion dubium*, *Trinema lineare*, *Centropyxis aerophila*, *Assulina muscorum, Difflugia lucida* and *Euglypha rotunda* are most frequent and most abundant (Smith 1987, Golemansky and Todorov 2004, Mieczan and Tarkowska-Kukuryk 2014).

Drivers of community structure

Abiotic factors

Unsurprisingly, diversity and abundance of Antarctic HSPs appear to be positively correlated with moisture gradients, ranging from wetter sites associated with productive moss covers to hyperarid unvegetated mineral soils (Smith 1974, Petz 1997). Fell et al. (2006) found the highest diversity of microeukaryotes (including fungus and micro-metazoa) between 3.1 and 4.9% soil moisture and some eukaryotes were found where soil moisture levels were as low as 0.2%. Flagellates (including most Cercozoa, many Excavates and various members of most other protist clades) and naked amoeba tend to dominate arid soils (Smith 1996, Bamforth et al. 2005, Bates et al. 2013), probably due to their smaller size (lower resource needs) and the fact that they can be mobile in far less water. Flagellates increased with increasing soil moisture (whether in terms of abundance or richness is not clear) but amoeba did not (Bamforth et al. 2005), potentially because amoebae are considered soil water film specialists and may not benefit from additional moisture (Geisen et al. 2014, Geisen et al. 2015). Ciliates are usually free swimming

and may be therefore restricted by lower moisture levels (Smith 1974, Bamforth et al. 2005). Velasco-Castrillon et al. (2014) found ciliates across a range of moistures, but most (~80%) were recovered from sites with 10% or greater moisture content. Conversely, a *Spathidium sp*. occurred far more frequently in arid soils (1 to ~8% moisture) than wetted ones (Niederberger et al. 2015). Perhaps the ability of *Spathidium* (and other hypotrichs) to crawl along surfaces explains their predominance in dryer soils, while they are outcompeted in wetter soils by faster moving ciliates. In temperate zones, testate amoeba are structured by moisture, but Royles et al. (2016) did not find this relationship in mosses from the NAP. A decreasing test size with increasing latitude along the peninsula in *Corythion dubium* could be an adaptive response to thinner water films in more southerly locations, but this relationship is hard to disentangle from confounding variables, e.g. trophic interactions, energetics or dispersal ability (Roland et al. 2017). Testate amoeba are restricted to moss habitats (Smith 1972, 1985, 1996) or occur in soils, but with a higher diversity in mosses (Sudzuki 1979, Petz 1997). Single, empty tests were at times found in mineral soils (Broady et al. 1987, Bamforth et al. 2005) and in soils associated with crustal algae or lichens (Broady et al. 1987).

Extreme low temperatures can be another limiting factor to many species because it limits molecular rates, promotes ice formation in the cytoplasm that can lyse cells, and reduces the availability of liquid water. Many Antarctic HSPs are known to exhibit varying degrees of psychrotolerance, though distinguishing between the local soil temperature measured and the actual temperature endured by the organisms is a challenge (Convey et al. 2018). *Heteromita globosa* and *Tetramitus rostratus* Perty, 1852 are facultative psychrophiles, and despite having optimal growth temperatures above 20°C both exhibit growth at or below 5°C (Smith 1996). The non-endemic ciliate *Colpoda maupasi* Enriques, 1908 has been found at -1.6°C in EA (Petz

1997), but additional investigation could reveal that the population is an unrelated cryptic species. A tendency towards a psychrotolerant rather than a psychrophilic lifestyle could result from the dramatic variation in Antarctic temperatures between seasons and even day to day (Sudzuki 1964). During the summer months, soil temperatures above 10°C are not uncommon, even at high latitudes, such as the McMurdo Dry Valleys (Doran et al. 2002). These temperatures could not give mesophiles the edge over true psychrophiles, given they are able to survive (probably through encystment) the harsher spring, fall and winter climate. It's also possible that culturing techniques are biased towards mesophile ranges and growth times (Janetschek 1963, Brown 1982, Bamforth et al. 2005).

Beyond moisture and low temperatures, pH, salinity, oligotrophy, increased UV radiation, and soil texture impose strong selection on community composition, especially in the more extreme mineral soils (Virginia and Wall 1999, Barrett et al. 2006a). Species representing most major clades have been recovered from soils ranging from pH 4 to pH 9 (Smith 1985, Petz and Foissner 1996, Petz 1997, Petz and Foissner 1997, Park et al. 2017b), and Smith (1992) observed that pH shaped testate amoeba community structure. Ciliate biomass correlated with pH in a study from Wilkes Land Antarctica (EA), potentially because pH is a major driver of their bacterial prey (Petz 1997). Although Bates et al. (2013) found no significant relationship between pH and protist diversity overall, mounting evidence of species-specific interactions between HSPs and their prey lends support to the connection (Glucksman et al. 2010, Saleem et al. 2013). Nutrient amendment studies show that limited carbon, nitrogen and phosphorus are drivers of microbial communities in Antarctic soils (Buelow et al. 2016, Aanderud et al. 2018), but do not explore the response of the HSP community. Virtually no stratification of organic carbon exists in dry soils farthest from streams and lake margins (Elberling et al. 2006),

suggesting that arid sites may be carbon limited due to decreased photosynthesis near the soil surface. This might select for smaller species than could survive in a more carbon rich environment. Velasco-Castrillon et al. (2014) reported that ciliates were limited to soils with an electrical conductivity range of 0.4 to 4.4 dS/m, much lower than reported in non-Antarctic soils (Kuppers et al. 2009). No work exists on the effects of soil salinity on flagellates (i.e. Cercozoa, Excavata and others), testate or naked amoeba in these ecosystems. To date, no studies have evaluated the effects of UV radiation on near-surface taxa or of pore size on community structure, though Gokul et al. (2013) suggested that smaller pores may shelter certain species during the harsh Antarctic winter.

The extreme variability of many of these parameters, notably water availability and temperature, has been cited as one of the harshest pressures for life of the continent (Peck et al. 2006, Chown and Convey 2007, Yergeau 2014). Some have suggested that the Antarctic environment has selected for small or medium-sized (5 – 50um) r-selected species which are best suited to deal with this variability, for example *Acanthamoeba sp.*, *Colpoda sp.* and *Corythion dubium* (Foissner 1996, Petz 1997, Bamforth et al. 2005). Species known to be k-selected are present, e.g. *Hartmanella sp.* and *Bodo sp.*, so how significant the skew towards r-selection actually is remains unclear.

Many HSPs form cysts to endure stressful environmental periods, but this is not a universal ability (Geisen et al. 2018). *Heteromita globosa* (Cercozoa) found in soils in the NAP, SVL, and TM (Sandon and Cutler 1924, Lawley et al. 2004, Bamforth et al. 2005) can encyst due to cold temperatures alone (Smith 1996) while *Bodo saltans* (Excavata) does not encyst and yet was found frequently in soil, moss and other habitats from the NAP and SVL (Smith 1978, Bamforth et al. 2005). Encysted protists have been found to be viable after even extremely long

periods of time (Lewis and Trainor 2012, Shmakova et al. 2016), and HSP communities in Antarctica may be able to persist in inhospitable conditions for many decades, centuries or even millennia (Matsuo et al. 2018). HSPs spend much of their time encysted, waiting for optimal conditions to arise before excysting to eat and reproduce. This can happen relatively quickly, altering community composition and structure on very brief time scales.

Encystment also allows for dispersal (especially aeolian) and contributes to community pools, yet it is largely unknown how HSP dispersal affects community structure in Antarctica. Smith (1985) discussed the effects of dispersal on fresh tephra on Deception Island in the late 1970s. They noted that a period of 10-30 months was required for any colonization to occur, and that small flagellates and amoeba were the first HSPs to arrive (Smith 1974, 1985) while testate amoeba were found only after a site had been vegetated for some time (Smith 1985). They also found that while time was correlated to an increase in protozoan diversity, a stronger driver was the arrival of moss propagules. This is likely due to moss-colonized soil retaining more moisture and providing higher niche diversity, greater nutrient concentrations (via photosynthesis) and an increase in pore size and variability (Smith 1985), itself a strong driver of HSP diversity (Rønn et al. 2012, Geisen et al. 2014). Despite the evidence for dispersal, some authors found local heterogeneity to be high (Smith 1974, Bamforth et al. 2005, Niederberger et al. 2015). Obbels et al. (2016) observed a high degree of shared diversity across habitat types but point out that closely related species might have been obscured by their methodologies. The degree of species heterogeneity across the landscape could itself vary, with more homogeneity near sources of water and less in more arid areas. Proximity to water could result in occasional aquatic dispersal as well as dispersal via moss propagules. Heterogeneity could also indicate more the inhospitableness of landing site conditions than the lack of dispersal. A systematic study of

heterogeneity in these ecosystems is needed as dispersal has a stabilizing effect on communities when local extinction rates are high (Sabelis and Diekmann 1988) (e.g. in extreme environments) but also encourages the invasion of non-native species (Lockwood et al. 2005).

Biotic drivers: co-occurrence and trophic interactions

It is debated whether biological drivers are a major factor in structuring communities in Antarctic soils (Hogg et al. 2006) and nematode abundance and bacterial diversity were observed to be unrelated in SVL soils (Barrett et al. 2006b). Most studies reviewed did not report co-occurrence between protozoa and other microbial groups (Table S2). Those that did reported the presence of nematodes, tardigrades and rotifers (the main groups of metazoa occurring in these ecosystems; Adams et al. (2006)), but few utilized deliberate methodology for exploring the correlation of these groups, and none examined the relationship between individual species. Bamforth et al. (2005)(SVL) reported that flagellates and amoebae co-occurred more frequently with nematode taxa than without, rarely with tardigrades and rotifers, and with ciliates only in the presence of other metazoan taxa. Decloître (1964)(AL) also noted an absence of tardigrades and rotifers where protozoa (testate amoeba from moss, in this case) were found. In other studies, ciliate and tardigrade taxa were found cooccurring in every sample examined while few rotifers were recovered, and nematodes were entirely absent (Steele et al. 1994). Bates et al. (2013)(SVL) noted a relatively weak correlation between protistan and bacterial communities, though this study drew these conclusions from a collection of globally distributed sites that included Antarctica, so whether this pattern holds true independently in Antarctic soils is uncertain. A number of studies report moss species associations with specific protozoan species

or community assemblages (Toriumi and Kato 1961, Sudzuki 1964, Mieczan and Tarkowska-Kukuryk 2014). Mieczan and Tarkowska-Kukuryk (2014) observed that moss species influenced ciliate body size, but concluded that microsite physicochemical parameters had a greater influence on ciliate abundance and diversity than did host moss species.

Interactions between HSP species and their respective prey can have a significant impact on community structure and ultimately ecosystem functioning (Corno and Jurgens 2008, Glucksman et al. 2010, Hünninghaus et al. 2017). Traditionally HSPs were viewed as uniformly bacterivorous, but recent work suggests that greater trophic diversity exists (e.g. facultative and obligate mycophagy, algavory, osmotrophy and predation) among even closely related HSP species (Petz and Foissner 1997, Bjørnlund and Rønn 2008, Glucksman et al. 2010, Geisen et al. 2018). In Antarctic soils, algavory has been observed in *Pseudonotohymena antarctica* Park, Jung, Min & Kim 2016 (Park et al. 2017b) and *Saccamoeba stagnicola* Page, 1974 (Bamforth et al. 2005). *Keronopsis helluo* Penard, 1922 (Ciliophora) – a consumer of rotifers and other large ciliates - has been isolated from King George Island (NAP) (Park et al. 2017a), although this behavior has not yet been observed in Antarctic populations. Fungivorous taxa, *Pseudoplatyophrya nana* (Ciliophora) from SVL and *Grossglockneria acuta* Foissner, 1980 from NAP and predatory species, such as *Urosomoida antarctica* Foissner, 1996, a ciliate from SVL which feeds on bacteria and possibly flagellates and naked amoeba (Foissner 1996) have also been reported. The predatory genus *Spathidium* (Ciliophora) occurred in three regions (NAP, EA, SVL – S1) and in a variety of habitats, more frequently recovered from guano sites than vegetated soils (Smith 1978). *Colpodella edax* (Klebs, 1892), found in SVL soils (Bamforth et al. 2005) and *Peranemopsis trichophora* (Ehrenberg, 1832) from NAP (Smith 1985), are known predators of colorless and photosynthetic flagellates (Simpson and Patterson 1996, Triemer

1997). Many bacterivores are also present, including the stramenopiles *Oikomonas termo* (Müller, 1773) and *O. mutabilis* Kent, 1880; the excavate *Tetramitus rostratus*; the cercozoan flagellates *Cercobodo agilis* (Moroff, 1904), *C. vibrans* (Sandon, 1927), *Cercomonas crassicauda* Dujardin, 1841, *C. longicauda* Dujardin, 1841 and *Heteromita globosa*; the cercozoan testate amoeba *Corythion dubium* and *Trinema lineare* Penard, 1890 and the ciliophoran *Colpoda steinii* and *C. cucullus.*

Methodological biases and challenges

The difficulty involved in accurately identifying protist species is perhaps the greatest challenge for assessing the function of HSPs in Antarctic soil communities. I briefly examine the implications that the known taxonomic diversity has on HSP functional diversity Morphological studies can suffer from a lack of distinguishing morphological characteristics and unknown culturing parameters (Boenigk 2008, Caron and Hu 2018). Distinguishing protists using only morphological traits has likely led to the "everything is everywhere" observation and hypothesis (e.g. Finlay (2002), as there are probably far fewer morphospecies than genetically distinct cryptic species (Foissner 2008). Thus, it is possible that some identifications made in these studies underestimate biodiversity (type II error; Adams (1998)) due to morphological convergence with well-known, globally distributed species. Alternatively, they were assumed to be cosmopolitan but are in fact themselves unresolved composites of cryptic species (Thompson et al. *in review*). Culture-based approaches are biased towards those taxa that respond best to the culturing conditions, regardless of their ecological significance or activity in the source environment, an especially acute problem when attempting to mimic the unique Antarctic

environment. Some authors used the most probable number (MPN) method for isolating living protists from their samples (Steele et al. 1994), which has been shown to seriously alter apparent community makeup by creating conditions that selectively suit some protists over others (Foissner 1987, Berthold and Palzenberger 1995). More accurate techniques exist, such as the flooded petri dish method, and were used in some but not all studies reviewed (Luftenegger et al. 1988, Petz 1997) but the lack of standardization is a concern. In addition, morphological identifications are challenging for the inexperienced, and the training required to be able to make such distinctions satisfactorily is time consuming – several authors did not identify protists past motility-based groupings, e.g. ciliates, flagellates and amoeba (Steele et al. 1994, Barman 2000, Velasco-Castrillon et al. 2014). Molecular tools provide additional characters in the form of nucleotides, do not suffer from culturing biases, are generally more standardized, are easier for the less experienced to carry out and have higher-throughput and greater data generation for less time invested (Caron et al. 2009). Molecular studies are still subject to biases: of the Antarctic studies that targeted the 18S ribosomal gene, none targeted the same region (Hodgson et al. 2010, Jung et al. 2015, Tyml et al. 2016, Park et al. 2017b). PCR amplification also alters community composition as do DNA extraction techniques (Santos et al. 2015) and species identification from DNA alone, not to mention single gene studies, can be misleading (Caron et al. 2009, Pawlowski and Burki 2009, Caron and Hu 2018). A paucity of adequate reference sequences in properly curated databases and relatively arbitrary clustering based off of sequence similarities can make identifications difficult and often unreliable (Lawley et al. 2004, Caron et al. 2009, Obbels et al. 2016). PCR bias can be mitigated by shotgun metagenomic studies, but differences in sequencer model and bioinformatic pipelines used can also bias results (Czechowski et al. 2016). Finally, dealing with singletons (real or artifacts) and decisions on

appropriate similarity binning cutoffs introduce a degree of subjectivity into these analyses that can lead to under or overestimating the true taxonomic diversity.

The assessment of protist diversity in Antarctica has experienced a wide range of methods utilized across most morphological and molecular studies. Differences include the amount of soil extracted, the number of replicates assessed, the culturing method (e.g. flooded Petri vs MPN) and temperature, the amount of time that passes between sampling and processing, the degree of taxonomic expertise, sampling depth, storage temperatures and transport. Many studies stemmed from opportunistic sampling, owing to the great difficulty involved in, especially in the beginning, successfully accessing the Antarctic continent (Richters 1907, 1908, Murray 1910, Penard 1911, Sandon and Cutler 1924). Other studies were more deliberate, involving well-designed sampling efforts across a wide range of locations and habitat types. Some were even exclusively focused on protists (Smith 1978, Sudzuki 1979, Ryan et al. 1989, Bamforth et al. 2005), though frequently studies only included protists as part of a broader assessment (Broady et al. 1987, Broady 1989, Schwarz et al. 1993, Obbels et al. 2016). Even among more methodical studies, sampling depth is not usually consistent nor often reported (Richters 1907, 1908, Murray 1910, Penard 1911, 1913, Sandon and Cutler 1924, Smith 1972, Smith 1987). When sampling depth is reported it ranges between 0 and 5cm (Smith 1974, 1978, 1985, Petz and Foissner 1996, Park et al. 2017b). Studies used a variety of storage temperatures and interim time prior to processing, but samples were always stored at temperatures consistent with their natural climate– i.e. between 20C and -20C. Freeze-thaw cycles have been shown to reduce survival of soil organisms in Antarctica (Knox et al. 2015) and while the effect of storage temperature and duration have not been specifically tested for these organisms, Mieczan and Tarkowska-Kukuryk (2014) and Petz (1997) processed their samples as quickly as possible after

sampling, an approach highly recommended by the latter. Another factor that can affect biodiversity estimates is the volume of soil used for subsequent processing – be it for culture-based examinations, live extraction or nucleotide extraction (Smith 1974, Smith 1987, Fell et al. 2006, Bates et al. 2013). Beyond sampling depth and extraction volume, descriptions of samples are frequently vague such that characterizing the difference between soil and moss taxa, for example, becomes ambiguous. Moreover, soils near lakes and streams, while apparently dry during sampling, can be unknowingly subject to greater input of liquid water than soils farther from these sources. In some cases, whether a sample was from near a lake margin or within it (Hada 1966, Chatterjee et al. 2000, Hodgson et al. 2010, Mieczan and Tarkowska-Kukuryk 2014) from under a moss bed or next to one, was unclear and as a result some of these studies could not be included in this study (Hada 1966, Chatterjee et al. 2000). A corollary to this challenge is a lack of sample coordinates or site description (Richters 1908, Murray 1910, Penard 1913, Sandon and Cutler 1924, Smith 1972, 1974, 1978, Smith 1987). Future assessments of diversity will need to include more detailed sample metadata if they are to be reliably included in broader diversity studies.

Insights and future studies

There has been a wide diversity of approaches to assessing diversity, both methodologically and logistically. Whether and how much this variety of methods has biased diversity estimates is unknown, but a standardized approach would facilitate more accurate and comparable diversity assessments between study sites and across biodiversity regions. This standard approach should consist of both morphological and molecular assessments (Roland et al. 2017, Geisen et al. 2018). Of 54 studies from across a continent roughly half again as large as Australia, 67% were based on morphology alone while 29% used only molecular data. Six

studies combined molecular and morphological data (Jung et al. 2015, Jung et al. 2016, Tyml et al. 2016, Park et al. 2017a, Park et al. 2017b, Park et al. 2018), yet none used both in a general diversity survey. Environmental DNA studies, especially high-throughput analyses of diversity like those in the burgeoning field of microbial metagenetics, are currently poorly equipped to generate data that inform morphology, physiology, and ecological function, especially in those microbial groups that have received less attention, like protists (Caron et al. 2004, Caron et al. 2009). Traditional methods in turn excel at these types of investigations, even considering the powerful potential of fields like environmental metagenomics and metatranscriptomics. Standardized sampling procedures should take into account habitat heterogeneity of field and the variability in permafrost depth across sites. Sampling vegetated soils must involve careful separation of above and below ground habitat space and in all cases careful description of sites and procedures used should be included in the methods of any associated publications. Sample processing should be carried out as soon as possible with limited storage and transport and include expert support. Removing and storing Antarctic soil samples for later analysis likely decreases the diversity of the samples, and thus sample analysis should be performed as soon after sampling as possible (Petz 1997, Adl and Gupta 2006). Both methods should use reliable techniques (i.e. direct counting over most probable number; Foissner (1987), Luftenegger et al. (1988), Foissner (1992), Berthold and Palzenberger (1995)), curated databases (e.g. PR2; Guillou et al. (2013)), consistent extraction protocols and unless unfeasible, sequencing approaches that reduce bias and inform ecology as well as diversity (e.g. metatranscriptomics and shotgun metagenomics). However, when targeted sequencing using primers is more reasonable, consider that current universal eukaryotic primers may not be appropriate for sampling all eukaryotic lineages equally. For example, Bates et al. (2013) recovered primarily organisms from the

AH/SAR supergroup (i.e. Plantae, Rhizaria, and Alveolata) but recovered little from the Excavata or Amoebozoan lineages. This is most striking in their study of the Dry Valleys, where a number of Amoebozoan species were recovered using a culture-based approach (Brown 1982, Bamforth et al. 2005). Care should be taken to verify that the primers being used can indeed sample all lineages sufficiently (Fell et al. 2006), or that studies target smaller taxonomic groupings.

Conclusions

Most of the identified 236 species of heterotrophic soil protists known from continental and peninsular Antarctica are concentrated in only a handful of regions, namely the North Antarctic Peninsula, East Antarctica and South Victoria Land. Overall, most of this diversity is regionally unique and as of yet the diversity shared between regions does not show strong trends. *Corythion dubium* is the most common species in soil and moss, while *Heteromita globosa* type cercozoan flagellates are the most widely distributed exclusively soil species. More protists have been found in moss than soil habitats. Understanding how HSP diversity relates to ecosystem functioning in Antarctica will rely on future investigations into community structure, food web interactions and functional redundancy. Characterizing food webs in the more extreme sites (e.g. South Victoria Land, Transantarctics, Ellsworth Land, Prince Charles Mountains) could be a feasible first step, owing to their relative lower diversity. Answering questions concerning the resiliency of soil ecosystems in the Antarctic in the face of climate change can be facilitated by understanding the specific contribution of key antarctic HSPs to nutrient cycling, the functional overlap between species, and the potential for local extinction of these players. A special focus on the effects of species-specific interactions on communities will also be important, including the susceptibility of key species to changes in temperature, moisture content and invasive

species. Such identification can be inferred from relative abundances (direct counting, metagenomics and metatranscriptomics), trophic interactions (determined by SIP; Crotty et al. (2012) and *in vitro* experiments using cultured isolates and mesocosms (Warren et al. 2003, Glucksman et al. 2010). Treating Antarctic terrestrial ecosystems as model systems for other microbial ecosystems worldwide (Priscu 2013) and recognizing their HSPs as important elements of those systems will deepen our understanding of community structure, stability and nutrient cycling in soils and aid in our ability to predict the effects of major environmental disturbances (i.e. climate change) on soil ecosystems.

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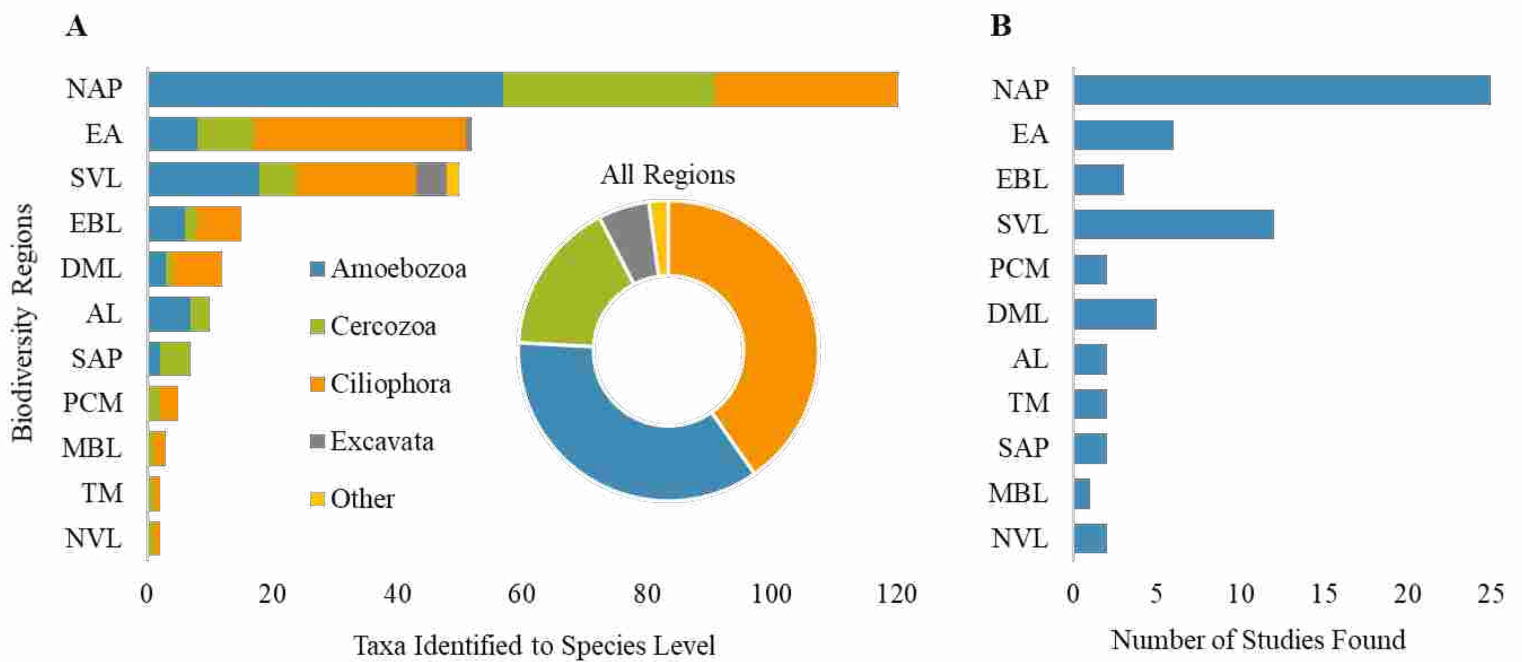

Figure 2.1: Alpha diversity and study count by biodiversity region. A: Phylum level species richness by biodiversity region as outlined by Terauds et al. 2016 using only taxa identified to the species level. Other protists include heterotrophic members of the Stramenopiles, Apicomplexa, Dinozoa, Nucleariids and Choanoflagellates. B: Number of studies to date for each region.

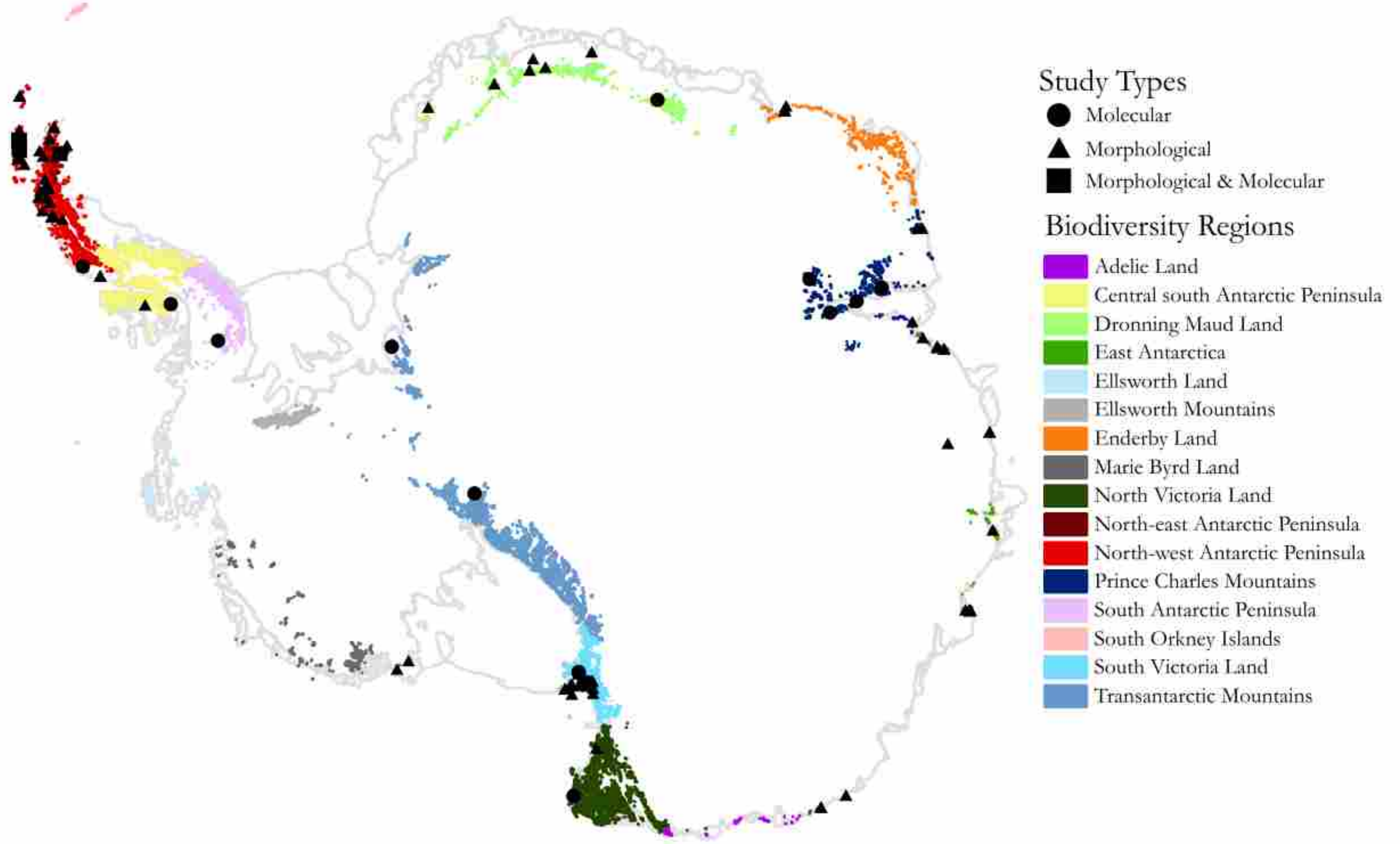

Figure 2.2: Study location and methodological type. Locations of regional study sites for all 54 studies, underlain by a modified Figure 1 from Terauds et al. 2016 showing all 16 Antarctic biodiversity regions. Triangles indicate morphology-only studies. Circles indicate studies that used molecular data. Squares indicate combined morphological and molecular studies.

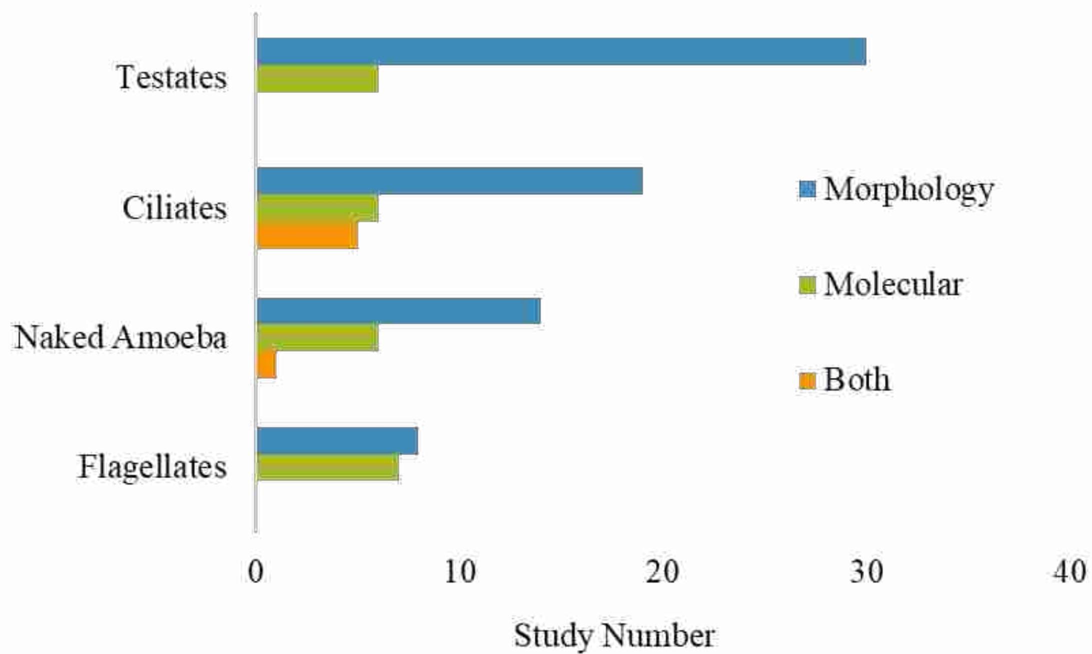

Figure 2.3: Overall study target bias by morphological group. Taxonomic targets of each study were determined and compared. In cases where intended targets were unclear, contextual clues were used to estimate the most likely intent.

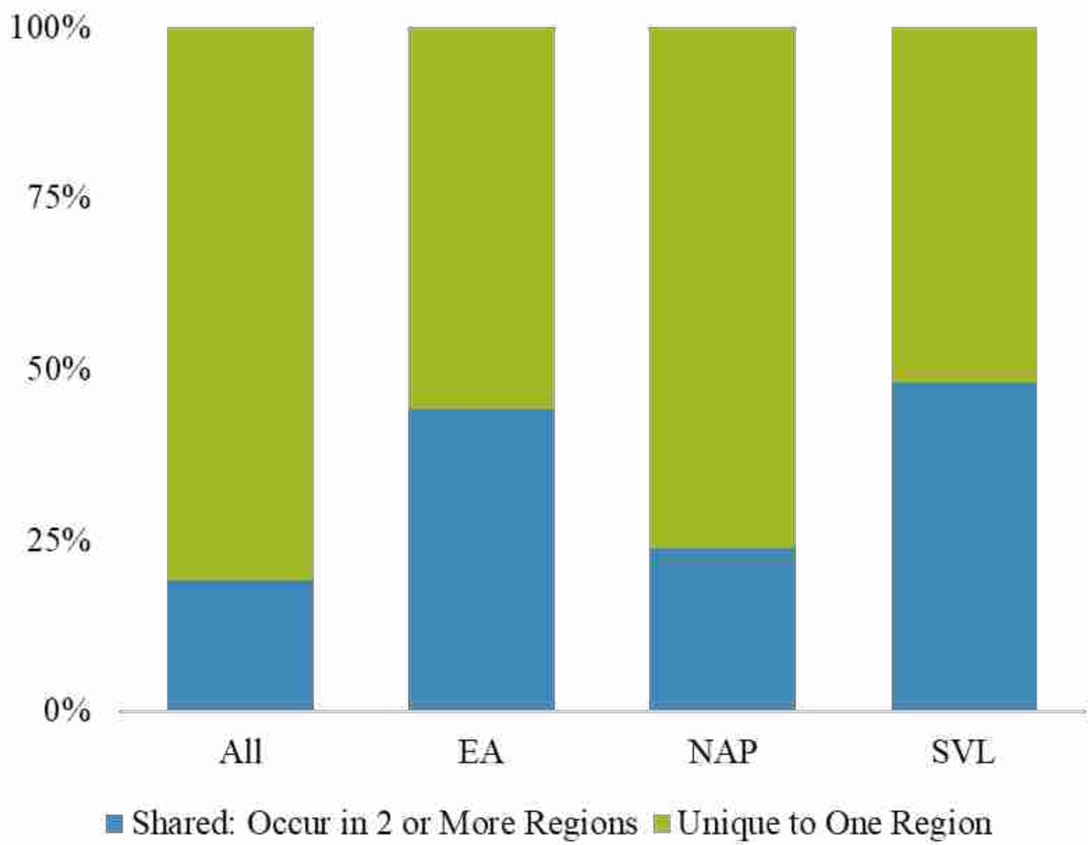

Figure 2.4: Ratio of shared to unique taxa by region. Compared lists of taxa identified to species from each region to determine which were shared between at least two regions. Regions with low overall diversity (<15 species) were excluded.

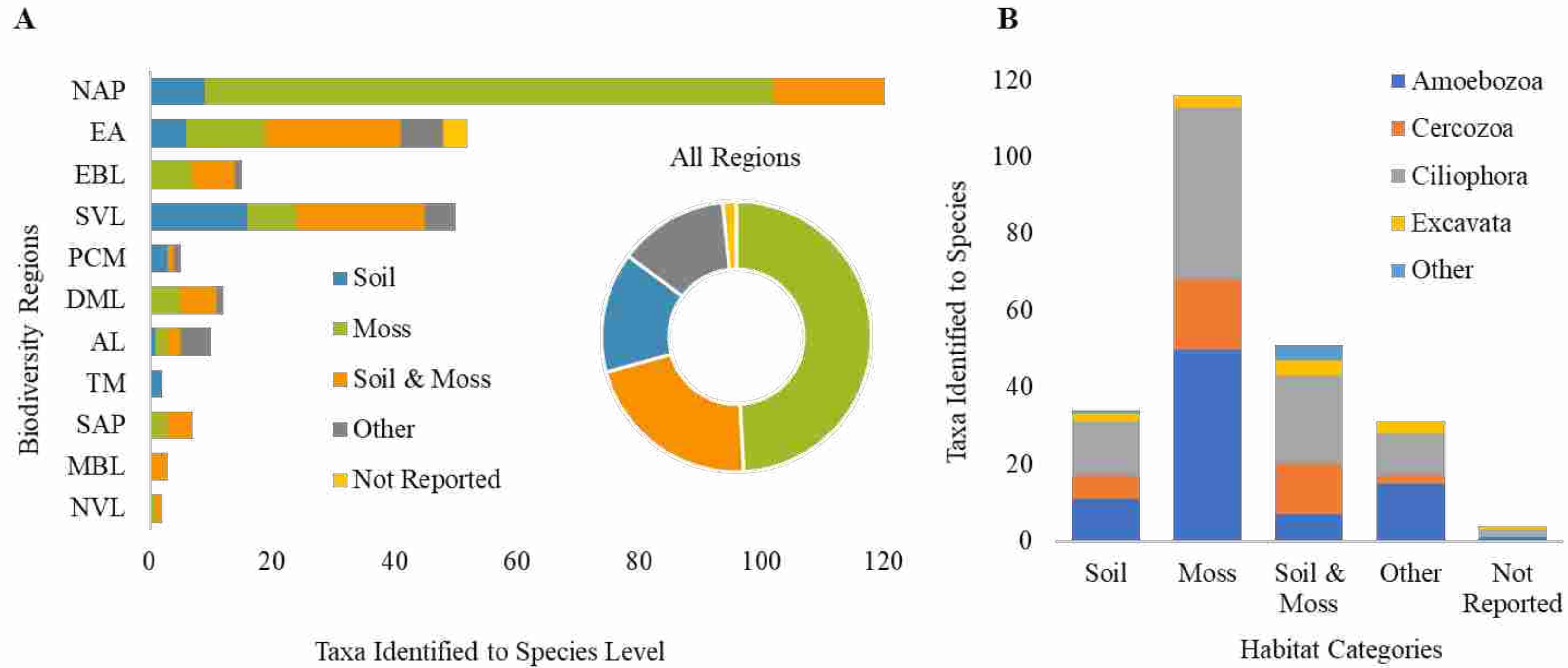

Figure 2.5: Taxa by habitat and by region. A: Breakdown of the number of taxa identified to species by habitat in each region. Taxa were assigned to categories based on sample and habitat descriptions from their original studies. "Soil" and "Moss" categories include only taxa recorded from soil or moss habitats. "Soil & Moss" are taxa found in both habitats in a single study or independently in both habitats from different studies. "Other" includes vegetated soils (not including moss-covered soils), ornithogenic derived habitats and ambiguously reported samples. Species with no associated or discernible habitat information are those "Not Reported". There are no taxa that occur in more than one category. B: Phylum-level composition of taxonomic diversity in each habitat category.

Supp. Table 2.1: Comprehensive list of studies on protozoan biodiversity in continental Antarctica. Overview of 54 studies found in literature review relating to assessing HSP diversity in continental and peninsular Antarctica, including the South Shetland Islands. Papers ordered by method of assessment, morphological, molecular or both. Earliest study 1907; most recent 2018.

Year	First Author(s)	Records	Biodiversity Region	Specific Sampling Location
Morphological				
1907	Richters	**2**	East Antarctica	Gaussberg
1908	Richters	**4**	Northern Antarctic Peninsula	Pointe-Beatrice, Seymour Island, Challenger Island, South Shetland Island, Hope Bay
1911	Penard	**10**	South Victoria Land	Cape Royds, stranded moraines
1913	Penard	**26**	Northern Antarctic Peninsula	King George Island, Goudier Island, Booth Wandel Island, Cap des Trois-Perez, Cap Fuxen, Cape Rasmussen, Berthelot Island, Argentine Islands, Petermann Island, Jenny Island, Marguerite Bay, Léonie Island
1924	Sandon & Cutler	**8**	Northern Antarctic Peninsula	Elephant Island
1960	Decloitre	**1**	Adélie Land	Not Reported
1960	Flint & Stout	**6**	South Victoria Land	Not Specified
1961	Toriumi & Kato	**7**	Enderby Land	Ongul Island, near Syowa station
1964	Decloitre	**9**	Adelie Land	Rochers Mathieu, Nunatak du Bon Docteur, Ile Lamarck, Ile des Petrels
1964	Sudzuki	**65**	Enderby Land	Station A and B
1966	Horak	**1**	Northern Antarctic Peninsula	Coughtrey Peninsula (Danco Coast)
1968a	Dillon	**6**	South Victoria Land	Cape Royds, Marble Point, Cape Evans, Kar Plateau

1972	Smith	**54**	Northern Antarctic Peninsula	Various locations around Elephant Island, South Shetland Islands (See paper)
1978	Smith	**57**	Northern Antarctic Peninsula	South Shetland Islands (Elephant Isl., Livingstone Isl., Deception Isl.), Argentine Islands (Galindez Isl.), Marguerite Bay (Avian Isl., Cone Isl., Pourquoi Pas Isl.) - Not specifically associated with taxon occurrence
1979	Sudzuki	**138**	East Antarctica	Bunger Hills, Langhovde, Vestfold Hills (Middle Tarn, Tryne Ford), Gaussberg
1982	Brown	**7**	South Victoria Land	Taylor Valley, Lake Vanda, Cape Evans, Cape Royds, Scott Base, McMurdo Station
1983	Ing and Smith	**1**	Northern Antarctic Peninsula	Cape Tuxen, Graham Coast
1985	Smith	**24**	Northern Antarctic Peninsula	Deception Island, South Shetland Islands
1986	Smith	**9**	Northern Antarctic Peninsula	Adelaide Island (Margeurite Bay)
1987	Smith	**7**	Northern Antarctic Peninsula	Brabant Island (Astrolabe Needle, Skua Point, Metchinkoff Point, Cairn Point)
1987	Broady	**1**	South Victoria Land	Mt. Melbourne, Terra Nova Bay
1989	Arambarri	**5**	Northern Antarctic Peninsula	Cierva Cove, San Martín Land
1989	Broady	**5**	Marie Byrd Land	All 23 nunataks in the Rockefeller and Alexandra mtns
1989	Ryan	**10**	Drønning Maud Land	Robertskollen
1992	Smith	**7**	South Victoria Land	Garwood Valley, Cape Royds, Lake Fryxell, Jutulsessen, Cape Bird, Cape Royds, Cape Crozier, Plogskaftet South, Plogen
1994	Steele	**1**	Drønning Maud Land	Vesleskarvet nunatak, Ahlmannryggen Mountains (near SANAE IV base)
1996	Foissner	**41**	South Victoria Land, Northern Antarctic Peninsula	Garwood Valley, West Beacon Mountains, Keble Valley, Lake Fryxell, Cape Bird, (SVL); Joinville Island, Astrolabe Island, Cuverville Island, Cape Tuxen, Andrée Island (Charlotte Bay), Graham Coast, Jenny, Lagotellerie, Dismal & Regufe Islands (Marguerite Bay), Livingstone Island, Cape Roquemaurel, Gamma Island, Melchior Islands (NAP)
1996	Petz & Foissner	**14**	East Antarctica	Casey Station

1997	Petz	**31**	East Antarctica	Casey Station, Hudson Island, Alexander Nunataks, Shirley Island (but most came from Casey station) - Not specified which species came from which locations
1997	Petz & Foissner	**16**	East Antarctica	Casey Station (Shirley & Beall islands (Windmill Islands),Whitney Point (Clark Pensinsula), Reeve Hill, near Casey Station)
2000	Barman	**9**	Drønning Maud Land	Schirmacher Oasis (Specifically Epsilon Lake, Lake 55, Lake 27, Zub Lake, all near Maitri)
2004	Golemansky	**44**	North Antarctic Peninsula	South Bay (Livingston Island)
2004	Putzke	**1**	North Antarctic Peninsula	Nelson Island (South Shetland Islands)
2005	Bamforth	**22**	South Victoria Land	Miers, Taylor (SS Lk Hoare, SS Lk Fryxell), Wright, Bull Pass, McKelvey, Victoria, Beacon, Linneaus Terrace, Lake Vanda, Meserve Glacier
2013	Royles	**12**	Central South Peninsula	Lazarev Bay
2014	Velasco-Castrillón	**1+**	East Antarctica, Enderby Land, Prince Charles Mountains	Casey Station, Vestfold Hills, Larsemann Hills-Broknes Peninsula, Hop Island, Mather Peninsual, Sansom Island, Framnes Mountains, Mawson Station
2014, 2015	Mieczan	**35**	Northern Antarctic Peninsula	Near Arctowski Station
2016	Royles	**13**	Northern Antarctic Peninsula	Elephant Island (South Shetland Islands), Ardley Island (South Shetland Islands), Norsel Point (Arthur Harbor), Green Island (Berthelot Islands)
2017	Roland	**1**	Northern Antarctic Peninsula	Elephant Island (South Shetland Islands), Ardley Island (South Shetland Islands), Green Island (Berthelot Islands)
Molecular				
2004	Lawley	**15**	Northern / Southern Antarctic Peninsula, Transantarctic Mountains	Mars Oasis, Coal Nunatak, LaGorce Mountains, Rothera Point, Sky Hi Nunatak

2006	Fell	**14**	South Victoria Land, Transantarctic Mountains	MDV (all over Taylor Valley and one spot in the labyrinth), Dufek Massif
2007	Stevens	**2**	North Victoria Land, South Victoria Land	Cape Hallet, Marble Point
2010	Hodgson	**2**	South Victoria Land	MDV (mostly Taylor Valley and one spot in the labyrinth)
2013	Gokul	**3**	South Victoria Land	Miers Valley
2015	Niederberger	**1**	South Victoria Land	Miers Valley
2016	Czechowski	**26**	Prince Charles Mountains	Lake Terrasovoje, Mawson Encarpment, Mount Menzies
2016	Obbels	**2**	Drønning Maud Land	Pingvinane, Utsteinen ridge, Utsteinen nunatak, Teltet, Duboisbreen, Tanngarden, Vikinghogda, Vengen, Svindlandfjellet, Yuboku Valley
Morphological & Molecular				
2015, 2016	Jung	**2**	Northern Antarctic Peninsula	King George Island South Shetland Islands
2016	Park	**1**	Northern Antarctic Peninsula	King George Island South Shetland Islands
2016	Tyml	**1**	Northern Antarctic Peninsula	James Ross Island, Graham Land
2017	Park	**1**	Northern Antarctic Peninsula	Robert Island
2018	Park	**1**	Northern Antarctic Peninsula	Greenwich Island

Supp. Table 2.2: Presence/Absence for Antarctic heterotrophic protists by region and habitat. Taxa are organized by habitat (habitat categories: 'Soil', 'Soil & Moss', 'Moss', 'Other', and 'Not Reported') and alphabetically by phylum. Species recorded from three or more biodiversity regions are in bold typeface. References to the ecological paper from which taxonomic information were extracted are listed. Taxonomic nomenclature was checked against the relevant literature on SCOPUS, Google Scholar, and Web of Science. Taxa identified to genus but not species in the original studies were included to aid in estimating the potential number of species identified. The singular epithet "sp." was retained when a single or multiple authors reported the same genus from the same region (e.g. Philaster sp., under 'Soil') and "spp." was used if at least one of the authors used it originally or if the same author listed "sp.1 & sp.2". If a genus was reported twice from the same study without specification (e.g. "sp.1 & sp.2"), then it was treated as a single species in the list. NAP = North Antarctic Peninsula, SVL=South Victoria Land, DML = Dronning Maud Land, EA=East Antarctica, EBL=Enderby Land, PCM = Prince Charles Mountains, TM = Transantarctic Mountains, AL = Adelie Land, MBL = Marie Byrd Land, SAP = South Antarctic Peninsula, NVL = North Victoria Land.

Soil	**NAP**	**SVL**	**DML**	**EA**	**EBL**	**PCM**	**TM**	**AL**	**MBL**	**SAP**	**NVL**	**Reference**
Amoebozoa												
Acanthamoeba castellanii (Douglas, 1930)		X										Brown 1982
Acanthamoeba polyphaga (Puschkarew, 1913)		X										Brown 1982
Acanthamoeba sp.		X										Bamforth 2005
Amoeba limicola Rhumbler, 1894		X										Dillon 1968
Centropyxis cassis (Wallich, 1864)	X											Golemansky 2004
Cyclopyxis sp.					X							Sudzuki 1979
Echinamoeba silvestris Page, 1975		X										Bamforth 2005
Heleopera sp.					X							Sudzuki 1979
Mayorella clavabellans Bovee, 1970		X										Dillon 1968

Mayorella vespertilio (Penard, 1902)		X										Dillon 1968
Microchlamys patella (Claparède and Lachmann, 1859)	X											Penard 1913, Golemansky 2004
Saccamoeba stagnicola Page, 1974		X										Bamforth 2005
Stenamoeba stenopodia (Page, 1969)		X										Bamforth 2005
Thecamoeba spp.		X										Dillon 1968
Trichameoba sp.					X							Sudzuki 1979
Trichamoeba osseosaccus Schaeffer, 1926		X										Dillon 1968
Vermamoeba spp.		X										Bamforth 2005, Brown 1982
Cercozoa												
Assulina sp.					X							Sudzuki 1979, Toriumi and Kato 1961 / Matsuda 1968
Biomyxa vagans Leidy, 1879	X											Sandon & Cutler 1924
Cavernomonas stercoris Vickerman, 2009 *in* Bass et al., 2009						X						Czechowski 2016
Cercomonas sp.		X										Bamforth 2005
Euglypha ciliata (Ehrenberg, 1848)	X							X				Decloitre 1964, Penard 1913, Golemansky 2004
Heteromita globosa (Stein, 1878)	X	X					X					Bamforth 2005, Lawley 2004, Sandon & Cutler 1924
Protaspa sp.						X						Czechowski 2016
Sainouron mikroteron Sandon, 1924	X											Smith 1978
Spongomonas sp.	X											Sandon & Cutler 1924

Trinema lineare var. *truncatum* Chardez, 1964	X											Golemansky 2004
Ciliophora												
Chilodonella sp.						X						Czechowski 2016
Ciliophrya sp.	X											Smith 1978
Colpoda maupasi Enriques, 1908				X								Petz 1997
Colpodella sp.		X										Fell 2006
Cyrtohymena citrina (Berger and Foissner, 1987)		X										Fell 2006
Dileptus sp.						X						Czechowski 2016
Enchelys polynucleata (Foissner, 1984)						X						Czechowski 2016
Epispathidium papilliferum (Kahl, 1930)						X						Czechowski 2016
Halteria grandinella (Müller, 1773)				X								Petz and Foissner 1997
Holosticha sp.		X										Bamforth 2005
Nassula sp.						X						Czechowski 2016
Nassula sp.				X								Petz 1997
Obertrumia sp.						X						Czechowski 2016
Odontochlamys sp.				X								Petz and Foissner 1996
Orthamphisiella breviseries Foissner, Agatha, and Berger, 2002		X										Fell 2006
Oxytricha granulifera Foissner and Adam, 1983							X					Lawley 2004
Oxytricha sp.					X							Sudzuki 1979
Philaster sp.	X											Smith 1972, Smith 1978
Platyophrya sp.						X						Czechowski 2016

Taxon	NAP	SVL	DML	EA	EBL	PCM	TM	AL	MBL	SAP	NVL	Reference
Pseudoholophrya terricola Berger, Foissner, and Adam, 1984				X								Petz and Foissner 1996
Pseudonotohymena antarctica Park, Jung, Min and Kim, 2016	X											Park 2016
Pseudoplatyophrya saltans Foissner, 1988				X								Petz 1997
Spathidium claviforme Kahl, 1930				X								Petz and Foissner 1996
Spathidium sp.		X										Bamforth 2005, Fell 2006, Niederberger 2015
Spathidium sp.				X								Petz and Foissner 1996, Sudzuki 1979
Spathidium sp.	X											Smith 1972, Smith 1978
Sterkiella histriomuscorum Foissner, Blatterer, Berger, and Kohmann, 1991				X								Petz and Foissner 1997
Tetrahymena rostrata Kahl, 1926		X										Bamforth 2005
Urosomoida antarctica Foissner, 1996		X										Foissner 1996
Excavata												
Naegleria gruberi (Schardinger, 1899)		X										Brown 1982
Parabodo caudatus (Dujardin, 1841)		X										Bamforth 2005
Vahlkampfia sp.		X										Brown 1982
Other Protists												
Nuclearia sp.		X										Bamforth 2005
Nuclearia sp.	X											Sandon & Cutler 1924
Actinomonas mirabilis Kent, 1880	X											Sandon & Cutler 1924
Moss	**NAP**	**SVL**	**DML**	**EA**	**EBL**	**PCM**	**TM**	**AL**	**MBL**	**SAP**	**NVL**	
Amoebozoa												
Amoeba discoides Schaeffer, 1916					X							Sudzuki 1964

Amoeba sp.					X							Sudzuki 1964, Sudzuki 1979, Toriumi and Kato 1961 / Matsuda 1968
Arcella arenaria var. *compressa* Chardez, 1965				X								Sudzuki 1979
Arcella arenaria var. *sphagnicola* Deflandre, 1928	X											Golemansky 2004
Arcella sp.					X							Sudzuki 1964, Sudzuki 1979, Toriumi and Kato 1961 / Matsuda 1968
Arcella vulgaris Ehrenberg, 1830	X									X		Smith 1978, Royles 2013
Astramoeba radiosa (Ehrenberg, 1830)	X											Smith 1978
Calomyxa metallica (Berk., 1837)	X											Arambarri 1989
Centropyxis aerophila var. *sphagnicola* Deflandre, 1929	X											Golemansky 2004
Centropyxis constricta (Ehrenberg, 1838)	X											Golemansky 2004, Smith 1978
Centropyxis elongata (Penard, 1890)	X											Golemansky 2004
Centropyxis spp.					X							Sudzuki 1964
Centropyxis sylvatica (Deflandre, 1929)	X											Golemansky 2004
Certesella certesi (Penard, 1911)	X											Smith 1978
Chaos sp.					X							Sudzuki 1964
Cochliopodium sp.					X							Sudzuki 1979
Cochliopodium tentaculatus				X								Sudzuki 1979
Cryptodifflugia apiculata (Cash, 1904)	X											Smith 1972

Cryptodifflugia compressa Penard, 1902	X											Golemansky 2004
Cryptodifflugia oviformis Penard, 1890	X									X		Royles 2013, Golemansky 2004
Cryptodifflugia sacculus (Penard, 1902)					X							Sudzuki 1964
Cryptodifflugia sp.	X											Royles 2016
Cryptodifflugia spp.					X							Sudzuki 1964
Cyclopyxis eurystoma Deflandre, 1929	X											Golemansky 2004
Diderma antarcticolum Horak, 1966	X											Horak 1966
Diderma crustaceum (Peck, 1873)	X											Arambarri 1989
Diderma niveum (Rostafinsky, 1874)	X											Ing and Smith 1983
Difflugia ampullula Playfair, 1918	X											Golemansky 2004
Difflugia bryophila (Penard, 1902)	X											Penard 1913, Mieczan 2015
Difflugia globulosa Dujardin, 1837	X											Richters 1908
***Difflugia lucida* Penard, 1890**	X	X	X									Penard 1911, Penard 1913, Barman 2000, Smith 1978, Smith 1987, Smith 1992, Golemansky 2004, Smith 1986
Difflugia manicata var. *langhovdensis* Sudzuki, 1964					X							Sudzuki 1964
Difflugia mica Frenzel, 1892	X											Smith 1986
Difflugia pristis Penard, 1902	X											Golemansky 2004, Royles 2016
Difflugia pulex Penard, 1890					X							Sudzuki 1964, Mieczan 2015
Difflugia sp.	X											Smith 1972
Difflugia spp.					X							Sudzuki 1964, Sudzuki 1979, Toriumi and Kato 1961 / Matsuda 1968

Difflugiella sp.	X											Smith 1978
Heleopera sylvatica Penard, 1890	X											Golemansky 2004
Hyalosphenia elegans (Leidy, 1874)	X							X				Decloitre 1964, Royles 2016
Hyalosphenia sp.	X											Royles 2016
Leptoderma megaspore Arambarri and Spinedi, 1989	X											Arambarri 1989
Mayorella sp.	X											Smith 1978, Smith 1985
Mayorella sp.					X							Sudzuki 1979
Metachaos sp.	X											Smith 1972, Smith 1978
Microcorycia radiata (Brown, 1912)	X											Royles 2016
Microcorycia sp.				X								Sudzuki 1979
Microcorycia spp.					X							Sudzuki 1964
Microcorycia tessellata (Penard, 1917)				X								Sudzuki 1979
Nebela collaris (Ehrenberg, 1848)	X											Richters 1908, Mieczan 2015
Nebela tincta (Leidy, 1879)	X											Richters 1908, Smith 1978
Oligonema dancoii Arambarri and Spinedi, 1989	X											Arambarri 1989
Padaungiella wailesi (Deflandre, 1936)	X											Smith 1986
Phalansterium solitarium Sandon, 1924	X											Smith 1972

Phryganella acropodia (Hertwig and Lesser, 1874)	X											Smith 1987, Smith 1972, Smith 1978, Smith 1986, Golemanksy 2004
Phryganella hemisphaerica (Penard, 1890)	X											Penard 1913
Plagiopyxis callida var. *grandis* Thomas, 1958	X											Golemansky 2004
Plagiopyxis labiata Penard, 1910	X											Royles 2016
Pyxidicula spp.					X							Sudzuki 1964
Thecamoeba sp.					X							Sudzuki 1964
Thecamoeba striata (Penard, 1890)	X											Golemansky 2004
Thecamoeba verrucosa (Ehrenberg, 1838)	X			X								Smith 1972, Sudzuki 1979
Trichia antarctica Arambarri and Spinedi, 1989	X											Arambarri 1989
Trichia varia (Pers., 1792)	X											Putzke 2004
Trigonopyxis arcula (Leidy, 1879)	X		X									Smith 1987, Smith 1992
Vannella mira (Schaeffer, 1926)	X											Smith 1972

Vannella simplex (Wohlfarth-Bottermann, 1960)	X											Golemansky 2004
Vermamoeba vermiformis (Page, 1967)	X											Golemansky 2004
Wailesella sp.					X							Sudzuki 1964
Cercozoa												
Amphitrema sp.	X											Mieczan 2015
***Assulina seminulum* (Ehrenberg, 1848)**	X											Richters 1908
Capsellina sp.					X							Sudzuki 1964
Corythion constricta (Certes, 1889)	X											Roland 2017
Corythion sp.					X							Sudzuki 1964
Corythion sp.					X							Sudzuki 1979
Euglypha bryophila Brown, 1911	X											Golemansky 2004
Euglypha compressa Carter, 1864	X	X										Penard 1911, Golemansky 2004, Mieczan 2015
Euglypha cristata Leidy, 1874	X											Golemansky 2004
Euglypha denticulata Brown, 1912	X											Golemansky 2004
Euglypha sp.	X											Foissner 1996, Penard 1913, Smith 1972
Euglypha sp.					X							Sudzuki 1979, Toriumi and Kato 1961 / Matsuda 1968
Euglypha strigosa (Ehrenberg, 1871)	X											Penard 1913, Smith 1985, Smith 1978

Euglypha strigosa f. glabra Wailes, 1898	X											Golemansky 2004
Euglypha tuberculata Dujardin, 1841								X		X		Decloitre 1964, Golemansky 2004, Mieczan 2015, Royles 2013, Royles 2016
Lecythium hyalinum Hertwig and Lesser, 1874	X											Smith 1972
Pseudodifflugia gracilis Schlumberger, 1845	X											Smith 1972
Pseudodifflugia sp.	X											Smith 1978
Spongomonas uvella Stein, 1878	X											Smith 1978
Trachelocorythion pulchellum (Penard, 1890)	X			X								Petz and Foissner 1997, Golemansky 2004
Trinema complanatum Penard, 1890	X											Golemansky 2004
Trinema contraria Decloitre, 1961				X								Sudzuki 1979
Trinema enchelys (Ehrenberg, 1838)	X	X										Penard 1911, Golemansky 2004, Smith 1978, Penard 1913
Valkanovia elegans Schönborn, 1964	X											Royles 2016

Ciliophora												
Acuholosticha paranotabilis (Foissner, Agatha and Berger, 2002)	X											Foissner 1996
Adumbratosticha tetracirrata (Buitkamp and Wilbert, 1974)			X									Ryan et al 1989
Blepharisma hyalinum Perty, 1849	X											Foissner 1996
Bryometopus sp.				X								Petz 1997
Bryophyllum loxophylliforme Kahl, 1931				X								Petz 1997
Caenomorpha spp.	X											Mieczan 2014
Choenia sp.	X											Smith 1972
Cinetochilum margaritaceum (Ehrenberg, 1831)	X											Mieczan 2014
Codonella cratera (Leidy, 1877)	X											Mieczan 2014
Colpoda californica Kahl, 1931					X							Sudzuki 1979
Colpoda ecaudata (Liebmann, 1936)	X											Foissner 1996
Colpoda sp.					X							Sudzuki 1964
***Colpoda steinii* Maupas, 1883**	X	X		X	X							Foissner 1996, Sudzuki 1979
Cyrtohymena candens (Kahl, 1932)	X											Foissner 1996
Cyrtolophosis acuta Kahl, 1926	X											Foissner 1996
Cyrtolophosis mucicola Stokes, 1885	X											Foissner 1996
Dichilum cuneiforme Schewiakoff, 1889	X											Smith 1978
Dileptus sp.	X											Smith 1972, Smith 1978
Dileptus sp.					X							Sudzuki 1964
Enchelys sp.	X											Smith 1972, Smith 1978
Euplotes sp.	X											Mieczan 2014, Smith 1972, Smith 1978,
Euplotes sp.				X								Petz 1997, Petz and Foissner 1996

Gastronauta derouxi Blatterer and Foissner, 1992				X								Petz 1997
Grossglockneria acuta Foissner, 1980	X											Foissner 1996
Halteria sp.	X											Mieczan 2014
Hemiurosomoida longa (Gelei and Szabodos, 1950)											X	Stevens 2007
Heterourosomoida lanceolata (Shibuya, 1930)		X										Foissner 1996
Holophrya sp.	X											Mieczan 2014, Smith 1972, Smith 1978
Holosticha pullaster (Müller, 1773)	X											Mieczan 2014
Kahlilembus attenuatus (Smith, 1897)	X											Mieczan 2014
Kahlilembus sp.	X											Foissner 1996
Lagynophrya sp.	X											Smith 1978, Smith 1985
Lamtostyla perisincirra (Hemberger, 1985)			X									Ryan et al 1989
Lamtostyla sp.		X										Foissner 1996

Lamtostylides edaphoni (Berger and Foissner, 1987)	X			X								Petz and Foissner 1997, Foissner 1996, Petz and Foissner 1996
Microdiaphanosoma arcuatum (Grandori and Grandori, 1934)	X											Foissner 1996
Microthorax simulans (Kahl, 1926)	X											Smith 1972
Microthorax sp.					X							Sudzuki 1979
Mycterothrix sp. nov.?			X									Ryan et al 1989
Nassula tuberculata Foissner, Agatha and Berger, 2002		X										Foissner 1996
Nivaliella plana Foissner, 1980	X											Foissner 1996
Opercularia curvicaule (Penard, 1922)			X									Ryan et al 1989
Opisthotricha sp.					X							Sudzuki 1964
Oxytricha setigera Stokes, 1981	X				X							Smith 1985, Smith 1978, Sudzuki 1979
Oxytricha sp.	X											Mieczan 2014
Paraenchelys terricola Foissner, 1984	X											Foissner 1996
Parafurgasonia sp.				X								Petz 1997
Paramecium putrinum Claparède and Lachmann, 1858	X											Mieczan 2014
Parasterkiella thompsoni (Foissner, 1996)				X								Petz 1997

Paroxytricha longigranulosa (Berger and Foissner, 1989)		X										Stevens 2007
Paruroleptus sp.					X							Sudzuki 1964
Pleuroplitoides smithi Foissner, 1996	X											Foissner 1996
Pleurotricha lanceolata (Ehrenberg, 1835)	X											Smith 1978
Prorodon sp.	X											Mieczan 2014
Pseudocohnilembus sp.				X								Petz 1997
Pseudocyrtolophosis alpestris Foissner, 1980	X											Foissner 1996
Pyxidium sp.					X							Sudzuki 1964
Rigidohymena quadrinucleata (Dragesco and Njiné, 1971)	X											Foissner 1996
Rurikoplites alpinus (Kahl, 1932)	X											Foissner 1996
Spathidium sp.					X							Sudzuki 1964
Sphaerophrya sp.					X							Sudzuki 1964
Sphaerophrya terricola Foissner, 1986		X										Foissner 1996
Strombilidium sp.					X							Sudzuki 1979
Stylonychia mytilus−complex	X											Mieczan 2014

Trithigmostoma sp.				X								Petz 1997
Trochilia minuta (Roux, 1899)	X											Mieczan 2014
Uroleptus (*Caudiholosticha*) *antarctica* Park, Min and Kim, 2018	X											Park 2018
Uroleptus sp.	X											Smith 1972, Smith 1978, Smith 1985
Uronema nigricans (Müller, 1786)	X											Smith 1985, Smith 1978
Urosomoida granulifera Foissner, 1996	X											Foissner 1996
Urotricha agilis (Stokes, 1886)	X											Smith 1978
Urotricha sp.	X											Mieczan 2014
Vorticella companula Ehrenberg, 1831	X											Mieczan 2014
Vorticella infusionum Dujardin, 1841				X								Petz 1997
Vorticella sp.					X							Toriumi and Kato 1961 / Matsuda 1968
Zosterodasys sp.	X											Mieczan 2014
Excavata												
Bodo globosus Stein, 1878				X								Sudzuki 1979
Bodo terricolus Martin	X											Smith 1972
Naegleria neopolaris De Jonckheere, 2006	X											Tyml 2016
Petalomonas angusta (Klebs, 1893)	X											Smith 1978
Soil & Moss	**NAP**	**SVL**	**DML**	**EA**	**EBL**	**PCM**	**TM**	**AL**	**MBL**	**SAP**	**NVL**	
Amoebozoa												
***Arcella arenaria* Greeff, 1866**	X	X			X							Penard 1911, Penard 1913, Sudzuki 1964, Golemansky 2004, Smith 1992, Mieczan 2015

***Centropyxis aerophila* Deflandre, 1929**	X	X	X		X							Penard 1913, Smith 1992, Sudzuki 1964, Smith 1987, Golemansky 2004, Mieczan 2015, Smith 1986
Diplochlamys timida Penard, 1909	X	X										Penard 1911, Penard 1913, Smith 1992
Heleopera petricola Leidy, 1879		X						X				Penard 1911, Decloitre 1960, Smith 1992,
***Microcorycia flava* (Greeff, 1866)**	X	X		X								Penard 1911, Penard 1913, Golemansky 2004, Richters 1907, Bamforth 2005
Schoenbornia viscicula Schönborn, 1964				X								Petz 1997
Vannella sp.	X											Smith 1978
Vannella sp.	X											Smith 1985
Cercozoa												
Allantion tachyploon Sandon, 1924	X											Smith 1978
***Assulina muscorum* Greeff, 1888**	X			X	X					X		Penard 1911, Penard 1913, Smith 1985, Foissner 1996, Smith 1987, Sudzuki 1964, Smith 1978, Sudzuki 1979, Petz 1997, Sudzuki 1979, Petz 1997, Golemansky 2004, Royles 2013, Royles 2016, Smith 1986, Mieczan 2015
Cercomonas agilis (Moroff, 1904)	X											Smith 1978
Cercomonas longicauda Dujardin, 1841	X			X								Smith 1978, Sudzuki 1979
Cercomonas vibrans (Sandon, 1927)	X											Smith 1985, Smith 1978

Corythion aerophila (Decloitre, 1850)	X			X								Sudzuki 1979, Golemansky 2004
***Corythion dubium* Taránek, 1881**	X	X	X	X				X	X	X	X	Decloitre 1964, Broady 1987, Penard 1913, Smith 1985, Foissner 1996, Smith 1987, Smith 1992, Smith 1978, Bamforth 2005, Petz 1997, Golemansky 2004, Broad 1989, Mieczan 2015, Royles 2013, Royles 2016, Smith 1986
Euglypha ciliata f. glabra Wailes, 1915	X											Golemansky 2004
Euglypha compressa f. *glabra* Cash, 1915	X											Golemansky 2004
***Euglypha laevis* (Ehrenberg, 1845)**	X	X			X							Penard 1911, Penard 1913, Sudzuki 1964, Smith 1978, Golemansky 2004, Mieczan 2015
***Euglypha rotunda* Wailes and Penard, 1911**	X			X		X				X		Smith 1972, Czechowski 2016, Royles 2013, Royles 2016, Penard 1913, Smith 1985, Petz 1997, Golemansky 2004, Smith 1978, Mieczan 2015
Euglypha sp.				X								Sudzuki 1979
Paracercomonas crassicauda (Dujardin, 1836)	X											Smith 1978, Sandon & Cutler 1924

Pseudodifflugia gracilis var. terricola Bonnet and Thomas, 1960				X								Petz 1997
***Trinema lineare* Penard, 1890**	X			X						X		Penard 1913, Golemansky 2004, Foissner 1996, Smith 1978, Sudzuki 1979, Mieczan 2015, Royles 2013, Royles 2016
Ciliophora												
Anteholosticha sigmoidea (Foissner, 1982)				X								Petz and Foissner 1997, Petz and Foissner 1996
***Colpoda cucullus* (Müller, 1773)**	X	X		X	X							Mieczan 2014, Sudzuki 1979, Petz and Foissner 1996, Petz and Foissner 1997, Foissner, 1996, Flint and Stout 1960
***Colpoda inflata* (Stokes, 1884)**	X		X	X	X							Foissner 1996, Ryan et al 1989, Petz and Foissner 1996, Petz 1997, Sudzuki 1979
Cyclidium glaucoma Müller, 1786	X											Smith 1985, Foissner 1996, Smith 1978
***Cyclidium muscicola* Kahl, 1931**	X	X		X								Petz 1997, Foissner 1996, Bamforth 2005
Drepanomonas revoluta Penard, 1922	X											Mieczan 2014, Foissner 1996
Drepanomonas sphagni Kahl, 1931		X										Foissner 1996

Fuscheria lacustris Song and Wilbert, 1989		X										Foissner 1996
Fuscheria terricola Berger, Foissner and Adam, 1983				X								Petz and Foissner 1997
Gonostomum affine (Stein, 1859)	X											Foissner 1996, Smith 1985, Smith 1987
***Homalogastra setosa* Kahl, 1926**		X	X	X								Petz and Foissner 1997, Ryan et al 1989, Foissner 1996, Flint and Stout 1960
Keronopsis helluo Penard, 1922	X											Park 2017
***Leptopharynx costatus* Mermod, 1914**		X	X	X					X			Foissner 1996, Ryan et al 1989, Petz 1997, Broady 1989, Petz and Foissner 1996
***Leptopharynx sphagnetorum* (Levander, 1900)**	X	X			X							Flint and Stout 1960, Smith 1978, Sudzuki 1979
Odontochlamys wisconsinensis (Kahl, 1931)				X								Petz and Foissner 1997

Oxytricha opisthomuscorum Foissner, Blatterer, Berger and Kohmann, 1991		X		X								Petz 1997, Foissner 1996
Platyophrya vorax Kahl, 1926	X			X								Mieczan 2014, Foissner 1996, Petz 1997
***Pseudoplatyophrya nana* (Kahl, 1926)**	X	X	X	X					X			Ryan et al 1989, Petz 1997, Broady 1989, Foissner 1996, Petz and Foissner 1996
Sathrophilus muscorum (Kahl, 1931)				X								Petz 1997, Petz and Foissner 1996
Tachysoma pellionellum (Müller, 1773)	X											Smith 1985, Smith 1978
Vorticella astyliformis Foissner, 1981		X		X								Foissner 1996, Petz 1997
Vorticella microstoma Ehrenberg, 1830	X											Smith 1978
Vorticella striata Dujardin, 1841	X											Smith 1985
Excavata												
Bodo saltans Ehrenberg, 1831	X	X										Smith 1978, Bamforth 2005
Colpodella edax (Klebs, 1892)	X	X										Smith 1985, Bamforth 2005
Paratrimastix pyriformis (Klebs, 1893)	X											Smith 1972
Peranemopsis trichophora (Ehrenberg, 1832)	X											Smith 1985
Vahlkampfia limax (Vahlkampf, 1905)	X	X										Smith 1978, Dillon 1968
Other Protists												

Oikomonas mutabilis Kent, 1880	X											Smith 1978, Smith 1985
Oikomonas termo (Müller, 1773)	X	X										Bamforth 2005, Sandon & Cutler 1924, Smith 1978
Polypseudopodius bacterioides Puschkarew, 1913	X											Smith 1978

Other habitats

Amoebozoa	**NAP**	**SVL**	**DML**	**EA**	**EBL**	**PCM**	**TM**	**AL**	**MBL**	**SAP**	**NVL**	
Centropyxis aculeata (Ehrenberg, 1832)		X										Penard 1911
Centropyxis minuta Deflandre, 1929	X											Golemansky 2004
Difflugia lanceolata Penard, 1890	X											Smith 1987
Difflugia sp.	X											Penard 1913
Diplochlamys gruberi Penard, 1909	X											Penard 1913
Diplochlamys sp.	X											Penard 1913
Diplochlamys vestita Penard, 1909	X											Penard 1913
Hyalosphenia elegans Leidy var. *major* Decloitre, 1964								X				Decloitre 1964
Hyalosphenia minuta Cash, 1891	X											Smith 1972
Hyalosphenia subflava Cash, 1909								X				Decloitre 1964

Nebela bohemica Taránek 1882 var. *adelia* Decloitre, 1964								X				Decloitre 1964
Padaungiella lageniformis (Penard, 1890)	X											Penard 1913, Mieczan 2015, Smith 1986, Golemansky 2004
Parmulina cyathus Penard, 1902								X				Decloitre 1964
Plagiopyxis declivis Thomas, 1955	X											Golemansky 2004
Plagiopyxis sp.	X											Penard 1913
Platyamoeba sp.		X										Brown 1982
Pyxidicula operculata (Agardh, 1827)								X				Decloitre 1964
Saccamoeba limax (Dujardin, 1841)		X										Gokul 2013
Vannella contorta (Moran and Anderson, 2007)		X										Gokul 2013
Cercozoa												
Cercomonas plasmodialis (Mylnikov, 1985)		X										Gokul 2013
Clathrulina elegans Cienkowski, 1867	X											Penard 1913
Euglypha -- var. minor Penard	X											Penard 1913
Heteromita sp.			X									Obbels 2016
Ciliophora												
Acineria uncinata Tucolesco, 1962	X											Foissner 1996

Anteholosticha rectangula Jung, Park and Kim, 2016	X											Jung 2016
Chaenea sp.	X											Smith 1978, Smith 1985
Cryptopharynx sp.	X											Smith 1985
Hemiophrys sp.	X											Smith 1985
Holosticha sp.	X											Smith 1978
Lacrymaria sp.	X											Smith 1978
Litonotus sp.				X								Petz 1997
Litonotus sp.	X											Smith 1985
Opercularia sp.				X								Petz 1997
Opercularia sp.			X									Ryan et al 1989
Oxytricha fallax Stein, 1859	X											Smith 1985, Smith 1978
***Paradileptus elephantinus* (Svec, 1897)**				X	X	X						Velasco-Castrillón 2014
Paraholosticha muscicola (Kahl, 1932)	X			X								Jung 2015, Petz and Foissner 1997
Plagiocampa difficilis Foissner, 1981				X								Petz and Foissner 1997
Protospathidium fraterculum Xu and Foissner, 2005				X								Petz and Foissner 1997
Protospathidium terricola Foissner, 1998				X								Petz and Foissner 1997

Pseudochilodonopsis mutabilis Foissner, 1981				X								Petz and Foissner 1997, Petz and Foissner 1996
Pseudocohnilembus pusillus (Quennerstadt, 1869)			X									Ryan et al 1989
Spathidium seppelti Foissner, 1997				X								Petz and Foissner 1997
Excavata												
Paravahlkampfia ustiana (Page, 1974)		X										Brown 1982
Petalomonas mediocanellata Stein, 1878	X											Smith 1985, Smith 1978
Tetramitus rostratus Perty, 1852	X											Smith 1978
Not Reported	**NAP**	**SVL**	**DML**	**EA**	**EBL**	**PCM**	**TM**	**AL**	**MBL**	**SAP**	**NVL**	
Amoebozoa												
Amoeba spp.				X								Sudzuki 1979
Astramoeba sp.				X								Sudzuki 1979
Centropyxis sp.				X								Sudzuki 1979
Cochliopodium sp.				X								Sudzuki 1979
Leptochlamys sp.				X								Sudzuki 1979
Physochila sp.				X								Sudzuki 1979
Pyxidicula sp.				X								Sudzuki 1979
Thecamoeba sp.				X								Sudzuki 1979
Thecamoeba terricola (Greeff, 1866)	X			X								Penard 1913, Richters 1907
Trichameoba sp.				X								Sudzuki 1979
Cercozoa												

Assulina sp.				X								Sudzuki 1979
Cercobodo sp.				X								Sudzuki 1979
Corythion sp.				X								Sudzuki 1979
Paulinella sp.		X										Hodgson 2010
Ciliophora												
Bryophyllum tegularum Kahl, 1931				X								Sudzuki 1979
Chilodonella sp.				X								Sudzuki 1979
Holosticha sp.				X								Sudzuki 1979
Keronopsis sp.				X								Sudzuki 1979
Microthorax elegans Giraud, 1863				X								Sudzuki 1979
Microthorax sp.				X								Sudzuki 1979
Oxytricha sp.				X								Sudzuki 1979
Paruroleptus sp.				X								Sudzuki 1979
Strombilidium sp.				X								Sudzuki 1979
Excavata												
Anisonema sp.				X								Sudzuki 1979
Bodo angustus (Dujardin, 1841)				X								Sudzuki 1979
Bodo spp.				X								Sudzuki 1979
Pleuromonas sp.				X								Sudzuki 1979
Tetramitus sp.				X								Sudzuki 1979

Chapter 3

Shotgun metagenomes reveal the diversity of heterotrophic soil protists in a model polar ecosystem

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Abstract

The McMurdo Dry Valleys of Antarctica are a model ecosystem for studying the dynamics of soil communities due to their low biological complexity and extreme physical environment. Very little is known of heterotrophic soil protist diversity and function in this system which hampers the predictive power of this model system. These organisms play crucial roles in mobilizing nutrients, regulating bacterial populations, and structuring communities in soils around the world. Here we discuss the results of analyzing the eukaryotic SSU of 18 shotgun metagenomes from a variety of habitats across this polar ecosystem. This is the first time such an approach has been used to study protist diversity in the Antarctic. We show a greater potential diversity of heterotrophic soil protists than previously known, increasing previous estimates of around 30 taxa to around 90 total. We also find a similar composition and structure of soil protist taxonomic groups (e.g. Ciliophora and Cercozoa) to that found in other sites at lower latitudes, including the wide distribution of Cercozoan family Sandonidae. Finally, species composition of arid sites are not just subsets of the diversity of moist sites, indicating that potentially important extremophile protists are at particular risk to warming, which will bring increased moisture to these ecosystems.

Introduction

Linking biodiversity to ecosystem processes in soils is an outstanding problem in soil ecology (Nielsen et al. 2011, Chakraborty et al. 2012) partly due to the challenge of unravelling the numerous interactions that occur between soil's myriad organisms (Bardgett 2002, Adams et al. 2006). The relative low biotic diversity in soil ecosystems in Antarctica's largest ice free region, the McMurdo Dry Valley (MDV), provides a unique opportunity to characterize the interactions between functional groups of soil-dwelling fauna and their combined influence on their ecosystem functioning (Adams et al. 2006, Wall 2007). Extreme cold, limited moisture, high salinity, low concentrations of key nutrients (e.g. nitrogen, phosphorus, and carbon) and short growing seasons in this environment have limited biotic diversity in the majority of soil communities to just a single nematode species, a few rotifer species, hundreds of bacterial taxa and an unknown number of soil protists (Adams et al. 2006, Barrett et al. 2006). Heterotrophic soil protists (HSP), motile and heterotrophic unicellular eukaryotes which include members of the Amoebozoa, Cercozoa, Discoba, Opisthokonta and Ciliophora, are integral components of soil food webs, serving as the primary means by which nutrients are transferred from bacteria to higher trophic levels (Geisen et al. 2018). Geophysical parameters of MDV soil, including irradiance, salinity, moisture input from precipitation (in the form of snow), glacier meltwater, and ambient and soil temperature have been correlated to distribution patterns of specific taxa and community dynamics for well over a decade, yet a comprehensive food web model does not exist for these communities and cannot without assessing HSP biodiversity.

HSPs play unique and essential functional roles in soil microbial ecosystems worldwide, including the regulation of bacterial populations, mobilization of nutrients, and structuring of soil communities (Crotty et al. 2012, Wilkinson et al. 2012). HSPs have diverse trophic preferences,

but studies have shown that their grazing on bacterial communities can increase the health of a bacterial population as well as increase the mineralization rates of nitrogen (Crotty et al. 2012, Saleem et al. 2012, Saleem et al. 2013, Geisen 2016). Some HSPs form an essential link between bacterial decomposers or primary producers and metazoan predators, as well as competing with metazoan bacterivores and algavores (e.g. tardigrades, rotifers, and nematodes) which are too large to exploit many micro-soil habitats where bacteria are capable of thriving (Wilkinson et al. 2012). Although HSPs certainly perform some of these same functions in MDV soil ecosystems, it is unknown whether their presence contributes to a multi-tiered food web with multiple layers of interactions, or is essential for the sustained stability of the food web, and whether changes in their composition and structure will lead to trophic cascades that could affect MDV ecosystem functioning. Answering these questions will require mapping the taxonomic diversity and functional roles of HSPs in the context of the whole MDV biotic community and their environmental drivers.

Currently, only a single systematic study of protozoan soil communities has been conducted in the MDV (Bamforth et al. 2005). Other studies have mentioned protozoan diversity in passing (Brown 1982, Fell et al. 2006, Gokul et al. 2013, Niederberger et al. 2015) or utilized dry valley soils in global surveys of the diversity of various protist groups (Foissner 1996, Bates et al. 2013) however to date a dedicated survey of molecular diversity is missing (Thompson et al., in review). Here we use shotgun metagenomics to generate eukaryotic SSU profiles because they are more efficient at capturing whole community diversity than targeted studies and can retain some information about raw abundance (Guo et al. 2016). Because of the high biotic diversity of soils (Bardgett 2002, Bardgett and van der Putten 2014, Nurk et al. 2017), obtaining eukaryotic SSU profiles from soil metagenomes requires much more depth than with amplicon

sequencing, but its relatively low biotic diversity makes the MDV a prime location for applying this approach. We also assemble longer sequences to better understand the taxonomic relationship between MDV protist species and their non-Antarctic relatives.

Methods

Sampling methods and locations

MDV soils are not homogenous at the landscape scale in their salinity, moisture content, organic nutrient content, or even temperature. Our collection aimed for the broadest diversity of biogeochemical parameters possible and includes 11 basins in 9 valleys across a latitudinal gradient of more than 2 degrees. The collection sites also encompass arid, moist and virtually inundated sites, low to high elevation sites, and sites that were both near (1km) and distant (>70km) to the Ross sea (Fig 1). Samples were taken during the 2014, 2015, 2016 and 2017 field seasons following the sampling procedure outlined in Freckman and Virginia (1993) from 87 sites across Southern Victoria land and pooled to form representative samples for each of as many landscape features as possible. Briefly, sterilized scoops were used to remove the top 10 cm (where possible) of soil into sterile whirlpak® bags, returned to McMurdo Station via helicopter at ambient air temperature and stepped down slowly over a week to -20°C for long-term storage. Eighteen sites were chosen to for sequencing: Beacon Valley (BV), Cliff Nunatak (CN), Canada Stream algal mat (CS), East Side Lake Fryxell (ESLF), Mount Gran (GR), Garwood Valley ET (GV), Hjorth Hill moss beds (HH), soils near Marr pond (Marr), soils near Benson Glacier and Flatiron (MG), Mount Murray (MGM), Mount Suess (MS), Miers Valley ET (MV), North Side Lake Hoare (NSLH), near Towle Glacier (TG), University Valley (UV), near Lake Brownworth in Wright Valley (WrB), Upper Wright Valley (WrU), and Wall Valley (WV). The most southern site was Miers Valley and the most northern Mount Murray. ESLF, HH, CS,

Marr and NSLH are all in Taylor Valley, the main focus of the MCM LTER research program; ESLF is in Fryxell basin and NSLH in the Lake Hoare basin, while HH is coastal. MG, MGM, MS, TG, GR and CN are all north of Victoria Valley and south of the Drygalski Ice Tongue. Additional DNA extraction was performed on samples from Virginia Valley (a hanging valley adjacent to Wall Valley), near lake Vanda in Wright Valley, and soils from the Lake Bonney Basin (south side of Lake Bonney, near Taylor Glacier), and Alatna Valley, but no detectable DNA could be extracted. Notable sites include Canada Stream algal mat samples (an Antarctic Specially Protected Area) and soils from University Valley, which is considered to be one of the most inhospitable terrestrial habitats on Earth (Goordial et al. 2016). Sites BV, WV, UV, GR, TG, and WrU were labeled as high elevation sites as they occurred around or above 1000m and belong to a distinct climate zone (Marchant and Head 2007). Samples from ESLF, NSLH, BV, UV, GR, and WV were typical mineral soils with no visible moisture or organic material (OM); soils from three sites had visible OM, referred to here after as productive sites (ϕ): HH (chunks of moss), CN, and CS (algal mat). These latter three, along with MGM, MG, MS, TG, WrB, WrU and Marr appeared moist to wet.

Environmental parameters

Individual (not pooled) soil samples (not including CS algal mat) were submitted to the Environmental Analytics Lab at Brigham Young University to measure moisture, pH, EC, total N, total C, total P, NO3-N, C:N ratio, and texture. To measure moisture content, soils were weighed before and after drying overnight at 50°C. pH was determined on a saturated paste (Rhodes 1982), EC was measured using a RC-16C Conductivity Bridge (Beckman Instruments, Brea, CA, USA), NO3-N was measured following (Sims and Jackson 1971), total C and N was measured on a TruSpec CN Determinator (LECO Instruments, St. Joseph, Mich., USA)

following (McGeegan and Naylor 1988), and total P was extracted with 0.5M sodium bicarbonate following Olsen et al. (1954). Texture was measured following Day (1965). Elevation (m), Distance to coast (km), and Aspect (°) were gathered for each individual sample (including CS) using Antarctic REMA explorer (Howat et al. 2019).

DNA extraction and sequence generation

Soils were slowly thawed over a week's time to 10°C for pooling and subsequent extraction of genomic DNA. Representative samples for each major landscape feature (e.g. Garwood Valley or Canada Stream) were pooled from 5g subsamples of individual samples into a total of 18 sites (Fig 1). Whole genomic DNA was extracted from each of these representative samples using the Dneasy PowerSoil Kit (Qiagen) following a modified protocol recommended by the manufacturer for use with soils with extremely low DNA content. Briefly, 1.8-2g of soil instead of 0.5 g were used per reaction, solution C3 (100ul instead of 200ul) was added immediately after solution C2 (100ul instead of 200ul) without an intervening incubation, and half the volume of eluate (50 ul instead of 100ul) was incubated at room temperature on the surface of the filter for 1 minute prior to elution. Additional DNA extractions were carried out for each sample until enough DNA had been recovered to avoid the need for an amplification step during library preparation (>2µg), which would have potentially obscured information on community structure. Hjorth Hills, Benson Glacier (MG), Canada Stream, and Cliff Nunatak sites had noticeably higher DNA content than the rest and each required only two extractions while samples from lower productivity areas required 12g to 20g total (see Table 2).

The concentration and quality of the extracted DNA was assessed using qubit and nanodrop respectively. Libraries were made using the NEBNext Ultra II DNA Prep kit (New

England Biolabs) with custom primers from IDT, and sequenced over one and a half lanes on an Illumina HiSeq in the Rapid Run mode with read lengths set to 2x250 and a total insert length of 500bp. Right and left reads from both lanes were concatenated such that each site had one fastq file for each paired end that included sequences from both lanes. FastQC (Andrews 2010) was used to determine where to trim reads and trimming was done using Trimmomatic with the following settings: LEADING 2, TRAILING 2, SLIDINGWINDOW 4:15 (default), MINLEN 30 (Bolger et al. 2014). Paired-end reads that passed trimming (Table S1) were merged using FLASH: -m 10 (min overlap) (default) -M blank (max-overlap) -x 0.20 (max mismatch density) -r 250 (average read length) (FastQC) -f 500 (fragment length) -s 50 (Magoč and Salzberg 2011).

Taxonomic assignment of reads

Accurate taxonomic assignment in molecular databases is constrained by the breadth and accuracy of reference databases. The Protist Ribosomal Database (PR2) is a manually curated database focusing on collecting and maintaining protist ribosomal sequences, but also includes many metazoan, chloroplast and mitochondrial sequences (Guillou et al. 2013). We searched the whole metagenome sequences for ribosomal rDNA using nhmmer (Wheeler and Eddy 2013) with the eukaryote hmm profile developed for the rRNA prediction software Barrnap and an e-value cutoff of 1e-5 (Seemann 2018). Recovered SSU sequences were converted to fasta format using the esl-reformat miniapp provided with the hmmer software package, version 3.2.1 (Eddy 2018), and blasted against the pr2 database using the command line version of ncbi-blast-2.7.1+ (Camacho et al. 2009). For each sequence, the hit with the highest bit score and lowest E-value was retained and sequences below 200bp were removed. 18S, 28S, 5S, and 5.8S sequences were separated and analyzed separately. Taxonomic assignments were checked by blasting the same extracted SSU sequences against the NCBI nt database (Camacho et al. 2009, NCBI 2018).

Lineage taxonomy for each assignment was updated using Mycobank, ITIS, Algaebase and the most recent classification revision for eukaryotes (Robert et al. 2013, Adl et al. 2018, Guiry and Guiry 2018, ITIS 2018). Finally, reads were normalized using the relative proportion of sequences that each sample received from the lane as a whole.

To extract and assign taxonomy to bacterial SSU sequences, we used the Metaxa2.2 default SILVA database using an e-value cutoff (-E) of 1e-5, --allow_single_domain 1e-5,0 and -N 1 (Bengtsson-Palme et al. 2015). Sequences with reliability scores lower than 80 (1,639 sequences), with length lower than 200bp (35,407 sequences), and with percent identity lower than 80% (38 sequences) were subsequently removed. There were 2,262 sequences for which reliability score, percent identity and sequence length were not reported, so these were also removed.

Analyses of extracted SSU sequences were done using the phyloseq (McMurdie and Holmes 2013) and vegan (Oksanen et al. 2016) packages in R version 3.2.2 (R Core Development Team 2016). To estimate whether sequencing depth was sufficient to reasonably recover diversity, we constructed rarefaction and species accumulation curves (Fig S1). Representative environmental parameter values for each site (except CS) were obtained by averaging measured values from individual samples for that site and used to perform analyses in R (Table S2). Permutational multivariate analysis of variance (PERMANOVA) was run on normalized abundance counts using Bray-Curtis distance matrices. Broad trophic function was assigned to all groups using general knowledge and the literature: consumer, parasite, phototroph, saprotoph, mixotroph and unsure. These designations were used to organize some taxonomic groups for convenient visualization. To simplify some whole eukaryotic community analyses, taxa were organized into supergroup levels, some of which are not official taxonomic

groups: Streptophyta, Chlorophyta (sometimes collectively Archaeplastida), Fungi, Metazoa, Heterotrophic protists, Photosynthetic protists, Parasites and Other Eukaryota. The group "Heterotrophic Protists" consists of the Ciliophora, Cercozoa, Amoebozoa (Tubulinea, Evosea, and Discosea), the Colpodellidae, Apusomonadida, Kathablepharidacea, Endomyxa, Euglenozoa, Discoba, Ancyromonadida, and some Stramenopiles. "Photosynthetic Protists" include the Bacillariophyta and most of the Ochryophyta; "Parasites" include Apicomplexa, Ichthyosporea, and Labyrinthulea. "Other Eukaryota" include taxa for which trophic function was uncertain, largely due to lack of higher taxonomic resolution e.g.: Dinoflagellata, Foraminifera, and some Stramenopiles.

Submission to MG-RAST

Unassembled forward and reverse reads from each sample were uploaded to MG-RAST using the MG-RAST API (Wilke et al. 2015) under the project name SVLSoil18Proj090218. Checksums for each file were calculated and checked to ensure proper upload and then paired ends were merged using MG-RASTs merging protocol. Screening for Homo sapiens sequences was performed, but dereplication was not (Nayfach and Pollard 2016), and the DynamicTrim (Cox et al. 2010) option with default parameters (15 and 5 respectively) was selected.

Results

In total, we recovered 281 eukaryote genera from 208 families (Figure 3A). Almost half (118) of the genera, but only 7% of the reads, were protists. Outside of CS (an algal mat), the richness of both eukaryotic generally and HSPs specifically are low. Most sites (12) had fewer than 10 HSP genera and no soil site had more than 20. Sites that had clearly visible OM during sampling (ϕ) were the most diverse for both HSPs and all eukaryotes while high elevation sites

were low in diversity (*). CS was by far the most taxonomically rich site for both HSPs and eukaryotes overall and had the highest abundance of HSP reads, but not eukaryotic reads. HH (moss covered soil) had more than twice the number of eukaryotic reads as any other site due to the presence of several highly abundant fungal taxa there. BV and WV did not have the lowest richness despite generally being considered among the most extreme environments among our samples (Marchant and Head 2007). Instead, WrU, WrB and GR had the lowest richness (Figure 2A). UV had more eukaryotic reads and OTUs than ESLF, WrU, WrB, and GR (Figure 3A), although it had the fewest bacterial SSU reads of any site, after normalizing. All but one of the five OTUs from UV are found in other sites, and three of these OTUs are among the most widely distributed (an unidentified Embryophyta, an unidentified Sandonidae, and an unidentified Rotifera), although they do not occur in all sites.

The most abundant taxon in our study was a *Glaciozyma* sp., probably *Glaciozyma antarctica* (Fell, Statzell, Hunter & Phaff, 1969), an obligate psychrophile (Turchetti et al. 2011) that has been isolated from a variety of locations and habitats around Antarctica (Turchetti et al. 2011, Tsuji et al. 2013, Bharudin et al. 2018, Connell et al. 2018). In terms of read abundance, this taxon dominated HH (49 %) but was present only as singletons in the other sites where it occurred.

Cercozoa are the most widely distributed eukaryotes, occurring in every site but one (Figure 3C). Fungi are both the most taxonomically diverse group and the most abundant in terms of normalized reads (53% of total). Archaeplastida are the next most abundant (38%), Metazoa and heterotrophic protists (most Ciliophora, Amoebozoa, Rhizaria and some Discoba) each make up 4% of reads, while 1% consist of Stramenopiles and putative parasites (Table 2). We found four genera of parasites at very low relative abundances and all but one occurred

exclusively in CS. Several reads were assigned to eukaryotes that have an unresolved relationship within Eukarya, including a member of the Opalozoan MAST-12C group, the Ancyromonadida *Fabomonas* sp., the Kathablepharidacean *Hatena* sp., and a species of Phylum Telonemia, a lineage that is so morphologically unique that no sister group has yet been identified (Yabuki et al. 2013) (Table 3).

Heterotrophic protists

Cercozoa are the most abundant group of heterotrophic protists by read count (34%), are relatively diverse phylogenetically (18 genera from 12 families), and are the most widely distributed, with two families (Sandonidae and an unidentified Rhogostoma lineage) occurring in 12 and 11 sites respectively (Figure 2D, Figure 3C). Fifty-six percent of cercozoan reads were classified as four genera within Sandonidae and 22% were assigned to an unidentified Rhogostoma-lineage. The only site that a cercozoan read was absent from was WrU, although NSLH, TG, MV, UV, WV, GR, and MG each had relatively low abundance.

Ciliophora were the most taxonomically rich protists and the second most abundant overall, however each taxon was recovered in low abundance (Fig 3D). The most abundant ciliate by reads (12% of ciliate reads) is an Oligohymenophoran in the order Peniculia, possibly a *Stokesia* sp. and the most widely distributed are reads that blast to Chilodonellidae, a family that possesses both predators as well as parasites. Amoebozoa account for only 1% of the total abundance (Table 2). Although most (89%) occurred in sites with relatively higher moisture - CN, CS, Marr and MG – they were almost absent from HH. Members of the Acanthamoebidae, a *Ptolemeba* sp., and a *Vermamoeba* sp. are the most widely distributed Amoebozoa.

Other eukaryotes

Fungal diversity is extremely uneven across our sites, with the majority of fungal reads (96%) assigned to just six genera: *Glaciozyma* (52%), *Rhodotorula* (30%), an unidentified *Microbotryomycetes* (7%), *Sporobolomyces* (5%) and *Sporidiobolus* (2%). All of these taxa are virtually restricted to a site with overlying moss beds (99% of reads attributed to these taxa occurred in HH) and few fungal reads occurred outside of Hjorth Hill (4%) (Table 2).

Archaeplastida are predominantly streptophyta (95%), which were essentially all (99.4%) recovered from moist sites – CN, CS, HH, MG, and MGM (Table 2, Figure 3B). Streptophytes dominate in abundance in CN, CS, MG and MGM, which are four of the top five sites with the highest moisture content (Figure 3AB). Reads blasting to members of the *Bryum* genus (30%) and to an unidentified Embryophyceae (36%) were by far the most common Streptophytes. Chlorophyta were much less abundant than Streptophyta (5%) but were more evenly distributed, occurring in all sites except for BV, GR, MV, NSLH, WrU, and WV (Table 2). Photosynthetic protists (Stramenopiles) are rare, more narrowly distributed and less diverse taxonomically compared to streptophytes and chlorophytes (Figure 3B, Fig 2C).

We recovered 23 genera from 13 metazoan families, including a single read belonging to a mite (*Dermonoton*) (Figure 3C, Table 3). The most abundant metazoan family was Rotifera, (53% of metazoan reads), followed by Tardigrada (38%) (Table 2). The most abundant identified Rotifer genus is *Adineta*, and the most abundant Tardigrade genus is *Acutuncus* (Taxonomic List?). Reads assigned to nematodes (Enoplea and Chromadorea) accounted for only 9% of metazoan reads and were absent from 11 of 18 sites (Table 2). The majority of our *Plectus* reads

were classified as *P. murrayi* (70%), but 15% were classified as an unidentified *Plectidae* sp., 10% as *Plectus aquatilus*, and 5% as *Plectus rhizophilus*.

Environmental drivers

When considering eukaryotic communities as a whole on a PCA, arid and high elevation samples separated out from more diverse, organic soils at lower elevations (CN, CS, MGM, MG) (Figure 4A). Significant drivers of differences between our samples were moisture, pH, elevation, and distance to coast (Adonis: moisture – $r2= 0.09108$, $p=0.046$; pH $r2= 0.08295$, $p=0.002$; elevation $r2= 0.09017$, $p=0.032$; distance to coast $r2= 0.10869$, $p=0.006$) (Table S2). Clustering of sites using only heterotrophic protist groups was less clear (Figure 4B). High elevation sites grouped together, but there is no obvious separation between sites based off of moisture and distance to coast. Indeed, unlike with eukaryotes overall, for HSPs moisture and pH were not found to be significant drivers although elevation and distance to coast were (Adonis: moisture $r2= 0.06982$, $p=0.314$; pH $r2= 0.07124$, $p=0.293$; elevation $r2= 0.11463$, $p=0.005$; distance to coast $r2= 0.11403$, $p=0.009$).

The productive sites (ɸ) (CS, CN, and HH) do not possess all the diversity of all other sites combined, or in other words communities at less productive or more arid sites are not just subsets of the diversity seen at the most productive site in the dry valleys. Reads were recovered from every site that blasted to taxa not included in CS's taxonomic list. The majority of this diversity comes from four fungal taxa in HH - *Glaciozyma, Rhodotorula, Microbotryomycetes*, and *Sporidiobolus* - which are also among the most abundant fungi by raw read count across all sites. The diversity of CS, CN, and HH combined misses 47 genera from 40 families, although each of these taxa possess two or fewer reads. The inclusion of Marr, MG, MS, and MGM, the

next most diverse sites (Figure 3A), still misses 10 genera from 10 families, including a Chilodonellidae sequence, the most widely distributed ciliate family in our data.

Discussion

MDV diversity trends

We recovered 90 genera of HSPs, almost one third of total eukaryotic diversity, although they only made up 5% of the overall read abundance. Overall, taxonomic richness for all groups is much lower than sites at lower latitudes (Geisen et al. 2015, Venter et al. 2018), consistent with the findings of Bates et al. (2013), which compared soil protist diversity across large geographic scales. Previous studies in the MDV, most cultivation based morphological studies, identified around one third as many taxa and also recorded a high richness of Ciliophora but not Cercozoa. (Brown 1982, Bamforth et al. 2005, Fell et al. 2006, Gokul et al. 2013, Niederberger et al. 2015). Our richness estimates could be overinflated as the result of intraspecific variation in the SSU region, but protist taxa in our study were diverse even at the family (83) and order (49) levels. Bamforth et al. (2005) recovered HSP abundances that were substantially higher than co-occurring nematode species, but our study does not support that observation. This discrepancy could in part be due to the fact that Bamforth et al. (2005) used culturing to selectively isolate protists, which could have altered the naturally occurring abundances. Although sequences of *Corythion dubium*, are present in the databases we searched, our study did not recover the cercozoan, despite the fact that it is thought to be the most widespread moss protist in Antarctica (Thompson in review).

At least one read classified as a heterotrophic protist was recovered from every site. This is not true of the metazoa, fungi, Archaeplastida, or photosynthetic protists, which were each

absent from five, three, three, and thirteen sites respectively (Figure 4A). Inherent sampling and sequencing biases aside, heterotrophic protists are a widely distributed, phylogenetically diverse, albeit seemingly rare component of MDV terrestrial ecosystems. That heterotrophic protists are widely occurring in MDV soils is consistent with previous findings (Bamforth et al. 2005) and provides additional support for their essential role as the main bacterivores in these sites.

Ciliophora has the highest taxonomic richness amongst the heterotrophic protists, but this richness is concentrated in the more moist, productive sites (ϕ), consistent with patterns seen elsewhere (Geisen et al. 2015). Conversely, cercozoan diversity is not overwhelmingly concentrated in one site, although productive sites (ϕ) (CN, CS and HH) together contain roughly half of cercozoan read abundance. Owing to their wide distribution and abundance (relative to other heterotrophic protists) sandonidae, a globally distributed family of gliding flagellates that are often dominant in soils (Howe et al. 2009, Venter et al. 2018) and an unidentified *Rhogostoma* lineage appear to be important members of MDV soil communities. *Rhogostoma* is a genus of bacterivorous testate amoeba with five known species, at least some of which are adapted for rapid desiccation tolerance (Dumack et al. 2017). Future efforts to isolate these potentially important taxa from MDV soils should note that these species are very small, and are not likely detectable using current methods in the MCM LTER.

Amoebozoa were lower in taxonomic diversity than either Cercozoa or Ciliophora and tended to occur more frequently in the more organic soils. Reads classified as *Acanthamoeba* were also recovered, albeit only from three sites and in low abundance. Some *Acanthamoeba* are pathogenic in humans, but many species are widespread and prominent members of soil communities (Geisen et al. 2014). Brown (1982) also recovered *Acanthamoeba* strains from MDV soils, some of which were capable of growing at 37°C. Future studies targeting this

species may help shed light on the apparent cosmopolitanism of this genus and the breadth of its functional role in soil communities.

The Foraminifera and choanoflagellida reads we recovered contribute to the growing consensus that these traditionally aquatic protist groups also have widespread terrestrial counterparts. The lack of parasites, however, is not surprising as potential hosts for parasites (microarthropods and plants) are low in diversity and occur only sporadically in Antarctic organic soils (Adams et al. 2006), which are far less abundant than the arid, oligotrophic mineral soils which dominate the landscape (Barrett et al. 2006).

Drivers of eukaryotic diversity in MDV soils

Moisture has been cited as a major driver of soil protist diversity (Bates et al. 2013), however our study did not confirm this trend directly. Instead, elevation and distance to coast were stronger predictors of HSP diversity. In the MDV both an increase in elevation and distance from the coast are correlated with a decrease in moisture (Marchant and Head 2007), so the significance of these variables in explaining HSP distribution is not necessarily uncoupled from moisture itself. It is also possible that additional drivers of HSP communities exist that are related indirectly to soil moisture, such as the appropriate abundance of suitable prey. Confirming whether moisture is a driver of HSP diversity in the MDV is critical to understanding the susceptibility of the food webs in these soils to impending climatic changes: increases in moisture due to thawing ice could select for protist groups that are more suited to wetted soils and threatens the biological simplicity of the arid, mineral soils that currently predominate.

Shotgun metagenomics in MDV soils presents unique benefits and challenges

Low read count for eukaryotes generally and protists specifically have likely influenced our analyses. Our shotgun metagenome approach allowed us to capture a significant proportion of the MDV soil biodiversity, yet our sequencing depth was still insufficient for all sites, especially highly diverse sites like CS and CN (Fig S1). DNA from CS, CN and HH was intentionally loaded onto the lane with twice the concentration of other sites (Table 1) to better capture their visibly higher diversity, but BV's and GR's relatively high and low read counts were unintentional. BV's higher than intended read count is instructive, as an increased read depth did not appear to increase the capture of novel taxa in arid sites. Our resulting read counts likely reflect lower concentrations of eukaryotic DNA in these samples. For future shotgun metagenome work on eukaryotes in the MDV, using increased amounts of source material in addition to deeper sequencing will be important. Normally, a few grams of soil are sufficient to capture local prokaryotic and micro-eukaryotic diversity (Santos et al. 2015, Guo et al. 2016), but in the MDV high habitat heterogeneity and low abundances may necessitate extracting more soil per sample as well as increasing the breadth of local sampling.

Conclusions

We found greater taxonomic richness of HSPs in the MDV than previously reported, but still much lower than non-polar sites. Interestingly, the relative composition of different HSP groups (i.e. Ciliophora, Amoebozoa, and Cercozoa) reflect what is found in more temperate soils, suggesting a conserved set of soil community functions. Thus, additional research into MDV HSP communities can benefit our understanding of the roles HSPs play in soil ecosystems

generally. Members of the cercozoan families Sandonidae and Rhogostomatidae warrant greater focus in future research, owing to their wide distribution in MDV sites and corresponding contributions to ecosystem functioning. Understanding the trophic interactions and habitat preferences of these organisms in particular could improve MDV food web models and inform predictions about the susceptibility of these food webs to the impact of warming in the Antarctic.

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Table 3.1: Site, sequencing and processing statistics. Total read count from whole shotgun metagenomes (R1 Reads (M)) is reported in millions of reads, and only for R1 reads. Metaxa2 with its custom Silva hmm profile and reference database was used to extract and classify prokaryotic SSU. The barrnap eukaryote hmm profile was used to extract eukaryotic 18S SSU and the protist ribosomal database (PR2) was used to classify them. Unfiltered eukaryotic SSU were summed with prokaryotic SSU to estimate the relative proportion of SSU in the full metagenomes. ϕ indicates sites with visible organic matter; * indicates sites at >1000m elevation. Sites are ordered from higher to lower moisture content, top to bottom.

Sample Information				**Sample Processing Statistics**						**Metaxa2 Silva hmm profile**						**Barrnap hmm profile + PR2 db**				**OTUs**	
Sample Name	**Sample ID**	**Samples Pooled**	**Sampling Year**	**Soil Extracted (g)**	**R1 Reads (M)**	**Proportion of Run**	**Normalize factor**	**Passed Trim (%)**	**Merged (%)**	**SSU (reads)**	**SSU (%)**	**Bacteria (reads)**	**Bacteria (% SSU)**	**Archaea (reads)**	**Archaea (% SSU)**	**Eukaryota (reads)**	**Eukaryota (% SSU)**	**Eukaryota 18S (>200 bp, raw)**	**Eukaryota 18S (>200 bp, normalized)**	**All Eukaryota**	**HSPs**
Canada Stream ϕ	CS	1	2013	1.75	21.8	7.96%	1.0	99.9%	75%	12674	0.06%	9357	74%	21	0.2%	3296	26.0%	2417	922	153	61
Mount Murray	MGM	3	2017	5.25	11.6	4.24%	0.5	99.9%	74%	5426	0.05%	5141	95%	22	0.4%	263	4.8%	188	133	28	4
Mount Suess	MS	3	2017	5.25	15.5	5.66%	0.7	99.8%	74%	8281	0.05%	8113	98%	71	0.9%	97	1.2%	64	44	31	8
Benson Glacier/Flatiron	MG	3	2017	3.5	16.3	5.96%	0.8	99.9%	77%	8091	0.05%	7770	96%	125	1.5%	196	2.4%	131	81	45	9
Cliff Nunatak ϕ	CN	2	2017	3.5	21.8	7.95%	1.0	99.8%	79%	12422	0.06%	9699	78%	19	0.2%	2704	21.8%	1720	646	91	19
Hjorth Hill ϕ	HH	5	2017	3.5	21.9	8.01%	1.0	99.8%	83%	28490	0.13%	18267	64%	5	0.0%	10218	35.9%	6488	2249	54	14
Garwood Valley	GV	6	2017	7	13.2	4.82%	0.6	99.8%	75%	6178	0.05%	6139	99%	15	0.2%	24	0.4%	18	17	13	6
Towle Glacier *	TG	3	2017	5.25	8.8	3.21%	0.4	99.8%	70%	4004	0.05%	3962	99%	18	0.4%	24	0.6%	17	17	9	2
Miers Valley	MV	6	2017	7	17.1	6.23%	0.8	99.8%	73%	7648	0.04%	7612	100%	14	0.2%	22	0.3%	12	10	10	5
Marr Pond	Marr	4	2016	5.25	13.3	4.84%	0.6	99.8%	79%	7246	0.05%	7181	99%	2	0.0%	63	0.9%	36	31	24	12
Mount Gran *	GR	1	2017	5.25	7.5	2.74%	0.3	99.9%	74%	3318	0.04%	3310	100%	5	0.2%	3	0.1%	2	2	2	1
Lower Wright Valley	WrB	8	2017	5.25	11.5	4.18%	0.5	99.9%	76%	6063	0.05%	6042	100%	6	0.1%	15	0.2%	3	3	3	1
East Side Lake Fryxell	ESLF	2	2015	5.25	12.8	4.68%	0.6	99.9%	74%	6080	0.05%	6063	100%	10	0.2%	7	0.1%	4	4	4	2
North Side Lake Hoare	NSLH	3	2015	5.25	16.0	5.83%	0.7	99.8%	74%	6498	0.04%	6470	100%	17	0.3%	11	0.2%	6	6	6	3
University Valley *	UV	7	2017	10.5	13.2	4.83%	0.6	99.9%	74%	4916	0.04%	4903	100%	3	0.1%	10	0.2%	7	6	5	2
Beacon Valley *	BV	14	2016	10.5	25.4	9.28%	1.2	99.9%	75%	12034	0.05%	12012	100%	2	0.0%	20	0.2%	13	9	8	5
Wall Valley *	WV	8	2017	7	13.2	4.83%	0.6	99.9%	76%	5846	0.04%	5825	100%	0	0.0%	21	0.4%	16	13	7	3
Upper Wright Valley *	WrU	8	2017	7	13.0	4.74%	0.6	99.8%	75%	5736	0.04%	5702	99%	24	0.4%	10	0.2%	6	5	4	3
Mean		5	2016	6	15.2	6%	1	99.84%	75.28%	8386	0.05%	7420	94.39%	21.06	0.29%	945	5.32%	619.33	233.22	28	9
Total		87	36296	103.25	273.8	N/A	N/A	99.84%	76%	150951	1%	133568	88%	379	0.25%	17004	11%	11148	4198	281	90

Table 3.2: Taxonomic breakdown of normalized SSU reads. Abundance of different taxonomic groups are arranged by site, organized to reflect lower and higher taxonomic resolution. Percent totals for each group are listed at the bottom of each column. Percent of total abundance for each site are listed in the far-right column. ϕ indicates sites with visible organic matter; * indicates sites at >1000m elevation. Sites are ordered from higher to lower moisture content, top to bottom.

	Amorphea											**Diaphoretickes**													*Incertae Sedis*			
	Amoebozoa			**Opisthokonta**								**Archaeplastida**				**SAR**									**Discoba**			
				Fungi			**Metazoa**					*Streptophyta*				**Stramenopiles**			**Alveolata**		**Rhizaria**							
	Discosea	*Evosea*	*Tubulinea*	*Ascomycota*	*Basidiomycota*	*Other*	*Tardigrada*	*Nematoda*	*Rotifera*	*Arthropoda*	*Other Amorphea*	*Bryopsida*	*Other*	*Chlorophyta*	*Rhodophyta*	*Bacillariophyta*	*Ochrophyta*	*Other*	*Ciliophora*	*Other*	*Cercozoa*	*Endomyxa*	*Foraminifera*	*Other Diaphoretickes*	*Euglenozoa*	*Heterolobosea*	*Other Incertae Sedis*	**Site Totals**
CS ϕ	55%	36%	61%	24%	0%	35%	25%	26%	35%	0%	100%	42%	57%	6%	0%	85%	35%	77%	65%	36%	0%	75%	0%	100%	11%	35%	0%	21.1%
MGM	0%	0%	0%	2%	0%	5%	6%	11%	0%	0%	0%	8%	6%	8%	0%	0%	0%	0%	2%	0%	0%	0%	0%	0%	0%	0%	0%	3.1%
MS	11%	0%	0%	0%	0%	12%	9%	17%	4%	0%	0%	0%	0%	9%	100%	11%	5%	11%	1%	0%	0%	0%	0%	0%	0%	0%	0%	0.8%
MG	10%	10%	11%	11%	0%	2%	1%	12%	2%	0%	0%	3%	3%	6%	0%	0%	7%	0%	4%	32%	0%	0%	100%	0%	0%	0%	0%	1.5%
CN ϕ	16%	43%	8%	56%	0%	1%	1%	21%	46%	100%	0%	37%	27%	51%	0%	0%	41%	0%	4%	12%	0%	0%	0%	0%	43%	0%	100%	15.1%
HH ϕ	8%	0%	0%	4%	99%	3%	49%	9%	7%	0%	0%	10%	6%	3%	0%	0%	8%	0%	3%	0%	0%	25%	0%	0%	11%	18%	0%	56.4%
GV	0%	0%	0%	2%	0%	0%	0%	0%	0%	0%	0%	0%	0%	3%	0%	0%	0%	0%	2%	0%	0%	0%	0%	0%	0%	0%	0%	0.3%
TG*	0%	0%	0%	0%	0%	29%	0%	0%	2%	0%	0%	0%	0%	2%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	27%	0%	0%	0.4%
MV	0%	0%	5%	0%	0%	6%	1%	0%	0%	0%	0%	0%	0%	0%	0%	0%	2%	0%	3%	0%	0%	0%	0%	0%	0%	23%	0%	0.1%
Marr	0%	12%	7%	0%	0%	7%	0%	0%	3%	0%	0%	0%	0%	7%	0%	3%	3%	13%	8%	20%	0%	0%	0%	0%	0%	0%	0%	0.5%
GR*	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0.1%
WrB	0%	0%	8%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	1%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0.0%
ESLF	0%	0%	0%	0%	0%	0%	0%	5%	0%	0%	0%	0%	0%	1%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0.1%
NSLH	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	1%	0%	0%	0%	0%	0%	0%	24%	0%	0.1%
UV*	0%	0%	0%	0%	0%	0%	0%	0%	1%	0%	0%	0%	0%	3%	0%	0%	0%	0%	2%	0%	0%	0%	0%	0%	0%	0%	0%	0.1%
BV*	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	9%	0%	0%	0.1%
WV*	0%	0%	0%	0%	0%	0%	8%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	3%	0%	0%	0%	0%	0%	0%	0%	0%	0.2%
WrU*	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	2%	0%	0%	0%	0%	0%	0%	0%	0%	0.1%
	0.1%	0.1%	0.2%	0.8%	52.0%	0.6%	1.7%	0.3%	2.3%	0.01%	0.03%	22.3%	14.2%	1.3%	0.01%	0.4%	0.5%	0.1%	0.9%	0.1%	1.7%	0.03%	0.01%	0.02%	0.1%	0.05%	0.01%	100%
	0.4%			**53%**			**4%**				**0.03%**	**38%**				**1%**			**1%**		**2%**			**0.02%**	**0.1%**		**0.01%**	100%

Table 3.3: Taxonomic assignment table (genus). Arrangement organizes heterotrophic protists from each sample according to SSU best hit assignment using the PR2 database. Taxonomic statements have been updated where possible. Unidentified reads are listed as unidentified members of the nearest level at which an identification was made (e.g. Order). Trophic levels are indicated: mixotrophs (*), parasites (**), uncertain (***), and consumers (not marked).

		Phylum	Class	Order	Family	Genus
Diaphoriticks	*Incertae sedis*	Telonemia				Unid. Telonemia
	Unresolved		Cryptista	Cryptophyceae	Kathablepharidacea	*Hatena**
	Alveolata	Apicomplexa	Unresolved	Gregarines		Unid. Gregarine**
		Dinoflagellata	Dinophyceae			Unid. Dinophyceae**
		Unresolved		Colpodellida		Unid. Colpodellida***
		Ciliophora	Colpodea	Bursariomorphida	Bryometopidae	*Bryometopus*
					Bursariidae	*Bursaria*
				Colpodida	Colpodidae	*Colpoda*
						Unid. Colpodidae
					Grossglockneriidae	*Pseudoplatyophrya*
					Hausmanniellidae	*Bresslauides*
				Cyrtolophosidida	Cyrtolophosididae	*Aristerostoma*
				Platyophryida	Woodruffiidae	*Etoschophrya*
					Unresolved	*Kuklikophrya*
			Litostomatea	Haptoria	Enchelyidae	*Enchelys*
					Lacrymariidae	*Phialina*
			Oligohymenophorea	Peniculia		Unid. Peniculia
					Stokesiidae	*Stokesia*
				Peritrichia	Astylozoidae	*Astylozoon*
					Opisthonectidae	*Opisthonecta*
				Scuticociliatia		Unid. Scuticociliata***
				Sessilida	Vorticellidae	*Vorticella*
						Unid. Vorticellidae
						Vorticellides
			Phyllopharyngea	Cyrtophoria	Chilodonellidae	Unid. Chilodonellidae***
						Pseudochilodonopsis
						Trithigmostoma
				Suctoria	Heliophryidae	*Heliophrya*
						Unid. Suctoria
					Tokophryidae	*Tokophrya*
			Spirotrichea	Euplotia	Euplotidae	*Euplotes*
				Hypotrichia	Cladotrichidae	*Engelmanniella*
						Unid. Hypotrichia

					Family	Genus
					Oxytrichidae	*Gonostomum*
						Oxytricha
						Unid. Oxytricha
						Stichotrichia

		Phylum	Class	Order	Family	Genus
Diaphoriticke s	Rhizaria	Endomyxa		Vampyrellida	sm27-lineage	Unid. sm27-lineage
						Unid. Vampyrellida
					Vampyrellidae	*Vampyrella*
		Cercozoa	Filosa-Imbricatea			Unid. Filosa-Imbricatea***
			Imbricatea	Marimonadida	Marimonadida	Unid. Marimonadida***
			Thecofilosea	Cryomonadida	Rhogostoma-lineage	Unid. Rhogostoma-lineage
			Unresolved	Cercomonadida	Cercomonadidae	*Cercomonas*
				Paracercomonadida	Paracercomonadidae	*Metabolomonas*
				Glissomonadida	Allapsidae	*Allantion*
						Unid. Allapsidae
						Group-Te
						Teretomonas
					Bodomorphidae	*Bodomorpha*
						Unid. Glissomonadida
					Sandonidae	*Flectomonas*
						Neoheteromita
						Sandona
						Unid. Sandonidae
		Foraminifera	Monothalamids	Monothalamids (Group 4)		Unid. Monothalamids (Group 4)***
	Stramenopiles	Ochrophyta	Chrysophyceae			Unid. Chryosphyceae (Clade C)***
						Unid. Chryosphyceae (Clade D)***
						Unid. Chryosphyceae (Clade E)***
				Ochromonadales	Unresolved	*Pedospumella*
					Chromuliniaceae	*Spumella*
		Opalozoa	MAST	MAST-12	MAST-12C	MAST-12C***
		Unresolved	Labyrinthulea	Thraustochytrida	Thraustochytriaceae	Unid. Thraustochytriaceae**
			Oomycota			Unid. Oomycota***
			Peronosporomycetes	Peronosporales	Unresolved	*Pythium***
			Bicoecea	Pseudodendromonadales		Unid. Pseudodendromonadales
			Labyrinthulomycetes	Amphitremida	Amphitraemidae	*Amphitrema**

		Phylum	Class	Order	Family	Genus
Amo rphe	Amoeb ozoa	Evosea	Variosea	Unresolved	Flamellidae	Unid. Flamella-lineage
				Cavosteliida	Unresolved	*Schizoplasmodiopsis*
				Fractovitellida	Schizoplasmodiidae	*Ceratiomyxella*

						Schizoplasmodium
					Soliformoviidae	*Grellamoeba*
						Unid. Variosea***
		Discosea		Vannellida	Vannellidae	*Vannella*
				Thecamoebida	Thecamoebidae	*Stenamoeba*
				Centramoebida	Acanthamoebidae	*Acanthamoeba*
						Protacanthamoeba
		Tubulinea		Himatismenida	Cochliopodiidae	*Cochliopodium*
				Unresolved	LKM74-lineage	Unidentifed LKM74-lineage
				Arcellinida	Difflugiidae	*Difflugia*
					Difflugiidae	*Arcella*
					Micrchlamyiidae	*Spumochlamys*
				Echinamoebida	Vermamoebidae	*Vermamoeba*
				Euamoebida	LOS7N/I-lineage	Unid. LOS7N/I-lineage
						Ptolemeba
				Leptomyxida	Flabellulidae	*Paraflabellula*
				Nolandida	Nolandellidae	Unid. Nolandellidae
	Obazoa	Holozoa	Choanoflagellata	Craspedida	Salpingoecidae	*Codosiga*
			Ichthyosporea	Dermocystida	Dermocystida_X	*Dermocystidium***
		Unresolved			Apusomonadida	*Amastigomonas*
Incertae sedis	Discoba	Euglenozoa	Diplonemea			Unid. Diplonemea
				Unresolved	Diplonemidae	*Diplonema*
			Euglenida	Unresolved		*Keelungia*
				Petalomonadales		Unid. Petalomonadales
					Unresolved	*Petalomonas*
			Kinetoplastea			Unid. Kinetoplastea
				Neobodonida	Unresolved	*Neobodo*
		Heterolobosea	Tetramitia	Unresolved	Neovahlkampfiidae	*Neovahlkampfia*
				Unresolved	Acrasidae	*Allovahlkampfia*
					Vahlkampfiidae	Unid. Vahlkampfiidae
	Unresolved		Ancyromonadida	Unresolved		*Fabomonas*

Figure 3.1: Map of study area. The McMurdo Dry Valleys are located at roughly S 77° E 162° in Southern Victoria Land and open towards the Ross Sea to the East. Samples were collected to best represent the variety of soil habitats that exist within and among the different valleys.

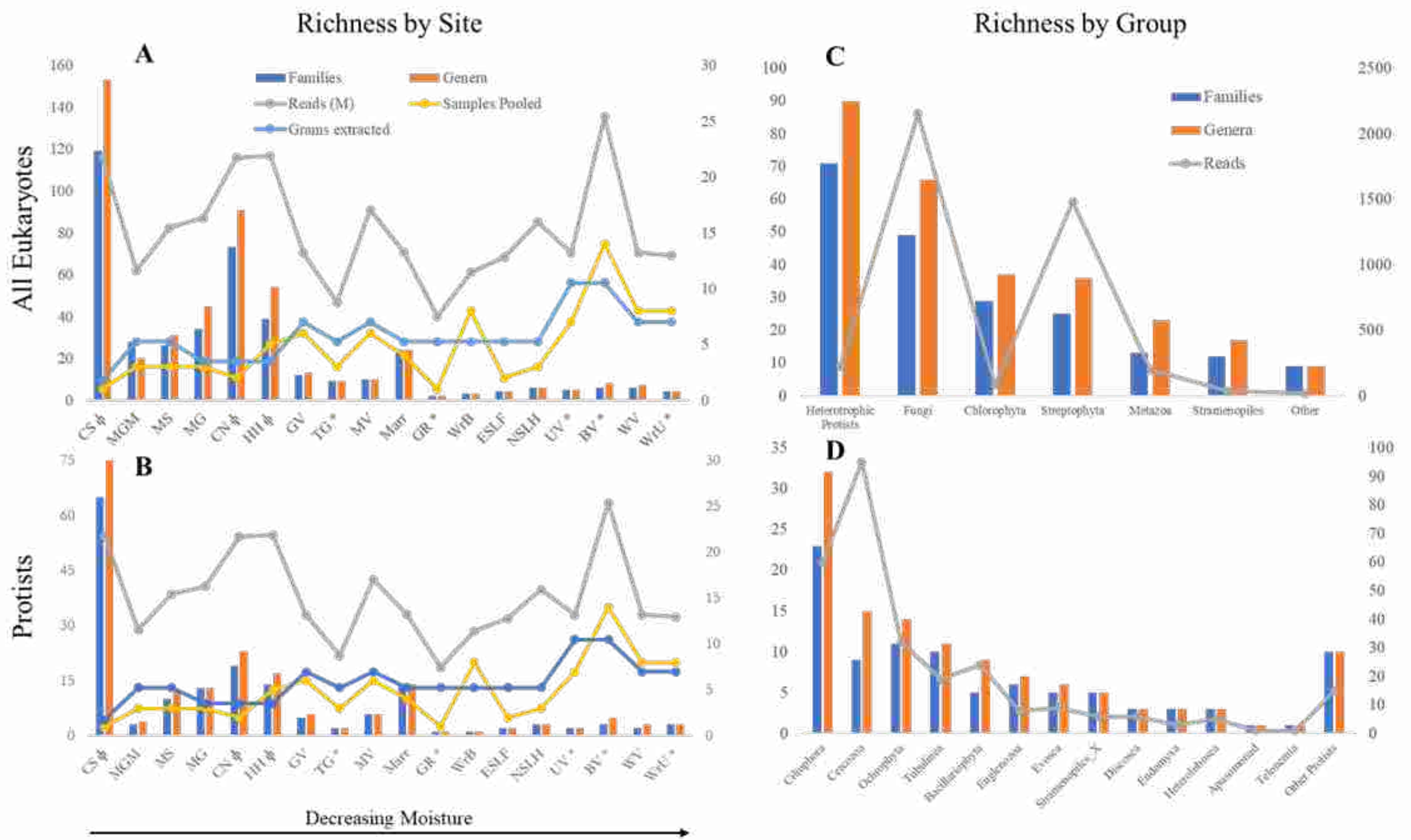

Figure 3.2: Richness by site and group. Alpha diversity by family and genus for all eukaryotes (A) and all protists (B) by site. Protists exclude metazoa, fungi, and archaeplastida (chlorophyta, streptophyta, and rhodophyta). Counts were made using read abundance data normalized to account for read depth. Alpha diversity is also plotted by generalized functional groups for all eukaryotes (C) and for protist phyla (D). Heterotrophic protists (C) include Ciliophora, Colpodellidae, Cercozoa, Amoebozoa, Euglenozoa, Heterolobosea, Cryptista, Vampyrellida, Choanaflagellata, Ancyromonadida, Apusomonadida, Telonemia, some Stramenopiles and some Ochrophyta. Whole shotgun metagenome read count (A,B) or total processed read count by group (C, D) is plotted (gray line). Number of samples pooled (yellow) and total grams of soil extracted (blue) is also plotted for each site (A,B). Reads, samples and grams soil all correspond to the right y-axis on their respective graph. Sites in AB are ordered from higher to lower moisture content, left to right.

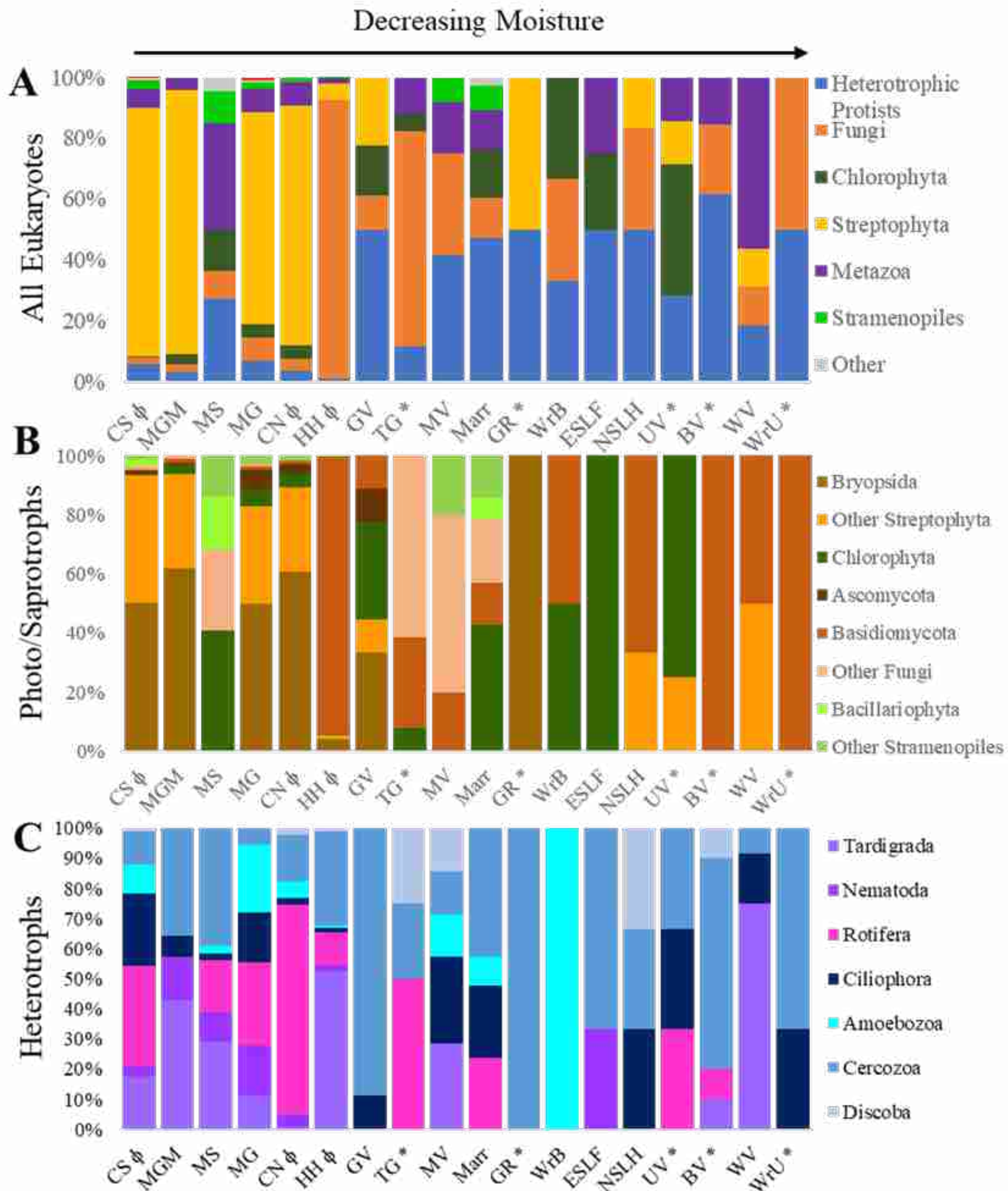

Figure 3.3: Relative abundance of normalized reads by site. Abundance graphs for Phototrophs/Saprotophs (B) and Heterotrophs (C) use the shades of the colors their respective groups were assigned in the All Eukaryotes graph (A). Metazoa are in purple hues, select protist groups in blue hues (C), Streptophyta in yellow hues, and fungi in orange hues (B). Chlorophyta is the same color in both A and B. Sites are ordered consistently in each graph from higher to lower moisture content, left to right.

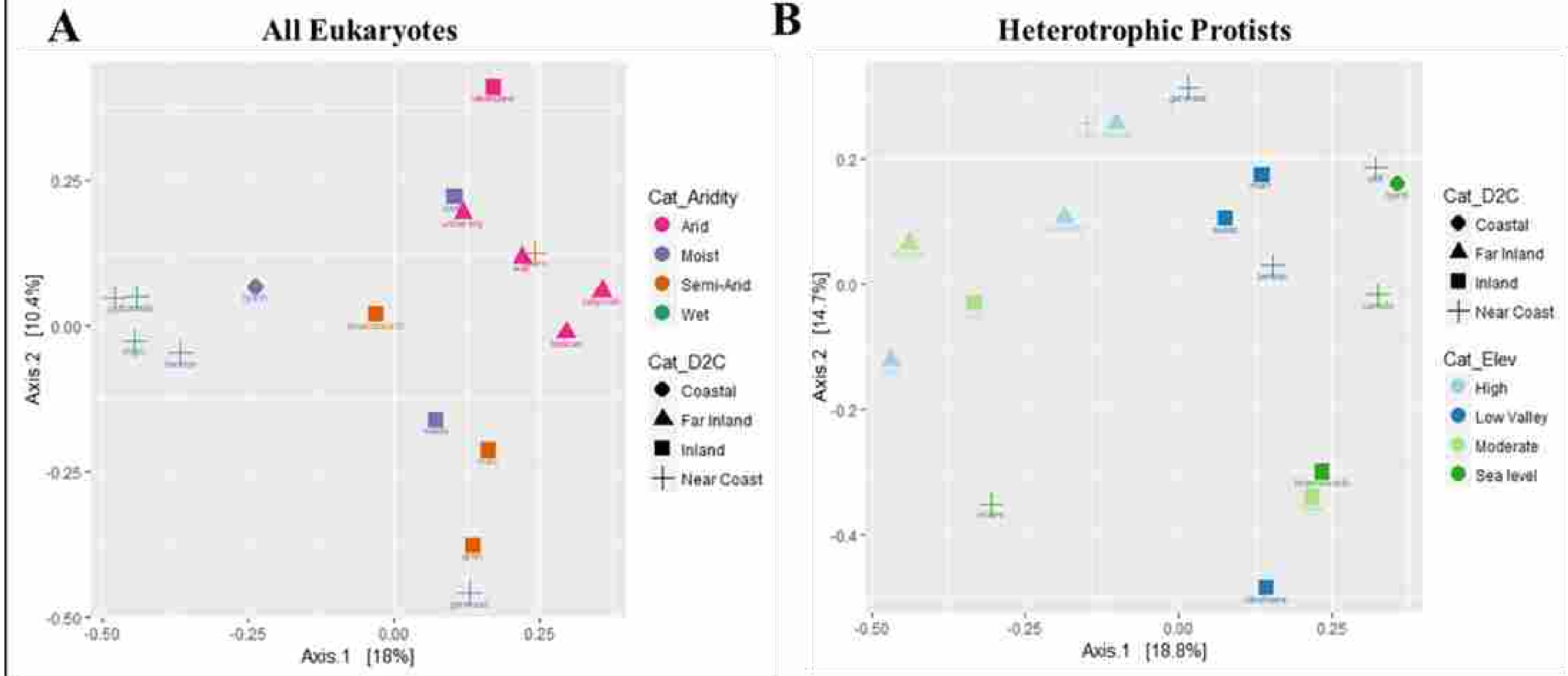

Figure 3.4: Site community composition comparisons. pCOAs were performed with Bray-Curtis distance matrices. Categories for aridity, elevation and distance to coast (D2C) were assigned using continuous environmental data.

Supplementals

Sequencing and post-processing

Our sequencing run produced 273.8 million reads, 99.84% of which passed our quality control standards. The eukaryote hmmer profile from barrnap recovered an average of 945 putative eukaryotic reads per sample, although CN, CS and HH were outliers with 2,704, 3,296, and 10,218 reads, respectively. Excluding HH, CS, and CN, there was an average of 52 SSU reads per site. Roughly 37% of eukaryotic SSU reads were less than 200bp: an average of 18 reads shorter than 200bp were removed from all sites except CN, CS, and HH, which had an average of 1,864 reads removed. An average of 258 reads per sample (after filtering for sequence length) were similar to the eukaryote *Berthella martensi*, a gastropod from the Indian ocean, by PR2. Additional blasting of these sequences using the NCBI database revealed them to be bacterial in origin and they were subsequently removed from our analysis. In addition, a total of 31 reads that were less than 80% similar to their associated reference sequence in the PR2 database were removed from the analysis as most of them blasted to archaea in the NCBI database. Of the remaining sequences, 496 reads or 4% of the total were <93% similar to their associated reference sequence in the PR2 database. Rarefaction and species accumulation curves show that virtually all sites were not sequenced to sufficient depth, and both diversity indices show high variability in species composition across sites (Table S3). We lost 228 of 287 OTUs (81%) when we performed sample rarefaction to make direct comparisons between site richness.

150,951 reads were identified as belonging to either the 16S or 18S rRNA gene target, or an average of 0.05% of reads that passed quality control. On average, 94.39% of these reads were bacterial and 5.32% were eukaryotic. Archaeal sequences were very rare, with an average of 0.28% across all sites with the highest proportion (1.5%, 125 reads) from Benson glacier/Flatiron (MG).

Some SSU reads extracted by hmmer were assigned to *Bipolaris sorokiniana* and *Berthella martensi* by the PR2 database but were subsequently determined to bacteria in origin.

Supp. Figure 3.1: Rarefaction curves for entire eukaryotic communities. A: Rarefaction on all sites, CS, CN, HH are labeled. B: Rarefaction of dataset where CS, CN and HH have been removed to better see the trends for the other sites.

Supp. Table 3.1: Averages for environmental variables for all sites. Includes categorical variables for Aridity (based on % moisture), Elevation, and Distance to Coast (Dist-to-Coast).

Site Name	Samples	% Moisture	pH	EC (dS/m)	% Clay	ppm NO3-N	ppm Total N	ppm Total C	C:N Ratio	ppm Total P	Elevation (m)	Dist-to-Coast (km)	Aspect (degrees)	Category: Aridity	Category: Elevation	Category: Dist-to-Coast
BV	14	0.984449	7.56	4.08	1.44	83.28	347.86	774.29	2.47	345.43	2023.33	74.84	145.36	Arid	High	Far Inland
CN	2	6.374647	6.67	4.08	8.54	2.53	640.00	3910.00	5.74	368.98	209.92	8.25	308.00	Moist	Sea level	Near Coast
CS	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	54.81	11.182	317.00	Wet	Sea level	Near Coast
F6	2	1.82763	8.53	1.11	2.00	1.30	465.00	1630.00	3.52	958.81	17.91	5.86	265.00	Arid	Sea level	Near Coast
GR	1	3.946002	7.56	1.70	0.72	3.07	300.00	1240.00	4.13	222.28	990.71	33.63	209.00	Semi-Arid	Moderate	Inland
GV	6	5.96875	8.24	0.70	0.62	2.38	435.00	9323.33	25.63	616.67	625.86	10.91	215.00	Moist	Low Valley	Near Coast
HH	5	5.99024	7.74	1.698	1.864	2.70	534	4012	6.87	971	119	1	144	Moist	Sea level	Coastal
Marr	4	4.068635	7.92	13.44	3.00	19.83	505.00	4522.50	7.68	410.99	731.62	22.94	137.75	Semi-Arid	Low Valley	Inland
MG	3	6.637262	7.31	5.70	4.52	2.99	666.67	4640.00	6.44	192.68	495.06	7.81	228.67	Moist	Low Valley	Near Coast
MGM	3	10.17755	7.44	0.34	7.45	2.32	450.00	1500.00	3.31	236.45	767.41	11.49	324.00	Wet	Moderate	Near Coast
MS	3	6.934245	7.71	0.32	3.02	1.30	416.67	2000.00	4.71	165.34	634.57	15.51	239.67	Moist	Low Valley	Inland
MV	6	4.171391	8.05	0.44	0.63	1.53	368.33	4506.67	12.26	558.20	214.88	9.84	272.00	Semi-Arid	Sea level	Near Coast
NSLH	3	1.682595	8.10	2.00	4.24	1.37	193.33	2021.67	12.11	437.98	304.10	15.39	243.00	Arid	Low Valley	Inland
TG	3	5.627354	7.76	0.22	1.17	1.62	293.33	596.67	2.13	188.07	1015.82	42.71	119.33	Semi-Arid	Moderate	Inland
UV	7	1.111279	7.22	3.29	5.42	119.33	465.71	572.86	1.40	95.82	1689.94	72.33	210.71	Arid	High	Far Inland
WrB	8	2.918142	8.00	10.48	3.72	50.06	391.25	1641.25	3.88	584.09	247.75	21.69	7.00	Semi-Arid	Sea level	Inland
WrU	8	0.695266	7.88	0.28	0.73	1.31	316.25	632.50	2.01	120.70	1050.11	61.80	169.00	Arid	Moderate	Far Inland
WV	8	0.715429	7.54	2.15	2.03	25.18	320.00	615.00	1.97	218.11	1542.60	65.97	178.38	Arid	High	Far Inland

Supp. Table 3.2: PERMANOVA output table. Parameters with significant p-values are in bold text. Results for "All Eukaryotes" are on left, results for "Heterotrophic protists" are on right.

All Eukaryotes

	Df	Sums Of Sqs	Mean Sqs	F. Model	R2	Pr(>F)
Moisture	**1**	**0.6329**	**0.63294**	**1.5031**	**0.09108**	**0.046 ***
Residuals	15	6.3162	0.42108	0.90892		
pH	**1**	**0.5764**	**0.57642**	**1.3568**	**0.08295**	**0.002 ****
Residuals	15	6.3727	0.42484	0.91705		
EC	1	0.4092	0.40917	0.93847	0.05888	0.585
Residuals	15	6.5399	0.43599	0.94112		
% Clay	1	0.5946	0.59455	1.4035	0.08556	0.061 .
Residuals	15	6.3545	0.42364	0.91444		
NO3-N (ppm)	1	0.4931	0.49307	1.1456	0.07095	0.197
Residuals	15	6.456	0.4304	0.92905		
Total N (ppm)	1	0.3782	0.37823	0.86343	0.05443	0.726
Residuals	15	6.5709	0.43806	0.94557		
Total C (ppm)	1	0.4061	0.40608	0.93094	0.05844	0.612
Residuals	15	6.543	0.4362	0.94156		
C:N Ratio	1	0.3919	0.39187	0.89642	0.05639	0.7
Residuals	15	6.5572	0.43715	0.94361		
Total P (ppm)	1	0.4386	0.43864	1.0106	0.06312	0.436
Residuals	15	6.5105	0.43403	0.93688		
Elevation (m)	**1**	**0.6266**	**0.62657**	**1.4865**	**0.09017**	**0.032 ***
Residuals	15	6.3225	0.4215	0.90983		
Dist-to-Coast (km)	**1**	**0.7553**	**0.75528**	**1.8291**	**0.10869**	**0.006 ****
Residuals	15	6.1938	0.41292	0.89131		
Aspect (degrees)	1	0.6892	0.68918	1.6514	0.09918	0.01 **
Residuals	15	6.2599	0.41733	0.90082		

Heterotrophic Protists

	Df	Sums Of Sqs	Mean Sqs	F. Model	R2	Pr(>F)
Moisture	1	0.4426	0.44263	1.126	0.06982	0.314
Residuals	15	5.8966	0.39311	0.93018		
pH	1	0.4516	0.45163	1.1506	0.07124	0.293
Residuals	15	5.8876	0.39251	0.92876		
EC	1	0.4538	0.45384	1.1567	0.07159	0.291
Residuals	15	5.8854	0.39236	0.92841		
% Clay	1	0.3027	0.30274	0.75228	0.04776	0.795
Residuals	15	6.0365	0.40243	0.95224		
NO3-N (ppm)	1	0.5128	0.51276	1.3201	0.08089	0.15
Residuals	15	5.8265	0.38843	0.91911		
Total N (ppm)	1	0.4414	0.44143	1.1227	0.06963	0.327
Residuals	15	5.8978	0.39319	0.93037		
Total C (ppm)	1	0.3764	0.37645	0.94699	0.05938	0.539
Residuals	15	5.9628	0.39752	0.94062		
C:N Ratio	1	0.3062	0.30624	0.7614	0.04831	0.788
Residuals	15	6.033	0.4022	0.95169		
Total P (ppm)	1	0.4972	0.49718	1.2765	0.07843	0.191
Residuals	15	5.8421	0.38947	0.92157		
Elevation (m)	**1**	**0.7266**	**0.72664**	**1.942**	**0.11463**	**0.005 ****
Residuals	15	5.6126	0.37417	0.88537		
Dist-to-Coast (km)	**1**	**0.7229**	**0.72287**	**1.9306**	**0.11403**	**0.009 ****
Residuals	15	5.6164	0.37443	0.88597		
Aspect (degrees)	1	0.4779	0.47794	1.2231	0.07539	0.214
Residuals	15	5.8613	0.39075	0.92461		

Chapter 4

Contributions of Heterotrophic protists to a soil food web in a model polar desert ecosystem: Evidence that biotic factors matter

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Abstract

Heterotrophic protists are a group of mostly unicellular eukaryotic microorganisms that occur in soils worldwide and play important roles in nutrient cycling and community structuring. Little is known of the specific ways in which heterotrophic protists interact with and contribute to the functioning of soil ecosystems, owing partially to the usually high species diversity of soils. The McMurdo Dry Valleys of Antarctica offer a unique opportunity to investigate these interactions because the extremeness of the physical environment creates a strong filter on biodiversity. Recent research indicates this system is structured more strongly by abiotic than biotic drivers, however heterotrophic soil protists have not been considered in these investigations. To better understand the degree to which heterotrophic protists play a role in structuring communities in these valleys, we used pair-wise co-occurrence tests, network analysis, and variation partitioning on 18S and 16S sequence data from 18 shotgun metagenomes. We found that our abiotic factors did not best explain the distribution of heterotrophic soil protists in the MDV, that most co-occurrence between OTUs are random but some species-specific interactions may occur, and that the phylum Cercozoa is the most interconnected with other organisms in this soil ecosystem. We also found no evidence for competition between consumers of the MDV soil microbiome and that bacteria were the most frequently associated taxa with MDV heterotrophic soil protists. We show that prey palatability may explain heterotrophic protist associations with bacteria, and that most associations are with fast-growing, more digestible bacteria. This study is one of few *in vivo* examinations of a whole soil food web with focus on the less studied heterotrophic protists and provides a starting point for untangling species-specific interactions between potentially prominent functional members of a model soil ecosystem.

Introduction

Heterotrophic soil protists (HSPs) are a highly diverse and prevalent component of soil communities in virtually every terrestrial biome on Earth (Ramirez et al. 2014, Seppey et al. 2017, Geisen et al. 2018). Recently, there has been an increased focus on understanding the diversity and function of these key soil organisms (Geisen et al. 2017, Geisen et al. 2018). HSPs are major grazers of bacteria in soil and promote bacterial diversity and growth as well as mobilize nutrients through soil food webs (Crotty et al. 2012, Saleem et al. 2012, Wilkinson et al. 2012). HSPs also regulate other eukaryotic grazers, including fungi, nematodes, and other HSPs via predation and competition for prey (Geisen 2016, Thakur and Geisen 2019). However, soil protist research is still nascent and many questions remain concerning their specific interactions with other species in soil ecosystems, and how these interactions contribute to nutrient cycling and overall ecosystem functioning (Geisen et al. 2017). For example, it is unclear what factors, both abiotic and biotic, contribute to the structuring of protist communities, how dominant and widely distributed HSP groups interact with each other and other bacterivores in the soil environment, and what controls HSP grazing preference in natural soil ecosystems (Geisen et al. 2017). Soils are one of the most biodiverse biomes on Earth (Wu et al. 2009, Decaëns 2010) and the complexity that results from this high species richness complicates efforts to disentangle biotic relationships (Bardgett 2002, Bardgett and van der Putten 2014). This makes studying ecological functions of HSPs in natural systems very challenging and most research is done in the laboratory with easily cultured HSPs and artificially small communities (Trap et al. 2016).

Soil ecosystems with highly reduced biodiversity, like the ice-free McMurdo Dry Valleys (MDV) of Antarctica, can be a useful tool for investigating the relationships soil protists have

with other soil biota. Extreme cold, ultraoligotrophic soils, short growing seasons, frequent freeze-thaw cycles, low soil moisture, basic pH and high soil salinity all contribute to an ecosystem devoid of all vascular plants and most multicellular eukaryotes (Adams et al. 2006, Barrett et al. 2006a, Wall 2007). Nematodes, rotifers and tardigrades comprise the highest trophic level at most sites (Adams et al. 2006), and even bacterial and fungal diversity are low relative to soils at lower latitudes (Cary et al. 2010, Fierer 2012). This reduced diversity leads to fewer interactions overall and simplifies efforts to characterize and relate this model ecosystem to soil food webs generally.

Indeed, the MDVs are so extreme that they are thought to be structured primarily by abiotic drivers (Hogg et al. 2006, Cary et al. 2010, Sokol et al. 2013), even when considering biotic variables (Caruso et al. 2019, Lee et al. 2019). However, analyses comparing the relative roles of abiotic and biotic drivers of community structure in the MDV have never considered HSPs. In this study we investigate whether HSPs are structured more by abiotic or biotic drivers, and how protists are specifically interacting with other biota. We hypothesize that co-occurrence of specific biota better explain HSP communities than abiotic drivers since HSPs form a key link between lower and higher trophic tiers (Thakur and Geisen 2019). Furthermore, we expect that HSPs will compete with generalist metazoan grazers than with other more specialized HSPs. Moreover, we predict that specialized feeding amongst MDV bacterivorous protists will reflect preferences for fast growing, nitrogen-rich bacteria and a deterrence from slow-growing, gram positive taxa. Examining co-occurrence patterns between protists and other MDV taxa we can gain additional insights into the relative importance of abiotic and biotic factors on community structure, the nature of HSP interactions in soil food webs, and the contribution of HSPs to ecosystem functioning.

Methods

Shotgun metagenome sequencing and SSU identification

We use SSU sequences extracted from shotgun metagenomes and environmental data generated in a previous study to test distribution patterns between HSPs, other eukaryotes, and bacteria for significant patterns of co-occurrence. Details of our sampling, library preparation, quality control, and bioinformatics have been detailed previously (Thompson et al. in review). Briefly, soil samples were collected from across the MDV over the span of several field seasons (2014-2017) and pooled to form 18 representative sites. The 18 sites included a wide variety of habitats, including several with visible organic matter (e.g. moss, algae). A shotgun metagenome library was prepared for each of these sites by extracting DNA from them using the Dneasy PowerSoil Kit (Qiagen), and then all 18 together were sequenced over one and one half illumina Hi-Seq RapidRun lanes. Reads were trimmed and merged using Trimmomatic (Bolger et al. 2014) and FLASH (Magoč and Salzberg 2011), respectively. We used the Metaxa2.2 default SILVA database to extract and assign taxonomy to bacterial SSU sequences (Bengtsson-Palme et al. 2015). Sequences with reliability scores lower than 80 (1,639 sequences), with length lower than 200bp (35,407 sequences), and with percent ID lower than 80% (38 sequences) were subsequently removed. Eukaryotic SSU sequences were found with the barrnap eukaryote hmm profile using nhmmer (Eddy 2018), and blasted against the pr2 database (Guillou et al. 2013) with ncbi-blast-2.7.1+ (Camacho et al. 2009). Reads were normalized to account for differences in sequencing depth (Thompson et al. in review). We divided our resulting sequences into multiple high-level groups for subsequent analysis: Cercozoa, Ciliophora, Amoebozoa, Discoba, other heterotrophic protists, bacteria, Fungi, Metazoa, Streptophyta, Chlorophyta, and Stramenopiles. The group other heterotrophic protists, consisting of a non-monophyletic group

of protists that fell outside of our other main groups, included: heterotrophic Stramenopiles, Colpodellida, Ancyromonadida, Apusomonadida, Telonemia, Cryptista, Choanoflagellida, and Endomyxa.

Physiochemical data for each sample site except the algal mat was obtained using standard soil analytic procedures (Thompson et al. in review). Moisture, texture, pH, electrical conductivity (EC), total N, total P, total C, C:N ratio, total NO3-N, Distance to coast, elevation, and aspect. Previous studies have correlated soil moisture and pH to HSP distribution in Antarctica (Petz 1997, Bates et al. 2013) and distance to coast in the MDV is considered a strong predictor of biodiversity (Lee et al. 2019). The rest of these variables are commonly associated with structuring soil communities in these valleys (Courtright et al. 2001, Barrett et al. 2006b, Aanderud et al. 2018).

Statistical analyses

In order to investigate HSP community structure we determine if particular pairs of OTUs were aggregated, segregated or random in occurrence with the use of a presence absence matrix that included all groups in a co-occurrence analysis implemented in the package "co-occur" (Griffith et al. 2016). This package evaluates the patterns of co-occurrence through probabilistic models to determine if pairwise interactions among all groups are positive, negative or random (Veech 2013). These models are based on combinatorial analysis which calculate the probability of the observed co-occurrence of OTUs assuming a random distribution of the community (Veech 2013, 2014). We used a significance level of 5% and a threshold to exclude pairs of OTUs that were expected to share less than one site (Griffith et al. 2016). To visualize the resulting associations, we built co-occurrence networks by converting the OTU by OTU pairs

into edge lists that were used to build unweighted unipartite networks using the Igraph package (Csardi and Nepusz 2005). We only did the network analysis for our five heterotrophic protist groups of interest: Cercozoa, Ciliophora, Amoebozoa, Discoba, and other heterotrophic protists.

After our co-occurrence analysis, we used variation partitioning to quantify the pure and shared contribution of abiotic and biotic factors in explaining the variation in the various protist groups at each sampled site (Peres-Neto et al. 2006, Borcard et al. 2018). We conducted a partial redundancy analysis (pRDA) using our normalized OTU abundance matrices for each protist group as the response. Each pRDA also included a biotic component and an abiotic component as explanatory variables. For the abiotic component we used all the soil physiochemical variables indicated above. However, to avoid collinearity among our abiotic variables we ran all pairwise correlations and excluded a variable from our analysis if it had a correlation above 0.70 (the variables "C:N ratio" and "Distance to Coast" were excluded in this way). We also checked the variables inflation factor (VIF) after pRDA and all abiotic variables were acceptable (below 10; Borcard et al. (2018)). We built a biotic component by running a partial least square regression between the presence absence matrix of the OTUs for each of the ten individual biotic groups (Ciliophora, Cercozoa, Amoebozoa, Discoba, Chlorophyta, Streptophyta, Bacteria, Fungi, Metazoa, Stramenopiles, and Other Heterotrophic Protists) plus an eleventh group consisting of all ten groups combined (All Biota) with the four HSP groups of interest. For each partial least square regression the response was always the HSP group as we were interested in explaining the effect of other groups on HSP. We retained either the first two components or those that accounted for more than 50% of the co-variation among the matrices for each of the pRDA (Wold and Eriksson 1995, Trivellone et al. 2017). The pRDA allows the total variation of the response variable (our five heterotrophic protist groups) to be partitioned into fractions that

correspond to the pure abiotic, pure biotic, shared fraction and unexplained variation. The variation explained by each fraction is reported as the adjusted coefficient of multiple determination (R^2_{adj}) to prevent inflation of R^2 values (Peres-Neto et al. 2006). The significance of each source of variation was tested trough a permutation (999 permutations) using the package vegan (Oksanen et al. 2016). All analyses were run in the R environment, unless otherwise specified (R Core Development Team 2016).

Results

Biotic vs. abiotic drivers

We used variation partitioning to determine the relative contribution of abiotic variables and co-occurrence with other biota to explain the distribution of our four HSP groups of interest (Ciliophora, Cercozoa, Amoebozoa, and Discoba). When comparing the influence of our measured abiotic variables against all biota combined, we found that the biotic component better explained the observed distribution than abiotic factors for all four of our HSP groups (Figure 1). However, this combined biotic component alone was statistically significant for only one group consisting of a single taxon: Discoba ($p = 0.009$). Instead, the influence of the biotic component and the shared component together was statistically significant in all HSP groups ($p = 0.001$ for all), and the combined influence of the biotic, shared, and abiotic components were significant in Ciliophora and Discoba ($p = 0.023$ and 0.014 respectively). The abiotic alone was never a significant explanation of Ciliophora, Cercozoa, Amoebozoa, or Discoba when considering all biota in the biotic component (Ciliophora: $p = 0.299$; Cercozoa: $p = 0.588$; Amoebozoa: $p = 0.905$; Discoba: $p = 0.395$). Even when considering individual group subdivisions as the biotic

explanatory variable, only once was abiotic alone a statistically significant explanation (Chlorophyta for Amoebozoa, $p = 0.041$) out of 40 cases (4 response groups and 10 explanatory biotic groups) (Table S1). At this higher resolution, the biotic and shared components were the most frequently significant (in 29 out of 40 combinations), while the influence of the abiotic, shared, and biotic components together was significant in 14 cases and the biotic alone was statistically significant in 15 cases (Table S1). Overall, the group All Biota accounted for >50% of the distribution patterns of each heterotrophic group (Amoebozoa: $R^2_{adj} = 0.5134$; Cercozoa: $R^2_{adj} = 0.7381$; Ciliophora: $R^2_{adj} = 0.6081$; Discoba: $R^2_{adj} = 0.9895$).

Only the biotic subdivisions Bacteria, Fungi and other heterotrophic protists were significant in explaining the occurrence of the response groups in almost all cases, but no one group monopolized explaining the distribution of heterotrophic protist groups in our samples (Table S1). Amoebozoa were explained significantly by a majority of biotic subdivisions: Ciliophora ($p = 0.01$), Discoba ($p = 0.008$), Chlorophyta ($p = 0.002$), Streptophyta ($p = 0.01$), Fungi ($p = 0.005$), Metazoa ($p = 0.025$), Stramenopiles ($p = 0.05$), and Other Heterotrophic protists ($p = 0.008$). Ciliophora ($p = 0.024$), Discoba ($p = 0.017$), Fungi ($p = 0.03$) and Stramenopiles ($p = 0.027$) were significant in explaining Cercozoan distribution, only All Biota was significant in explaining Discoban distribution, and only Stramenopiles ($p = 0.035$) and Other Heterotrophic Protists ($p = 0.046$) explained Ciliophora distribution. Individual biotic groups were variable in how much HSP occurrence they explained. A large proportion of Amoebozoan distribution was explained by all biotic groups (mean and median $R^2_{adj} = 0.5135$, 0.5395) except Cercozoa, but only a few groups for Discoban taxa (mean and median $R^2_{adj} = -0.025$, 0.1488). Cercozoa (mean and median $R^2_{adj} = 0.5135$, 0.5395) was explained well ($R^2_{adj} > 0.40$) by Ciliophora, Discoba, Fungi and Stramenopiles, while only other heterotrophic protists

accounted for more than 40% of Ciliophora distribution (mean and median R^2_{adj} = 0.2237, 0.3097). Residuals accounted for almost one third of all variation for Ciliophora (R^2_{adj} = 0.300) and for Amoebozoa (R^2_{adj} = 0.267) but were much lower for Cercozoa (R^2_{adj} = 0.109) and Discoba (R^2_{adj} = 0.074).

General composition of co-occurrence types

Overall, the vast majority of co-occurrence between our OTUs are random (Figure 2). Overall, 26 of 90 HSP OTUs have non-random associations with other OTUs and the majority of non-random associations are positive (80%). Cercozoa have the most non-random associations and have the highest ratio of OTUs with non-random associations to those with only random associations (73%). Amoebozoa have the next highest ratio of OTUs with non-random associations (35%), then Ciliophora (19%), and Discoba (8%). Although positive associations are the most abundant non-random association, there are more HSP OTUs with only negative associations (13) than there are with only positive (5) or both positive and negative (8) (Figure 2). Most cercozoan and amoebozoan associations are positive while most ciliophoran associations are negative (Figure 3, 4, and 5).

Trends in network analyses

Most heterotrophic protist interactions in the MDV are with bacteria (Figures 3, 4, 5 and 6). Bacteria in the networks belong to 14 phyla, but the majority (77%) are Proteobacteria (41%), Actinobacteria (17%), Chloroflexi (11%), and Acidobacteria (8%) (Figure 7). Fourteen of 26 HSP OTUs were associated with Proteobacteria and most of these associations were positive (92%) (Figures 3, 4, 5 and 6; Table S2). Only the ciliophoran *Pseudochilodonopsis* (OTU 23) was negatively associated with Proteobacteria (Figure 4). The majority of associations between

HSPs from all groups and Actinobacteria are negative (76%), half of the associations between HSPs and Chloroflexi were negative, and most associations between HSPs and Acidobacteria were positive (77%) (Figures 3, 4, 5 and 6; Table S2). These 14 phyla consist of 74 families, of which only four show up in every network: Burkholderiaceae (Proteobacteria, OTU 337), Rhizobiaceae (Proteobacteria, OTU 501), Trueperaceae (Deinococcus-Thermus, OTU 545), and group wr0007 in the Rhodospirillales (Proteobacteria, OTU 642) (Table S2). The first two are always positively associated, but Trueperaceae (OTU 545) shows up exclusively in negative associations with Amoebozoa OTUs 43, 37, and 46 (*Stenamoeba*, *Schizoplasmodiopsis*, and *Cochliopodium*); Ciliophora OTUs 17 and 22 (unidentified Chilodonellidae and *Opisthonecta*); Cercozoa OTU 248 (*Metabolomonas*); and with Discoban OTU 134 (*Keelungia*).

Associations between protists and eukaryotic groups were fewer than associations between protists and bacteria. No cercozoan, ciliophoran, discoban, or amoebozoan had associations with an OTU from its own phylum or supergroup (Figures 3, 4, 5 and 6). No HSP group had associations with Ciliophora, even though Ciliophora and other groups co-occurred frequently (Thompson et al in review). The only HSP to HSP associations were positive and occurred between a single cercozoan (*Sandona*, OTU 257) and an Amoebozoan (*Acanthamoeba*, OTU 44), and the same cercozoan and a Stramenopile (*Spumella*, OTU 275). Streptophyte OTUs 101 and 117 (*Bryum* and *Lygodium*) show up in every HSP network analysis except for Discoba, which only has one association (Figure 6). These streptophyte OTUs are positively associated with the heterotrophic Stramenopile *Spumella* (OTU 275), two Cercozoa (*Sandona* and an unidentified Allapsidae; OTUs 257 and 250 respectively), one Ciliophoran (an unidentified Oxytrichidae, OTU 33), and an Amoebozoan (*Protacanthamoeba*, OTU 45) (Figures 3, 4, 5, and 5). The rotifer *Adineta* (OTU 224) is the most common metazoan to have any association with

HSPs, and is positively associated with a cercozoan, ciliophoran, an amoebozoan, and the Stramenopile *Spumella* (OTU 275) (Figures 3, 4, 5, and 6). *Adineta* and an unidentified rotifer (OTU 231) are the only metazoan to occur outside of the Cercozoa network. Interestingly, *Adineta* is not associated with the cercozoan *Sandona* (OTU 257) despite the fact that *Sandona* has the most associations of any HSP in our samples (Figure 3). A single nematode OTU (*Plectus*, OTU 218) was positively associated with the Cercozoa *Sandona* and an unidentified Allapsidae (OTU 250), while a tardigrade (*Macrobiotus* OTU 235) was positively associated with the Cercozoa *Sandona, Neoheteromita* (OTU 256) and *Flectomonas* (OTU 255). Only a single fungal taxon (*Sporolobomyces*, OTU 188) aggregates with any HSP, the cercozoan *Sandona* (Figure 3) (Figure 3). Most associations between HSPs and other taxa are positive, except for among the ciliates, where only one ciliate taxon has exclusively positive associations (OTU 33, an unidentified Oxytrichidae) (Figure 4). Cercozoa have proportionally fewer negative associations with bacteria than either Ciliophora or Amoebozoa, and all negative associations in all networks are with bacteria. All eukaryote to eukaryote interactions are positive. For a full list of the taxonomic identifications for each OTU in the network analyses, see Table S2.

Discussion

The MDV are an ideal ecosystem for studying the complex interactions that occur in soil, yet current knowledge suggests that abiotic factors contribute more to structuring these unique communities than biotic (Caruso et al. 2019, Lee et al. 2019). It is clear from our variation partitioning that the environment alone does not play a statistically significant role in the distribution of heterotrophic protists at higher levels of taxonomy (e.g. Ciliophora, Amoebozoa,

Cercozoa and Discoba) in the MDV. It also seems that while the biotic component alone often plays an important role in structuring these communities, it is an interaction between the biotic and shared components that is most important for explaining the distribution patterns observed in our samples.

We cannot control for the influence of abiotic factors on specific association in our networks, but we can infer from the results of our pRDA that many of the association patterns we recovered are predominately structured by biotic factors (Figure 1). Moreover, we acknowledge that while non-active microbial species can co-occur without ever interacting our methods may create an artefactual association between co-occurring but non-interacting taxa. However, we consider it likely that our relatively low sampling depth (Thompson et al in review) and the differential success in extracting DNA from encysted organisms compared to active ones (Santos et al. 2015) has recovered largely true co-occurrence patterns between functionally significant members of the MDV soil communities.

The structural complexity of soil food webs depends on the relative abundance of species-specific to generalist interactions. In one co-occurrence study done on soil protists, the proportion of random to non-random interactions was group dependent (Seppey et al. 2017), indicating a relationship between trophic specificity and randomness in co-occurrence patterns. Most associations in our study were random (Figure 2), which may be consistent with a food web model with few species-specific interactions and wide trophic generalism amongst MDV taxa. There are a few patterns that do suggest species-specific associations. A cercozoan (*Sandona sp.*), an amoebozoan (*Acanthamoeba sp*.) and a heterotrophic Stramenopile (*Spumella sp*.) are the only HSPs to have non-random interactions with other HSPs. All three share positive associations with two proteobacteria OTUs (642, and 501), and all of *Acanthamoeba's* and half

of *Spumella's* positive bacterial associations are shared with one of the other two protist OTUs. Since each of these species are known bacterivores (Grossmann et al. 2016, Adl et al. 2018), it is plausible that this represents a network of specific protists grazing specific MDV bacteria. Both *Sandona sp.* and *Spumella sp.* are positively aggregated with a moss OTU (*Bryum sp.*) suggesting that this aggregation forms a core community in high productivity soils in the MDV, which the mosses promoting suitable habitat for both the protists and their bacterial prey. The absence of association between *Acanthamoeba sp.* and this moss may be the effect of sampling bias or indication that the bacteria grazed by these three consumers are not restricted to high productivity sites and play key roles in more arid sites that dominate the MDV landscape. *Macrobiotus sp.* (Tardigrada, OTU 235) and a *Plectus* sp. (Nematoda, OTU 218), both of which are known to occur in higher productivity sites with higher moisture (Adams et al. 2006), were also associated with *Sandona sp.* and another streptophyte associated Cercozoa, an unidentified Allapsidae (OTU 250). Exploring the true nature of these associations will require studies that isolate these taxa of interest and conduct *in vitro* assessments of environmental tolerances and feeding preferences (Newsham et al. 2004, Knox et al. 2015, Majdi et al. 2019).

Predation and competition between consumers of the soil microbiome have not been well documented in context of the soil community as a whole (Thakur and Geisen 2019). Recent isotopic work in the MDV shows that *Eudorylaimus* sp. in the Dry valleys likely act as predators or perhaps scavengers (Shaw et al. 2018), and one possibility is that this nematode species is feeding on HSPs. No OTU attributed to *Eudorylaimus* sp. appeared in the networks, suggesting that if it is a predator of HSPs then it is only opportunistic. *Eudorylaimus* sp. have a limited distribution in the MDV and were rare in the original metagenome dataset (Thompson et al. in review); detecting any relationship between this potential predator and HSP consumers will

require focused study in sites where they co-occur. Another nematode to not appear in the networks was *Scottnema lindsayae*, a dominant and a key bacterivore in arid MDV sites. The absence of negative associations between *S. lindsayae* and putative arid site HSP consumers suggests bacterivore competition in dry soils is minimal. That no positive associations exist between these taxa points to a lack of overlap of suitable habitat or prey types, providing evidence instead for niche partitioning. Instead, the rotifer *Adineta* and an unidentified Rotifera OTU were present in every protist network, and were the only metazoa to aggregate with non-cercozoan protists. As their associations were always positive, this too appears to be evidence for low competition. We consider it likely that prey sources are not strongly overlapping between metazoan and HSP bacterivores, or that there is temporal displacement between HSP and metazoan grazing, and that top down predation on HSPs is facultative and occurs only in sites that support populations of potential predators (i.e. *Eudorylaimus* sp.).

As expected, most HSP associations were with bacterial taxa. The predominance of positively associated Proteobacteria in the networks suggests preferential grazing on fast-growing and easily digestible (i.e. "palatable") bacterial prey. Likewise, the negative associations with the slow-growing, less palatable phylum Actinobacteria and family Trueperaceae (phylum Deinococcus-Thermus) suggests that environments that are suitable for these bacteria are either too harsh for MDV protists or do not provide suitable habitat for preferred prey bacteria. Trueperaceae is highly desiccation-tolerant (Albuquerque et al. 2005, Ujaoney et al. 2017) and has been correlated with increasing EC in MDV soils (Feeser et al. 2018). That Trueperaceae forms no positive associations with any HSP taxon and appears to prefer highly arid, saline sites lends support to our interpretation. Only the cercozoan testate amoeba *Rhogostoma* sp. and the Amoebozoan *Protocanthamoeba* are positively associated with Actinobacterial families,

suggesting that species represented by these OTUs may be important grazers of the less productive and extreme soil habitats that dominate the MDV landscape. However, *Protacanthamoeba* is also associated with the same moss that *Sandona* sp. and *Spumella* sp. are, indicating that this Amoebozoan inhabits multiple habitat types or has specialized to eat non palatable food sources in high productivity sites to avoid competition with faster moving grazers. *Rhogostoma* sp. is unique among HSPs in the network in its broad and even distribution across sites (Thompson et al. in review) and in its apparent preference for non-proteobacterial prey. Of five positive associations with bacterial taxa, only one is with a more digestible proteobacteria and two are with Acidobacteria and one with Actinobacteria. This testate amoeba may therefore be specially adapted to arid soil environments where specialized grazing of slow-growing, less palatable prey with lower nutritional value are required adaptations. Ciliophora OTUs 23, 17, 31, and 10 (*Pseudochilodonopsis*, *Opisthonecta*, *Gonostomum*, and *Etoschophrya* respectively), were each only negatively associated with bacteria (but not the same bacteria). Among all HSP response groups, Ciliophora had the highest adjusted R^2 for its residuals (Figure 1). Our biotic data is arguably comprehensive of all abundant soil taxa excepting viruses, indicating that Ciliophora are also being driven by abiotic variables not accounted for in our study. These observations suggest that environmental filtering is stronger for Ciliophora than Cercozoa or Amoebozoa and broad and opportunistic feeding preferences for MDV ciliates.

Conclusions

Our study shows that biotic factors may be more important drivers of community structure in MDV soil food webs than previously thought. MDV HSPs appear to be structured primarily by the shared influence of biotic and an as yet unaccounted for variable. This challenges previous research that concluded abiotic factors were a more important driver of

community structure here than biotic factors. Investigating the function of individual HSP species in this ecosystem will be key to understanding the full functioning of these soil communities. Cercozoa are fundamental bacterial consumers in both productive and arid MDV soil habitats and appear to selectively graze fast-growing, palatable bacterial taxa. Future research should focus on clarifying the relative roles of abiotic and biotic factors driving the associations between Cercozoan bacterivores, their prey, and their potential competitors amongst the Amoebozoa, Ciliophora and heterotrophic Stramenopiles. Doing so will enable future researchers to conduct more comprehensive studies of the resiliency of this ecosystem to future ecological disturbances, such as anthropogenic climate change.

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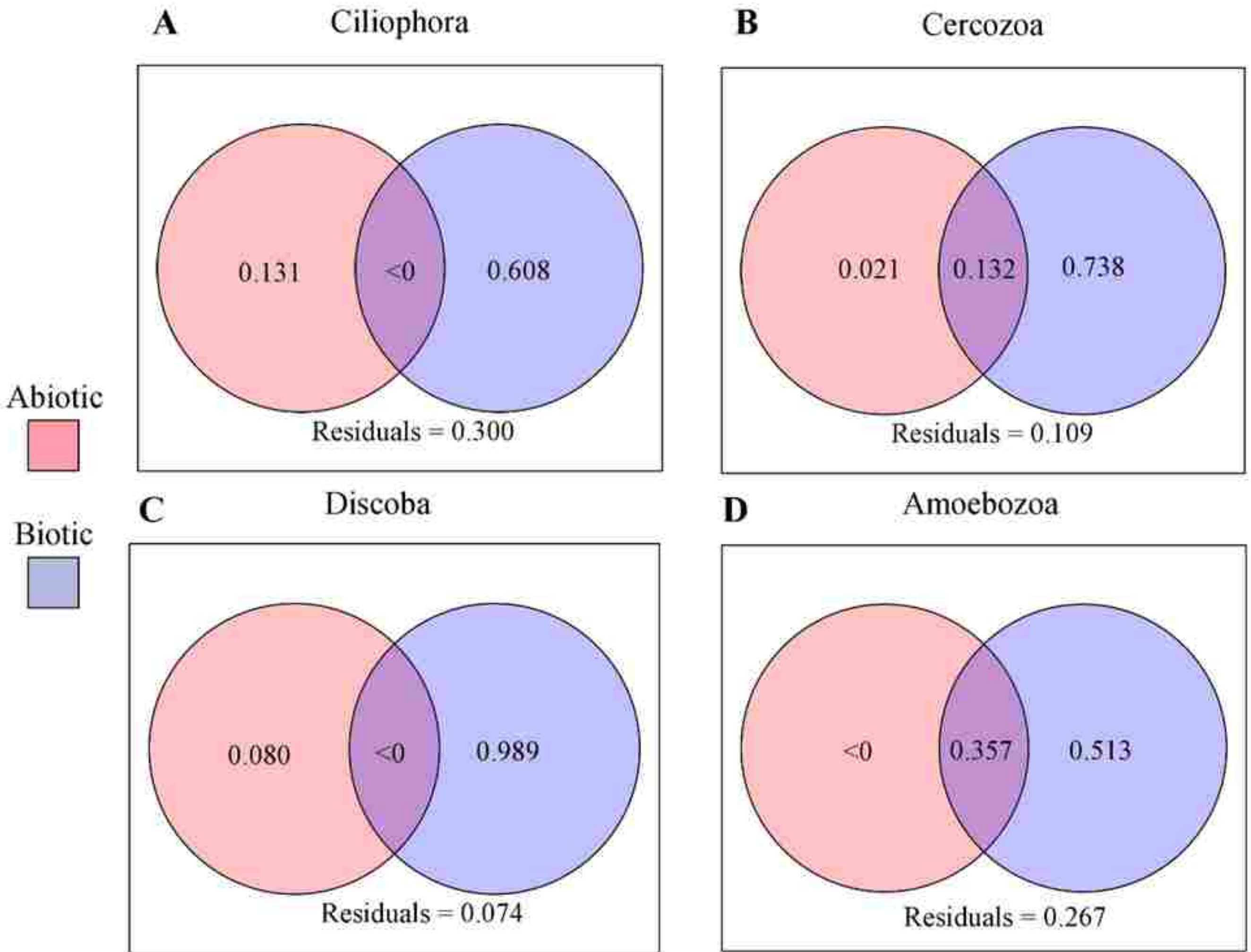

Figure 4.1: Variation Partitioning with All Biota as biotic component and environmental variables as abiotic component. Adjusted R^2 values for abiotic (left side), biotic (right side), shared, or residual proportion of variation. Four heterotrophic groups (A: Cercozoa, B: Ciliophora, C: Amoebozoa, D: Discoba) were tested against a set of environmental variables including moisture, pH, EC, total P, N, C, NO3-N, C:N ration, % clay, elevation, distance to coast, and aspect. Biotic component includes all eukaryotic and bacteria taxa in study.

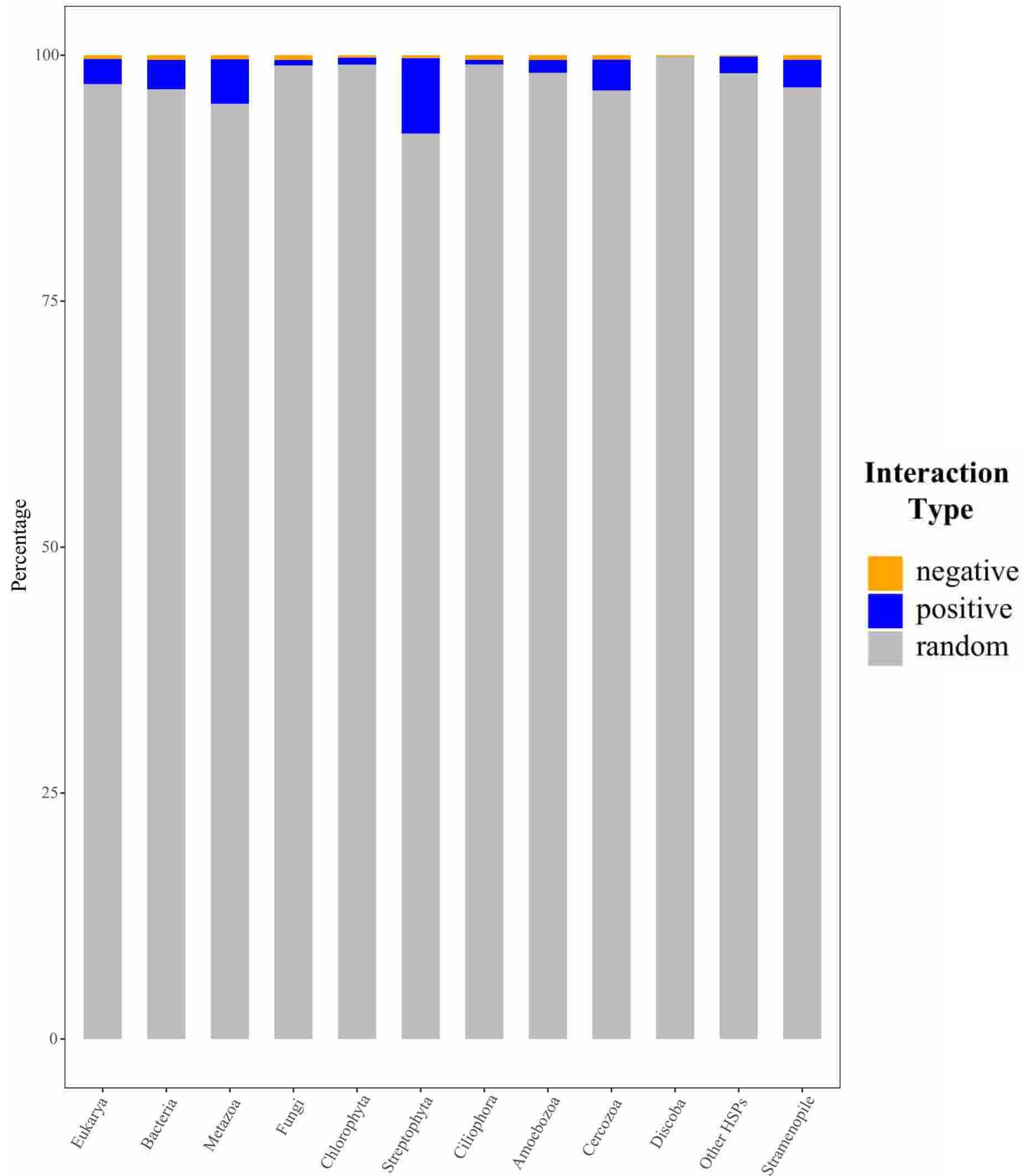

Figure 4.2: Proportion of association types among OTUs of all groups given by the pairwise analysis of co-occurrence. Pairwise co-occurrence analysis of all OTUs in the 18S and 16S datasets. Gray represents statistically random co-occurrence, blue represents positive associations, and yellow represents negative associations. Co-occurrence for supergroups and key heterotrophic protist groups presented. Other heterotrophic protists include those heterotrophic protists that do not fall under Ciliophora, Amoebozoa, Discoba or Cercozoa (e.g. Apusomonadida and Telonemia).

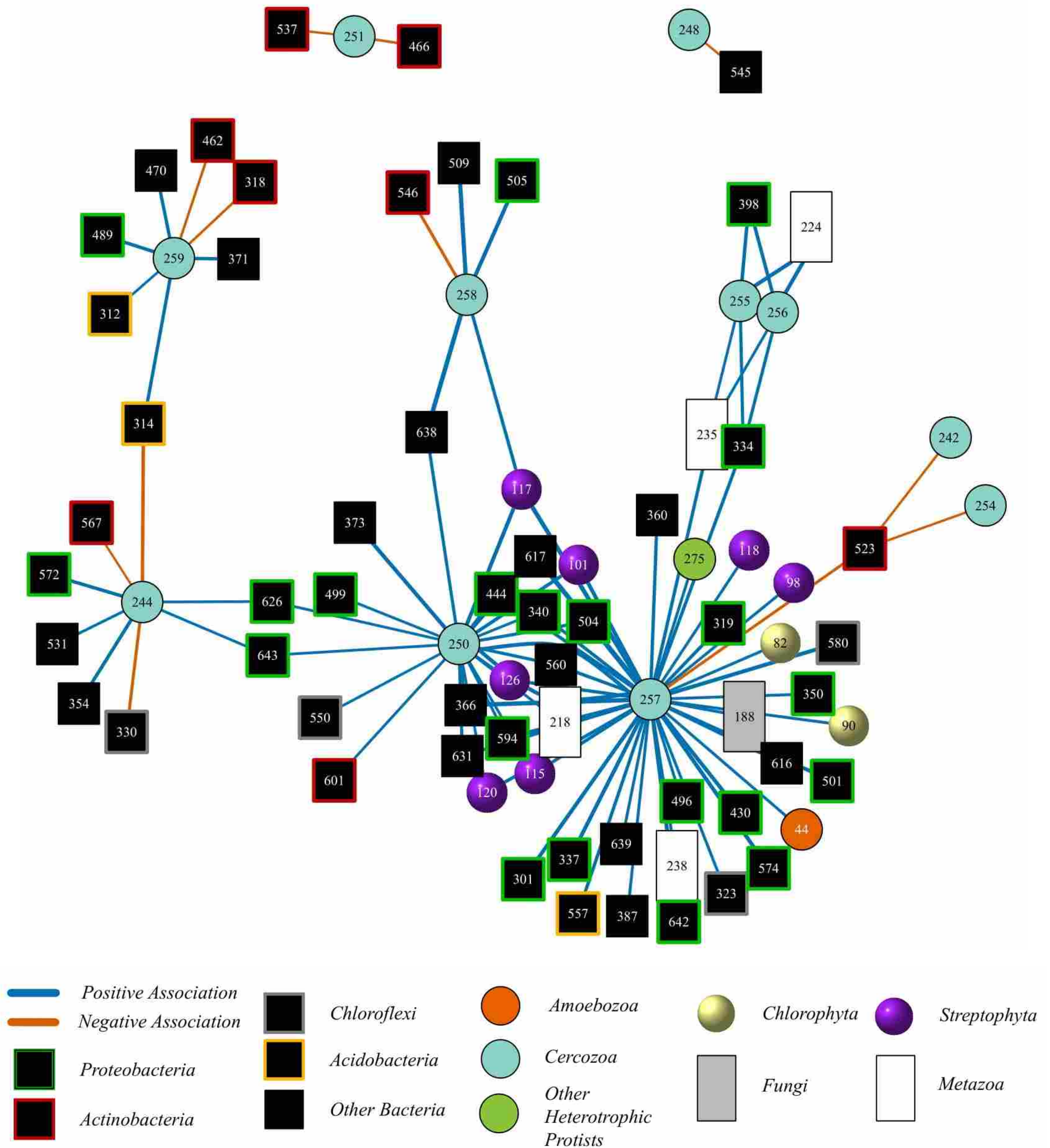

Figure 4.3: Network of pairwise co-occurrence associations for Cercozoa. Visualization of the non-random positive and negative interactions resulting from our pairwise co-occurrence analysis. Line length has no meaning, but line thickness indicates effect size.

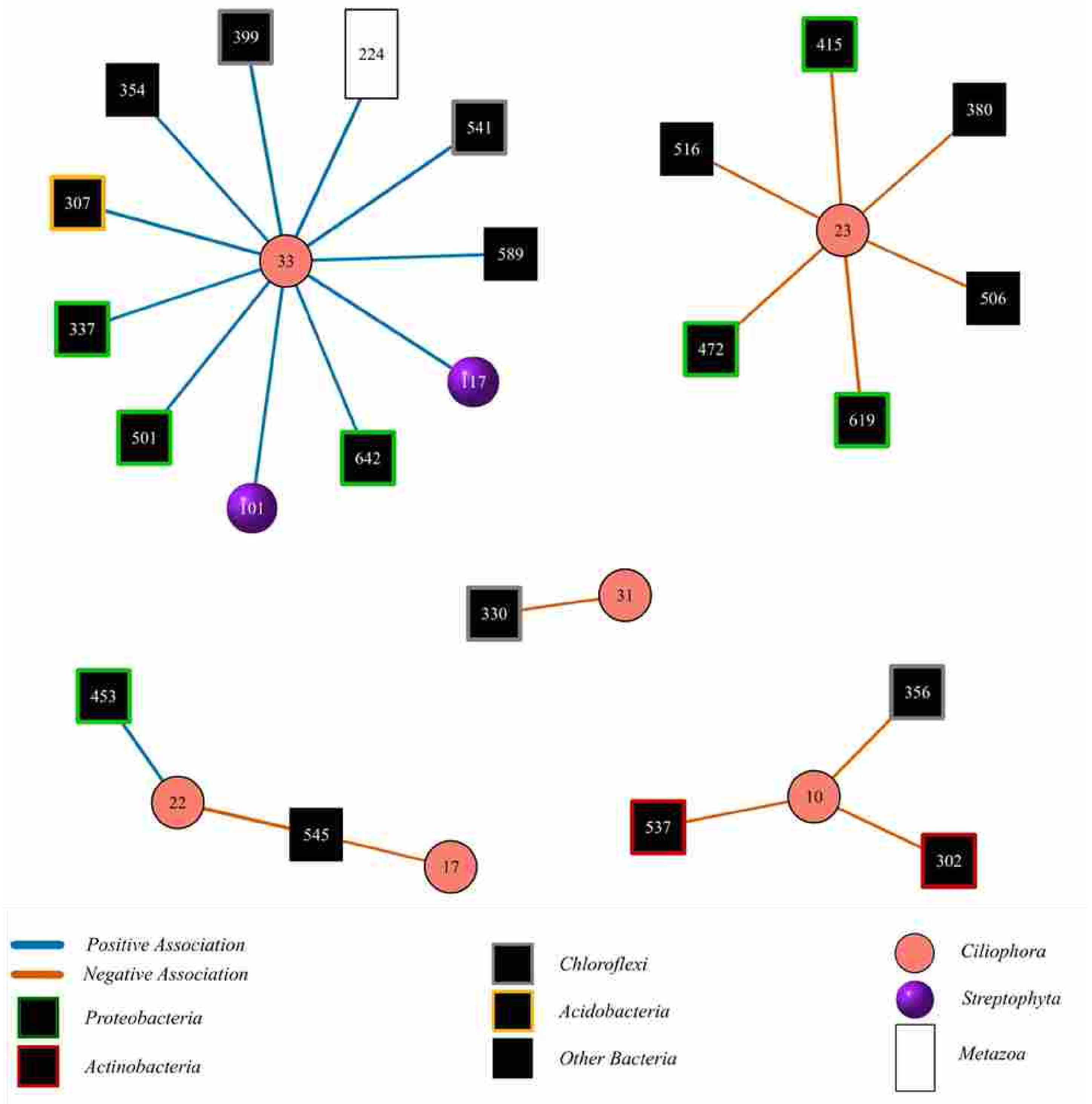

Figure 4.4: Network of pairwise co-occurrence associations for Ciliophora. Visualization of the non-random positive and negative interactions resulting from our pairwise co-occurrence analysis. Line length has no meaning, but line thickness indicates effect size.

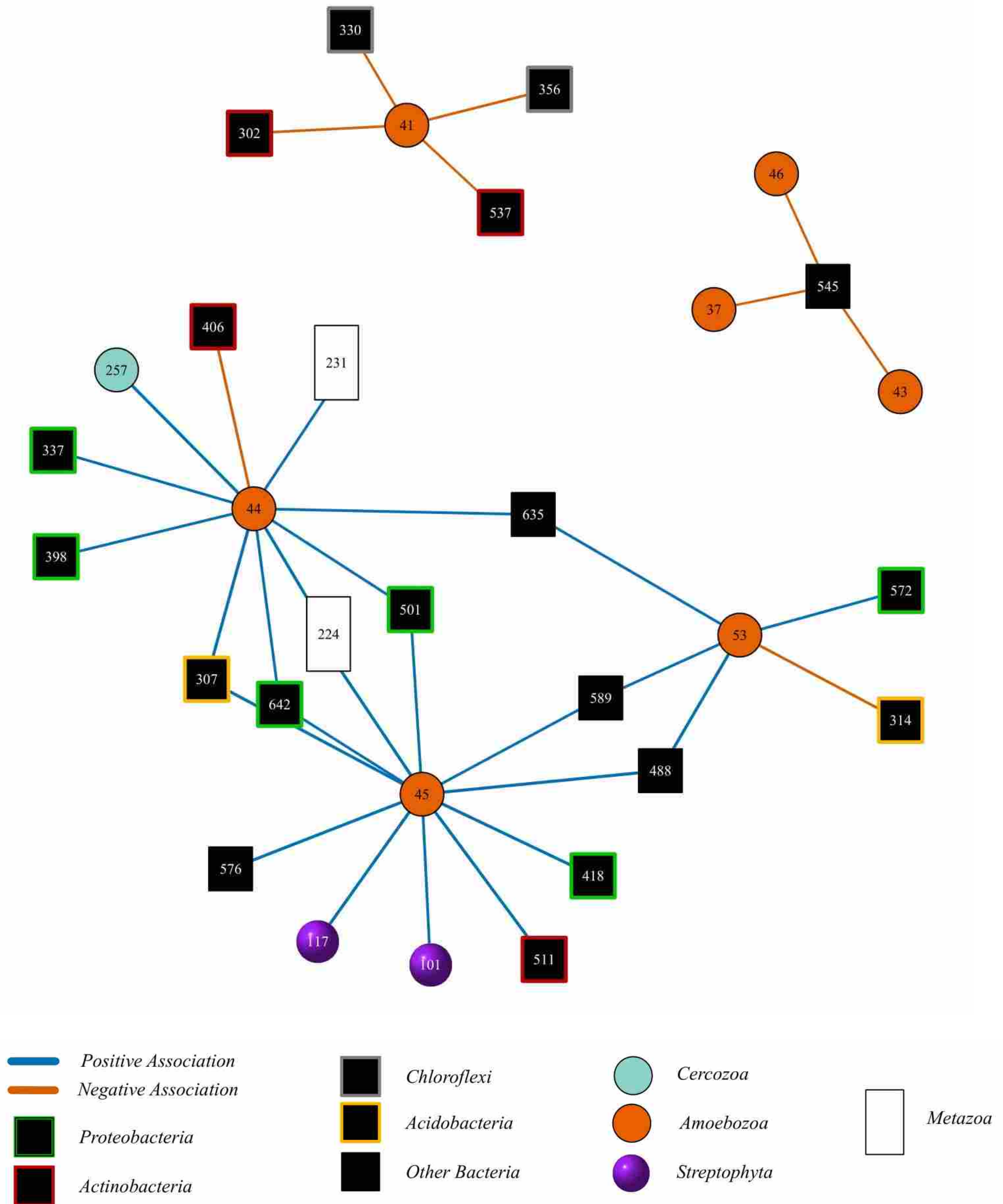

Figure 4.5: Network of pairwise co-occurrence associations for Amoebozoa. Visualization of the non-random positive and negative interactions resulting from our pairwise co-occurrence analysis. Line length has no meaning, but line thickness indicates effect size.

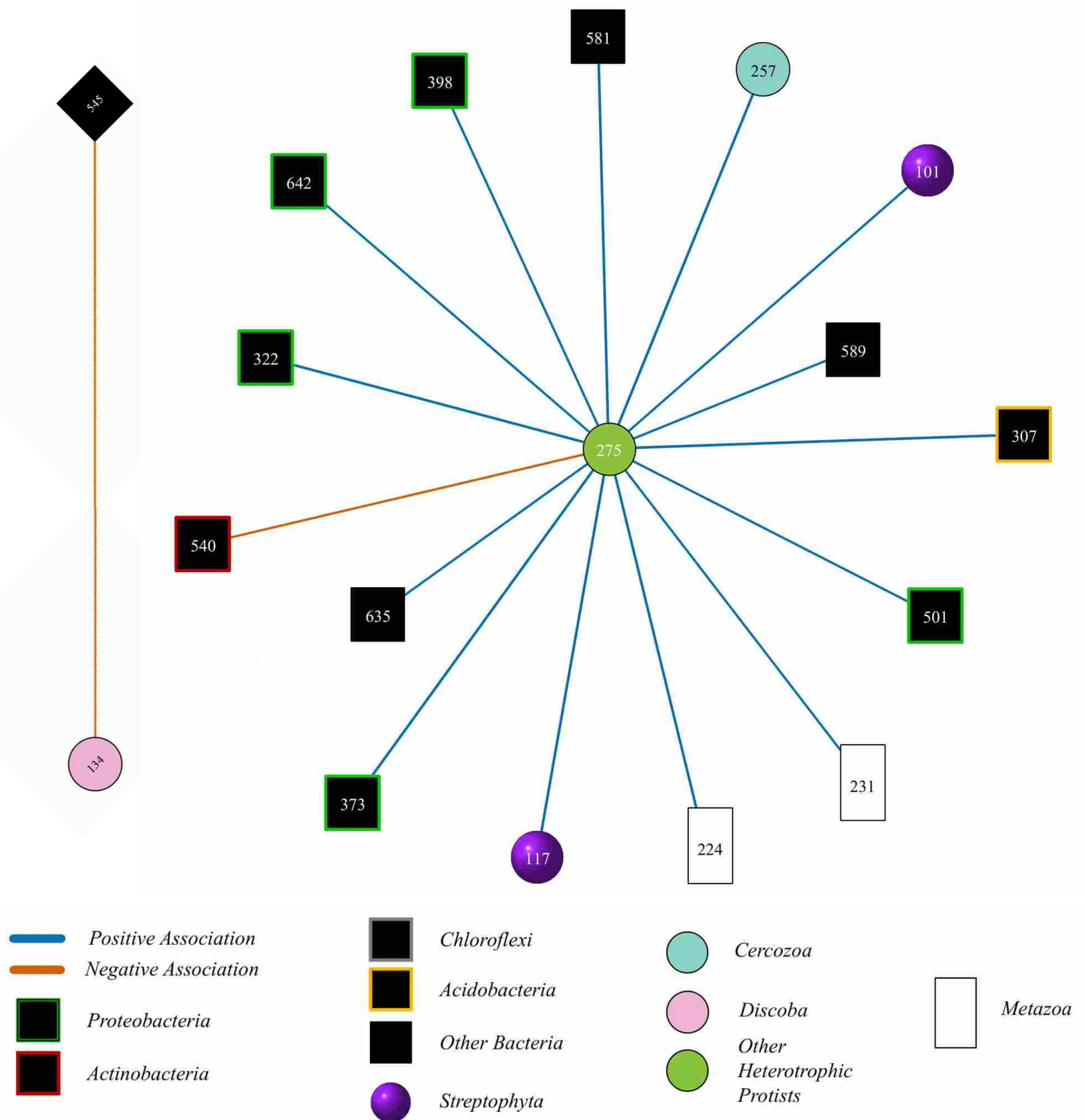

Figure 4.6 - Network of pairwise co-occurrence associations for Discoba and other heterotrophic protists. Visualization of the non-random positive and negative interactions resulting from our pairwise co-occurrence analysis. Line length has no meaning, but line thickness indicates effect size. Other heterotrophic protists include those heterotrophic protists that do not fall under Ciliophora, Amoebozoa, Discoba or Cercozoa (e.g. Apusomonadida and Telonemia).

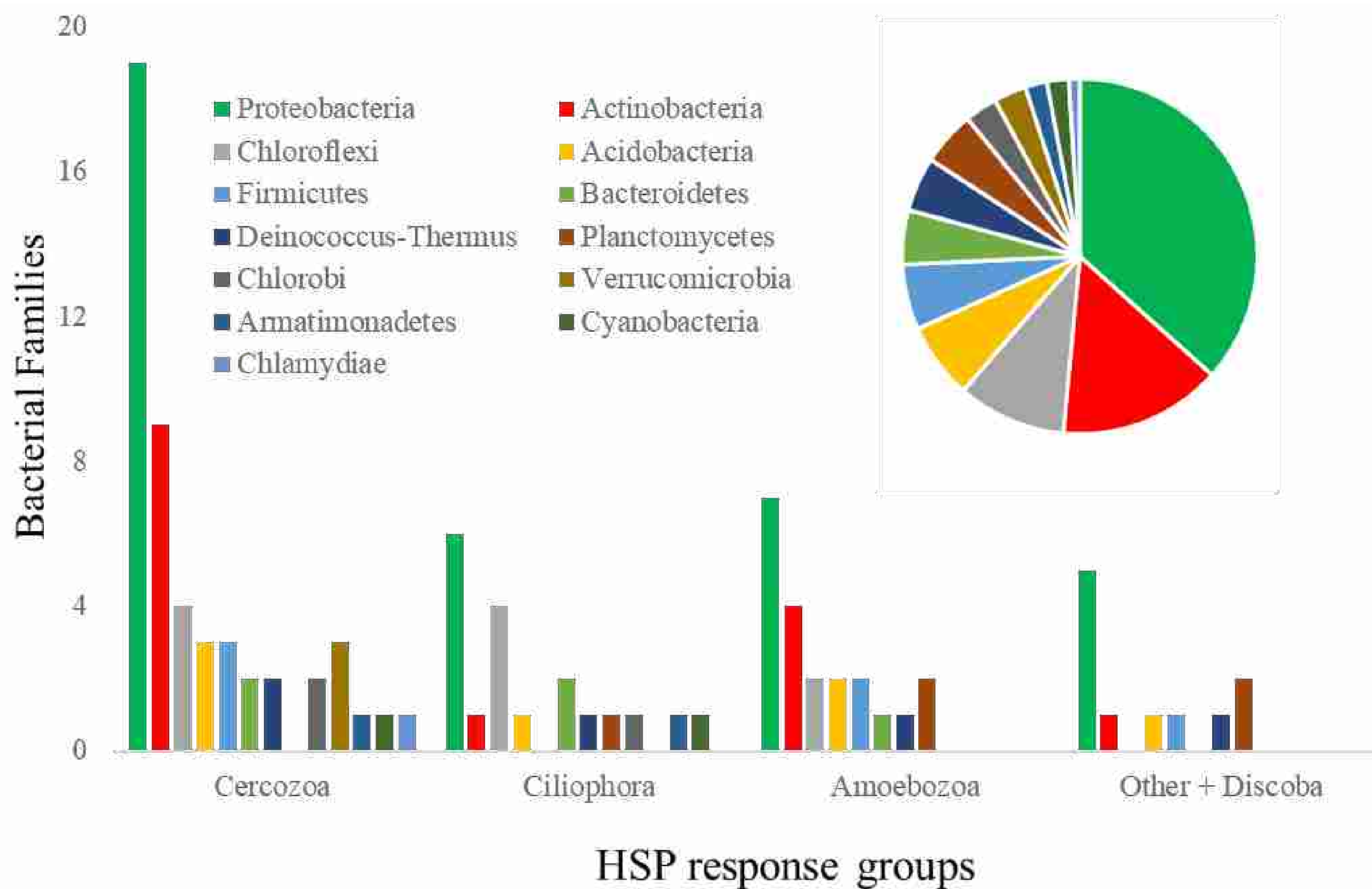

Figure 4.7: Proportion of bacterial phyla associated with HSP response groups. Bacterial taxonomy was assigned using Metaxa2.2 with the Silva reference release 111. Bacteria are color-coded by phylum, y-axis values are of families per phylum. Venn diagram shows overall proportion of bacterial phyla associated with all HSP response groups combined. Bar graph shows proportion of bacterial phyla per HSP response group (e.g. Cercozoa).

Supp. Table 4.1: Partial redundancy analysis results by biotic subdivision and statistical component for each HSP response group. Response groups are: Amoebozoa, Cercozoa, Ciliophora and Discoba. There are 10 biotic subgroups plus results for All Biota combined. Test components “Environment & Shared”, “Biotic & Shared”, “Environment, Shared & Biotic”, “Environment”, “Shared”, “Biotic”, and “Residuals” denote different combinations of Biotic, Shared, and Environment variables.

Cercozoa

	All Biota				***Ciliophora***				***Amoebozoa***			
Test Components	**DF**	**F-value**	**P-value**	**Adj. R^2**	**DF**	**F-value**	**P-value**	**Adj. R^2**	**DF**	**F-value**	**P-value**	**Adj. R^2**
Environment & Shared	10	1.2894	0.384	0.15316	10	1.2894	0.396	0.15316	10	1.2894	0.433	0.15316
Biotic & Shared	5	22.465	**0.001**	0.87026	4	5.9096	**0.07**	0.55105	2	2.4341	0.13	0.15201
Environment, Shared & Biotic	15	9.7437	0.057	0.89127	14	8.6485	**0.031**	0.87	12	0.9636	0.59	-0.02807
Environment	10	1.2126	0.588	0.02101	10	3.9443	0.115	0.31896	10	0.7548	0.684	-0.18008
Shared	0	NA	NA	0.13215	0	NA	NA	-0.16579	0	NA	NA	0.33325
Biotic	5	9.1463	0.092	0.73811	4	9.2714	**0.024**	0.71684	2	0.4712	0.769	-0.18123
Residuals	NA	NA	NA	0.10873	NA	NA	NA	0.13	NA	NA	NA	1.02807
	Discoba				***Chlorophyta***				***Streptophyta***			
Test Components	**DF**	**F-value**	**P-value**	**Adj. R^2**	**DF**	**F-value**	**P-value**	**Adj. R^2**	**DF**	**F-value**	**P-value**	**Adj. R^2**
Environment & Shared	10	1.2894	0.409	0.15316	10	1.2894	0.416	0.15316	10	1.2894	0.378	0.15316
Biotic & Shared	2	27.654	**0.01**	0.76915	2	2.9155	0.138	0.19318	2	5.4334	**0.087**	0.35657
Environment, Shared & Biotic	12	4.94	**0.056**	0.74716	12	2.1967	0.198	0.473	12	1.2594	0.42	0.16286
Environment	10	0.8782	0.61	-0.02199	10	1.7433	0.29	0.27982	10	0.676	0.769	-0.19371
Shared	0	NA	NA	0.17515	0	NA	NA	-0.12665	0	NA	NA	0.34687
Biotic	2	8.0478	**0.017**	0.59399	2	2.8207	0.132	0.31983	2	1.0348	0.478	0.0097
Residuals	NA	NA	NA	0.25284	NA	NA	NA	0.527	NA	NA	NA	0.83714
	Bacteria				***Fungi***				***Metazoa***			
Test Components	**DF**	**F-value**	**P-value**	**Adj. R^2**	**DF**	**F-value**	**P-value**	**Adj. R^2**	**DF**	**F-value**	**P-value**	**Adj. R^2**
Environment & Shared	10	1.2894	0.383	0.15316	10	1.2894	0.386	0.15316	10	1.2894	0.401	0.15316
Biotic & Shared	6	17.802	**0.001**	0.86302	2	29.588	**0.008**	0.78135	2	4.6566	0.096	0.31369
Environment, Shared & Biotic	16	NA	NA	NA	12	6.0675	**0.039**	0.79169	12	1.3235	0.39	0.19523
Environment	10	NA	NA	NA	10	1.0695	0.501	0.01034	10	0.7939	0.68	-0.11846
Shared	0	NA	NA	NA	0	NA	NA	0.14282	0	NA	NA	0.27162

Biotic	6	NA	NA	NA	2	10.196	**0.003**	0.63853	2	1.1568	0.41	0.04207
Residuals	NA	NA	NA	NA	NA	NA	NA	0.20831	NA	NA	NA	0.80477
	Stramenopiles				***Other Heterotrophic Protists***							
Test Components	**DF**	**F-value**	**P-value**	**Adj. R^2**	**DF**	**F-value**	**P-value**	**Adj. R^2**				
Environment & Shared	10	1.2894	0.419	0.15316	10	1.2894	0.434	0.15316				
Biotic & Shared	2	14.549	**0.021**	0.62876	3	8.0059	**0.005**	0.56777				
Environment, Shared & Biotic	12	3.4541	0.13	0.64796	13	1.5897	0.355	0.32393				
Environment	10	1.0764	0.495	0.0192	10	0.5311	0.866	-0.24385				
Shared	0	NA	NA	0.13396	0	NA	NA	0.39701				
Biotic	2	5.2165	**0.027**	0.4948	3	1.5052	0.308	0.17076				
Residuals	NA	NA	NA	0.35204	NA	NA	NA	0.67607				
Ciliophora												
	All Biota				***Amoebozoa***				***Cercozoa***			
Test Components	**DF**	**F-value**	**P-value**	**Adj. R^2**	**DF**	**F-value**	**P-value**	**Adj. R^2**	**DF**	**F-value**	**P-value**	**Adj. R^2**
Environment & Shared	10	1.1611	0.335	0.09149	10	1.1611	0.314	0.09149	10	1.1611	0.305	0.09149
Biotic & Shared	5	5.2184	**0.001**	0.56864	3	2.1869	**0.026**	0.18204	2	2.7586	**0.08**	0.18021
Environment, Shared & Biotic	15	3.4835	**0.023**	0.69954	13	1.5699	0.109	0.31649	12	1.5362	0.14	0.2868
Environment	10	1.4792	0.299	0.1309	10	1.2557	0.25	0.13445	10	1.2092	0.336	0.1066
Shared	0	NA	NA	-0.03941	0	NA	NA	-0.04296	0	NA	NA	-0.01511
Biotic	5	3.4285	0.076	0.60805	3	1.6584	0.173	0.225	2	1.8216	0.122	0.19532
Residuals	NA	NA	NA	0.30046	NA	NA	NA	0.68351	NA	NA	NA	0.7132
	Discoba				***Chlorophyta***				***Streptophyta***			
Test Components	**DF**	**F-value**	**P-value**	**Adj. R^2**	**DF**	**F-value**	**P-value**	**Adj. R^2**	**DF**	**F-value**	**P-value**	**Adj. R^2**
Environment & Shared	10	1.1611	0.314	0.09149	10	1.1611	0.337	0.09149	10	1.1611	0.315	0.09149
Biotic & Shared	2	2.1975	0.14	0.1302	2	3.5473	**0.014**	0.24151	2	1.9431	0.101	0.10546
Environment, Shared & Biotic	12	1.4745	0.162	0.26246	12	1.4218	0.153	0.24032	12	1.099	0.389	0.0691
Environment	10	1.2511	0.346	0.13226	10	0.9978	0.529	-0.00119	10	0.9453	0.589	-0.03636
Shared	0	NA	NA	-0.04078	0	NA	NA	0.09268	0	NA	NA	0.12785
Biotic	2	1.6954	0.172	0.17097	2	1.5877	0.189	0.14883	2	0.9278	0.504	-0.02239
Residuals	NA	NA	NA	0.73754	NA	NA	NA	0.75968	NA	NA	NA	0.9309
	Bacteria				***Fungi***				***Metazoa***			
Test Components	**DF**	**F-value**	**P-value**	**Adj. R^2**	**DF**	**F-value**	**P-value**	**Adj. R^2**	**DF**	**F-value**	**P-value**	**Adj. R^2**

Environment & Shared	10	1.1611	0.328	0.09149	10	1.1611	0.332	0.09149	10	1.1611	0.321	0.09149
Biotic & Shared	5	5.8025	**0.001**	0.60013	2	3.317	**0.034**	0.22458	2	2.1018	**0.072**	0.12105
Environment, Shared & Biotic	15	1.9543	0.198	0.47221	12	1.9891	**0.037**	0.42588	12	1.2551	0.285	0.16062
Environment	10	0.7334	0.749	-0.12792	10	1.4909	0.148	0.2013	10	1.066	0.466	0.03957
Shared	0	NA	NA	0.21941	0	NA	NA	-0.10981	0	NA	NA	0.05192
Biotic	5	1.8656	0.252	0.38072	2	2.7473	**0.038**	0.33439	2	1.2471	0.341	0.06913
Residuals	NA	NA	NA	0.52779	NA	NA	NA	0.57412	NA	NA	NA	0.83938

	Stramenopiles				***Other Heterotrophic Protists***			
Test Components	**DF**	**F-value**	**P-value**	**Adj. R^2**	**DF**	**F-value**	**P-value**	**Adj. R^2**
Environment & Shared	10	1.1611	0.327	0.09149	10	1.1611	0.315	0.09149
Biotic & Shared	2	3.1555	**0.025**	0.21225	3	4.9699	**0.001**	0.42672
Environment, Shared & Biotic	12	1.8932	**0.053**	0.40116	13	2.3156	**0.01**	0.51666
Environment	10	1.4416	0.152	0.18891	10	1.2419	0.231	0.08993
Shared	0	NA	NA	-0.09742	0	NA	NA	0.00156
Biotic	2	2.5513	**0.035**	0.30967	3	2.7593	**0.046**	0.42517
Residuals	NA	NA	NA	0.59884	NA	NA	NA	0.48334

Amoebozoa

	All Biota				***Ciliophora***				***Cercozoa***			
Test Components	**DF**	**F-value**	**P-value**	**Adj. R^2**	**DF**	**F-value**	**P-value**	**Adj. R^2**	**DF**	**F-value**	**P-value**	**Adj. R^2**
Environment & Shared	10	1.45	0.289	0.21951	10	1.45	0.286	0.21951	10	1.45	0.276	0.21951
Biotic & Shared	5	22.504	**0.001**	0.87047	3	16.027	**0.002**	0.73806	2	3.8945	**0.049**	0.26569
Environment, Shared & Biotic	15	3.927	0.215	0.73291	13	5.5182	**0.016**	0.78591	12	2.5233	0.054	0.53326
Environment	10	0.4335	0.905	-0.13756	10	1.2906	0.357	0.04785	10	1.8026	0.12	0.26757
Shared	0	NA	NA	0.35707	0	NA	NA	0.17166	0	NA	NA	-0.04806
Biotic	5	3.3066	0.2	0.5134	3	6.2914	**0.01**	0.5664	2	3.0166	0.086	0.31375
Residuals	NA	NA	NA	0.26709	NA	NA	NA	0.21409	NA	NA	NA	0.46674

	Discoba				***Chlorophyta***				***Streptophyta***			
Test Components	**DF**	**F-value**	**P-value**	**Adj. R^2**	**DF**	**F-value**	**P-value**	**Adj. R^2**	**DF**	**F-value**	**P-value**	**Adj. R^2**
Environment & Shared	10	1.45	0.295	0.21951	10	1.45	0.279	0.21951	10	1.45	0.294	0.21951
Biotic & Shared	2	11.987	0.034	0.57866	2	16.291	**0.001**	0.65653	2	14.36	**0.002**	0.62547
Environment, Shared & Biotic	12	5.4114	**0.003**	0.7679	12	6.353	**0.002**	0.80059	12	4.2757	**0.006**	0.71071
Environment	10	2.1415	0.077	0.18925	10	2.0114	**0.041**	0.14406	10	1.4125	0.161	0.08524
Shared	0	NA	NA	0.03026	0	NA	NA	0.07545	0	NA	NA	0.13427

	DF	F-value	P-value	Adj. R^2	DF	F-value	P-value	Adj. R^2	DF	F-value	P-value	Adj. R^2
Biotic	2	8.0883	**0.008**	0.54839	2	9.7419	**0.002**	0.58108	2	6.0939	**0.01**	0.4912
Residuals	NA	NA	NA	0.2321	NA	NA	NA	0.19941	NA	NA	NA	0.28929

	Bacteria				***Fungi***				***Metazoa***			
Test Components	**DF**	**F-value**	**P-value**	**Adj. R^2**	**DF**	**F-value**	**P-value**	**Adj. R^2**	**DF**	**F-value**	**P-value**	**Adj. R^2**
Environment & Shared	10	1.45	0.294	0.21951	10	1.45	0.268	0.21951	10	1.45	0.299	0.21951
Biotic & Shared	5	16.613	**0.001**	0.82991	3	14.268	**0.006**	0.71329	2	10.56	**0.01**	0.54443
Environment, Shared & Biotic	15	4.3592	0.156	0.75899	13	5.9442	**0.011**	0.80068	12	3.3177	**0.011**	0.63481
Environment	10	0.6763	0.772	-0.07091	10	1.57	0.294	0.08739	10	1.3465	0.273	0.09038
Shared	0	NA	NA	0.29042	0	NA	NA	0.13212	0	NA	NA	0.12913
Biotic	5	3.6862	0.169	0.53948	3	6.8316	**0.005**	0.58117	2	4.4117	**0.025**	0.4153
Residuals	NA	NA	NA	0.24101	NA	NA	NA	0.19932	NA	NA	NA	0.36519

	Stramenopiles				***Other Heterotrophic Protists***			
Test Components	**DF**	**F-value**	**P-value**	**Adj. R^2**	**DF**	**F-value**	**P-value**	**Adj. R^2**
Environment & Shared	10	1.45	0.28	0.21951	10	1.45	0.286	0.21951
Biotic & Shared	3	9.7286	**0.007**	0.62073	4	13.162	**0.001**	0.75251
Environment, Shared & Biotic	13	3.6269	**0.045**	0.68095	14	7.8071	**0.006**	0.85624
Environment	10	1.2454	0.384	0.06023	10	1.8659	0.195	0.10374
Shared	0	NA	NA	0.15928	0	NA	NA	0.11577
Biotic	3	3.8927	**0.05**	0.46144	4	7.6439	**0.008**	0.63673
Residuals	NA	NA	NA	0.31905	NA	NA	NA	0.14376

Discoba

	All Biota				***Ciliophora***				***Cercozoa***			
Test Components	**DF**	**F-value**	**P-value**	**Adj. R^2**	**DF**	**F-value**	**P-value**	**Adj. R^2**	**DF**	**F-value**	**P-value**	**Adj. R^2**
Environment & Shared	10	0.905	0.63	-0.0631	10	0.905	0.608	-0.0631	10	0.905	0.617	-0.0631
Biotic & Shared	5	18.574	**0.001**	0.84596	4	2.6847	0.093	0.29636	2	3.2552	**0.069**	0.21991
Environment, Shared & Biotic	15	14.423	**0.014**	0.92639	14	0.6256	0.794	-0.48718	12	0.8004	0.705	-0.17601
Environment	10	2.2017	0.395	0.08042	10	0.3678	0.884	-0.78354	10	0.5287	0.899	-0.39592
Shared	0	NA	NA	-0.14352	0	NA	NA	0.72044	0	NA	NA	0.33282
Biotic	5	17.13	**0.009**	0.98949	4	0.5723	0.804	-0.42408	2	0.712	0.604	-0.11291
Residuals	NA	NA	NA	0.07361	NA	NA	NA	1.48718	NA	NA	NA	1.17601

	Amoebozoa				***Chlorophyta***				***Streptophyta***			
Test Components	**DF**	**F-value**	**P-value**	**Adj. R^2**	**DF**	**F-value**	**P-value**	**Adj. R^2**	**DF**	**F-value**	**P-value**	**Adj. R^2**
Environment & Shared	10	0.905	0.618	-0.0631	10	0.905	0.6	-0.0631	10	0.905	0.632	-0.0631

Biotic & Shared	2	2.9775	**0.084**	0.1982	2	3.7866	0.133	0.25834	2	3.2422	**0.081**	0.21892
Environment, Shared & Biotic	12	0.8873	0.601	-0.09233	12	1.058	0.509	0.04168	12	0.903	0.614	-0.07846
Environment	10	0.6276	0.847	-0.29052	10	0.6835	0.756	-0.21665	10	0.614	0.858	-0.29738
Shared	0	NA	NA	0.22742	0	NA	NA	0.15355	0	NA	NA	0.23427
Biotic	2	0.9197	0.532	-0.02922	2	1.328	0.338	0.10479	2	0.9573	0.481	-0.01536
Residuals	NA	NA	NA	1.09233	NA	NA	NA	0.95832	NA	NA	NA	1.07846

	Bacteria				***Fungi***				***Metazoa***			
Test Components	**DF**	**F-value**	**P-value**	**Adj. R^2**	**DF**	**F-value**	**P-value**	**Adj. R^2**	**DF**	**F-value**	**P-value**	**Adj. R^2**
Environment & Shared	10	0.905	0.606	-0.0631	10	0.905	0.601	-0.0631	10	0.905	0.631	-0.0631
Biotic & Shared	6	36.286	**0.001**	0.92974	2	4.2175	0.103	0.28683	3	2.6866	0.119	0.24026
Environment, Shared & Biotic	16	NA	NA	NA	12	0.949	0.559	-0.03973	13	0.9101	0.604	-0.0788
Environment	10	NA	NA	NA	10	0.5603	0.802	-0.32656	10	0.6155	0.75	-0.31906
Shared	0	NA	NA	NA	0	NA	NA	0.26346	0	NA	NA	0.25595
Biotic	6	NA	NA	NA	2	1.0674	0.412	0.02337	3	0.9709	0.539	-0.0157
Residuals	NA	NA	NA	NA	NA	NA	NA	1.03973	NA	NA	NA	1.0788

	Stramenopiles				***Other Heterotrophic Protists***			
Test Components	**DF**	**F-value**	**P-value**	**Adj. R^2**	**DF**	**F-value**	**P-value**	**Adj. R^2**
Environment & Shared	10	0.905	0.613	-0.0631	10	0.905	0.63	-0.0631
Biotic & Shared	3	2.8356	0.132	0.25605	4	3.3574	**0.044**	0.37081
Environment, Shared & Biotic	13	1.0323	0.493	0.0256	14	1.1144	0.436	0.09102
Environment	10	0.6925	0.737	-0.23046	10	0.6306	0.779	-0.27979
Shared	0	NA	NA	0.16736	0	NA	NA	0.21669
Biotic	3	1.1821	0.421	0.0887	4	1.2543	0.444	0.15412
Residuals	NA	NA	NA	0.9744	NA	NA	NA	0.90898

Supp. Table 4.2: Taxonomic reference for OTUs in network diagrams. Bacterial OTUs are listed on the left, eukaryotic on the right. For bacteria, both the family and phylum level taxonomy are listed. For the Eukarya only genus is listed. OTUs with an unidentified Family (Bacteria) or Genus (Eukarya) designation are listed as “Ud.” plus the lowest resolution taxonomic available (e.g. Order).

Cercozoa Network

	Bacteria		*OTU*	*Family*	*Phylum*		**Archaeplastida**
OTU	*Family*	*Phylum*	504	Rhodocyclaceae	Proteobacteria	98	*Anomobryum*
312	Acidobacteriaceae	Acidobacteria	505	Rhodospirillaceae	Proteobacteria	101	*Bryum*
314	Acidobacteriaceae	Acidobacteria	509	Ruminococcaceae	Firmicutes	115	*Imbribryum*
318	Actinospicaceae	Actinobacteria	523	Streptosporangiaceae	Actinobacteria	117	*Lygodium*
319	Alcaligenaceae	Proteobacteria	531	Thermaceae	Deinococcus-Thermus	118	*Marchantia*
323	Anaerolineaceae	Chloroflexi	537	Thermoleophilaceae	Actinobacteria	120	*Mielichhoferia*
330	Ud. Thermomicrobia	Chloroflexi	545	Trueperaceae	Deinococcus-Thermus	126	*Rosulabryum*
334	Beijerinckiaceae	Proteobacteria	546	Tsukamurellaceae	Actinobacteria		**Metazoa**
337	Burkholderiaceae	Proteobacteria	550	Ud. Anaerolineales	Chloroflexi	218	*Plectus*
340	Candidatus_Alysiosphaera	Proteobacteria	557	Ud. Candidatus_Solibacter	Acidobacteria	224	*Adineta*
350	Caulobacteraceae	Proteobacteria	560	Ud. Chlamydiales	Chlamydiae	235	*Macrobiotus*
354	Chlorobiaceae	Chlorobi	567	Ud. Corynebacteriales	Actinobacteria	238	Ud. Tardigrada
360	Chthonomonadaceae	Armatimonadetes	572	Ud. Desulfovibrionales	Proteobacteria		**Amoebozoa**
366	Clostridiaceae	Firmicutes	574	Desulfuromonadales	Proteobacteria	44	*Acanthamoeba*
371	Corynebacteriaceae	Actinobacteria	580	Ktedonobacterales	Chloroflexi		**Cercozoa**
373	Cryomorphaceae	Bacteroidetes	594	Ud. Rhodospirillales	Proteobacteria	242	Ud. Filosa-Imbricatea
387	Ud. Chthoniobacterales	Verrucomicrobia	616	Ud. Cyanobacteria	Cyanobacteria	244	*Cercomonas*
398	Desulfuromonadaceae	Proteobacteria	617	Ud. Cytophagia	Bacteroidetes	248	*Metabolomonas*
430	Hyphomicrobiaceae	Proteobacteria	626	Ud. Xanthomonadales	Proteobacteria	250	Ud. Allapsidae
444	Methylocystaceae	Proteobacteria	631	Ud. Chlorobi	Chlorobi	251	Allapsidae Group-Te
462	Nitriliruptoraceae	Actinobacteria	638	Veillonellaceae	Firmicutes	254	Ud. Glissomonadida
466	Nocardiaceae	Actinobacteria	639	Verrucomicrobiaceae	Verrucomicrobia	255	*Flectomonas*

OTU	Family	Phylum	OTU	Family	Phylum	OTU	Taxon
470	Opitutaceae	Verrucomicrobia	642	wr0007 (Rhodospirillales)	Proteobacteria	256	*Neoheteromita*
489	Proteobacteria	Polyangiaceae	643	Xanthobacteraceae	Proteobacteria	257	*Sandona*
496	Pseudomonadaceae	Proteobacteria	601	Ud. Streptomycetales	Actinobacteria	258	Ud. Sandonidae
499	Incertae Sedis (Rhodospirillales)	Proteobacteria		**Fungi**		259	Ud. Rhogostoma-lineage
501	Rhizobiaceae	Proteobacteria	188	Sporobolomyces			

Ciliophora Network

			Bacteria				**Ciliophora**
OTU	*Family*	*Phylum*	*OTU*	*Family*	*Phylum*	10	Etoschophrya
302	480-2 (Solirubrobacterales)	Actinobacteria	472	Oxalobacteraceae	Proteobacteria	17	*Opisthonecta*
307	Order_incertae sedis	Acidobacteria	501	Rhizobiaceae	Proteobacteria	22	Ud. Chilodonellidae
330	Ud. Thermomicrobia	Chloroflexi	506	Rhodothermaceae	Bacteroidetes	23	*Pseudochilodonopsis*
337	Burkholderiaceae	Proteobacteria	516	Sphingobacteriaceae	Bacteroidetes	31	*Gonostomum*
354	Chlorobiaceae	Chlorobi	537	Thermoleophilaceae	Actinobacteria	33	Ud. Oxytrichidae
356	Chloroflexi Incertae Sedis	Chloroflexi	541	Thermosporotrichaceae	Chloroflexi		**Streptophyta**
380	Subsection III (Cyanobacteria)	Cyanobacteria	545*	Trueperaceae	Deinococcus-Thermus	101	*Bryum*
399	Dehalococcoidetes Incertae Sedis	Chloroflexi	589	Ud. Planctomycetales	Planctomycetes	117	*Lygodium*
415	Geobacteraceae	Proteobacteria	619	Ud. Deltaproteobacteria	Proteobacteria		**Metazoa**
453	Moraxellaceae	Proteobacteria	642	wr0007 (Rhodospirillales)	Proteobacteria	224	*Adineta*

Amoebozoa

			Bacteria				**Amoebozoa**
OTU	*Family*	*Phylum*	*OTU*	*Family*	*Phylum*	37	*Schizoplasmodiopsis*
302	480-2 (Solirubrobacterales)	Actinobacteria	545	Trueperaceae	Deinococcus-Thermus	41	Ud. Variosea
307	Order_incertae sedis	Acidobacteria	572	Ud. Desulfovibrionales	Proteobacteria	43	*Stenamoeba*
314	Acidobacteriaceae_K22	Acidobacteria	576	Ud. Flavobacteriales	Bacteroidetes	44	*Acanthamoeba*

330	bacterium_Ellin6505 (Thermomicrobia)	Chloroflexi	589	Ud. Planctomycetales	Planctomycetes	45	*Protacanthamoeba*
337	Burkholderiaceae	Proteobacteria	635	Ud. Planctomycetes	Planctomycetes	46	*Cochliopodium*
356	Chloroflexi_Incertae_Sedis	Chloroflexi	642	wr0007 (Rhodospirillales)	Proteobacteria	53	*Ptolemeba*
398	Desulfuromonadaceae	Proteobacteria					**Streptophyta**
406	Euzebyaceae	Actinobacteria				101	*Bryum*
418	GR-WP33-58 (Desulfuromonadales)	Proteobacteria				117	*Lygodium*
488	Planococcaceae	Firmicutes					**Metazoa**
501	Rhizobiaceae	Proteobacteria				231	Ud. Rotifera
511	Sanguibacteraceae	Actinobacteria				224	*Adineta*
537	Thermoleophilaceae	Actinobacteria					**Cercozoa**
						257	*Sandona*

Other Heterotrophic Protists and Discoba Network

Bacteria			**Streptophyta**	
OTU	*Family*	*Phylum*	101	*Bryum*
307	Order_incertae sedis	Acidobacteria	117	*Lygodium*
322	Alteromonadaceae	Proteobacteria		**Metazoa**
373	Burkholderiaceae	Proteobacteria	224	*Adineta*
398	Desulfuromonadaceae	Proteobacteria	231	Ud. Rotifera
501	Rhizobiaceae	Proteobacteria		**Other HSPs**
540	Thermomonosporaceae	Actinobacteria	275	*Spumella*
545	Trueperaceae	Deinococcus-Thermus		**Discoba**
581	Ud. Lactobacillales	Firmicutes	134	*Keelungia*
589	Ud. Planctomycetales	Planctomycetes		**Cercozoa**
635	Ud. Planctomycetes	Planctomycetes	257	*Sandona*
642	wr0007 (Rhodospirillales)	Proteobacteria		

Chapter 5

Searching for complex unicellular life in the Universe: Are key evolutionary innovations repeatable?

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Abstract

Testing the processes that lead to key evolutionary innovations on Earth, like the origin of life, the development of the cell, the evolution of the complex unicell, and the development of an intelligent, civilization-building species are difficult because we currently have only one case study to evaluate. Investigating whether these traits are chance occurrences or expressions of fundamental ecological and evolutionary laws that we don't yet fully understand would be facilitated greatly by the discovery of an independent lineage of life on another planet. Current efforts are focusing on the search for any life like Earth's on Mars. However, searching for complex unicellular life is also critical since the search for life is in part an attempt to answer whether we as a socially complex, civilization-building and intelligent species are alone. Here, I evaluate whether there is any theoretical basis for looking for complex unicellular life outside of Earth and what that life might look like. I also present practical considerations for finding that life on Mars, the most likely place that in situ searches for extraterrestrial life will occur in the next decades. I propose that complex unicellular life is likely to be a universal concept due to ecological and evolutionary tendencies seen in Earth's biological systems and that it could even be possibly found on Mars. Recognizing the subjectivity of these considerations, an intended outcome of this paper is the increased discussion around this important topic in Astrobiology.

Introduction

One of the biggest questions to ever face humanity is whether or not we are alone in the universe. Implicit in this overarching question is whether there is not only life itself beyond Earth's bounds, but life akin to humankind – that is intelligent, biologically complex life. The logical first step in this undertaking is to understand the evolution of biological complexity on Earth. In this context, we define biological complexity as the number of unique interactions that can occur within an organism and between an organism and its environment – a mouse is not necessarily less complex than an elephant (size alone does not predict complexity), but a bacterium is less complex than a ciliate. On Earth, major increases in biological complexity have been associated with the transition of pre-biotic life to ancestral cellular life, from ancestral prokaryotes to ancestral unicellular eukaryotes, and with the evolution of multicellular eukaryotes (Bengtson 2002, Lane and Martin 2010, Heim et al. 2017). The search for life has focused on the origin of life, the habitat preferences and physical limits of extremophile prokaryotes (Rothschild and Mancinelli 2001, Weber et al. 2007) and the search for extraterrestrial intelligence (SETI) institute (Drake 2011) (Figure 1). The first two focuses bias our search towards environments that are conducive only to the least complex forms of life known (Cavicchioli 2002) while the third possesses an inherently high risk of obtaining a false negative. Intelligent, civilization-building organisms can persist without the emission of electromagnetic radiation as has been the case for the vast majority of human history on Earth. These approaches therefore strongly bias against the detection of complex life. Here I focus on the evolutionary innovation of the unicellular eukaryotic cell, a major transition in cellular complexity that has been relatively un-explored in context of the search for life outside of Earth.

The discovery of complex unicellular life on other planets in our solar system would contribute key insights into the nature of fundamental evolutionary and ecological processes and inform our search for complex life akin to ourselves beyond our solar system. Searching for complex unicellular life, or "eukaryote-analogs", requires first determining whether complex unicellular life is a universal concept or a morphological experiment that occurred uniquely on Earth. If universal, then a better understanding of what constitutes complex unicellular life in the broadest possible sense is needed. We can then select appropriate targets in and beyond our Solar System for searching for complex unicellular life and identify universal biomarkers of non-Earth complex unicells. I specifically discuss the search for complex unicells in context of Mars due to its logistical accessibility, relatively Earth-like environment (Shapiro and Schulze-Makuch 2009) and the growing scientific and political momentum behind its exploration. The history of Mars offers many challenges to the evolution of complex unicells, such as its proximity to Earth (false positive) (Mileikowsky et al. 2000), a relatively short period of initial habitability (Cabrol 2018), and physically extreme surface (Rummel et al. 2014). However, I show that despite these challenges, searching for complex unicells on Mars can be a fruitful endeavor.

The universality of complex unicellular life

I predict that eukaryotes are a Terran iteration of a distinct functional evolutionary innovation that can occur repeatedly across independent lineages of life. I reach this conclusion because of the following aspects of eukaryogenesis and evolutionary ecology: 1) eukaryogenesis may only appear to be a rare trait on Earth due to a biased fossil record owing to temporal constraints, 2) the precursor behaviors that led to eukaryogenesis (i.e. predation or parasitism) are

likely to be common aspects of any ecological system, 3) eukaryogenesis encountered and exploited a distinct and advantageous niche space that will exist elsewhere given a minimum level of free energy in the environment, and 4) that the exploitation of this niche space requires a combination of functional traits that can be identified as belonging distinctly to complex unicells.

The rarity of eukaryogenesis

Though eukaryotes are now very common on earth, current evidence shows that these traits appeared in combination only once in the history of earth. This has led some to conclude that eukaryotic evolution is a rare event owing to the incredible number of innovations associated with this feature (Booth and Doolittle 2015). Compare eukaryogenesis with the evolution of powered flight, differentiated multicellularity, and the transition from aquatic to terrestrial environments. These functional innovations have each occurred a multitude of times in a variety of deeply divergent lineages notwithstanding the number of innovations required for making these transitions. Perhaps the algorithmic nature by which evolution produces solutions more frequently and readily than others is in part a result of relatively uncommon and complex sets of circumstances. Alternatively, this apparently ultra-rare trait might only appear to be so because of sampling bias - the emergence of the original eukaryotes probably occurred so anciently that any trace of competing lineages has arguably been lost (Schopf 2006, Dacks et al. 2016).

Eukaryogenesis via predation or parasitism

There are two main competing hypotheses for what led to the acquisition of the mitochondrion in the proto-eukaryote, arguably the defining feature and driving force behind the development of eukaryotic cellular complexity (Lane and Martin 2010, Dacks et al. 2016). The first

(via predation) proposes that phagocytosis developed in an archaeal ancestor which then acquired the bacterium that would become the mitochondrion. The second proposes that the ancestral eukaryotic organism was physically invaded by the ancestral mitochondrion in a case of prokaryote-on-prokaryote intracellular parasitism (Martijn and Ettema 2013, Booth and Doolittle 2015). Neither parasitism nor predation are uncommon means of acquiring energy in prokaryotes or eukaryotes today and would have likely been present far in advance of the evolution of eukaryotes on Earth (Davidov and Jurkevitch 2009). Negative interactions between prokaryotes must have occurred frequently due to limited resources (including space) giving rise to chemical and physical warfare that ended in death and (cellular) dismemberment (Granato et al. 2019). As the step from absorbing detritus organic matter (OM) from the environment to killing and then absorbing said OM is not a difficult one to imagine, the pre-conditions for endosymbiotic formation of a functional eukaryote-analog would likely be widespread in environments with cellular life that competes for resources.

Universal ecological niche space

Evidence for a distinct niche space occupied by complex unicells on Earth lies in the differences in cellular energetics and size between prokaryotes and unicellular eukaryotes, as well as unicellular eukaryotes and multicellular eukaryotes (Lane and Martin 2010, Heim et al. 2017). This niche space arises from the ability to acquire more resources from the environment, possibly through whole-cell predation (cytotrophy) which was likely the ancestral state of early unicellular eukaryotes (Yutin et al. 2009). As this niche space is related to predation and energetics, two fundamental ecological concepts (Gillooly and Allen 2007, Davidov and Jurkevitch 2009), then the available niche space required for the evolution of complex unicells will be nearly ubiquitous in

extraterrestrial environments where there is enough free energy in the environment. What the minimum amount of free energy in the environment might be to support organisms in this niche space requires further research that is beyond the scope of this paper.

Eukaryotes on Earth evolved when proto-eukaryotes encountered a novel ecological niche space allowing for more efficient energy acquisition at some point before 1.8 bya (Dacks et al. 2016). Whether major evolutionary innovations are repeatable has been the subject of some debate (Gould 1990, Morris 2011). The tendency of evolution to converge on similar functional solutions to similar ecological problems through unrelated structures (i.e. convergent evolution) indicates that seemingly rare, complex biological innovations (like eukaryote-analogs) could emerge fairly readily on other planets, moons, and planet-like bodies given sufficient time and appropriate ecological conditions (i.e. sufficient free energy) (Morris 2011). Since functional innovations are repeatable through evolution, this process would be expected to occur again, given proper environmental conditions and time for the appropriate number of generations.

Functional eukaryotes are identifiable

Evolution converges on functionally similar solutions using unique and potentially unrelated morphologies. The search for complex unicells requires defining the range of functional traits necessary for occupying the same niche space complex unicells do on Earth. Eukaryotes possess a membrane bound region where their genomic DNA is kept, organized, replicated and expressed, a much higher degree of cellular complexity than prokaryotes (organisms without a nucleus), cytoskeletal structures based on microtubules and actin complexes, complex intracellular signaling, extensive compartmentalization of cellular functions via organelles (e.g. Golgi apparatus,

endoplasmic reticulum, plastids, mitochondrion, phagosomes, etc.) and relatively complex genetics (introns, chromosome structure, meiosis) (Davidov and Jurkevitch 2009, Booth and Doolittle 2015, Lane and Martin 2015). Many of the traits considered exclusively eukaryotic have in recent years been found in modified form in prokaryotic organisms (Lane and Martin 2010), including structures similar to a nucleus (Lindsay et al. 2001), cytoskeletal structures (Vats et al. 2009), and sterols (Wei et al. 2016), however full compartmentalization (i.e. organelles), unique gene to protein synthesis energetics, and endocytosis remain uniquely eukaryotic. Therefore, I suggest that behaviors relating to these functions be targeted when searching for functional eukaryote-analogs.

Future research will need to comprehensively explore the boundaries of the theoretical range of traits associated with universal eukaryote-analogs, including a minimal set of traits and alternative pathways for evolving them, if they exist. For instance, though one might assume that the acquisition of a mitochondrion, and the cellular innovations that allow for this acquisition, are a universally defining feature, alternative pathways for either acquiring an endosymbiont or for obtaining increased energy yields are likely possible. For example, some prokaryotes have heavily invaginated cell membranes increasing membrane surface area and energy production to cell volume ratios, though this appears to still be strongly limiting (Lane and Martin 2010). Separating energy production from the cell membrane allows for increasing cell volume without sacrificing energy transport efficiencies. Coupled with additional cellular innovations, such invaginations could potentially lead to an alternative pathway for exploiting eukaryote-analog niche space.

Testing for functional eukaryote analogs

Of the traits related to eukaryote complexity listed previously, we can design practical tests for compartmentalization and whole cell phagocytosis. Compartmentalized unicells would have a higher ratio of biogenic lipid membranes to cytosolic macromolecules than would unicells without organelles. Lipid-based cellular membranes are considered probable for life because of their ubiquity in the environment and utility for creating energy gradients vital for life as we know it (Georgiou and Deamer 2014). This approach assumes an upper limit to prokaryote-analog cytosol to membrane ratios and that this value does not overlap with the lower limit of eukaryote-analog ratios. Estimating a lower limit for a functional eukaryote cytosol to membrane ratio could be done surveying these ratios in some of the smallest modern eukaryotes and comparing them to those from a range of modern prokaryotes (Derelle et al. 2006). Additional research into these ratios as they compare between the three domains of life on Earth and how to accurately measure them could highlight useful patterns for the detection of compartmentalized unicells elsewhere.

So far as known, formal endocytosis does not occur in prokaryotes although functionally similar processes have been observed in at least one lineage of bacteria (Fuerst and Sagulenko 2010), albeit on a smaller scale. As endocytosis of whole prey items could hypothetically lead to the acquisition of greater energy needs via the adoption of an endosymbiont, targeting organisms that possess the ability to endocytose whole organisms could be a strategic way to identify functionally analogous but structurally distinct evolutionary innovations. Magnetic beads have been used to study this process in ciliates (Voskühler and Tiedtke 1993, Maicher and Tiedtke 1999) and a similar approach could be applied to isolate endocytic organisms from extraterrestrial environments (Figure 2). This would involve the incubation of magnetic beads of shapes and sizes similar to prokaryote cells with sample material (aquatic or a soil slurry) in situ. Introduction of a large magnet after

incubation would then extract the magnets and recover any organism that may have ingested them whole. Although this approach is practical and straightforward, using it on Mars specifically would be challenging owing to the presence of magnetite in its surface regolith. This approach also assumes trophic generalism, that prey detection does not require recognition of biological surface antigens, and would entirely miss obligate phototrophs and saprotrophs. Additional research is needed to develop these approaches in the field and additional approaches for other universal biomarkers of complex unicells, including those that capture cells no longer adapted for whole-cell phagocytosis.

Complex unicellular life on Mars

Evidence has been mounting that early Mars was a warmer, wetter world than previously known, even to the point of being habitable enough for life to arise and evolve (Cabrol 2018). Assuming that an independent origin of life occurred on Mars, one then can ask whether 1) conditions of habitability persisted long enough for life to accumulate the traits necessary for the development of a eukaryote-analog, 2) if these analogs could have persisted as Mars became less habitable, and 3) if there are locations on Mars currently that could be habitable to such analogs.

The initial evolution of eukaryote-analogs on Mars: Time and oxygen

To predict whether complex unicells could have evolved on Mars, one can use what is known about eukaryote evolution on Earth. The fossil record gives an estimate of the evolution of eukaryotes between 1.5 and 2.8 billion years ago, though conservative estimates rarely predate 1.8

bya (Dacks et al. 2016). Current estimates suggest Mars was habitable by standard Terran definition for approximately 0.5-1 billion years at the start of the planet's history (Cabrol 2018), which some might interpret to mean that there would not have been enough time for such a transition on Mars, even if life did originate there. However, direct fossil evidence of microfauna of any domain from the Paleoproterozoic and the Archaean is scarce relative to more recent eras (Schopf 2006), and it is possible that eukaryotic life might have evolved much earlier than the present fossil record suggests. In addition, this evolutionary step is probably dependent on a correct combination of environmental conditions and existing biological traits and not only the result of a specific amount of elapsed time. Moreover, since the distinct niche occupied by complex unicells is tightly associated with energy acquisition, an environment that provides more energy than that of early Earth could encourage certain evolutionary innovations to occur more rapidly. In theory, a broad sampling of independent lineages of life (perhaps across inhabited planets) might reveal a minimum and maximum temporal range for the evolution of eukaryote-analogs. Before this range one might assume too little time could elapse, even with the shortest generation times, to evolve the traits necessary; beyond this range one could assume that environmental conditions (e.g. available free energy) are simply not conducive to the evolution of additional complexity. If this is the case, and it seems likely that it is considering the dynamic nature of both evolutionary processes and environmental equilibria, then the differences in the span of habitable conditions on early Earth and Mars may not be a reason to dismiss the development of eukaryote-analogs on Mars. In other words, we should be careful to not assume that since unicellular eukaryotes took upwards of two billion years to evolve on Earth such a transition would require a similar amount of time to evolve elsewhere.

Another aspect of eukaryotic evolution on Earth is oxygen: Mars lacks oxygen on the surface and aerobic organisms or evolutionary pathways associated with the metabolism of oxygen

would likely be precluded from surviving in Mars present atmosphere. That eukaryotes probably evolved after the oxygenation of the atmosphere (Dacks et al. 2016) and that this transition has largely been attributed to the acquisition of an aerobic endosymbiont implies that oxygen and the development of biologically complex cells are intimately linked (Dacks et al. 2016). Moreover, many anaerobic eukaryote lineages do exist (Stairs et al. 2015) indicating that basic eukaryotic structures and functions (i.e. compartmentalization, complex genetics, and a complex cytoskeleton) can exist without the energetics of an aerobic metabolism. The evolution of eukaryotes on Earth would have been stepwise, and certain models suggest that early eukaryotes (i.e. cells with complex cytoskeletons capable of phagocytosis) existed before later descendants acquired the mitochondrion (Martijn and Ettema 2013). In addition, although oxygen is absent in the Martian atmosphere, some research suggests that certain microenvironments, such as liquid brines, could contain enough O2 to support some aerobic metabolism (Stamenković et al. 2018). Even if oxygen was an absolute pre-requisite for eukaryote-analogs, aerobic microenvironments on Mars might still be able to support them.

Evolutionary limits in extreme environments

Assuming that the appropriate conditions and time intervals existed for the evolution of eukaryote analogs, the persistent harshness of the Martian environment over the 3 billion years since its relative habitability (Cabrol 2018) would have enacted a strong purifying selection on Martian organisms, likely encouraging specializations for a multitude of extremes, although the evolution of these polyextremophile phenotypes are still not well understood (Seckbach and Rampelotto 2015). The magnitude and frequency of local, regional and global disturbance events are thought to be

important determinants of extinction risk for a given species (Muscente et al. 2018), especially highly specialized communities (Day et al. 2016) such as those potentially found on Mars. If disturbances relevant to Martian microeukaryotes (e.g. climatic changes, freeze-thaw cycles, biological competition) were more frequent during this period, it would have maintained variation and thereby adaptive flexibility. Less frequent disturbances may have allowed for a greater degree of specialization over time and a subsequent higher risk of extinction. Determining which scenario was more likely to have influenced the evolution of any Martian organisms over time is challenging because Mars possesses many potential microhabitats and microclimates, each subject to unique disturbance types and frequencies. Infrequent but consistent climatic disturbances over long timescales owing to the high obliquity of Mars' axis (relative to Earth) (Kreslavsky and Head 2005, Schorghofer 2008) could also pose a threat to highly specialized Martian life, even though theoretically such cycles might render the surface more habitable during that time (Jakosky et al. 2003, McKay et al. 2013). The surface regolith is an extremely hostile environment (Rummel et al. 2014) and it has been suggested that Martian life would most likely persist in subterranean cavities or subsurface sediments (Jepsen et al. 2007, Cabrol 2018). Although less hostile in conventional terms, these environments would also be subject to a lower frequency of disturbances (Rummel et al. 2014), decreasing the adaptive potential of their indigenous biotic communities.

Understanding the limits of eukaryotes on Earth

One common approach to the search for life involves conducting surveys of extremophile diversity on Earth and applying what is known about their physiological tolerances to aspects of Martian surface conditions (Courtright et al. 2001, Rothschild and Mancinelli 2001, Council 2007).

For the majority of the year over the majority of its surface, Mars is far colder and dryer than anywhere on Earth, although a few locations make good approximations (e.g. Antarctic ice-free soils and the Atacama Desert) (Wentworth et al. 2005, Warren-Rhodes et al. 2019). Martian regolith is highly saline and possesses salt species (e.g. perchlorates) that are powerful oxidizers, while the top meter or more is bombarded by the whole spectrum of UV radiation as well as cosmic rays, conditions that do not occur naturally on Earth (Rummel et al. 2014, Cabrol 2018). Most surveys of extremophile diversity focus on Earth prokaryotes (Cvetkovska et al. 2017, Buzzini et al. 2018), but microeukaryote extremophiles are more prevalent than some may realize, especially at the limits of cold and desiccation tolerance (McKay and Davila 2014). A review of the diversity of microeukaryotes in extreme environments is beyond the scope of this paper (but see (Weber et al. 2007, Buzzini et al. 2018, Santiago et al. 2018)), however in general more information is needed about microeukaryote extremophiles, especially heterotrophic protists (Harding and Simpson 2018).

One drawback to the survey approach is that there are phenotypes that are evolutionarily possible yet not present on Earth today (Hoffmann 2014). How extensive this missing diversity of phenotypes is and how to explore it are questions that have largely been unexplored in the context of the search for life outside Earth. Earth's unique physical parameters (e.g. mass, spin, orbital cycle, position in the solar system and axial tilt), as well as its climatic and geological history, including the frequency, duration and extent of its recent ice ages, the frequency of major and minor extinction events, and even the possibility of multiple snowball Earths (Hoffman and Schrag 2002) have placed a set of constraints on the range of phenotypes life on Earth could evolve. For example, a planet's mass and position in the solar system can impact the relative abundances of biologically relevant compounds in its crust and mantle, the amount of liquid water accessible by a surface biosphere, and the strength of its magnetosphere which is crucial for the development of a stable

atmosphere (Bergin et al. 2015). Continent formation and movement are linked to fundamental evolutionary processes (e.g. allopatric speciation, facilitation of migration) (Lindsay and Brasier 2002, Molina-Venegas et al. 2015) and are a significant driver of changes in climate (e.g. through volcanic activity), which itself is a well-known filter and driver of species diversification on Earth (Benton 2009). Finally, extinction events can disturb ecological equilibria on various scales, allowing for adaptive radiation in some surviving lineages and exploration of novel phenotypic space (Feduccia 2014). Moreover, evolution's efficiency at exploring phenotypic space varies with scale (probably less efficient over short time intervals, more so over longer ones) but it will likely never be fully comprehensive because it is constrained by stochastic variables (Orr 2005, Hoffmann 2014). Thus, there should be phenotypes discoverable through directed evolution experiments that could not occur naturally on Earth but might occur elsewhere (Romero and Arnold 2009).

Testing for non-independent lineages of life on Mars

The discovery of functional eukaryote-analogs on Mars would not be considered clear evidence of an independent origin of biological complexity until verified that it was not related to Earth life. Asteroid strikes large enough to eject Earth organisms into space could have (eventually) transferred Earth eukaryotes to Mars in a process called lithopanspermia (Mileikowsky et al. 2000, Nicholson 2009). In addition, trans-planetary contamination during present-day scientific missions to Mars could also introduce eukaryotes, especially fungi (Rummel et al. 2014). Controlling for the presence of Earth life on Mars would involve evaluating the chirality and composition of the biomolecules in the putatively independent Martian lineage. The degree to which certain biomolecules (e.g. DNA) would evolve repeatedly across multiple lineages of life is not known, but

it probably follows similar rationale as described previously for the evolution of other biological traits. The discovery of biomolecules identical to our own wouldn't necessarily indicate contamination from Earth due to convergent evolution. Likewise, the discovery of different biomolecules wouldn't necessarily indicate a distinct lineage since early biotic chemistry on Earth may have originally had multiple sister lineages of which only one left any detectable trace (i.e. life as we know it). Panspermic events may have been more common early in Earth's history (Wesson 2010) and may have transferred examples of basal Earth life lineages, possibly with somewhat distinct biochemistries, to the Martian surface where they were able to survive while their counterparts on Earth did not. Careful study of biomolecules within isolated complex unicells from Mars will be needed, but without additional research into the potential biochemical diversity of very early lineages of life on Earth a definitive conclusion may be difficult to reach.

Conclusions

So far, most of the search for extraterrestrial life has focused on researching the conditions required for the origins of life and the limits of prokaryotes on Earth. However, in doing so we in fact bias our efforts against finding complex life. The complexity inherent in intelligent, civilization-building species like ourselves is not likely to arise on another planet or moon without the important precursor step of complex unicells. Here I proposed a theoretical framework for searching for complex unicells by establishing that their distinct functional role is one that will reasonably be encountered repeatedly across multiple independent lineages of life. I also proposed distinct functional aspects of eukaryote-analogs that can be used to design practical tests in extraterrestrial environments. Finally, I find that the case for Martian eukaryote-analogs is not an

immediately dismissible one. Incorporating tests for the existence of eukaryote-analogs on Mars into upcoming scientific missions could result in astounding discoveries that provide the means of confirming fundamental evolutionary processes as well as informing our selection parameters of potential exoplanets for interplanetary missions.

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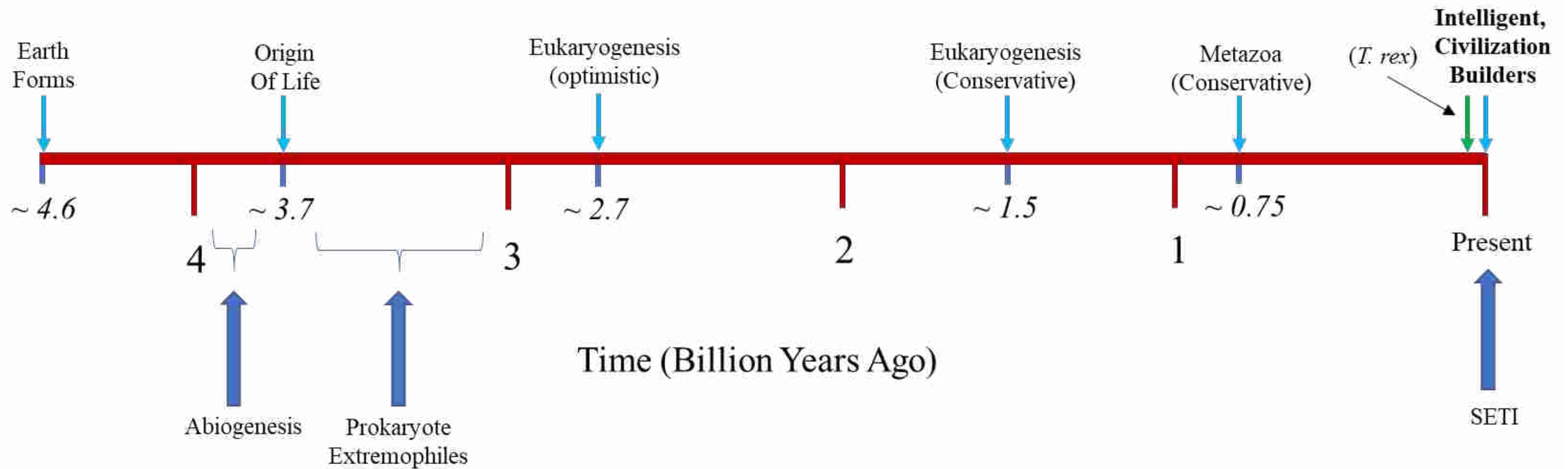
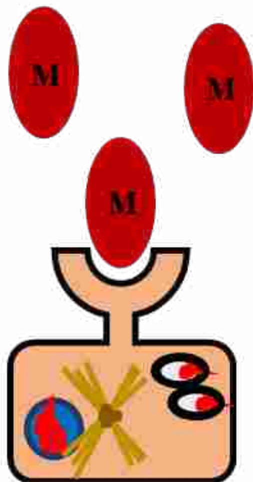
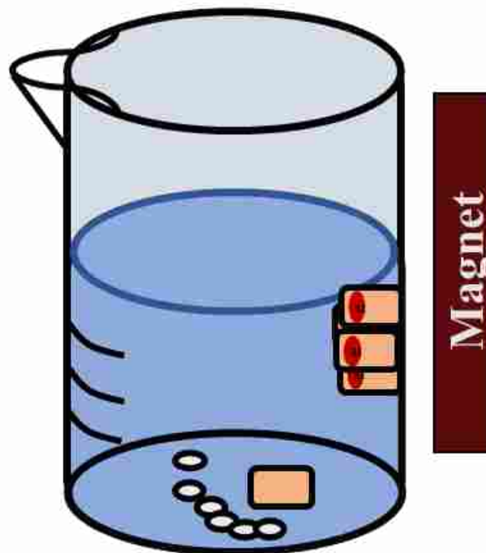


Figure 5.1: Common targets in the search for life against evolutionary time. Diagram showing common targets in the search for life and when they evolved on Earth. The timeline (in red) is in billions of years. Key evolutionary innovations are listed above the timeline (e.g. Origin of Life), and approximate times for their appearance are listed below. A green arrow marks the approximate point at which *Tyrannosaurus rex* existed, for scale. Large blue arrows below the timeline show the three main groups targeted in research programs searching for life: abiogenesis, prokaryotes (generally extremophiles), and the search for extraterrestrial intelligence (SETI).

Targeting phagotrophic complex unicells

1) Incubation **2) Isolation**





Magnetic Bead

Extraterrestrial prokaryote-analog

Extraterrestrial eukaryote-analog

Figure 5.2: Schematic for isolating extraterrestrial phagotrophic unicells. Red Ovals represent magnetic beads introduced into the sample, grey ovals represent an extraterrestrial prokaryote-analog, light red rounded square represents an extraterrestrial eukaryote-analog. Diagram shows two general steps, 1) Incubation and 2) Isolation.