# Exploring the Diversity and Activity of Microeukaryotes from Modern and Ancient Ecosystems from the Atacama Desert

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# Contents

## Abstract

The crescent interest of microbiologists on the ecology of protists is revealing new community assemblages in the natural ecosystems, changing the paradigms on the habitability of extreme environments. Consequently, questions on the ecology of microorganisms as the protists have been refined and supported by high-throughput sequencing (HTS). This approach can be used to uncover previously unknown global patterns of microbes, aiming to extend our knowledge on the biogeography of protists, even in challenging environments. The continual refinement of these technologies has enabled the detection of microbial communities relying on the environmental sequencing of hypervariable regions of gene sequences, commonly used as phylogenetic markers (e.g., ribosomal genes). Therefore, monoclonal cultures of the unicellular protists are urgently needed to check such results. The progressive curation of reference databases has helped to increase the confidence on the use of environmental sequencing and techniques involving the amplification of marker genes from the genetic pool found in nature (e.g., metabarcoding). Such approaches must consider parallel cultivation approaches, aiming to back up the detection of protist lineages across areas of unknown diversity as the natural laboratories found across the ancient Atacama Desert.

As recently uncovered, the Atacama Desert can be inhabited by the primordial prokaryotes (bacteria and archaea), in unique habitats distributed in this desert, sustaining life even at the dry limit. Biotic factors controlling their populations, as the recent free-living protists, are understudied in the most arid desert on Earth, and the functions and structure of their communities is yet to be studied. In this study, the Atacama Desert was used as a model system for the study of potentially extremophile protists, including understudied areas as arid soils, biofilms and microbial mats. The relative abundance of the protists communities in the arid Atacama could hide a great gap in the fundamental knowledge of the microbial ecology surviving in the vicinity of areas considered to be analogous to the neighbor planet, Mars. We hypothesized that the uncultivable protists detectable by metabarcoding and the structure of their communities, correlate with the environmental

gradients and that their active physiological state can be detected in microhabitats of this desert. We focus on protists due to earlier findings describing a novel diversity of species from diverse groups as heterotrophic flagellates and ciliates, as well as to the description of protist communities in hypersaline systems by metabarcoding. As the ancient Atacama covers an extensive area, we focused on microbial habitats which could serve as a good source of protist diversity, as those found at the limits of the arid zones. The coastal hills facing the Pacific Ocean and the sparse areas covered by biological crusts and biofilms could hide the keys to adaptation, enduring scarce water regimes. Interesting microbial structures growing at the benthic sediments of Andean Lagoons represent hotspots of biodiversity and could serve as a reservoir of protist life. We studied the diversity of protists constituting these sediments and studied their stratification at a millimeter-scale to assess the microbial guilds at a small scale (e.g., ciliates, flagellates and amoebae). Additionally, we tested for correlation of protist taxa with the water column properties at five remote basins found across the Atacama and the Andean plateau aiming evaluate their association to abiotic factors. Finally, the occurrence of these groups in datasets obtained by the modern metabarcoding approach was estimated to infer their potential interaction based on correlation patterns across different habitats (e.g., soils and benthic microbial mats). The identification of such significant correlated taxa could shed light on the members of these communities which could help the human to prioritize on potential keystone species in conservation initiatives. The microbial life able to thrive an extreme environment as the Atacama Desert could hide the adaptation mechanisms useful to understand the population dynamics in remote microhabitats threatened by the current climate change scenario.

## General Introduction

### Diversity and Function of Protists in Nature

An important fraction of the ecosystems is filled by microbial life, distributed ubiquitously along habitats, playing a paramount role in environmental processes part of Earth's biogeochemical cycles since primal ages (Nutman et al., 2016). Biochemical and physiological properties allow bacteria, archaea and microeukaryotes to thrive every imaginable niche, even in the presence of extreme factors including temperature, pressure, pH, salinity, geological scales and geographical barriers, solar radiation, chemical extremes, scarce nutrition, osmotic barriers (Hoehler and Jørgensen, 2013; Živaljić et al., 2018; Wierzchos et al., 2018). Under different conditions, the microeukaryotes known as protists, can exist as free-living organisms occupying a vast variety of habitats ranging from hyperarid soils in desert, down to the depths of the oceans (Fernández, 2015; Schoenle et al., 2022). Different to prokaryotes, protists are evolutionarily closer to humans, raising modern questions on their evolution of multicellularity (Tikhonenkov et al., 2020; Pöggeler and James, 2023).

The study of these microbes has been greatly affected by the development of new technologies for their study, starting with the early observations of Antoni Van Leeuwenhoek (1676), developing the first microscopes. Such technique enabled the morphological classification of protists giving birth to later phylogenies of the microorganisms (Haeckel, 1866). More recently, the development of the sequencing technologies, allowed scientists to classify life, first in three domains including Archaea, Bacteria and Eucarya based on ribosomal information (Woese et al., 1990). Furthermore, the advent of high-throughput technologies for gene sequencing, mainly from environmental samples, introduced the possibility to detect an enormous diversity of uncultured microbes (Taberlet et al., 2012; Ji et al., 2013). Furthermore, metagenomic sequencing (i.e., sequencing of environmental molecules), revealed the Asgard archaea, those archaea group coding diverse eukaryotic signature proteins than other archaea (MacLeod et al., 2019).

This helped to partly overcome agar plate paradoxes in cultivation, that could not resolve the real abundance patterns in nature, given the specificity of culture media, enriching a small fraction of species in environmental samples. Nevertheless, these approaches are not error-proof and diversity and co-occurrence patterns detected by metagenomics, metatranscriptomics or metabarcoding should be studied with caution and supported by the parallel isolation of phylotypes in culture (Kirchman, 2018).

The diversity of protists is currently in the focus of microbial ecologists, increasing constantly the complexity of the eukaryotic branch of the tree of life (Figure 1, Burki *et al.*, 2020).

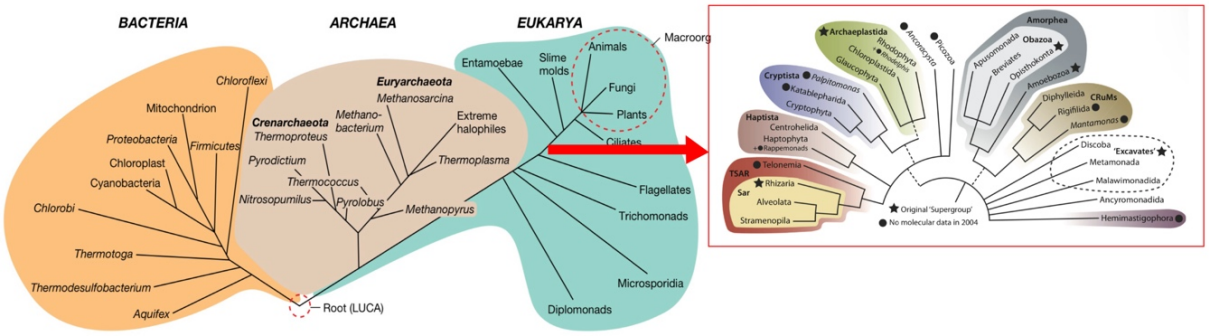


**Figure 1.** View of the phylogenetic relationships of the diverse lineages in the tree of life. Left side: evolutionary relationships and the phylogenetic tree of life including Domains as Bacteria, Archaea and Eukarya (Extracted and modified from Madigan et al., 2019). Right side: The eukaryotic tree of life after (Burki *et al.*, 2020).

Functionally, the protists are mainly linked to the carbon cycle at the base of the microbial loop, grazing on bacteria, predating potential pathogens at the roots of plants (Bonkowski, 2004). They can include heterotrophs and phototrophs and even organisms being both. Furthermore, in direct or indirect relation to phototrophs, heterotrophic protists obtain the elements necessary for their nutrition from the byproducts of photosynthesis. In terrestrial ecosystems, a particular group, Cercozoa, has been linked to plants, as determinant of their fitness through the phagocytosis of potential pathogens (Xiong *et al.*, 2020). Protists can also predate microalgae, viruses and other protists as well, or being even found inhabiting macroorganisms, including metazoans (Geisen *et al.*, 2015). Overall, these

microbes are a key building block in the microbial foodwebs and consequently linked to the functioning of the biogeochemical cycles. Moreover, although they can be restricted at certain habitats (e.g., hyperarid zones), the proliferation of resting stages or cysts, help these microbes to persist in the environment. During recent years, vast knowledge on the biogeography of protists across Earth habitats has revealed a huge diversity and abundance across the globe (Burki, et al., 2021). Nevertheless, the diversity of protists conforming the microbial ecology in extreme environments awaits to be further elucidated (Rappaport and Oliverio, 2023).

**Protists in Dry Environments**

Between the habitats on the planet, terrestrial ones are vastly diverse compared to those in aquatic ecosystems, due to the heterogeneity of the soil matrix. Nevertheless, aquatic ones can still show a quite high diversity, leading to the "paradox of the plankton (Hutchinson, 1961). This pointed out the large number of microbial species that seem to be competing for a small number of resources in physically simple and unstructured environments. Soils, on the other side, are being increasingly recognized to have a crucial underlying role in the ecosystem processes and to host a vast diversity of eukaryotic microbes (Geisen et al., 2020).This emphasizes the idea of studying the protists as the biotic factors that can change across different conditions aiming to study their inter-association and ecologic roles sustaining ecosystem services in the diverse and complex soil habitats.

Under the current climate change, both habitats on lands or in waters can be affected, promoting a redistribution of the species (Pecl *et al.*, 2017). Thus, the desertification can affect the activity of essential biological-based processes mediated by soil microbes as the stability of ecosystems in the case of the most sensitive and fragile ecosystems as those found in arid and hyper-arid environments (Zeng *et al.*, 2018). Interestingly, the activity of microbial communities of protozoans has been studied under temperature variations, showing high productivity in their populations, different to species from higher trophic levels in the trophic chains (Arndt and Nomdedeu, 2016). These findings, support the idea of a possibility to encounter highly adapted protists in dry

habitats. Nevertheless, assessments of these ideas in the extreme environments located in desert remain to be studied, mainly due to the remoteness of dry areas, as in the case of polar and non-polar deserts. Depending mainly on water, protists can profit from the surroundings, even if it constitutes the fluids of other species, including ranges from freshwater to salty water at their habitats. Additionally, the ability to form cysts, is a faculty that these microbes use to persist when water is absent or in critical amounts, surviving the dehydration in habitats subjected to long and harsh water regimes. One of the deserts on Earth, which could help us to understand the assembly of highly specialized extremophile protists, and which has been poorly investigated is the most arid one, the Atacama Desert.

**Protist Life in the Atacama**

The Atacama Desert, located in the North of Chile, is the oldest and driest non-polar desert on Earth and is often used as a model system to investigate the dry and ultra-violet limits for life (Warren-Rhodes et al., 2006; Pérez et al., 2017a; Schulze-Makuch et al., 2018). It extends along the coast of northern Chile from the Pacific shore to the western slopes of the Andes (Bull *et al.*, 2018). The main feature, aridity, may have persisted in this region for the past 90 million years, with an onset of hyperaridity in a core area, tracked to the middle to late Miocene, in association with the uplift of the Andes (Rech *et al.*, 2010).

This remote place represents a unique extreme environment to explore microbial diversity given the challenging condition for life and the multiple ecosystems within. Some of Atacama's ecosystems are endorheic basins generated during the geologic periods Tertiary and Quaternary, when the area was exposed to intense volcanic and sedimentary activity. As a result, extended lacustrine systems evolved into evaporitic basins of diverse size; certainly, this process continues, shaping lakes, saline lagoons, wetlands and salt flats. Such salt deposits, remnant of former paleolakes (late Pleistocene), were originated by the alteration of volcanic and sedimentary rocks as also by the dissolution of ancient evaporites, as by oxidation of sulfur minerals (Tapia et al., 2018). Recently, researchers have started the study of the monocellular protists in the Atacama, revealing a novel diversity, including different major divisions of these microeukaryotes as Stramenopiles, Alveolata and

Opisthokonta (e.g., Schiwitza et al., 2018; Arndt et al., 2020; Rybarski et al., 2021). Between these groups, they have been postulated to have different times of colonization in this desert, being the placidid stramenopiles, those showing a deeper divergence from other clades already more than hundred million years ago (Arndt et al., 2020).

Such pioneer works on the protistology in the Atacama, pinpoint this area as rich in natural laboratories, especially in the inland waters conforming the saline to hypersaline lagoons distributed there. Nevertheless, the role in the interaction to endemic plants and animals, together with specific microbe-protist interactions involving parasitism, predation or syntrophy, remain unknown in this area. The features of the unicellular protists, make them ideal model organisms to combine studies on their biogeography and cultivability. As biogeography is a key aspect to understand the evolution of species, information on their colonization patterns across this arid desert, could shed light on distribution patterns across varied scales. This includes the vast area occupied by arid to hyperarid soils, fog oasis sustaining the heterogeneously distributed flora and isolated water bodies as ponds and lagoons mainly found in the Andean range. Thus, we could resolve interesting questions on protist lineages turn-over across habitats, as well as to test if all of them are everywhere (Baas Becking, 1934), or if they possess a moderate endemicity (Foissner, 2007).

## Aims

The main objective of this study was to investigate the diversity and functionality of the protists in different soils and microbial mats from the Atacama Desert using a metabarcoding approach. To do this, we sampled soils and microbial mats at different latitudes of the Atacama Desert, including millimeter-scale profiles of sediments and different terrestrial habitats (e.g., soils crusts, fog oasis soil). We focused on resolving their spatial distribution and ecological adaptations to different environmental settings by studying the correlation of water parameters to the protist lineages found at the top layer of sediments. We support our environmental sequencing approach by isolating monoclonal cultures of protists and by comparing their marker gene sequence to genotypes obtained by metabarcoding. We aimed to better understand their biogeographical distribution patterns in this desert and to determine ecological and evolutionary processes supporting these patterns.

We addressed the following questions:

1. How distinct to described species are protist strains isolated from the Atacama Desert?
2. Is the structure of the protist communities, due to their geographical isolation or habitat type, unique across habitats in the Atacama Desert?
3. Are the genotypes obtained by PCR-based environmental sequencing closely related to those obtained from strains isolated from this study area?
4. Are there large- and small-scale community patterns changes detectable by metabarcoding?

To answer these questions, protists were enriched from environmental samples where these microorganisms could be thriving (e.g., plants, soils), isolated in axenic protist cultures, sequenced their 18S rDNA gene (and ITS gene sequence when necessary), studied their phylogeny and described their morphological characteristics (Chapter 1). Soil

communities from different latitudes in the Atacama Desert, were investigated with the aim to resolve distribution and co-occurrence patterns across habitats, including terrestrial and benthic microbial communities from this desert. The description of such communities and the study of their dissimilarity is supported by phylogenetic inference of the hypervariable region V9 from isolated strains and metabarcodes (Chapter 2). Additionally, five different ecosystems were selected for the study of microbial mats protists and their communities, aiming to extend the knowledge of the uncultivable taxa inhabiting these understudied structures and their association to environmental factors as the overlaying water physic-chemical composition. Additionally, we extend our investigation at the millimeter-scale, studying the groups exclusively detected along a vertical transect in microbial mats (Chapter 3).

**Summary of Chapters**

The findings presented in the framework of this thesis are structured in three chapters. Each chapter intended to be complementary to each other focusing on the sequencing of the ribosomal gene 18S (rRNA), in specific the hypervariable region V9. The recovery and sequencing of those amplicons were used as an environmental proxy to the protists which could be detected in templates as DNA, RNA, and the DNA from isolates. The proxies used to depict the protist communities are known as amplicon sequence variants (ASVs). The first chapter is a description work of protists found in endemic cacti of the Atacama. The second chapter is a metabarcoding study based on ASVs found across terrestrial and aquatic ecosystems, including cultivation of strains isolated from the study sites. The third chapter is a metabarcoding assessment of the protist communities across five microbial mats of Andean lagoons in the Atacama Desert.

**Chapter 1**: **Novel cercozoan and heterolobosean protists from the rhizosphere and phyllosphere of two endemic cacti from the Atacama Desert**

As a starting point of this work, protist strains were isolated in monoclonal culture from the spines of two endemic cacti from the Atacama Desert, the columnar *Browningia candelaris* (Meyen) and the shrubby *Eulychnia taltalensis* (F. Ritter). We could obtain the complete 18S rDNA gene sequence of the strains to assess the phylogenetic distance to species described across different lineages. By this approach, we effectively described five novel species, as we found differences in morphological and molecular information compared to reference information. Furthermore, we highlighted that these local strains possess the sequence of one of the two widely used phylogenetic markers (hypervariable V9 region 18SSU), which were planned to be used in our upcoming metabarcoding investigations. *Published in European Journal of Protistology, DOI: 10.1016/j.ejop.2023.126034*

**Chapter 2: Evidence of protists colonization in terricolous lichens, coastal hills and microbial mats highlight abundant molecular signatures related to the genera *Rhogostoma*, *Euplotes* and *Neobodo***

The metabarcoding approach was used in three different stations, including soils showing the presence of lichenized soil crusts (19°S), soil from a fog oasis part of a coastal hill (25°S, up to 682 m a.s.l.) and from a microbial mat (23°S). The differences in the structure of the communities represented by genotypes amplified from the environmental DNA and cDNA was analyzed. Although no significant differences were found, this study constitutes the first metabarcoding of protists in Atacama Desert soils, revealing abundant genotypes related mainly to the taxa *Rhogostoma*, *Euplotes* and *Neobodo*. Interestingly, we could isolate in parallel, monoclonal cultures, which in preliminary identification using BLAST, were related to *Euplotes* (HFCC988) and *Neobodo* (HFCC986). This allowed us to infer the phylogeny aiming to compare ASVs and the phylogenetic marker, finding that the ASVs amplified from local areas are closer to Atacama strains than to other species. Additionally, we used the sequence from *Rhogostoma olyaorum* (HFCC860, described during **Chapter 1**), corroborating it closeness to our ASVs obtained from environmental samples. We included

strict filtering steps in our pipeline, assessing our hypothesis using a highly curated dataset. Finally, as we calculated the co-occurrence of the ASVs, we could depict the ASVs showing correlation, giving us a clue of potential interaction between these biosignatures and examined the assemblages obtained from terrestrial and aquatic habitats. *Manuscript intended for European Journal of Protistology*

**Chapter 3: Protist communities of microbial mats from extreme environments of five saline Andean lagoons at high elevation in the Atacama Desert**

In this study we investigated the diversity and distribution of benthic protists communities in microbial mats found at five different lagoons across the Andes, using the amplification of the V9 region on the 18S rDNA. We included studies on cDNA in parallel to extend our knowledge on the physiological state of the studied protist communities. Thereby, it was found that protist community composition significantly differed across stations and giving furthermore, insights on their composition uniqueness at the millimeter-scale. Additionally, we enumerate the genotype overlap between stations, finding a core microbiome for each millimeter depth and genotypes occurring exclusively at each layer from each station. Overall, we detected genotypes related to *Rhogostoma* and *Euplotes* by DNA and cDNA, confirming their presence and potential active metabolic state. Moreover, diatoms dominated uppermost layers, confirming the typical structure of microbial mats subjected to high UV-radiation. Additionally, we used the molecular information obtained during **Chapter 1** and **Chapter 2** from monoclonal cultures. This helped us to compare our ASVs obtained by metabarcoding and to assess their closeness to reference sequences of the strains HFCC860 (*Rhogostoma*) and HFCC988 (*Euplotes*), supporting their detection by metabarcoding. *Submitted to Frontiers in Microbiology, Special Issue Exploring Microbial Mats Communities in Extreme Environments*

# Chapter 1: Novel Cercozoan and Heterolobosean Protists from the Rhizosphere and Phyllosphere of Two Endemic Cacti from the Atacama Desert

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# Novel cercozoan and heterolobosean protists from the rhizosphere and phyllosphere of two endemic cacti from the Atacama Desert

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ABSTRACT

Cercozoans and heterolobose amoebae are found across terrestrial habitats where they feed on other unicellular microbes, including bacteria, fungi and microalgae. They constitute a significant fraction of soil ecosystems and are integral members of plant microbiota. Here, we present the results on the isolation of protozoans from the rhizosphere and phyllosphere of *Browningia candelaris* (Meyen) in the Andean Altiplano and *Eulychnia taltalensis* (F. Ritter) from the Coastal Cordillera of the Atacama Desert, both endemic to this ancient desert. We identified a new heterolobose amoeba species of the genus *Allovahlkampfia* isolated from cactus soil, three new species of the different glissomonad genera *Allapsa*, *Neoheteromita*, *Neocercomonas* and one new thecofilosean amoeba of the genus *Rhogostoma* isolated from the phyllosphere of one studied cactus. In addition, one bacterivorous flagellate was isolated from cactus spines and identified as a member of the non-scaled imbricatean family Spongomonadidae (*Spongomonas*). The isolation of protists from cactus spines extends the knowledge on the habitat ranges of taxa typically found on plant leaves or soils. The molecular data presented here is a prerequisite for further investigations on the ecology and diversity of protists including next-generation sequencing of microhabitats in plants and the rhizosphere, allowing for deeper taxonomic classification.

## 1. Introduction

Heterotrophic flagellates and amoebae inhabit plants and soils and are increasingly recognized as key components of microbial food webs, predating prokaryotes and fungi and hence playing important roles in mineralization and organic matter cycle (Geisen et al., 2018). In fact, the diverse unicellular eukaryotes are considered determinant of plant fitness, predating rhizobacteria, actively participating in the microbial loop (Bonkowski, 2004; Guo et al., 2022). In particular gliding protists as those from the phylum Cercozoa, have been described not only as major members of the soil microbiota but also as part of the rhizosphere (Sapp et al., 2018) and phyllosphere (Dumack et al., 2017a; Flues et al., 2018; Ploch et al., 2016). A functional trait of these protists is the consumption of bacteria through phagotrophy, a feeding mode that has been linked to plant health at the roots, likely shifting microbial communities and mitigating the pathogen success on the plant's surface (Xiong et al., 2020). Further, functions of protists in the plant microbiome are yet to be unveiled. Their phenotype traits can only be studied with cultured strains that are increasingly becoming available for experiments (Dumack et al., 2021).

The Atacama Desert, the driest place on Earth, likely hides functional communities of microbes inhabiting the phyllosphere and rhizosphere of desertic plant hosts as the cacti. These succulent plants have a highly reduced or even lost ability to produce leaves. Instead of basal branches they possess clusters of spines called areoles concentrating water droplets in eventual fog oases (Nyffeler, 2002). Thus, species from the family Cactaceae have adapted to the arid landscape in the Atacama, showing allopatric distribution throughout a complex orography and exhibiting a surprisingly rich diversity of desertic flora (Merklinger et al., 2021). At oasis referred to as "lomas" at the coast as well as at valleys hillsides in the Altiplano, the shrubby species of the genus *Eulychnia* and the columnar cacti from the genus *Browningia* can be found (Charles, 2000; Hoxey and Klaassen, 2011; Merklinger et al., 2021). Arid and hyperarid soils are currently the predominant habitat in the Atacama Desert. With low precipitation and high evaporation rates, these hostile habitats originated millions of years ago (mid- to late Miocene) associated to the uplift of the Andes (Rech et al., 2010). However, the scarce humid periods are sufficient to sustain the endemic diversity of plants as cacti, shrubs as well as epiphytic and terricolous lichens surviving ever since, now occurring mainly at the borders of the arid core (Böhnert et al.,

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2019; Jung et al., 2020; Merklinger et al., 2021).

The ecology of protists at a global scale remains to be further studied to be fully understood, nevertheless in the Atacama Desert, several species have been described as being endemic, reflecting the local geologic and climatic evolution (Arndt et al., 2020; Schiwitza et al., 2018). Increasing attention on the microbiology of this desert aiming to recover protists in pure culture, resulted in studies of amoebae (Salazar-Ardiles et al., 2022) and of flagellates and their potential habitat ranges (Carduck et al., 2021; Rybarski et al., 2021; Schiwitza et al., 2018, 2021; Schoenle et al., 2022), describing new protozoans in formerly unknown hotspots of biodiversity. Since the advent of high-throughput sequencing, the questions on the ecology of protists have been refined, requiring curated molecular information on type strains, crucial for taxonomy assignment in massive sequencing surveys in plants (Dumack and Bonkowski, 2020). Studies focused on the endemic microbiome of plants in this ancient desert may give insights into reciprocal differentiation processes between hosts and microbiomes implicated in the adaptation to persist in the driest place on Earth. As postulated by Zilber-Rosenberg and Rosenberg (2008) the hologenome theory of evolution can be investigated in inter-domain associations. The small size and short generation times of protists may lead to a rapid fixation of beneficial mutations in this inhospitable desert which is potentially reflected in their speciation. In this study, we isolated and described six new protist species, aiming to increase our understanding on their diversity and ecology within the microbiome of plants in the Atacama Desert. This novel morphological and molecular information on microeukaryotes is essential to support future massive sequencing projects assessing the structure of protists communities throughout this remote desert.

## 2. Material and methods

### 2.1. Sampling

The cacti *Browningia candelaris* (Meyen) Britton & Rosse, found at slopes of the Quebrada Aroma (Fig. 1B) and *Eulychnia taltalensis* (F. Ritter) Hoxey, growing at the nearby of the coastal locality of Paposo (Fig. 1C), were selected to study the protozoans of the phyllosphere (Table 1). Soil surface samples (20 g from the upper 5 cm) were taken in a radius of 1 m around the plant *B. candelaris* and stored in 50 ml sterile plastic tubes (Sarstedt AG, Nümbrecht, Germany). The phyllosphere samples from cacti included spines taken from each studied cactus under sterile conditions (i.e., sample collection using a sterile cotton hydrated by sterile dd$H_2$0). The samples were stored in 50 ml sterile plastic tubes with silica gel (Carl Roth GmbH, Karlsruhe, Germany) covered with a sterile tissue, to avoid fungal contamination.

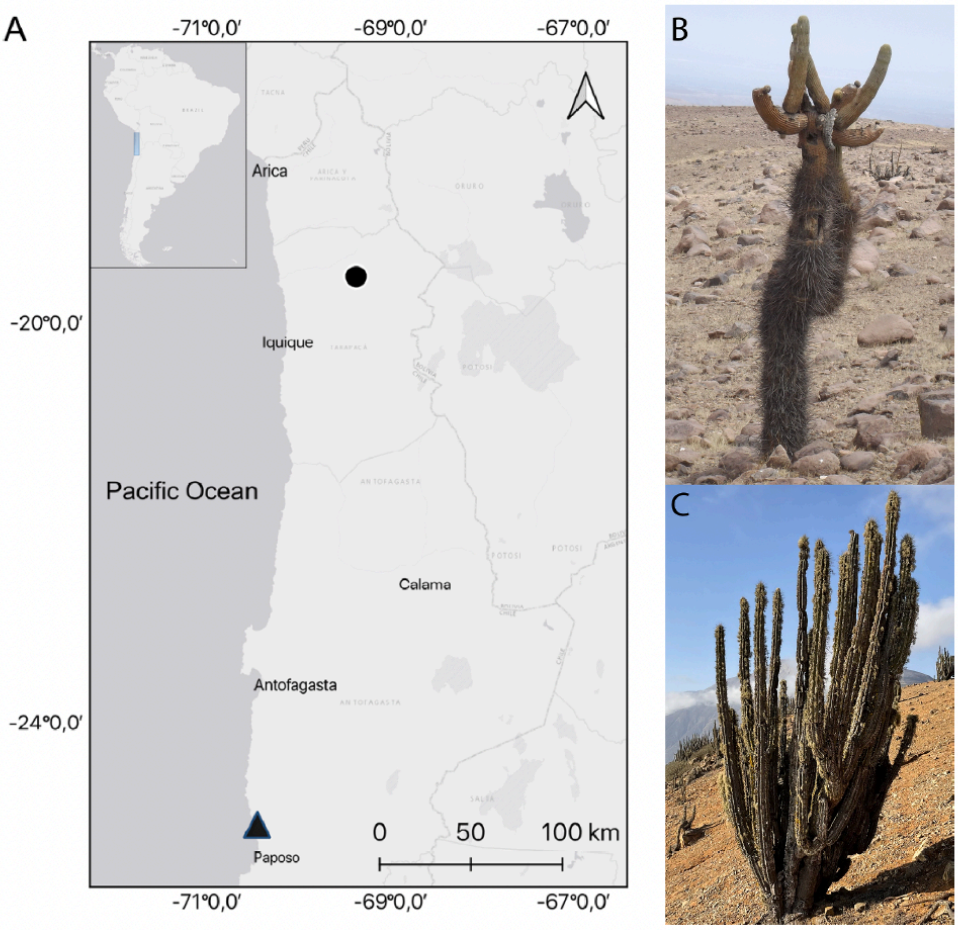


**Fig. 1.** A: Map indicating the sampling sites for the isolation of protists from the cacti B: *Browningia candelaris* (circle) and C: *Eulychnia taltalensis* (triangle).

**Table 1**
Origin of samples used for the enrichment and isolation of protists.

| Species | Isolate | Geographic location | Altitude (m a.s.l.) | Sample type | Accession number (NCBI) |
|---|---|---|---|---|---|
| *Allapsa antofagastaensis* | HFCC 838 | 25°00'29.6"S,70°27'01.8"W | 691 | Spine of *Eulychnia taltalensis* | OR731268 |
| *Spongomonas* sp. | HFCC 856 | 19°31'45.9"S,69°22'26.1"W | 2,720 | Spine of *Browningia candelaris* | OR731270 |
| *Neoheteromita paposoensis* | HFCC 857 | 25°00'29.6"S,70°27'01.8"W | 691 | Spine of *Eulychnia taltalensis* | OR731273 |
| *Neocercomonas granulosum* | HFCC 858 | 25°00'29.6"S,70°27'01.8"W | 691 | Spine of *Eulychnia taltalensis* | OR731277 |
| *Rhogostoma olyaorum* | HFCC 860 | 19°31'45.9"S,69°22'26.1"W | 2,720 | Spine of *Browningia candelaris* | OR775727 |
| *Allovahlkampfia pisaguaensis* | HFCC 979 | 19°31'45.9"S,69°22'26.1"W | 2,720 | Soil around *Browningia candelaris* | OR750521 |

### 2.2. Enrichment of protists

For the enrichment of protists from cactus spines we used the Wright's Chu medium (WC, Guillard and Lorenzen, 1972), transferring one cactus spine (ca. one gram) in 30 ml of culture medium in 50 ml culture flasks (Starstedt AG, Nümbrecht, Germany). On the other hand, one gram of soil was rehydrated in 30 ml of sterile Schmaltz-Pratt medium (28.15 g/l Na Cl, 0.67 g/l KCl, 5.51 g/l MgCl2 × 6 H2O, 6.92 g/l MgSO4 × 7 H2O, 1.45 g/l CaCl2·H2O, 0.1 g/l KNO3 and 0.01 g/l K2HPO4·3H2O in deionized water; final salinity 5 practical salinity units, PSU) contained in 50 ml culture flasks. These culture media were used to foster the growth of protists based on earlier experiences on the enrichment of protists from environmental samples across the Atacama. To foster the growth of bacteria, the food source for the grown protists, one sterile wheat grain was added as carbon source to the cultures. Samples were stored at room temperature and observed once per day using an inverted light microscope.

### 2.3. Isolation of protists and light microscopy

The protist growth was detected after 7–10 days and different morphotypes were isolated from mixed cultures through the liquid aliquot method (LAM) (Butler' and Rogerson, 1995). Aliquots of 1.5 µl of mixed culture were inoculated in 24-well plates filled with 2 ml of liquid media and including one quinoa seed as carbon source. An inverted light microscope (Axiovert S100, Zeiss, Oberkochen, Germany), equipped with phase contrast, was used to observe the cultures and to select cells for clonal cultures. The taxonomy of the isolated phylotypes was based on the eukaryotic classification proposed by Adl et al., (2019).

### 2.4. Electron microscopy

For scanning electron microscopy (SEM), samples were prepared as follows: 5 ml of fresh culture were fixed in 2.5 % glutaraldehyde prepared with the culture medium and cacodylate buffer (final concentration of 0.05 M) for 30 min at 4 °C. Subsequently, 1 % osmium tetroxide was added to the mixture for 10 min at room temperature. The samples were transferred into a filtration unit and filtered using polycarbonate filters of 2 µm pore size and dehydrated washing with increasing ethanol concentrations of 30 %, 50 %, 70 %, 80 %, 90 %, and twice of 96 %, maintaining the samples for 10 min in each ethanol concentration. Following this, a 50/50 hexamethyldisilazane (HMDS)-ethanol solution was added and maintained for 10 min, followed by a washing step with 100 % HMDS for 5 min as an alternative for critical point drying (Nitsche and Arndt, 2008). The samples were investigated morphologically at 20 kV using a scanning electron microscope (SEM; FEI Quanta 250 FEG).

### 2.5. Morphological characterization and protists measurements

An inverted research microscope (Axio-Observer A1, Zeiss, Oberkochen, Germany), equipped with differential interference contrast (DIC) was used for detailed morphological characterization. Allen Video Enhanced Contrast (AVEC) was used consisting of an analogous camera (Hamamatsu C6489) with contrast amplification and noise suppression (Argus-20, Hamamatsu, Japan) connected to the microscope. Pictures were taken from the videos using VirtualDub-1.10.4 and edited with the software ImageJ (version 1.50i). For the size measurements of the protist isolates, AxioVision Rel. 4.8 was used and boxplots were drawn using the obtained measurement data using the ggplot2 package (version 3.4.2) in R (version 4.1.2).

### 2.6. Molecular analysis (DNA extraction, amplification and sequencing)

For DNA extraction, clonal strains were pre-cultivated for 10 days and cells were detached from the culture flask using a cell scraper and concentrated by centrifugation (4000 × g for 20 min at 4 °C, Megafuge 2.0 R. Heraeus Instruments). The DNA was extracted using the QuickgDNA$^{TM}$ MiniPrep kit (Zymo Research, USA). For amplification of the 18S rRNA gene, the primer combination 18S-For (5′ -AACCTGGTTGATCCTGCCAGT- 3′) and 18S-Rev (5′ -TGATCCTTCCGCAGGTTCACCTAC- 3′) was used (Medlin et al., 1988). The PCR reactions were performed using 12 µl of $ddH_2O$, 5 µl of each primer (1 µM), 25 µl of 2 × PCR Master Mix (VWR, Radnor, USA) and 3 µl of the genomic DNA from the isolates. The PCR conditions were set as follow: pre-denaturation at 98° C for 2 min, 35 cycles of 98 °C for 30 s, 55 °C for 45 s and 72 °C for 2 min 30 s; final extension at 72° C for 10 min. For the amplification of the Internal Transcribed Spacer (ITS), the primer combination JITS-For (5′ -GTCTTCGTAGTGAACCTGC- 3′) and JITS-Rev (5′ -CCGCTTACTGATATGCTTAA- 3′) for heteroloboseans (De Jonckheere and Brown, 2005) and 1259F (5′ -GGTCCRGACAYAGTRAGGATTGACAGATTGAAG- 3′) and 28Sr1 (5′ -CGGTACTTGTTCGCTATCGG- 3′) for *Neoheteromita* (Howe et al., 2009) were applied. The amplicons were purified with the FastGene Gel/PCR Extraction Kit, (Fast Gene, Japan) according to the manufacturer's instructions. The samples were Sanger sequenced at Eurofins Scientific using the PCR primers.

### 2.7. Phylogenetic analysis

The 18S rDNA gene sequences of the new strains were used as query in BLAST (www.ncbi.nlm.nih.gov). The closest hits were used as a guide to the latest published phylogeny. The used reference alignments were found in the following publications: For the *Allapsa* and *Neoheteromita* genera, Howe et al., (2009), for the *Spongomonas* genus, Howe et al., (2011), for the *Neocercomonas* genus, Flues et al., (2018), for the *Rhogostoma* genus, Pohl et al., (2021) and for *Allovahlkampfia* genus, the alignment from Geisen et al., (2015), complemented with sequences from Gao et al., (2022). The sequences were imported into BioEdit (Hall, 1999) and aligned using the accessory application ClustalW Multiple alignment (Thompson et al., 2003) with default parameters. The unambiguous alignments were used for the best substitution model selection (GTRIG) under the Akaike Informed Criterion using MrAic (Nylander, 2004). Maximum-likelihood (ML) phylogenies were calculated using RaxML in the Cyberinfrastructure for Phylogenetic Research (CIPRES), (Miller et al., 2010) with 1000 bootstrap replicates each. The Bayesian Posterior probabilities were calculated locally using MrBayes (version 3.2.5) (Ronquist and Huelsenbeck, 2003). The parameters of the likelihood model were set, as suggested by MrAic, to the General time reversible (GTR) model, using a gamma-distributed rate variation across sites and a proportion of invariable sites using 4 rates categories. For the Bayesian analysis, simultaneous Markov chains Monte Carlo

(mcmc) were run for 1.000.000 generations (nchains = 4) and trees were sampled every 10 generations using a burning number of 25 % of the generations. FigTree version 1.4.4 was used to view resulting trees and the final layout was prepared with Adobe Illustrator version 27.7 (Adobe Systems, USA).

## 3. Results

### 3.1. Light microscopy and SEM

We recovered five different protist morphotypes from the phyllosphere of the studied cacti which were all observed and measured by light microscopy, while it was possible to obtain SEM pictures only for the strains HFCC838, 856, 860 and 858 (Fig. 2 and Fig. 3). Out of the total six strains, three are heterotrophic biflagellates bearing a beating anterior flagellum and gliding on the substratum by adhering to the long posterior flagellum, typical features of cercomonads (HFCC838, 857 and 858). One is a biflagellated imbraicatean (HFCC856) and one a testate amoeba (HFCC860). In addition, one heterolobose amoebae (HFCC979) was isolated from the soil surrounding the cactus *Browningia candelaris*.

### 3.2. Strain HFCC838

**Description**: Rounded semi-rigid cells of 2.4–3.9 µm length and 1.9–2.8 µm width (Fig. 4F), often elongated according to the position of the cell body due to spasms during locomotion. Presence of one anterior flagellum of 1.4–2.8 µm and one non-acronematic posterior flagellum (4.4—9.1 µm) laying alongside the cell, trailing passively behind exhibiting curls depending on its attachment to the substrate (Fig. 3H, I, J, K and L). Strain grown in WC medium.

### 3.3. Strain HFCC857

**Description**: Elongated biflagellate cell of 3.8–7.2 µm in length and 1.8–3.1 µm in width. Anterior flagellum of 3.4—8.6 µm and posterior flagellum of 7.7–11.1 µm in length (Fig. 4D). Nucleus of ca. 0.5 µm. Twists during locomotion lead to a curvature of the cell approximately in the middle of the cell body; Cytoplasm is granular (Fig. 3D, E, F and G). Strain grown in WC medium.

### 3.4. Strain HFCC858

**Description**: Cell body of 10.6–21.4 µm in length and 6.8–12.4 µm in width and a nucleus of 1.3–2.4 µm diameter (Fig. 4E). The cell shape appears to be ovoid with exceptions during the locomotion of the cell which appears to change the shape to an irregular, rather triangular form. The cell exhibits one jiggling anterior flagellum of 11.8–23.7 µm and one passive posterior flagellum, 15.9–33.7 µm. The strain produces cysts of ca. 10 µm (Fig. 3B). The cell moves gliding on the substrate originating a tail at the back end of the cell body, changing the cell shape (Fig. 3A and C). The cytoplasm is rich in granules. Strain grown in WC medium.

### 3.5. Strain HFCC860

**Description**: Thecate amoeba with a spherical and slightly flattened hyaline test, measuring 9.4–14.6 µm in length and 7.9–11.2 µm in width (Fig. 4B) exhibiting lamellipodia. The aperture for prey ingestion is about 3.6–5.4 µm in diameter. Food vacuoles containing prey items as well as contractile vacuoles are observed (Fig. 2F, G, H, I, J, K and L). Strain grown in WC medium.

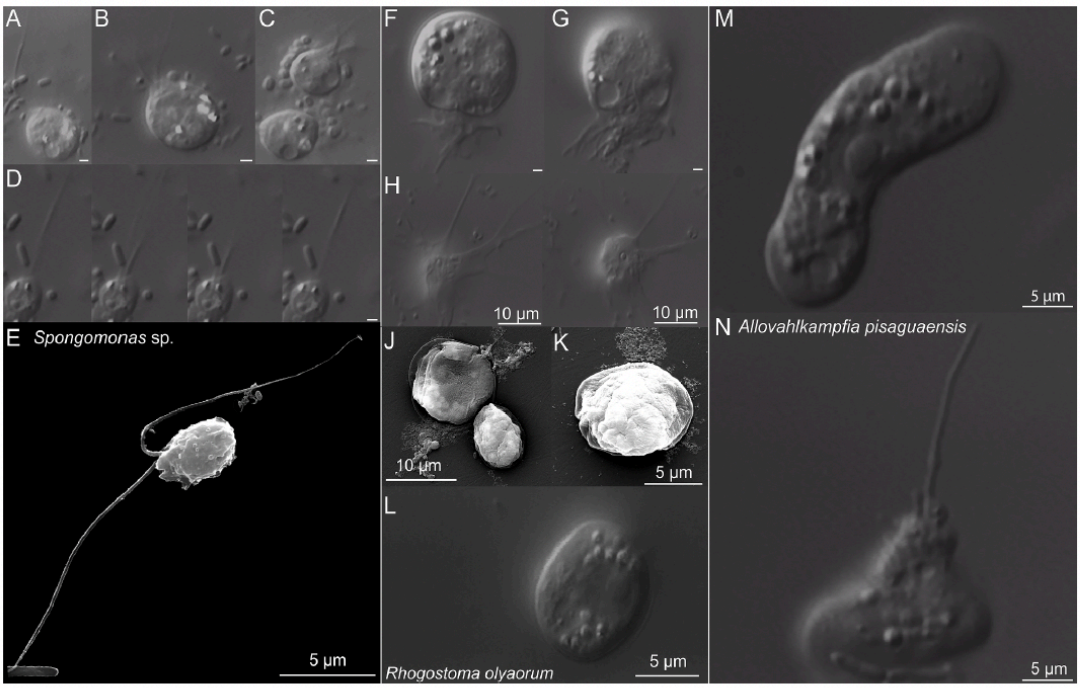


**Fig. 2.** Micrographs of the protist species isolated from *Browningia candelaris*. A-E: Light microscopy and electron micrographs of *Spongomonas* sp. F-L: Light and electron micrographs of *Rhogostoma olyaorum*. M–N: Light micrographs of *Allovahlkampfia pisaguaensis*. Scale bar size represent 1 µm unless specified.

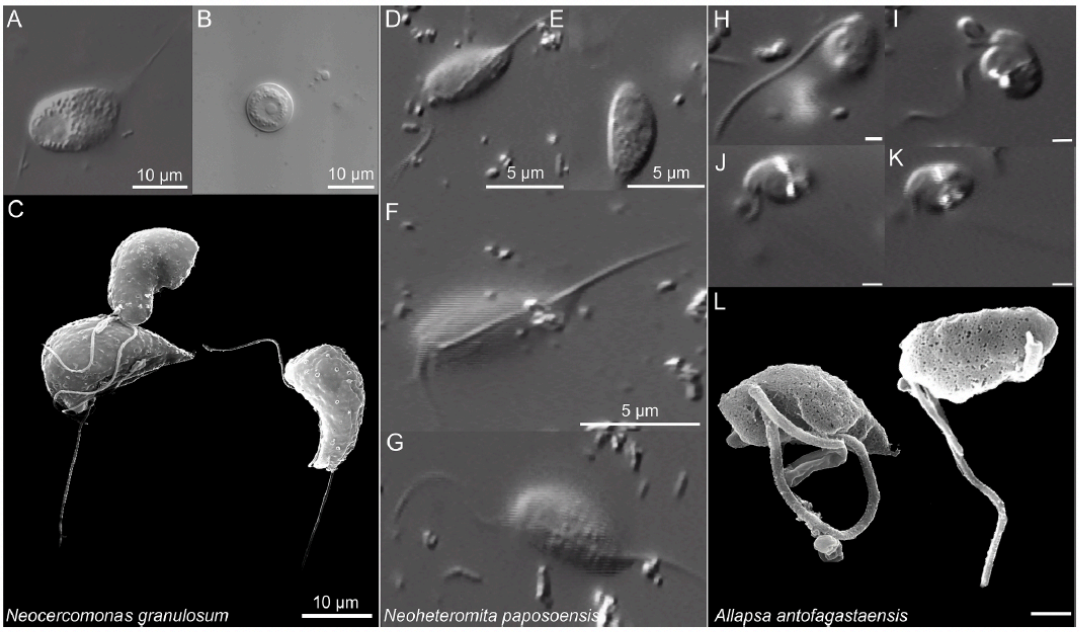


**Fig. 3.** Micrographs of the protist species isolated from *Eulychnia taltalensis*. A-C: Light and electron micrographs of *Neocercomonas granulosum*. D-G: Light and electron micrographs of *Neoheteromita paposoensis*. H-L: Light and electron micrographs of *Allapsa antofagastaensis*. Scale bar size represent 1 µm unless specified.

### 3.6. Strain HFCC856

**Description**: Spherical cells with irregular margin of 4.4–6.5 µm length and 2.7–5.5 µm width, with two naked heterodynamic flagella of roughly equal size (10.0–12.4 µm) emerging from a small protuberance resembling a flagellar pocket (Fig. 4A). The two flagella appear to be stiffed and curved during beating and in constant movement (Fig. 2A, B, C, D and E). The isolate displayed stationary as well as swimming forms in cultures. Strain grown in WC medium.

### 3.7. Strain HFCC979

**Description**: Heterolobose amoeba exhibiting pseudopodia originated by eruptive locomotion of the limax (Fig. 2M). Trophozoite length between 5.4 and 26.6 µm and width between 4.4 and 13.4 µm and a nucleus diameter of 0.9–4.4 µm (Fig. 4C). In culture, cells tend to encyst after 72–96 h. Some individuals exhibit the formation of uroidal filaments. At the back part of the cell these filaments attach to the substrata until the distance limit of up to ca. 10 µm (Fig. 2N). No flagellated stage was observed. Strain grown in Schmaltz-Pratt medium in a concentration of 5 PSU.

### 3.8. Phylogenetic results

The phylogeny inferred using maximum likelihood (ML) and Bayesian analysis (Bayesian inference posterior probabilities, biPP) of the studied 18S rRNA gene sequences show a similar topology. Four isolates cluster together with cercozoans from the monadofilose groups with high support (mlBP 89 %, biPP 1.00, Fig. 5). The isolate HFCC838 has a p-distance of 3.96 % to *Allapsa vibrans* (AF411265), both strains branch together with high support (100 % mlBP, biPP 1.00) in the major clade including species of the *Allapsa* genus (100 % mlBP, biPP 1.00). The isolate HFCC856, has a p-distance of 0.54 % to *Spongomonas solitaria*, both sharing a clade (71 % mlBP, biPP 1.00), clustering within the highly supported *Spongomonas* genus clade (Imbricatea) (100 % mlBP, biPP 1.00). The isolate HFCC857 has a p-distance of 1.3 % to *Neoheteromita hederae* (EU709211) and is part of the monophyletic *Neoheteromita* genus clustering within the Sandonidae family including other genera as *Sandona*, *Flectomonas* and *Teretomonas*. Additionally, the p-distance of HFCC857 and its closest relative *N. hederae* based on the ITS2 region is 4 %, sharing both a branch in the ITS phylogeny in a highly supported clade (100 % mlBP) (Fig. 8). The isolate HFCC858 has a p-distance of 1.96 % to *Neocercomonas epiphylla* (MG775605), part of the Cercomonadidae family, clustering together in a moderately supported clade (70 % mlBP, biPP 0.90). The last cercozoan strain we recovered is HFCC860 which has a p-distance of 0.36 % to *Rhogostoma tahiri* (Öztoprak et al., 2020), both strains cluster together in a moderately supported clade (76 % mlBP, biPP 1.00) of the *Rhogostoma* genus (mlBP 99 %, biPP 1.00) (Fig. 6). The sequence of the isolate HFCC979 is placed within the *Allovahlkampfia* genus (Acrasida), together with other species isolated from soils (Geisen et al., 2015). The closest species relative to HFCC979 within the acrasids according to our phylogeny is *Allovahlkampfia spelaea* (p-distance 1.03 %) with moderate support (80 % mlBP, biPP 1.00) (Fig. 7). Furthermore, the p-distance between HFCC979 and its closest relative, *A. spelaea* based on the ITS region is 6 %, clustering in a highly supported clade (90 % mlBP) (Fig. 9), away from the groups number 2 and 3 according to Gao et al. (2022) for this genus considering the whole ITS sequence.

### 3.9. Taxonomic summary

Genus *Allapsa* Howe & Cavalier-Smith 2009
***Allapsa antofagastaensis*** sp. nov. Acosta, Nitsche et Arndt
**Diagnosis**: Free-living, rounded-elongated biflagellate exhibiting one dynamic (beating) anterior flagellum (AF) of ca. 2 µm and one posterior flagellum (PF) of ca. 7 µm gliding passively alongside the cell body as described for other members of the genus *Allapsa*.
**Etymology**: The species name refers to the sampling area (Antofagasta Region) of the Atacama Desert, origin of the sampled host cactus *Eulychnia taltalensis*.

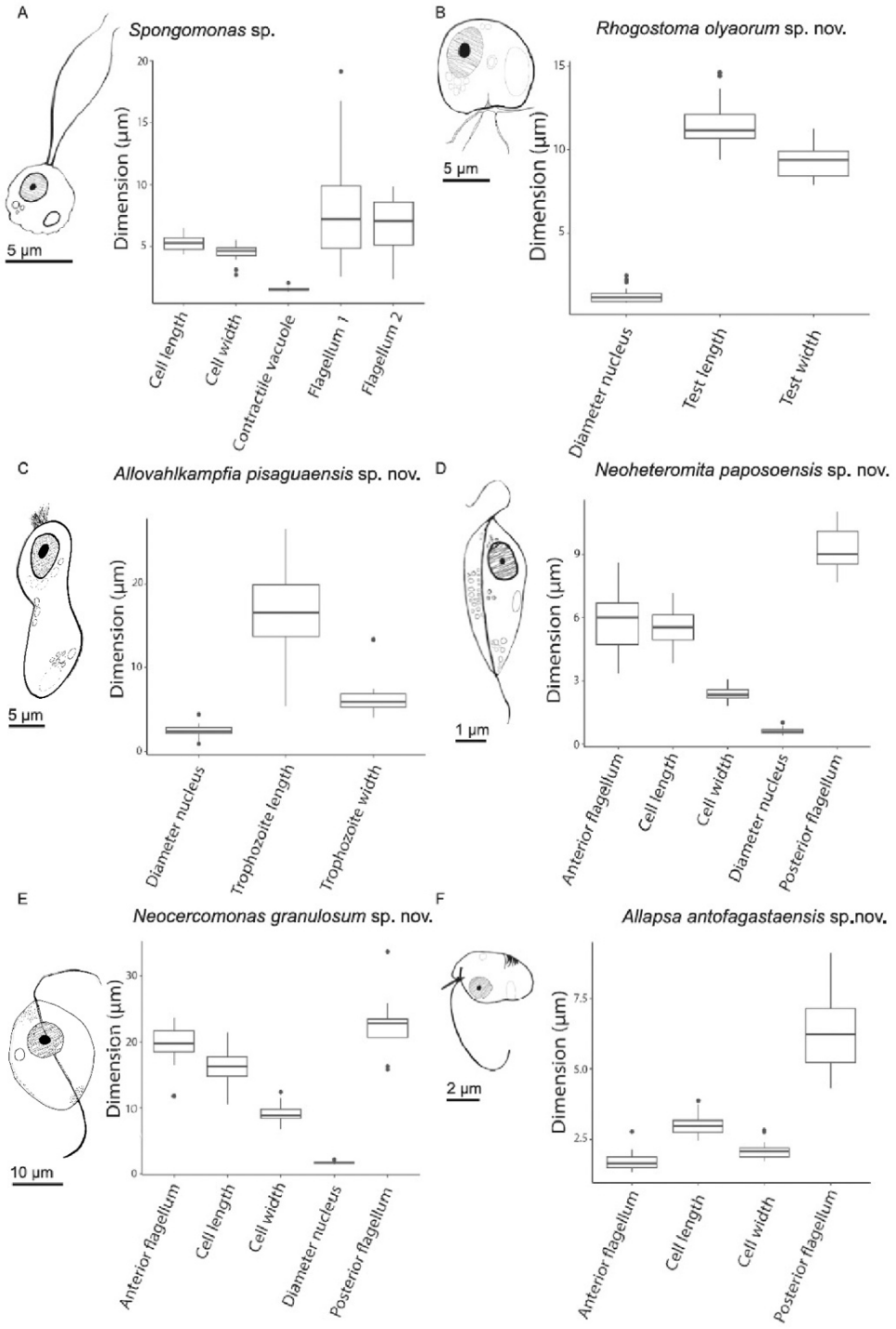


**Fig. 4.** Boxplots showing the morphological measurements of the new species obtained during this study. Under the letter identifying each boxplot a drawing of the species is included. Number of observations n = 25.

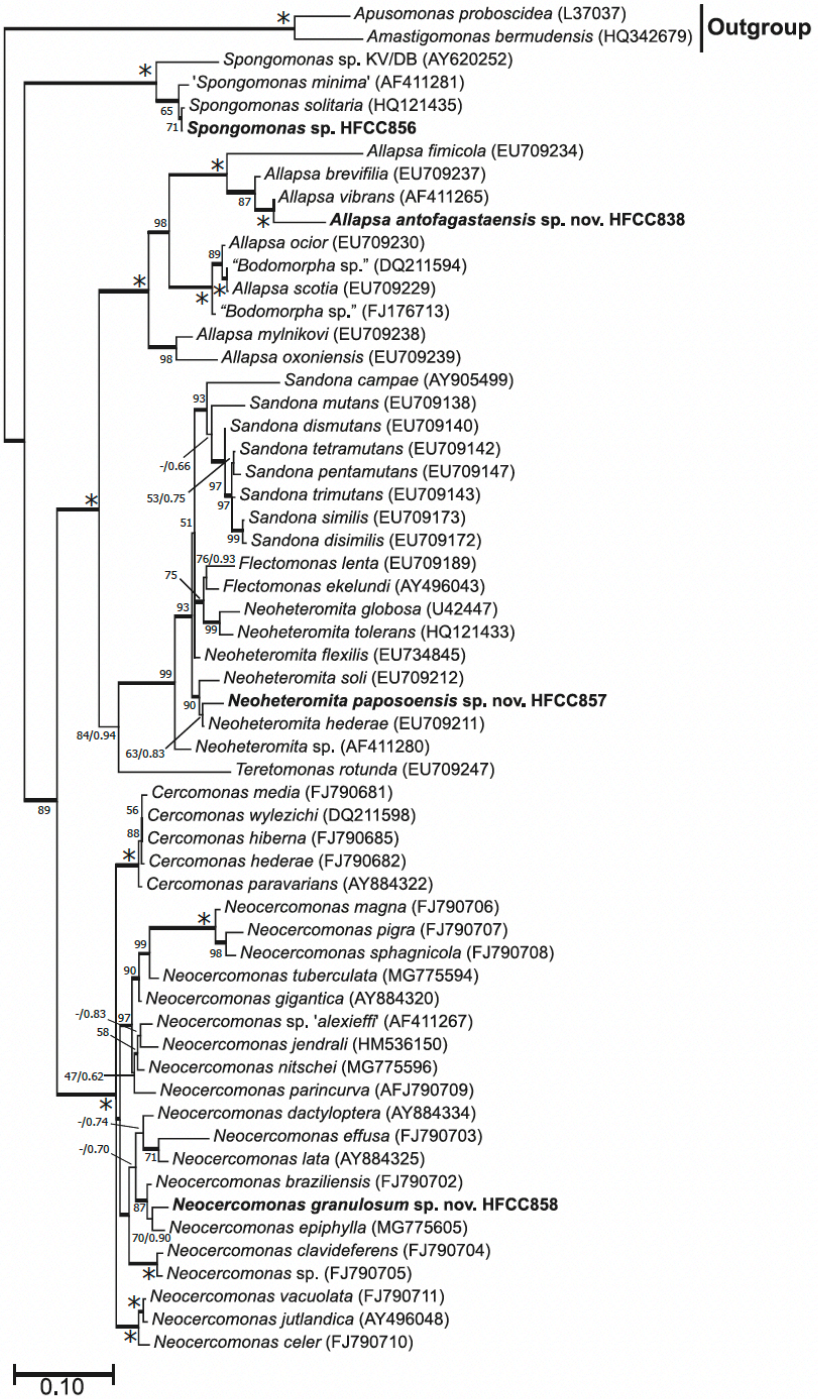


**Fig. 5.** Phylogenetic analysis, based on a manual corrected alignment of 1973 nucleotides, of the reference cercozoans and HFCC838, HFCC856, HFCC857 and HFCC858. Maximum likelihood tree shown for the analysis (ML: GTR + Γ model). A Bayesian Inference (BI) using the same model yielded a similar topology. Numbers on branches represent ML bootstrap percentages (100 replicates); asterisks indicate bootstrap percentages of 100 %; values lower than 50 % are not shown. Nodes supported by Bayesian posterior probabilities (MrBayes) ≥ 0.95 are shown by thick lines. Scale bar represents 0.10 expected substitution/site in the ML analysis. The names of the isolated strains in this study are highlighted in bold.

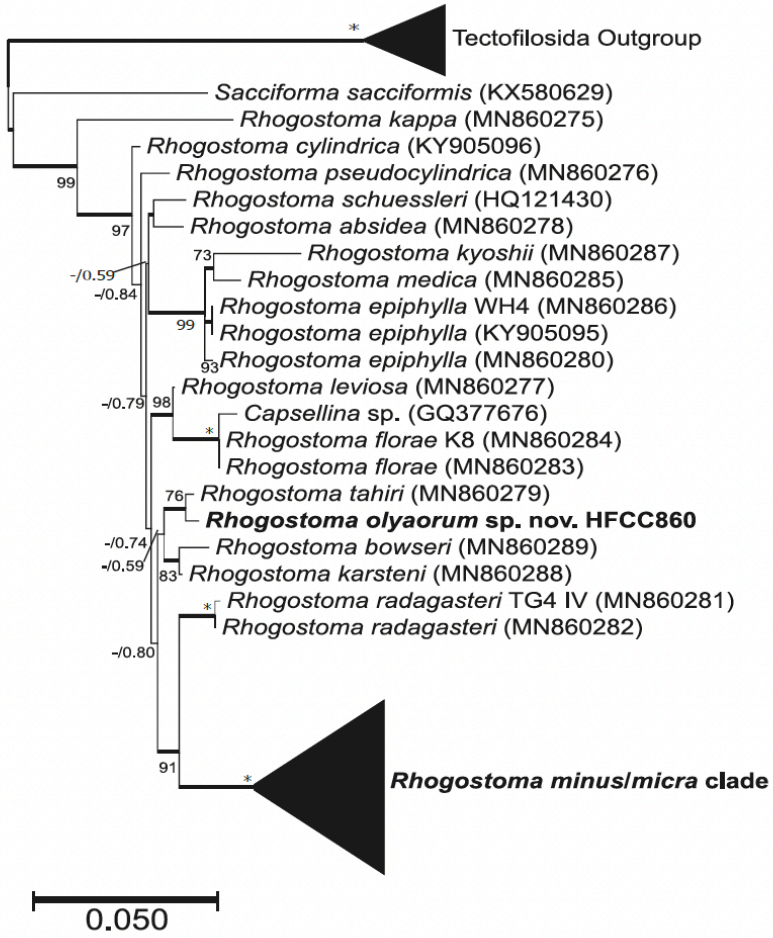


**Fig. 6.** Phylogenetic analysis, based on a manual corrected alignment of 1870 nucleotides, of the reference tectofilosea amoebae and HFCC860. Maximum likelihood tree shown for the analysis (ML: GTR + Γ model). A Bayesian Inference (BI) under the same model yielded a similar topology. Numbers on branches represent ML bootstrap percentages (100 replicates); asterisks indicate bootstrap percentages of 100 %; values lower than 50 % are not shown. Nodes supported by Bayesian posterior probabilities (MrBayes) ≥ 0.95 are shown by thick lines. Scale bar represents 0.050 expected substitution/site in the ML analysis. The names of the isolated strains in this study are highlighted in bold.

**Holotype**: Type of *Allapsa antofagastaensis* sp. nov. is shown in Fig. 3H.
**Type strain**: Heterotrophic Flagellate Collection Cologne (HFCC) strain 838.
**Type sequence data**: The 18S rDNA gene sequence of HFCC838 has the accession number OR731268 in NCBI.
**Type locality:** Isolate originated from the culture enrichment of a spine of one specimen cacti *Eulychnia taltalensis*, found at Paposo, Chile.
**Zoobank registration:** urn:lsid:zoobank.org:act:DC0B1CF9-AAD7-47E2-BCAF-D3D561259BCC

**Remarks**: *Allapsa antofagastaensis* sp. nov. cells reach only half the length of its closest relative *Allapsa vibrans* (p-distance of 18S rRNA = 3.96 %; AF411265). However, the cell features as shape and proportion of length of flagella with respect to cell length appear to be similar to *A. vibrans*. As no ecological data are available for *A. vibrans* (species of unknown locality and isolation date), comparisons regarding their habitats are not possible. Other species described by Howe et al. (2009), overlap slightly in size with *A. antofagastaensis,* but differ significantly regarding their 18S rRNA (p-distance to *A. oxoniensis* = 11.6 % and to *A. mylnikovi* = 8.05 %)

Genus *Neoheteromita* Howe, Bass, Vickermann, Chao and Cavalier-Smith 2009
***Neoheteromita paposoensis*** sp. nov. Acosta, Nitsche et Arndt
**Diagnosis**: Elongated gliding cells with two flagella. Anterior flagellum is beating, catching prey items and particles, posterior flagellum trails below the cell. The cell moves in pulses, shaking during glide on the substrate and eventually changing slightly the cell shape while twisting.
**Etymology**: Species named after the locality Paposo, Chile, where the species was found on the cactus *Eulychnia taltalensis*.
**Holotype**: Type of *Neoheteromita paposoensis* sp. nov. is shown in Fig. 3D.
**Type Strain**: Heterotrophic Flagellate Collection Cologne (HFCC) strain 857.
**Type sequence data**: The 18S rDNA gene sequence of HFCC857 has the accession number OR731273 in NCBI.

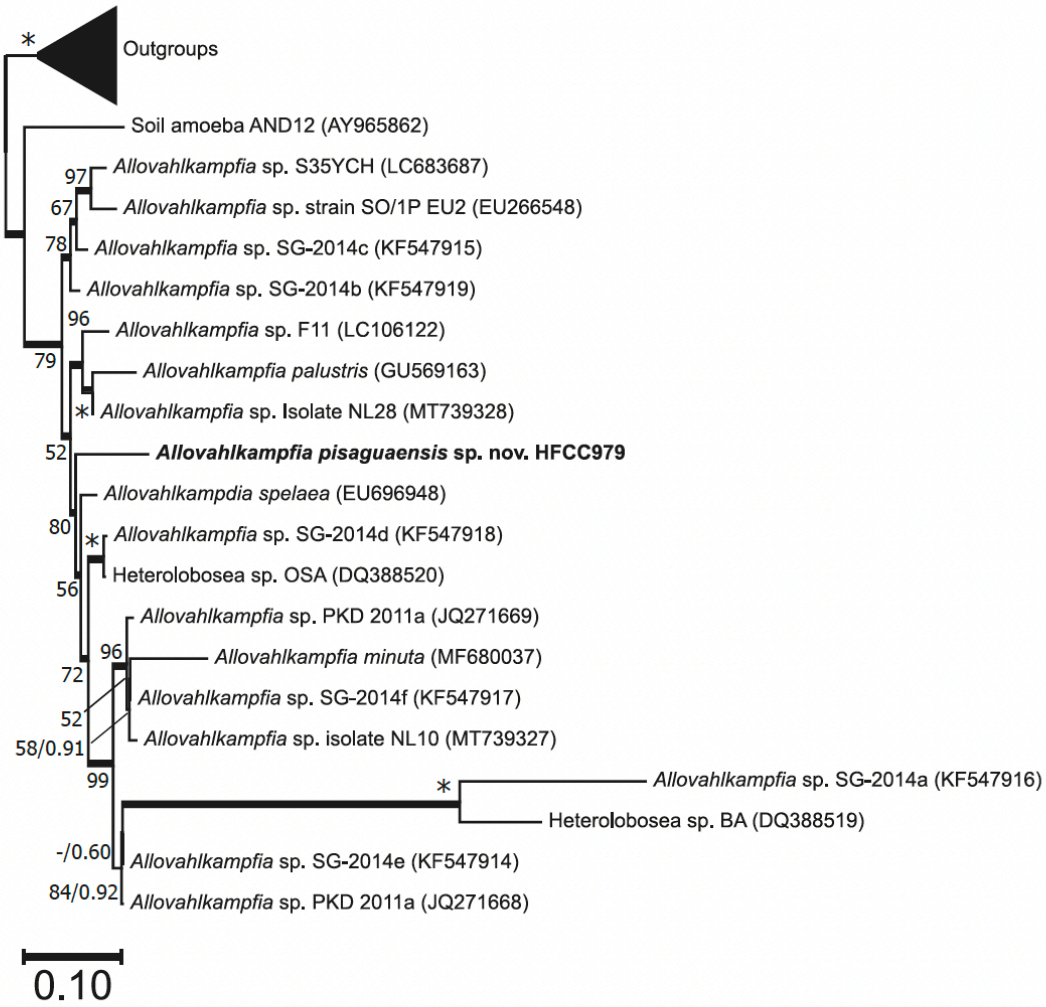


**Fig. 7.** Phylogenetic analysis, based on a manual corrected alignment of 2238 nucleotides, of the reference heterolobosean and HFCC979. Maximum likelihood tree shown for the analysis (ML: GTR + Γ model). A Bayesian Inference (BI) under the same model yielded a similar topology. The numbers on branches represent ML bootstrap percentages (100 replicates); asterisks indicate bootstrap percentages of 100 %; values lower than 50 % are not shown. Nodes supported by Bayesian posterior probabilities (MrBayes) ≥ 0.95 are shown by thick lines. Scale bar represents 0.10 expected substitution/site in the ML analysis. The names of the isolated strains in this study are highlighted in bold.

**Type locality**: The species was found after the enrichment of a spine of the cactus *Eulychnia* sp., found at Paposo, Chile.

**ZooBank registration:** urn:lsid:zoobank.org:act:20CFDE54-8831-4ABA-8E3B-90045521D451

**Remarks**: *Neoheteromita paposoensis* sp. nov. shares morphocharacters of this genus with other described species, including an elongated rigid cell body and exhibiting slight changes in shape during locomotion (gliding). *N. paposoensis* sp. nov. is closely related to *N. hederae* (p-distance = 1.3 %, EU709211) isolated from ivy leaf surface, however, *N. paposoensis* sp. nov. is smaller in cell length (3.8–7.2 μm vs. 6.5–10 μm). Other species slightly overlap in size with *N. paposoensis* sp. nov. but differ significantly regarding their 18S rRNA (p-distance to *N. caudratti* = 3.07 % and to *N. globosa* = 2.48 %). Other species of this genus reach larger cell lengths. *N. paposoensis* has a p-distance of 4 % to *N. hederae* based on the ITS2 sequences.

Family Cercomonadidae Kent 1880, sensu Karpov et al. 2006

Genus *Neocercomonas* (Ekelund et al., 2004)

***Neocercomonas granulosum*** sp. nov. Acosta, Nitsche et Arndt.

**Diagnosis**: Ameboid cells, exhibiting rounded-oval shapes. Cell body with two flagella emerging from the frontal end of the cell body. One anterior flagellum beating towards both sides of the cell body and one posterior flagellum below the cell used for gliding. Contains granules dispersed across cell plasm.

**Etymology**: Species name "granulosum" (Latin), referring to the notable granules across the cytoplasm.

**Holotype**: Type of *Neocercomonas granulosum* sp. nov. is shown in Fig. 3A.

**Type Strain**: Heterotrophic Flagellate Collection Cologne (HFCC) strain 858.

**Type sequence data**: The 18S rRNA gene sequence of HFCC858 has the accession number OR731277 in NCBI.

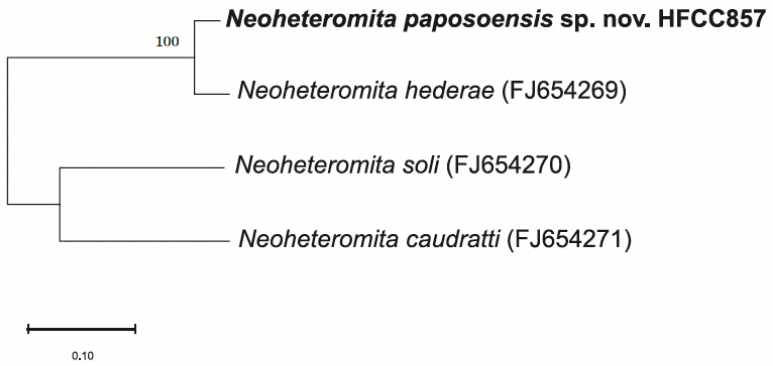


**Fig. 8.** Maximum likelihood unrooted tree shown for the analysis (ML: GTR + Γ model) for the ITS2 sequence in the genus *Neoheteromita*. The numbers on branches represent ML bootstrap percentages (100 replicates). Scale bar represents 0.10 expected substitution/site in the ML analysis. The name of the isolated strain in this study is highlighted in bold.

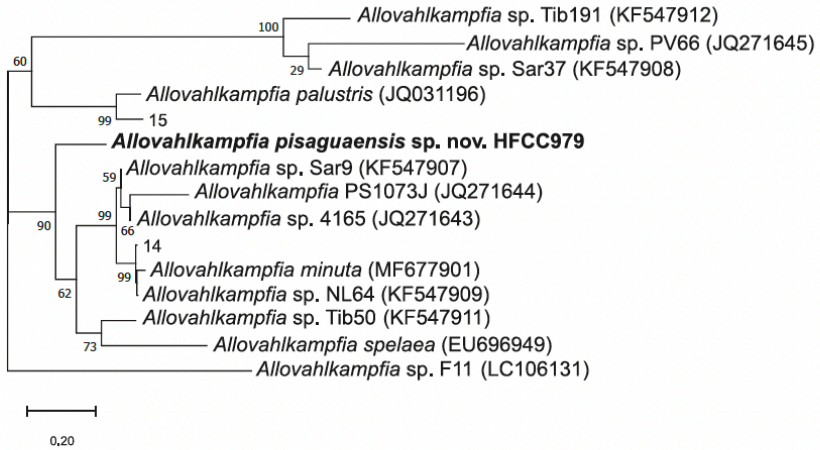


**Fig. 9.** Maximum likelihood unrooted tree shown for the analysis (ML: GTR + Γ model) for the entire ITS sequence in the genus *Allovahlkampfia*. The numbers on branches represent ML bootstrap percentages (100 replicates). Scale bar represents 0.20 expected substitution/site in the ML analysis. The name of the isolated strain in this study is highlighted in bold.

**Type locality**: This isolate was found in an enrichment culture of a spine of the cactus *Eulychnia* sp., found at the locality of Paposo, Chile.
**ZooBank registration:** urn:lsid:zoobank.org:act:8F974FC4-4A65-4F9C-BF50-4F8524C52EE7

**Remarks:** *Neocercomonas granulosum* sp. nov. is closely related only to *N. epiphylla* (p-distance = 1.96 %, MG775605), all other described species are even more genetically distant based on 18S rRNA. *N. granulosum* sp. nov. is smaller than *N. epiphylla* (10.6–21.4 μm vs. 17.3–25.8 μm), *N. epiphylla* was recorded from *Plantago* leaves from the boreal region (Flues et al., 2018). Both species share cellular features as shape and locomotion as well as a granule-rich cell plasma. Strain forming cysts of approx. 10 μm, slightly smaller than those observed for *N. epiphylla* (11 μm).

Genus *Rhogostoma* Belar 1921
***Rhogostoma olyaorum*** sp. nov. Acosta, Nitsche et Arndt
**Diagnosis**: Amoebae exhibiting a hyaline spherical test with a smooth surface positioned on lamellipodia originating from the basal side of the test and branching as pseudopodia. Protoplast moves attached to the substrate enlarging and changing shape and size of the filopodia from the lamellar structure (up to 8 μm). The nucleus is approximately 4 μm in diameter, displaying a hyaline nucleoplasm and a granular nucleolus. Bacterial preys are engulfed though the filopodia and ingested entering through the aperture.
**Etymology**: Species-group named "*olyaorum*" after Julia Oly and her family who gave great support to Eduardo Acosta S., during his stay in Germany.
**Holotype**: The type of *Rhogostoma olyaorum* sp. nov. is shown in Fig. 2G
**Type Strain**: Heterotrophic Flagellate Collection Cologne (HFCC) strain 860
**Type sequence data**: The 18S rRNA gene sequence of HFCC860 has the accession number OR775727 in NCBI.
**Type locality**: The isolate HFCC860 was obtained from an enrichment culture of a spine of the cactus *Browningia candelaris*
**ZooBank registration:** urn:lsid:zoobank.org:act:B917BC62-C256-4AFD-B052-863E4CB72325

**Remarks**: *Rhogostoma olyaorum* sp. nov. is closely related to *R. tahiri* (p-distance = 0.36 %, MN860279) which cluster together in a monophyletic clade in the presented Rhogostomidae phylogeny. Öztoprak et al. (2020) described the test, the cell length to width

ratio and the length of the invagination of the aperture as useful characters for species delimitation. These traits differed between the two *Rhogostoma* species as *R. tahiri* isolated from the phyllosphere in Europe (Cologne, Germany), exhibit a more cylindrical shape, while *R. olyaorum* isolated from a cactus in the South America (Atacama, Chile) appears to be slightly more elliptic. Additionally, the cell length to width ratio of *R. olyaorum* is 1.22 (SD = 0.1), which is significantly larger than this ratio for the closest relative, *R. tahiri* is 1.08 (SD = 0.09) ($p < 0.05$) and furthermore, the invagination of the aperture for *R. olyaorum* has a mean value of 4.48 (SD = 0.52), which is significantly different to the one from *R. tahiri*, 2.38 (SD = 0.64) ($p < 0.05$).

Genus *Spongomonas* Stein 1878

***Spongomonas*** sp.

**Diagnosis**: Mostly sessile, spherical cells attached to the substrate exhibiting two heterodynamic flagella of apparent equal size, highly acronematic. Single cells have irregular shape and are often covered with particles of detritus and bacteria.

**Strain**: Heterotrophic Flagellate Collection Cologne strain 856

**Strain sequence data**: The 18S rRNA gene sequence of HFCC856 has the accession number OR731270 in NCBI.

**Locality**: The strain was isolated in enrichment culture of a spine of the endemic cactus *Browningia candelaris* at Quebrada Aroma, Chile.

**ZooBank registration**: urn:lsid:zoobank.org:act:0B47406E-A8E9-43DF-90B0-F6093ED93401

**Remarks**: *Spongomonas* sp. differs in its 18S rRNA gene to both related species *S. solitaria* (p-distance = 0.54 %) and *S. minima* (p-distance = 0.36 %). The cell length of the strain *Spongomonas* sp. is slightly smaller when compared to the closest relative *S. solitaria* (4.4–6.5 μm vs. 4–9 μm). All these species share the rigid membrane exhibiting an irregular-circular cocoon shape with two heterodynamic flagella.

Genus *Allovahlkampfia* Walochnik et Mulec 2009, emend. Geisen 2015

***Allovahlkampfia pisaguaensis*** sp. nov. Acosta, Fincke, Nitsche et Arndt

**Diagnosis**: Amoebae with eruptive formation of pseudopodia from the limax stage displaying the typical shape of heterolobose amoebae. Cells measure about 15 μm in length, with a nucleus and digestive vacuoles. Uroidal filaments are formed at the end of the cell after locomotion and are difficult to capture through light microscopy recordings.

**Etymology**: Species name given after the Pisagua Internment Camp and the grave of political prisoners and missing persons in the region of the sampling site.

**Holotype**: The type of *Allovahlkampfia pisaguaensis* sp. nov. is shown in Fig. 2M.

**Type Strain**: Heterotrophic Flagellate Collection Cologne (HFCC) strain 979.

**Type sequence data**: The 18S rRNA gene sequence of HFCC979 has the accession number OR750521 in NCBI.

**Type locality**: Strain found after the enrichment of soil samples from the rhizosphere of the cactus *Browningia candelaris* at Quebrada Aroma, Tarapacá, Chile.

**ZooBank registration**: urn:lsid:zoobank.org:act:BB2349F7-5BFE-439B-A9E1-9730C843C150

**Remarks:** *Allovahlkampfia pisaguaensis* sp. nov. isolated from cactus soil is part of a clade including the closest relative *A. spelaea* as well as *A. minuta* and various other allovahlkampfiid strains isolated from soils. *A. pisaguaensis* sp. nov. significantly differs in its 18S rRNA gene to both related species (p-distance to *A. spelaea* = 1.03 % and to *A. minuta* 7.33 %). The p-distance of *A. pisaguaensis* sp. nov. to its closest relative (*A. spelaea*) based on the ITS region is 6 %. The cell length of *A. pisaguaensis* sp. nov. has a wide range, reaching sizes even smaller than *A. minuta*.

## 4. Discussion

In this study we aimed to contribute to the knowledge on protist diversity inhabiting xeric plants and the surrounding soil, describing new species from diverse protist clades found on two endemic cacti across the Atacama Desert. We isolated two new cercozoans from cactus spines, one belonging to the class Imbricatea, one to Thecofilosea and one heterolobose amoeba species from the soil of the columnar cacti *B. candelaris* from high altitudes (Fig. 1A). On the other hand, we isolated three new cercozoans of two main clades, Glissomonadida and Cercomonadida, from the spines of the shrubby cactus *Eulychnia taltalensis*, inhabiting a fog oasis in the Coastal Cordillera (Fig. 1B). By enrichment and isolation of protists phylotypes, we detected cercozoan clades known to have close relation to the plant microbiome and their surrounding soils (Howe et al., 2009, 2011; Flues et al., 2018; Dumack et al., 2017a; Gao et al., 2022). Our results underline the benefits of classical cultivation techniques complemented by molecular identification of protists in order to investigate the protist diversity that might play a functional role in the plant microbiome. By sequencing the complete 18S rRNA gene, we aimed to obtain the hypervariable regions V4 and V9 for identifying taxonomic units in further metabarcoding studies in the Atacama Desert.

We isolated a new strain of *Spongomonas* (Imbricatea), a genus that was initially observed by Stein (1878). Such morphotypes were described to form colonies on particles or substrate, while the new strain of *Spongomonas* sp. was rarely observed in colonies, occasionally sharing space on substrata. Based on molecular data, our spongomonad phylotype, isolated from the cactus *B. candelaris*, clusters together with *S. solitaria*, found and isolated from a dried freshwater creek sediment in North America (Arizona, USA) (Howe et al., 2011). The species *Spongomonas solitaria* was the first type strain of this genus classified as Cercozoa based on molecular information (18S rRNA). The similar morphological characters shared between species of *Spongomonas* makes it difficult to distinguish the different species based on features observed by light microscopy. Phylogeny based on the rDNA has led to the disentanglement within this group (Howe et al., 2011). Morphologically, our isolate is slightly smaller than *S. solitaria* and the p-distance between the two isolates is relatively low (0.36 %). The p-distances to *S. minima* and *Spongomonas* sp. are even larger, 1.2 and 6.5 %, respectively (Fig. 2). Overall, the formally described species appear to be closely related, with exception of the strain *Spongomonas* sp. KV/DB. Thus, further isolation of phylotypes could help to better resolve the phylogeny within this group.

Another protist phylotype we retrieved from the cactus *Eulychnia taltalensis* is *Allapsa antofagastaensis* sp. nov. The genus *Allapsa* (Glissomonadida) include the type strain *Allapsa vibrans* from which the locality and the isolation date are unknown, nonetheless, other members of this genus (up to six species) were registered as isolated from Europe and Russia (Howe et al., 2009). The p-distance (3.96 %) between *A. antofagastaensis* sp. nov. and *A. vibrans* is high (Fig. 4) and moreover, *A. vibrans* (4–6.5 μm) is about double the size of our isolate (2.4–3.9 μm). The genus *Neoheteromita*, known to inhabit soils and the phylloplane (Flues et al., 2018), is also a glissomonad and sister clade of the genus *Allapsa*. To characterize the phylogenetic position of our new *Neoheteromita* species, we included representatives of the genera *Flectomonas*, *Teretomonas* and *Sandona*, all members of the family Sandonidae, due to their close relation and low morphological diversity (Fig. 5). Within this group, our new phylotype *Neoheteromita paposoensis* sp. nov. isolated from *Eulychnia taltalensis* has a p-distance of 1.3 % to *N. hederae*, the closest relative in our 18S rRNA phylogeny which is significantly larger in size. To obtain a higher resolution for species delimitation, we sequenced the ITS2 region of rRNA, observing a great p-distance of 4 % to *N. hederae* (Fig. 8), supporting the description of *N. paposoensis* as a

new species. The genera *Allapsa* and *Neoheteromita* have been suggested to be an integral part of the phyllosphere microbiome (Flues et al., 2018). Probably, due to morphological differences in the feeding apparatus, these two glissomonad genera have a specific impact on the composition and abundance of the bacterial communities, as suggested for a variety of other cercozoan strains including *Allapsa* and *Sandona* (Glücksman et al., 2010).

Bacterivorous amoeboflagellates as the family Cercomonadidae include soil inhabitants and active plant-microbiome predators which received increasing attention in recent ecological studies to understand their function in the rhizosphere and phyllosphere (Flues et al., 2018). The closest relative to our isolate *Neocercomonas granulosum* sp. nov. is the phyllosphere inhabitant *N. epiphylla* with a p-distance of 1.96 % to the new species and being larger with up to 25.8 µm vs. up to 21.4 µm cell length of *N. granulosum* sp. nov. The genus *Neocercomonas* has been consistently detected in the phyllosphere of *Plantago*, *Trifolium* sp., and *Arabidopsis thaliana*, feeding on bacteria (Flues et al., 2018, Sapp et al., 2018; Ploch et al., 2016). With the identification of *Neocercomonas granulosum* sp. nov., we contribute to disentangle branching patterns between closely related species by providing a new 18S rRNA gene sequence and morphological data of this genus from remote desertic cacti. *N. granulosum* sp. nov. forms cysts of spherical shape with a mean size of 10 µm (N = 25) (Fig. 3B), smaller than those produced by *N. epiphylla* (11 µm).

Within the Cercozoa, the thecofilosean amoebae as those from the family Rhogostomidae have been found in a variety of natural and human-based environments ranging from soils and even the phyllosphere to activated sludge (Dumack et al., 2017b; Öztoprak et al., 2020; Pohl et al., 2021). Our isolate *Rhogostoma olyaorum* sp. nov. has a 0.36 % p-distance to its closest relative *R. tahiri.* These species overlap in test sizes ranging from 9.6 to 12.6 µm but exhibit a slightly different shape. As already shown by Öztoprak et al. (2020), *Rhogostoma* phylogeny does not appear to form species radiation according to their habitat (i.e., freshwater, soil, phyllosphere) or either have a morphological trait to give advantage to persist in inhospitable conditions. Interestingly, we found a difference that could shed light in speciation reflected in the morphology. The varying cell length to width ratio, suggested as a morphological trait that could be used in species delimitation (Öztoprak et al., 2020), supports the status of *R. olyaorum* as a new species. Our isolate cluster in a moderately ML bootstrap-supported clade (Fig. 6) which is fully supported by Bayesian posterior probability. Thus, we report the occurrence of the genus *Rhogostoma* from an additional harsh habitat as the Atacama Desert for the first time.

The new isolate *Allovahlkampfia pisaguaensis* sp. nov. belongs to the diverse heterolobose amoebae known from soil microbiota (Geisen et al., 2015; Gao et al., 2022). Other vahlkampfiid species were also detected in vegetation-covered soils in deserts (Pérez-Juárez et al., 2018). Our new isolate shares a clade in our inferred phylogeny of allovahlkampfiids with the closest relative *A. spelaea*, an inhabitant of cave soils with a p-distance of 1.03 % which is much larger (up to 40 µm) compared to our isolate *A. pisaguaensis* sp. nov. (up to 26.6 µm). The high p-distance and the phylogenetic pattern found in our phylogeny show a clear separated clade of *A. pisaguaensis* sp. nov. from the type strain *A. spelaea* and other *Allovahlkampfia* species for the 18S and ITS sequences (Figs. 6 and 9).

The hypervariable region V9 (alternatively V4) of the 18S rRNA gene is frequently used as a diversity marker in the metabarcoding of protists due to the high variability and short sequence length (Choi and Park, 2020). We successfully obtained the hypervariable region V9 from all isolates, except for *Allapsa antofagastaensis*, and made it available for taxonomy assignation in further metabarcoding and metagenomic projects.

## 5. Conclusion

In the present culture-dependent approach, we described new species of bacterivorous protists found in an extreme environment. Our phylogenetic studies confirm the novelty of strains isolated from cacti of the desert and assignment to different classes of Glissomonadida, Imbricatea, Thecofilosea and Heterolobosea (Discoba). Further efforts to investigate the function of cercozoans for xeric plant fitness might be useful in biodiversity conservation initiatives, for example of endangered plant species under the current climate change scenario and biodiversity loss in the ecosystems. Our results shed light on the so-called dark matter of protists life in cacti, providing valuable insights for upcoming metabarcoding projects. This study highlights the significant role that these protists could play in energy transfer within the plants and soils of one of the Earth's most extreme biotopes, the Atacama Desert.

## CRediT authorship contribution statement

**Eduardo Acosta:** Data curation, Formal analysis, Investigation, Visualization, Writing – original draft. **Victoria Fincke:** Data curation, Formal analysis, Investigation, Writing – review & editing. **Frank Nitsche:** Methodology, Conceptualization, Investigation, Supervision, Writing – review & editing. **Hartmut Arndt:** Conceptualization, Investigation, Supervision, Funding acquisition, Project administration, Writing – review & editing.

## Data availability

Data will be made available on request.

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# Chapter 2: Evidence of Protists Colonization in Terricolous Lichens, Coastal Hills and Microbial Mats Highlight Abundant Molecular Signatures Related to the Genera *Rhogostoma*, *Euplotes* and *Neobodo*

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Research Paper

# Protist diversity and co-occurrence patterns obtained by metabarcoding of terricolous lichens, coastal cliffs and a microbial mat in the Atacama Desert, northern Chile

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ABSTRACT

Protists can endure challenging environments sustaining key ecosystem processes of the microbial food webs even under aridic or hypersaline conditions. We studied the diversity of protists at different latitudes of the Atacama Desert by massive sequencing of the hypervariable region V9 of the 18S rRNA gene from soils and microbial mats collected in the Andes. The main protist groups in soils detected in active stage through cDNA were cercozoans, ciliates, and kinetoplastids, while the diversity of protists was higher including diatoms and amoebae in the microbial mat detected solely through DNA. Co-occurrence networks from soils indicated similar assemblages dominated by amplicon sequence variants (ASVs) identified as *Rhogostoma*, *Euplotes*, and *Neobodo*. Microbial mat networks, on the other hand, were structured by ASVs classified as raphid-pennate diatoms and amoebae from the genera *Hartmannella* and *Vannella*, mostly negatively correlated to flagellates and microalgae. Additionally, our phylogenetic inferences of ASVs classified as *Euplotes*, *Neobodo*, and *Rhogostoma* were supported by sequence data of strains isolated during this study. Our results represent the first snapshot of the diversity patterns of culturable and unculturable protists and putative keystone taxa detected at remote habitats from the Atacama Desert.

## 1. Introduction

The Atacama Desert located in the North of Chile is the oldest and driest non-polar desert on Earth and is often used as a model system to study the dry and ultra-violet radiation exposure limits of life (Davila et al., 2008; Hernández et al., 2016; Pérez et al., 2017). In this desert, the access to water is limited to diel water condensation cycles and rare precipitation events (i.e., rainfall), occurring between long dry periods, imposing constraints on life even for microbial species (Davila et al., 2013; Houston, 2006). This dry environment extends along the coast of northern Chile, from the Pacific coast to the western slopes of the Andes in which aridity has persisted for the past 90 million years (Bull et al., 2018; Rech et al., 2010). In this complex orography, the onset of hyperaridity is concentrated in a core area between the Coastal Range and the Altiplano, currently considered the best analog of the neighboring planet Mars (Warren-Rhodes et al., 2019).

At the driest limits for its existence, life is represented mainly by microbes, persisting even inside salt rocks, as halites and soils affected gradually by the aridity and macro-scale elevation changes (Finstad et al., 2017; Knief et al., 2020). Other isolated microbial ecosystems in the Atacama include endorheic basins, harboring salty lakes as well as saline and hypersaline lagoons, locally known as "salares" (Chong, 1994). The extremophiles registered in the Atacama have been extensively investigated focusing mainly on prokaryotes (bacteria and archaea; Davila et al., 2008; Demergasso et al., 2003; Dorador et al., 2008; Fernandez et al., 2016; Rasuk et al., 2014; Schulze-Makuch et al., 2018), fungi and, into a minor degree, on photosynthetic eukaryotes (e.g., diatoms; Farías, 2020 and references cited therein). Unicellular protists, on the other hand, have been greatly neglected in studies of microbial life in such extreme habitats. Just recently, diverse species of heterotrophic protists including alveolates, stramenopiles, rhizaria, and opisthokont flagellates, among other groups, have been found in the Atacama Desert using culture-dependent techniques (Acosta et al., 2023; Arndt et al., 2020; Hohlfeld et al., 2023; Schiwitza et al., 2018, 2019, 2021). In the areas at the limits of the arid core, biodiversity is represented by endemic species of cacti, shrubs as well as epiphytic and terricolous lichens, surviving in fog oases usually found at the coastal borders and the inland slopes known as "lomas" (Böhnert et al., 2019;

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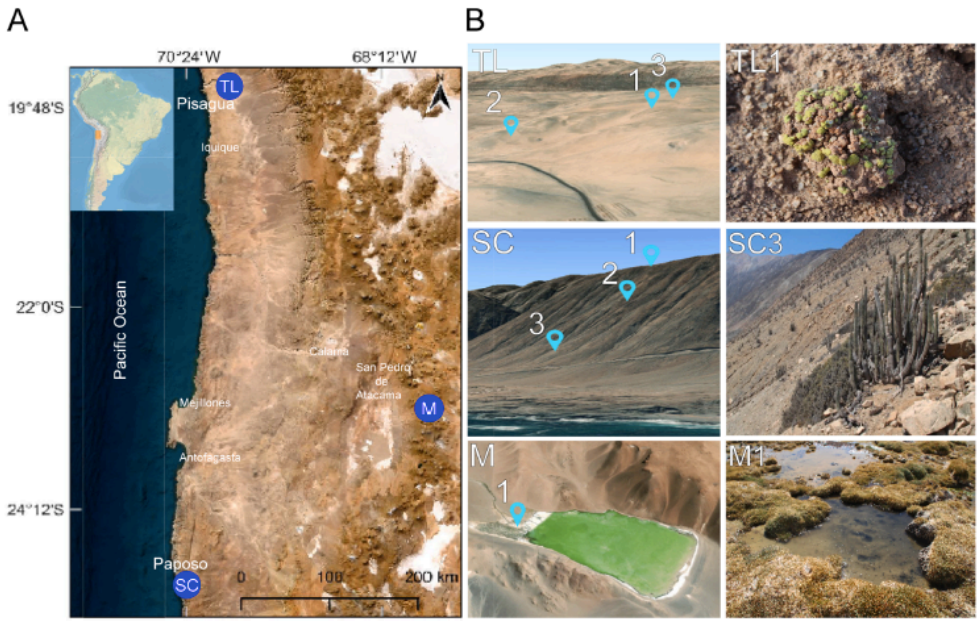


**Fig. 1.** Overview of the study sites across the Atacama Desert. (A) Map showing the location of the stations across the Atacama Desert. (B) Overview of the study sites at each station, with representative photographs of some stations. TL, Terricolous lichen (study sites 1–3); SC, Soil coastal cliff (study sites 1–3); M, Microbial mat (study site 1). Map produced and adapted from QGIS ver. 3.28.5 – Firenze. Overviews obtained with ArcGIS Earth iOS ver. 2.1.0.

Jung et al., 2019, 2020; Merklinger et al., 2021). Recent works started to link the phyllosphere of columnar and shrubby species of cacti to a diversity of ciliates, flagellates, and naked as well as thecate amoebae (Acosta et al., 2023; Arndt et al., 2020). These microbes play paramount roles in natural ecosystems, regulating bacterial populations, decomposing organic matter, providing nutrients to plants, and even influencing soil aggregates improving water retention and aeration in soils (Bonkowski, 2004; Geisen et al., 2018).

The ongoing development of sequencing technologies used for the identification of microbes in the environment has revealed a wider extent of protist diversity in terrestrial habitats and the phyllosphere than formerly known (Geisen et al., 2018; Jauss et al., 2021; Öztoprak et al., 2020; Venter et al., 2017, 2018). The ability to form dormant stages and rapid evolution, features found across protist taxa, could be crucial for survival until the next suitable period of humidity in the Atacama. In the present work, we uncover the diversity of free-living protists detected by metabarcoding across different microbial habitats (soils, soil crusts, and microbial mats) and study their community assemblages based on their abundance and occurrence. Additionally, the metabarcoding approach used to identify environmental genotypes was supported by the cultivation of strains obtained from the environmental samples collected from these desertic habitats.

## 2. Material and methods

### 2.1. Soil and microbial mats sampling

During the austral summer of 2022, we collected samples from three stations (Fig. 1, Table 1): samples for "Terricolous lichen" were taken at three different sites being a few hundred meters apart from each other in the vicinity of Pisagua, with three samples at each site; samples for "Soil coastal cliff" were collected from three different sites along the coastal cliff at different elevations near Paposo with three samples at each site; samples for "Microbial mat" were taken from one site of Amarilla Lagoon in the Andean Altiplano at an altitude of 4541 m a.s.l., with three samples at that site. Overall, 21 samples were analyzed. The soil samples consisted of 5 g from the upper 2 cm of soil. The microbial mat samples used in this study consisted of the first two millimeters of sediments. All samples included in this study were taken using sterile metal ware and gloves. Body coveralls were used to keep cross-contamination to a minimum. Aiming to preserve the environmental DNA and RNA, the samples were collected into 15 ml sterile centrifuge tubes priorly filled with sterile RNAlater (Invitrogen, U.S.A.) and stored at 4 °C until further analyses according to Wang et al. (2018).

**Table 1**
Station description, studied template, altitude and geographic location of investigated stations.

| Station | Template | Altitude (m a.s.l.) | Geographic location |
|---|---|---|---|
| Terricolous lichen 1 | DNA and cDNA | 1,099 | S19°33'24.1" W70° 04'48.0" |
| Terricolous lichen 2 | DNA and cDNA | 1,053 | S19°33'15.1" W70° 04'40.7" |
| Terricolous lichen 3 | DNA and cDNA | 1,111 | S19°33'28.6" W70° 04'48.0" |
| Soil coastal cliff 1 | DNA | 200 | S25°00'29.9" W70° 27'30.8" |
| Soil coastal cliff 2 | DNA | 441 | S25°00'32.8" W70° 27'18.1" |
| Soil coastal cliff 3 | DNA | 682 | S25°00'28.9" W70° 27'03.6" |
| Microbial mat 1 | DNA | 4,541 | S23°13'11.7" W67° 36'13.6" |

### 2.2. Mock community preparation and sequencing

To assess the accuracy of the used classification method on our sequencing libraries, we included an artificial control (mock community). This control was composed of equimolar concentrations of the PCR-amplified V9 region from nine species from the Heteroflagellate Collection Cologne (HFCC) (Dünn and Arndt, 2023). The control samples included strains from the six following protist supergroups: Alveolata (represented by *Protocruzia* sp. MT355146 and *Aristerostoma* sp. MT081566), Ancyromonadida (*Fabomonas tropica* MT355148), Rhizaria (*Massisteria* sp. MT355122), Discoba (*Neobodo* sp. MT355124 and Rhynchomonadidae sp. MT355133), Stramenopila (Bicosoecida sp. MT355117), and Opisthokonta (*Cafeteria burkhardae* MN315604 and *Ministeria vibrans* MT355150). DNA was extracted from these strains by transferring clonal cultures (30 ml) into 50 ml centrifuge tubes (Sarstedt, Nümbrecht, Germany) and centrifuging at 4000 *g* and 4 °C per 20 min. After discarding the supernatant, DNA was extracted from the cellular pellet using the Quick gDNA Mini-Prep Kit (Zymo Research Corporation, CA, U.S.A.) following the manufacturer's instructions. An aliquot of 3 µl of DNA of each strain was used as a template in a PCR reaction using 5 µM of the universal 18S primer set: EukA (5′-AAC CTG GTT GAT CCT GCC AGT-3′) and EukB (5′-TGA TCC TTC GCA GGT TCA CCT AC-3′) (Medlin et al., 1988). The PCR conditions were the following: a denaturation step at 96 °C for 2 min, then 34 cycles of 96 °C for 30 s, 55 °C for 45 s, 72 °C for 2.5 min, and a final elongation step for 7 min at 72 °C. The PCR products were purified using the FastGene Gel/PCR Extraction Kit (Fast Gene, Japan) and sequenced using the 18S rRNA primer sets at GATC Biotech, Germany. Sequences were analyzed using the Basic Local Alignment Search Tool (BLAST, NCBI).

### 2.3. DNA and RNA extraction and metabarcoding of protists communities

Trizol (Invitrogen, U.S.A.) was used to extract the DNA and RNA from the samples as recommended by the manufacturer with minor modifications. Namely, the samples were homogenized in 1 ml of Trizol reagent per 10 min as a lysis step using a vortex adaptor. Final DNA was resuspended in 100 µl nuclease-free water and immediately used for the amplification of the target gene. RNA was resuspended in 50 µl nuclease-free water for downstream analyses. The total RNA was used as template for cDNA synthesis using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher, U.S.A.) with random hexamer primers. The total DNA-, RNA-, and cDNA concentrations were measured using an ND-1000 nanodrop (Peqlab Biotechnologie, Germany). The environmental DNA, the cDNA samples, as well as the DNA from the strains included in the mock community, were adjusted to a concentration of 5 ng per microliter for the amplification of the V9 hypervariable region of the nuclear small ribosomal subunit (18S) gene, using the multiplex identifier-tagged (MIDs) primers 1389F and 1510R (Amaral-Zettler et al., 2009). We selected this hypervariable region for the detection of protists due to comparability to other datasets obtained from this desert by our working group. The primers were used in 10 µM concentration. PCR was conducted using the Taq DNA Polymerase Master Mix (VWR, Germany) under the following conditions: 98 °C for 30 s; 25 cycles of 10 s at 98 °C, 30 s at 57 °C, 30 s at 72 °C; and a final step at 72 °C for 10 min. Amplicons were purified using the FastGene Gel/PCR Extraction Kit (Fast Gene, Japan). Equimolar concentrations of all samples were pair-end sequenced (2 × 150 bp) in an Illumina NovaSeq 6000 sequencer (Illumina, U.S.A.) at the Cologne Center of Genomics (CCG) (https://www.ccg.uni-koeln.de).

### 2.4. Data analysis

The raw forward and reverse sequences in ".fastq" format were imported into the software Quantitative Insights Into Microbial Ecology 2 (QIIME2) (Bolyen et al., 2019). The MIDs and primer sequences were removed from the libraries using the "cutadapt trim-paired" function and sequences with an error rate higher than zero were discarded. The raw forward and reverse reads were trimmed to exclude primer sequences, dereplicated, denoised, and merged into amplicon sequencing variants (ASVs) calling the "denoise-paired" function from the DADA2 plugin (Callahan et al., 2016). This method includes a chimera-filtering step on the ASVs using the "consensus" chimera detection method on all replicates and sequences found chimeric in a sufficient fraction of replicates were removed (method: isBimeraDenovo). To discern between protist taxa, the ASVs were classified using a cut-off value = 0.8 and a 97 % identity to the database through the function "feature-classifier classify-consensus-blast" (Camacho et al., 2009). For this, we used the Protist Ribosomal Reference database ver. 4.14.0 ($PR^2$, Guillou et al., 2012) amended with the V9 region of 18S rRNA gene sequences from the Heteroflagellate Collection Cologne (HFCC) (Dünn and Arndt, 2023). The ASVs classified as Metazoa, Archaeplastida, Fungi, or unassigned were excluded from further analyses.

### 2.5. Statistics and reproducibility

The complete dataset for our high-throughput sequencing libraries is deposited as a Sequence Read Archive (SRA) in the National Library of Medicine (NCBI) under the accession number PRJNA1065327. The alpha and beta diversity metrics were obtained through the function "qiime diversity core-metrics-phylogenetic" rarefying to the lowest number of sequences obtained between all samples and classified as protists using a 97 % identity to the database. Samples were rarefied to a minimal number equal to 5025 sequences. The significance of the alpha diversity metrics (observed number of ASVs and the Shannon index) was assessed with the Kruskal–Wallis test for paired groups (study site-study site and DNA-cDNA) using the function "qiime diversity alpha-group-significance". Additionally, significant dissimilarity in the beta diversity metrics (Jaccard distance) across study sites was tested with a permutational analysis of variance (PERMANOVA, 999 permutations) through the function "qiime diversity beta-group-significance". The box plots showing the overall observed ASVs per station and their Shannon diversity index as well as the clustering patterns shown in a Principal Coordinates Analysis (PCoA) were illustrated using the "microeco" package in R (Liu et al., 2021).

### 2.6. Network analyses

The following files from QIIME2: ASV representative sequences, ASV frequency table, assigned taxonomy, phylogenetic tree, and a metadata table, were imported into R ver. 4.3.0, using the "file2meco" package and processed using the "microeco" package. The function "trans_network" was used on the microtable object (R6 class) of the dataset of each study site to test for co-occurrence between ASVs classified using a 97 % identity to the database. Co-occurrence networks were built for each study site based on correlation inference using the method "Sparse Correlations for Compositional data (SparCC)" (Friedman and Alm, 2012). A null model was included to infer modularity between nodes represented by the correlated genotypes in the networks using 100 bootstraps. The modules and their names were assigned using the function "cal_module" with a *p*-value threshold of 0.01 for significant correlations represented by edges between the nodes. Additionally, to study the importance of the nodes in the co-occurrence networks, we included the betweenness centrality to measure the extent to which a node lies on paths between other nodes, as represented by its size. The networks were illustrated in Gephi ver. 0.9.2 and graphically processed in Adobe Illustrator ver. 27.7 (Adobe Systems, U.S.A.).

### 2.7. Cultivation of protists

Environmental samples (1 g) were collected from soils and microbial mats and inoculated to 50 ml centrifuge tubes containing liquid medium (Schmaltz–Pratt medium: 28.15 g/l NaCl, 0.67 g/l KCl, 5.51 g/l

$MgCl_2{\cdot}6H_2O$, 6.92 g/l $MgSO_4{\cdot}7H_2O$, 1.45 g/l $CaCl_2{\cdot}H_2O$, 0.1 g/l $KNO_3$, and 0.01 g/l $K_2HPO_4{\cdot}3H_2O$ in 1 l deionized water; final concentration 23 per mille), with one sterile wheat grain to feed bacteria as a food source for protists. After 7 days, the raw liquid cultures were inoculated into culture flasks containing fresh medium with a wheat grain as a carbon source for bacteria, and protist morphotypes were selected from mixed cultures using the Liquid Aliquot Method (LAM) (Butler and Rogerson, 1995). Monoclonal cultures of two protist species were cultivated for 2–5 days. Subsequently, cells were detached using a cell scraper from the culture flask and concentrated by centrifugation (4000 *g* for 20 min at 4 °C, Megafuge 2.0 R, Heraeus Instruments). The genomic DNA was extracted using the Quick-gDNA MiniPrep kit (Zymo Research, U.S.A.). For amplification of the 18S rRNA gene, the EukA and EukB primers were used (see above). The PCR reactions were performed using 12 µl of $ddH_2O$, 0.5 µl of each primer (1 µM), 25 µl of PCR 2x PCR Master Mix (VWR, Radnor, PA, U.S.A.), and 3 µl of the genomic DNA from the isolates. PCR conditions were as follows: pre-denaturation at 98 °C for 2 min; 35 cycles of 98 °C for 30 s, 55 °C for 45 s and 72 °C for 2.5 min; and final extension at 72 °C for 10 min. Five microliters of the purified product were pooled together with five microliters of each used forward and reverse primer and Sanger sequenced at Eurofins Scientific. The 18S rRNA gene sequences of the new strains were used as a query in the BLAST search (https://www.ncbi.nlm.nih.gov).

### 2.8. Phylogeny of the V9 region

The V9 region sequences of the 18S rRNA gene of isolates and ASVs obtained from the metabarcoding were aligned in SeaView ver. 5.0.4. These alignments included one for HFCC 986 (*Neobodo* sp.), one for HFCC 988 (*Euplotes* sp), and one for HFCC 860 (*Rhogostoma olyaorum*, see Acosta et al., 2023), together with sequences from the $PR^2$ database classified as the genera *Neobodo*, *Euplotes*, and *Rhogostoma*, respectively. The maximum likelihood phylogenies were inferred using the "PhyML" option in SeaView, with a bootstrap value of 100. In the program, parameters were set to the General time reversible (GTR) model, a gamma-distributed rate variation across sites, four gamma rate categories, and a proportion of invariable sites.

## 3. Results

### 3.1. Environmental sequencing

We amplified and sequenced the target gene region from all samples included in the DNA metabarcoding except for two replicates from "Soil coastal cliff" station, which could not get amplified. Our efforts to amplify this gene from cDNA were successful only for the samples collected at the station "Terricolous lichen". In total, over 122.5 million reads were obtained for all stations. The DNA metabarcoding of the station "Terricolous lichen" included over 46.6 million raw reads from which 98.75 % were found barcode clipped and further denoised into 7755 ASVs associated with ca. 36 million sequences (Table 2). Sequences of the cDNA metabarcoding at the station "Terricolous lichen" accounted for over 31 million reads from which 96.05 % were barcode clipped and further denoised into 5409 ASVs associated with over 25.7 million sequences. From a total of over 25 million reads obtained from the environmental DNA of the station "Soil coastal cliff", 99.16 % were found as barcode clipped and later denoised into 6331 ASVs associated with ca. 19.1 million sequences. From the station "Microbial mat", the number of sequences accounted for over ca. 19.9 million sequences from which 97.93 % were barcode clipped and later denoised into 4578 ASVs associated with over 17.1 million sequences. Additionally, we assessed the accuracy of the classification method using the artificial sample of known composition (so-called mock community). We detected all nine strains included in this artificial control (Supplementary Table 1). The sequences included in the mock communities accounted from 6.2 % up to 17.54 % of the total relative abundance of sequences of the mock community classified using a 97 % identity to the database. Additionally, we detected low-frequency ASVs in the mock community classified as taxa that were not included in the artificial sample. Such ASVs were found in a frequency ranging from 0.03 to 0.05 % of the total frequency of the ASVs across the three mock communities from each sequencing library. These percentages were used to define a threshold of minimal frequency, aiming to exclude potential spurious sequences from our sequencing libraries.

Our DNA- and cDNA-based assessment (station "Terricolous lichen") showed that a high proportion of the sequences obtained by metabarcoding are related to taxa classified as eukaryotes (DNA = 75.6 %, cDNA = 88.5 %) at a level of 97 % identity (Fig. 2). The metabarcoding of the station "Soil coastal cliff" also showed a high proportion of classified protist sequences, reaching over 75.4 % of the sequences. Contrastingly, classified sequences obtained from the metabarcoding of the station "Microbial mat" reached only 24.1 % protist sequences. After the frequency filtering, our target study group, the protists, was assigned to a total of 195 ASVs associated with over 28 million sequences from "Terricolous lichen" DNA, while from "Terricolous lichen" cDNA, 125 ASVs were associated with over 13 million sequences. The number of ASVs from "Soil coastal cliff" was 77 ASVs associated with over 4.01 million sequences, while from "Microbial mat" DNA, 139 ASVs were associated with over 15 million sequences. Among the major protist lineages, Rhizaria were the main group assigned to the ASVs obtained from the stations "Terricolous lichen" and "Soil coastal cliff". The main lineages assigned for ASVs found in "Microbial mat" corresponded to Stramenopiles, Amoebozoa, and Alveolata (Fig. 2). As for "Terricolous lichen" and "Soil coastal cliff", the recovered ASVs were mainly classified as part of the genera *Rhogostoma* (phylum Cercozoa), *Euplotes* (phylum Ciliophora) and, in minor proportions, to *Neobodo* (phylum Discoba). In contrast, protist taxa of the station "Microbial mat" mainly belonged to pennate diatoms (Bacillariophyta) and other heterotrophic protists including different ciliates (Euplotidae, Cyrtolophosidida), flagellates (Planomonadidae, Bicoecea, Massisteriidae, Cafeteriaceae), and amoebae (Ministeriidae, Vermamoebidae) (Fig. 3).

**Table 2**
Results of metabarcoding studies. Total numbers of amplicon sequencing variants (ASV) obtained and the number of ASVs left after critical filtering steps in the bioinformatic analyses.

| Template | Station | Number of replicates | Total number of ASVs | Total number of reads | Number of ASVs after filtering | Number of reads after filtering | Number of protist ASVs after filtering | Number of protists reads after filtering |
|---|---|---|---|---|---|---|---|---|
| DNA | Terricolous lichen | 9 | 7,755 | 46,608,043 | 955 | 35,981,990 | 195 | 28,056,807 |
| | Soil coastal cliff | 7 | 6,331 | 25,024,895 | 810 | 18,630,461 | 125 | 13,039,427 |
| | Microbial mat | 3 | 4,578 | 19,961,608 | 562 | 16,463,271 | 77 | 4,017,207 |
| RNA | Terricolous lichen | 9 | 5,409 | 31,089,720 | 529 | 25,220,402 | 139 | 15,026,648 |

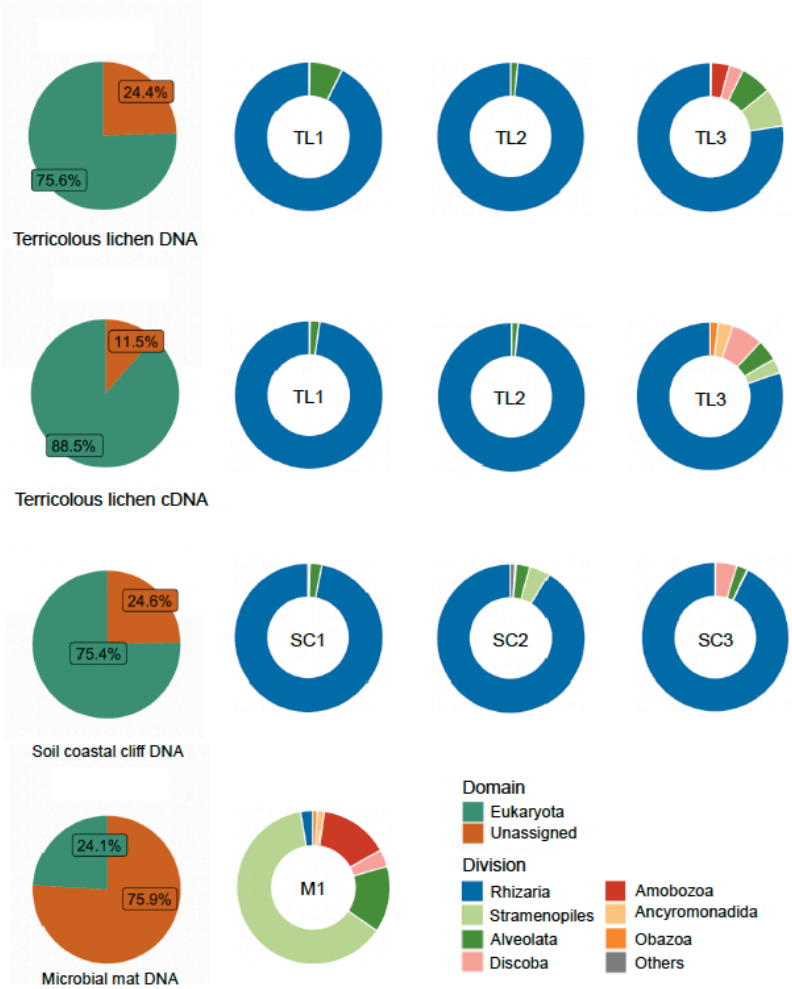


Fig. 2. The relative abundance of classified sequences across stations. Left side: Piecharts of the relative abundance of sequences classified as Eukaryota versus those unclassified. Right side: Closed ring plots showing the relative abundance of the major protist groups detected at each study site of the three stations. Letters refer to the study sites shown in Fig. 1.

### 3.2. Alpha and beta diversity

The mean number of ASVs observed in "Terricolous lichen" was 21 (DNA) and 15 (cDNA) per sample. For the "Soil coastal cliff" and the station "Microbial mat", the mean number of ASVs observed was 17 and 25, respectively (Fig. 4A). The Shannon diversity registered in the "Terricolous lichen" was 0.69 for the DNA and 0.67 for the cDNA metabarcoding, and 0.64 for the "Soil coastal cliff" (DNA) and 2.54 for the "Microbial mat" (DNA). When comparing the study sites from each station in balanced comparisons ($n = 3$), no significant differences could be detected between the measured dissimilarity metrics.

The Principal Coordinates Analysis (PCoA) explained 43.1 % of the total variation of the studied protists communities across stations (PCoA axis 1 = 33.4 %, PcoA axis 2 = 9.7 %, Fig. 4B). This visual inspection suggested a similar clustering pattern between the samples from the stations "Terricolous lichen" (DNA and cDNA) and "Soil coastal cliff" (DNA), distinct to samples from the station "Microbial mat". Nevertheless, the assessment of dissimilarity of the Jaccard distances in balanced comparisons ($n = 3$) showed that the stations were not significantly different in their protist community composition.

### 3.3. Co-occurrence network analyses

To obtain a graphic visualization of potential relationships between the different genotypes of the protist communities, we calculated four co-occurrence networks for the stations "Terricolous lichen" DNA and cDNA, "Soil coastal cliff" DNA and "Microbial mat" DNA ("Terricolous lichen" DNA and "Microbial mat" shown in Fig. 5; for properties, see Supplementary Table 2). Overall, the co-occurrence network "Terricolous lichen" DNA (Fig. 5A) was represented by 14 nodes and 47 edges from which 83.0 % (39 edges) showed positive correlations and 17.0 % (8 edges) showed negative correlations. Furthermore, the betweenness

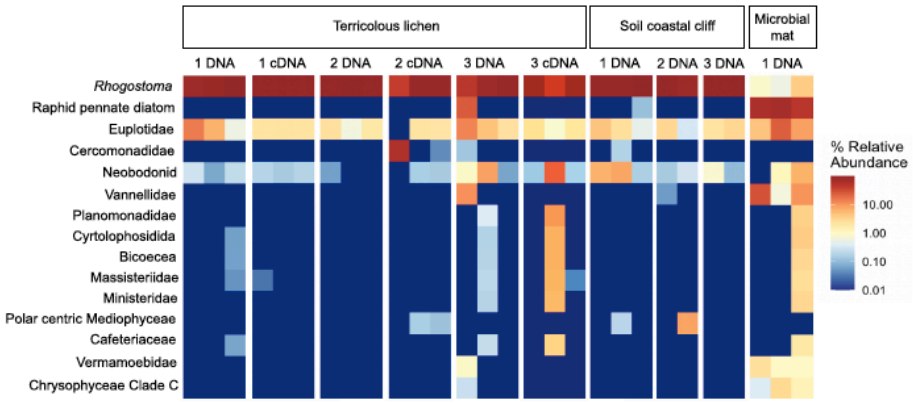


**Fig. 3.** Heatmap indicating the relative abundance of reads of protist groups detected across the different study sites for the 15 most abundant ASVs detected by DNA and cDNA analyses.

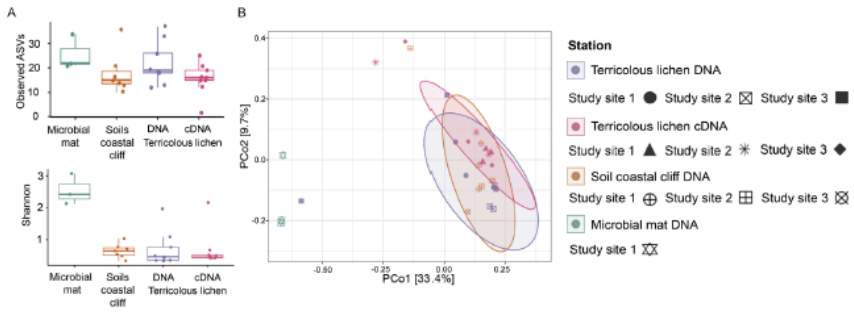


**Fig. 4.** Alpha and beta diversity of the protist communities calculated on the base of read abundance across stations. (A) Boxplots showing the amount of ASVs and the Shannon diversity for the different stations. (B) Principal coordinates analysis based on the Jaccard distances including all stations.

centrality of the nodes included in this network ranged between 0.2 and 10. Contrastingly, the co-occurrence network "Microbial mat" DNA (Fig. 5B) was represented by 33 nodes and 73 edges from which 55.7 % (41 edges) showed positive correlations and 44.3 % (32 edges) showed negative correlations. The betweenness centrality values ranged from 5 up to 130 in this network. The co-occurrence network "Soil coastal cliff" (DNA) comprised 16 nodes and 109 edges from which 58 % (63 edges) showed positive correlations and 42 % (46 edges) showed negative correlations (Supplementary Fig. 5A). Additionally, the betweenness centrality in this network ranged from 0.25 up to 25. On the other hand, the co-occurrence network from the station "Terricolous lichen" (cDNA) was represented by 19 nodes and 65 edges from which 65.4 % (42 edges) showed positive correlations and 34.6 % (23 edges) showed negative correlations (Supplementary Fig. 5B). The betweenness centrality for this network ranged from 0.7 up to 40. Negative correlations were predominantly observed between nodes classified as *Rhogostoma* and *Neobodo* and between genotypes classified as *Neobodo* and *Euplotes* in the networks obtained from soil stations. In the network "Microbial mat" (DNA), negative correlations were observed between genotypes classified as *Nitzschia*, *Ochromonas*, *Massisteria*-like, and *Hartmannella*.

### 3.4. Cultivation experiments

In our search to identify important protists of these communities, we were able to isolate two strains from two out of the three studied stations. One strain isolated from samples collected at the station "Soil coastal cliff" corresponded to the strain HFCC986 (Supplementary Fig. 1) and the other isolate from the station "Microbial mat" corresponded to the strain HFCC988 (Supplementary Fig. 2). Additionally, we used the 18S rRNA gene sequence from a new species of *Rhogostoma* (*R. olyaorum*), which was already described by Acosta et al. (2023). The closest hit in BLAST of the 18S rRNA gene sequence of the strain HFCC986 was *Neobodo designis* isolate Dune2 (AY53623) with a query coverage of 100 % and a percentage of identity equal to 90.5 %. On the other hand, the closest hit in BLAST for this gene sequence from the strain HFCC988 was *Euplotes rariseta* isolate QD-2 (FJ423449) with a query coverage of 99 % and 94.4 % of identity.

The phylogenetic analysis of the V9 region of the strain HFCC986 indicated that it belongs to one of the neobodonid clusters with very close relations to several *Neobodo* ASVs obtained during this study (Supplementary Fig. 1). In this phylogeny, the closest sequence corresponded to *N. designis* (AY53623) and the closest ASV was ASV66d6. The phylogenetic analysis of the strain HFCC988 revealed that it belongs to a branch of the phylogenetic tree including diverse species registered for this genus (Supplementary Fig. 2). In this branch, we found sequences representing *Euplotes* species such as *E. orientalis* and *E. woodruffi* as well as the ASVf479.

In addition, as we detected the genus *Rhogostoma* as abundant across the soil stations, we calculated a phylogenetic tree of the ASVs related to this group by our metabarcoding study. This phylogenetic tree was highly supported by maximum likelihood bootstrap values. We found there two large clusters, one composed exclusively of reference

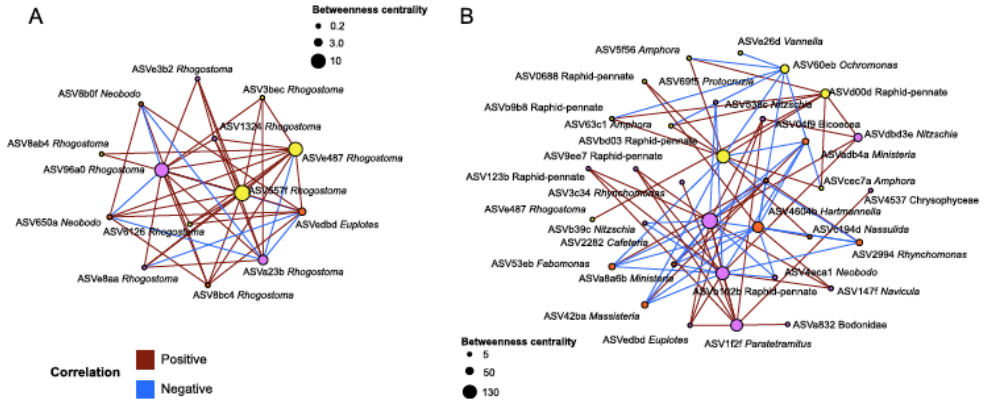


**Fig. 5.** Co-occurrence networks for protist genotypes (ASVs). (A) Station "Terricolous lichen" (based on DNA data). (B) Station "Microbial mat" (DNA data). Each node represents one ASV, the size of the nodes indicates the betweenness centrality, and the colors of the edges indicate the type of correlation between ASVs. The colored nodes indicate ASVs co-occurring more frequently with each other than with other genotypes (see Supplementary Fig. 5 for additional networks).

sequences from public databases and another one that included reference sequences as well as six ASVs from our metabarcoding study and also the sequence of an isolate we had already described from this area (*R. olyaorum* HFCC860, Acosta et al., 2023) (Supplementary Fig. 3).

## 4. Discussion

### 4.1. Protist assemblages in soils and microbial mats from the Atacama Desert

While prokaryote diversity is already well studied in the Atacama (Davila et al., 2008; Demergasso et al., 2003; Dorador et al., 2008; Fernandez et al., 2016; Rasuk et al., 2014; Schulze-Makuch et al., 2018), we assessed for the first time the unculturable protist diversity represented by amplicon sequence variants obtained from soils at different latitudes of the Atacama Desert (Figs. 2 and 3). To support the detection of protist lineages by metabarcoding (V9 region of the 18S rRNA gene), we amplified this molecular marker from cDNA to detect the active fraction of these groups (e.g., "Terricolous lichen", Fig. 2). Nevertheless, as RNA can be found also in resting stages persisting in the environment, we interpreted protists lineages detected by cDNA (synthesized from RNA) as genotypes of potentially active protists. Overall, the novelty of microbial diversity was indicated by the high degree of unassigned sequences when compared to public databases (Supplementary Fig. 2). The percentages of classified sequences from soil stations were similar, ranging between 75.4–75.6 % from the DNA metabarcoding and 88.5 % in "Terricolous lichen" cDNA, using a common identity threshold in microbial ecology studies (97 % of identity). The low fraction of classified sequences that we found especially in the microbial mat datasets (24.1 %) characterizes this habitat as a black box of microbial diversity (see also Burki et al., 2021). As a contribution to shed a bit of light on this black box, we tried to isolate strains from field samples of our metabarcoding studies. We were successful in cultivating three strains of bacterivorous protists. Sanger sequencing of these strains (representatives of the genera *Euplotes*, *Neobodo*, and *Rhogostoma*) revealed V9 regions, which were very similar to ASVs obtained by metabarcoding supporting the employed classification (Supplementary Figs. 1, 2, and 3). We constructed co-occurrence networks of ASVs assemblages across the studied habitats (Fig. 4 and Supplementary Fig. 5), deciphering potential interaction patterns in the protist communities, and highlighting ASVs that could represent keystone taxa.

We underline the use of an aliquot of known composition (i.e., mock community) in the sequencing libraries to assess potential spurious sequences in PCR-based approaches as recommended by Schloss et al. (2011). Later studies adapted this quality filter as an essential component of the metabarcoding of protists in soils (Fiore-Donno et al., 2019; Roshan et al., 2021). In the Atacama, studies using the same phylogenetic marker (V9 region) and similar frequency threshold controls have been used in the metabarcoding of hypersaline lagoons (Rybarski et al., 2023) and for the analysis of Andean microbial mats (Acosta et al., 2024). The use of this frequency threshold in our study affected mainly the number of ASVs, but a high number of sequences remained for further analyses (Table 2). With our results, we confirm the use of mock community-deduced thresholds as a backbone of studies on the protist communities in different environments by metabarcoding (Fiore-Donno et al., 2019; Rybarski et al., 2023; Sachs et al., 2023).

### 4.2. Alpha diversity and taxonomy

As protist biota is concentrated in regions with the presence of organic matter and commonly also in association with growing roots of plants, we hypothesized the presence of protists in the two selected soil stations. Furthermore, as microbial mats have been documented as a potential hotspot of biodiversity (Rasuk et al., 2016), we selected microbial mats of an Andean lagoon, expecting a high protist diversity. However, our results on ASV numbers observed were not greater than 30 ASVs per sample and Shannon diversity was only up to 2.5 ("Microbial mat"). We detected a similarly low number of ASVs also from the other environments (Fig. 4A). Differences in the diversity of the three different stations occurred, although they were not significant. Comparing our study with similar approaches for the study of free-living protists, similar values (1–2) were reached in aquatic habitats of hypersaline lagoons of the Atacama Desert using the hypervariable region V9 of the 18S rRNA gene (Rybarski et al., 2023). Employing the same phylogenetic marker, Rybarski et al. (2023) detected different protist groups inhabiting the water column, indicating only little turnover between the water column and the benthic communities of the microbial mat detected in the present study. Furthermore, the number of shared ASVs reported by Rybarski et al. (2023) was higher than the number of shared genotypes observed in our study. For example, the number of shared ASVs between the stations in this study reached up to 16 ASVs ("Terricolous lichen" DNA and "Microbial mat" DNA, Supplementary Fig. 4), versus 44 ASVs obtained by Rybarski et al. (2023).

Regarding the species composition, the samples from soil habitats are mainly dominated by the cercozoan genus *Rhogostoma*. Members of the family Rhogostomidae have been described as abundant in

terrestrial ecosystems exhibiting species turnover in beta diversity assessments (Öztoprak et al., 2020). The main result of the metabarcoding in the present study is the high abundance of the genus *Rhogostoma* across the stations "Terricolous lichen" (DNA and cDNA) and "Soil coastal cliff" (DNA), suggesting that it is an active component of the soil microbiome in formerly undocumented habitats in this desert. Recently, Acosta et al. (2023) described a novel diversity of cercozoans inhabiting the phyllosphere of endemic cacti of the Atacama Desert, including the new species *Rhogostoma olyaorum*. The ASVs we retrieved during the present study were highly similar to the V9 region sequence of *R. olyaorum* (Supplementary Fig. 3, only one or two nucleotide difference), validating the detection of this genus by our metabarcoding studies of Atacama soils and microbial mats.

Lichens are biological soil crusts occurring mainly in drylands. They represent a mutualistic symbiosis of microalgae and fungi, already documented as hidden diversity distributed at different surfaces across the arid Atacama (Jung et al., 2019). In our soil samples, we obtained sequences classified as fungi, mainly from the study sites at "Terricolous lichen" (DNA and cDNA). These sequences were classified into the class Lecanoromycetes (Ascomycota) (Baloch et al., 2010). These complementary results confirm the presence of an ostropalean fungi typically associated with aerial algae. In such lichenized biocrusts, organic carbon is produced by microalgae, partly explaining the detection of amoebae bearing an organic test (e.g., *Rhogostoma*).

Two additional important groups isolated during this study and present also in our metabarcoding dataset were the ciliate *Euplotes* (Alveolata, Spirotrichea) and the heterotrophic flagellate *Neobodo* (Discoba, Kinetoplastida). Our metabarcoding datasets suggest the presence of these taxa in lichens, which is a novel result and remains to be further elucidated, given that our cultivation experiments were successful in one of the studied soil stations ("Soil coastal cliff"). On the other hand, ciliates have been recognized as members of the protist biota related to cacti (Arndt et al., 2020) and a part of the protist community in hypersaline lagoons (Acosta et al., 2024; Rybarski et al., 2023; Saghaï et al., 2017). Our *Euplotes* isolate HFCC988 from "Microbial mat" in BLAST shared a low identity (90.4 %) with published sequences in the GenBank, evidencing either the novelty of this species with a potentially high degree of adaptation or the BLAST search limitation due to a scarce coverage in the employed database for protist phylotypes isolated from the study region. Spirotrichs, common members of aquatic and terrestrial habitats (e.g., Syberg-Olsen et al., 2016), have also been detected by environmental sequencing in the vicinity of our study sites, such as in hypersaline lagoons of the Atacama (Rybarski et al., 2023). A second isolate obtained from the station "Soil coastal cliff" represented a novel phylotype of the genus *Neobodo*, as indicated by its low identity (90.5 %) with the closest hit in the BLAST search (*Neobodo designis* isolate Dune2, AY753623). The large genetic distance to all other genotypes of *Neobodo* highlights the remote uniqueness of Atacama protists (Arndt et al., 2020).

As we collected samples for metabarcoding from different terrestrial habitats at different altitudes in the Atacama, we expected a potential variability in their community structures. However, our metabarcoding analyses revealed relatively similar protist communities, even when compared to a microbial mat growing in a shallow pond (Fig. 4). Nevertheless, the protist communities appeared to be mainly composed of cercozoans in soils and of diverse amoebae and algae in the microbial mat (Fig. 3). Therefore, we inspected the shared and unique taxa detected across all habitats to determine potential unique genotypes (Supplementary Fig. 4). Such unique genotypes for the station "Microbial mat" (DNA) comprise diatoms, amoebae, and flagellates, while at the terrestrial habitats, mainly cercozoans and ciliates were found (Fig. 3). Additionally, two ASVs occurred independent of the type of habitat, classified as *Rhogostoma* and *Euplotes*, which suggests that they represent a core group in the protist microbiome. Moreover, one genotype found across all stations studied is related to the genus *Vannella*. This cyst-forming naked amoebae is a known member of aquatic habitats and occurs also in soils and biofilms (Lasjerdi et al., 2011), partly explaining its presence in our dataset.

### 4.3. Microbial associations of genotypes

Aiming to understand the potential interaction among the taxa represented by genotypes classified as protists, we calculated correlation networks (Fig. 5, Supplementary Fig. 5). As these networks included both positive and negative correlations, they indicate competitive interactions at all stations (Montoya et al., 2006). Despite the use of an algorithm designed for sparse data in the study of correlation patterns and the stringent data filtering included in our methodology (e.g., mock community and low correlation significance), we interpret such correlations with caution, as recommended by Goberna and Verdú (2022). Although these results do not indicate a direct trophic interaction, they shed light on potential relationships between these genotypes (Deng et al., 2012). Our network analyses described a putative interdependence between the ASVs found to be common across soil stations and classified as *Rhogostoma*, *Neobodo*, and *Euplotes* (Fig. 5A, Supplementary Fig. 5). Although still present, the importance of these taxa decreased in the network calculated from the station "Microbial mat", as amoebae and diatoms were the groups with the highest betweenness centrality (Fig. 5B). Nevertheless, the betweenness centrality is often associated with organisms necessary to sustain the ecosystems. They are known as keystone species and generally show a low betweenness centrality (Berry and Widder, 2014). ASVs as ASVedbd, classified as related to the genus *Euplotes*, are present in the network from "Terricolous lichen" (DNA) and "Microbial mat" (Fig. 5). This was also observed for the genus *Rhogostoma* through ASVe487, suggesting a wide distribution of these genotypes among the Atacama ecosystems. Diverse networks with a similar number of interacting nodes in the soil habitats (14 nodes in "Terricolous lichen" and 16 nodes in "Soil coastal cliff") were simpler than those obtained from the station "Microbial mat" (33 nodes). We detected a richer correlation in cDNA-based networks compared to DNA-based networks correlating ASVs classified as *Massisteria*, *Ministeria*, *Protocruzia*, and *Fabomonas* (Supplementary Fig. 5). These taxa have not yet been obtained in cultures from this region, and should be further investigated as potential transitory marine taxa transported by birds and other organisms (Arndt et al., 2020). Remarkably, the genotype ASVe487, classified as *Rhogostoma*, is highly connected in the network "Terricolous lichen" (DNA), showing mostly positive correlations. The same genotype (ASVe4878 *Rhogostoma*) was also observed in the network "Microbial mat" (DNA), with positive correlation to the ASVd00d, classified as a raphid-pennate diatom (Fig. 5). On the other hand, the ASVedbd classified as *Euplotes* and the ASVs557f and ASVe8aa, classified as *Rhogostoma*, were detected in both DNA and cDNA-based networks. From these, the ASVsedbd (*Euplotes*) and ASV557f (*Rhogostoma*) show a positive correlation among DNA and cDNA data, suggesting mutualism. However, the correlation of ASVedbd with other ASVs classified as *Rhogostoma* can be negative (DNA network, Fig. 5). Moreover, in the network "Terricolous lichen" (DNA), negative correlations were observed between ASVs classified as *Rhogostoma*. Further co-cultivation experiments could shed light on yet undocumented competitive or exclusive relationships between, or even within these protist taxa. Overall, this first attempt to understand interactions through the co-occurrence of genotypes in different habitats points to a turnover in these assemblages depending on the type of habitat (terrestrial or aquatic). As aridity has been linked to the complexity degree of microbial networks, our result supports the initial hypothesis of guilds of microbes highly adapted to these harsh environments (see also Chen et al., 2022). It has been stated that stress factors such as drought could influence the structure of microbial associations, increasing positive correlations in community networks under stress scenarios based on the Stress Gradient Hypothesis (Deng et al., 2012).

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## CRediT authorship contribution statement

**Eduardo Acosta:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Frank Nitsche:** Writing – review & editing, Supervision, Methodology, Data curation, Conceptualization. **Hartmut Arndt:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Data curation, Conceptualization.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejop.2024.126108.

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# Supplementary Information

Protist diversity and co-occurrence patterns obtained by metabarcoding of terricolous lichens, coastal cliffs and a microbial mat in the Atacama Desert, northern Chile

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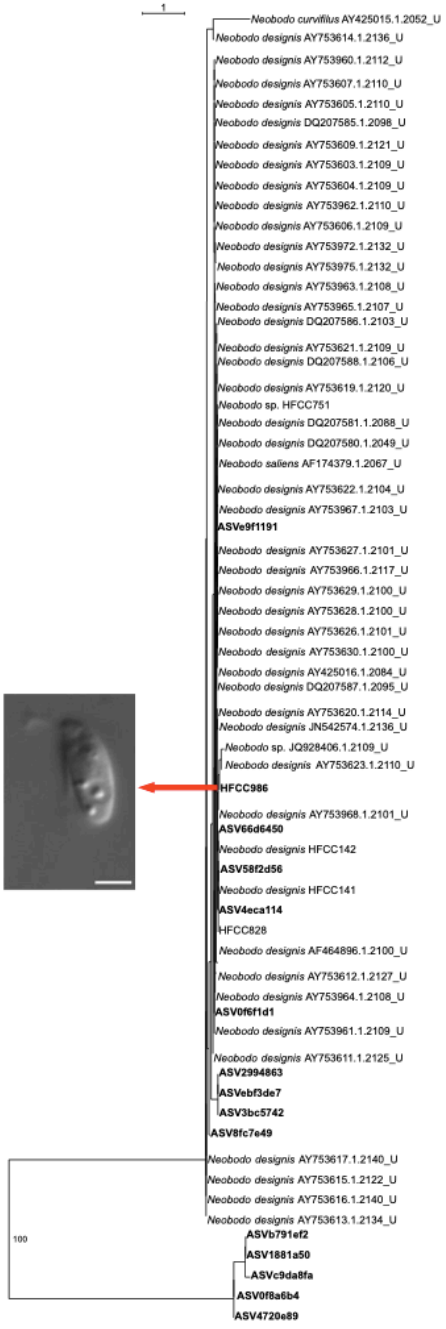
Supplementary Table 1. The amounts of sequences and ASVs obtained from the metabarcoding of the mock communities included in the sequencing libraries included in this work.

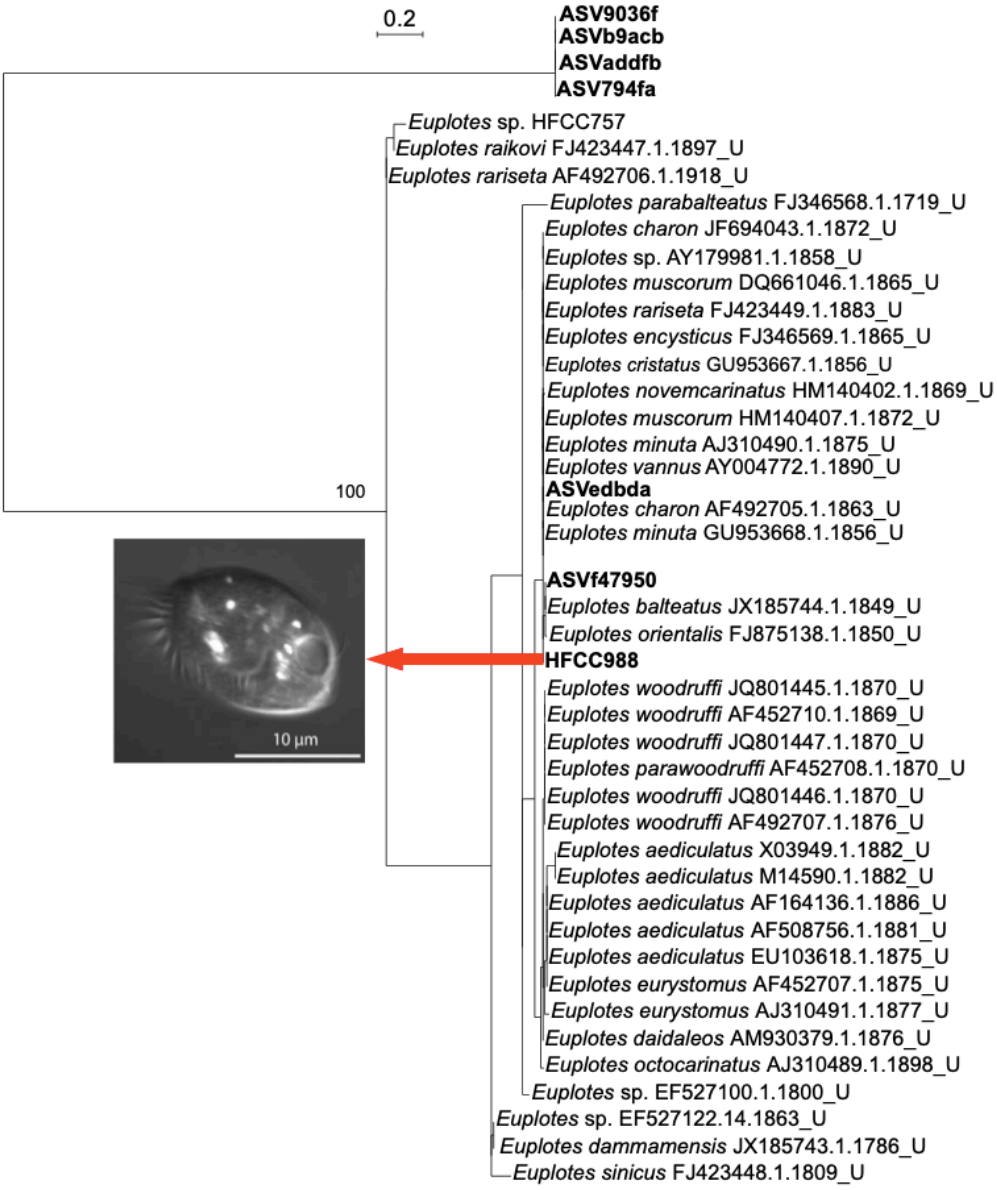
| Strain number | Taxonomy of strain | Mock1 | Mock2 | Mock3 |
|---|---|---|---|---|
| 1 | ASVs *Neobodo* | 47 | 80 | 32 |
| | Sequences Neobodo | 1,386,871 | 3,185,261 | 2,041,880 |
| 2 | ASVs *Fabomonas* | 37 | 44 | 27 |
| | Sequences *Fabomonas* | 1,139,189 | 1,933,743 | 1,466,362 |
| 3 | *Aristerostoma* ASVs | 27 | 28 | 8 |
| | Sequences *Aristerostoma* | 1,173,877 | 1,168,564 | 713,009 |
| 4 | *Rhynchomonas* ASVs | 12 | 30 | 3 |
| | Sequences *Rhynchomonas* | 491,609 | 884,280 | 477,237 |
| 5 | *Massisteria* ASVs | 29 | 28 | 2 |
| | *Massisteria* Sequences | 705,753 | 1,058,806 | 412,602 |
| 6 | *Protocruzia* ASVs | 22 | 17 | 8 |
| | *Protocruzia* Sequences | 547,074 | 562,760 | 464,741 |
| 7 | Ministeria ASVs | 39 | 37 | 12 |
| | *Ministeria* Sequences | 985,069 | 1,106,443 | 723,602 |
| 8 | *Cafeteria* ASVs | 10 | 8 | 1 |
| | *Cafeteria* Sequences | 528,380 | 573,348 | 406,835 |
| 9 | Bicoecea ASVs | 38 | 42 | 14 |
| | Bicoecea Sequences | 945,630 | 1,179,017 | 722,295 |

Supplementary Table 2. Topographic properties of the co-occurrence networks calculated at each study stie.

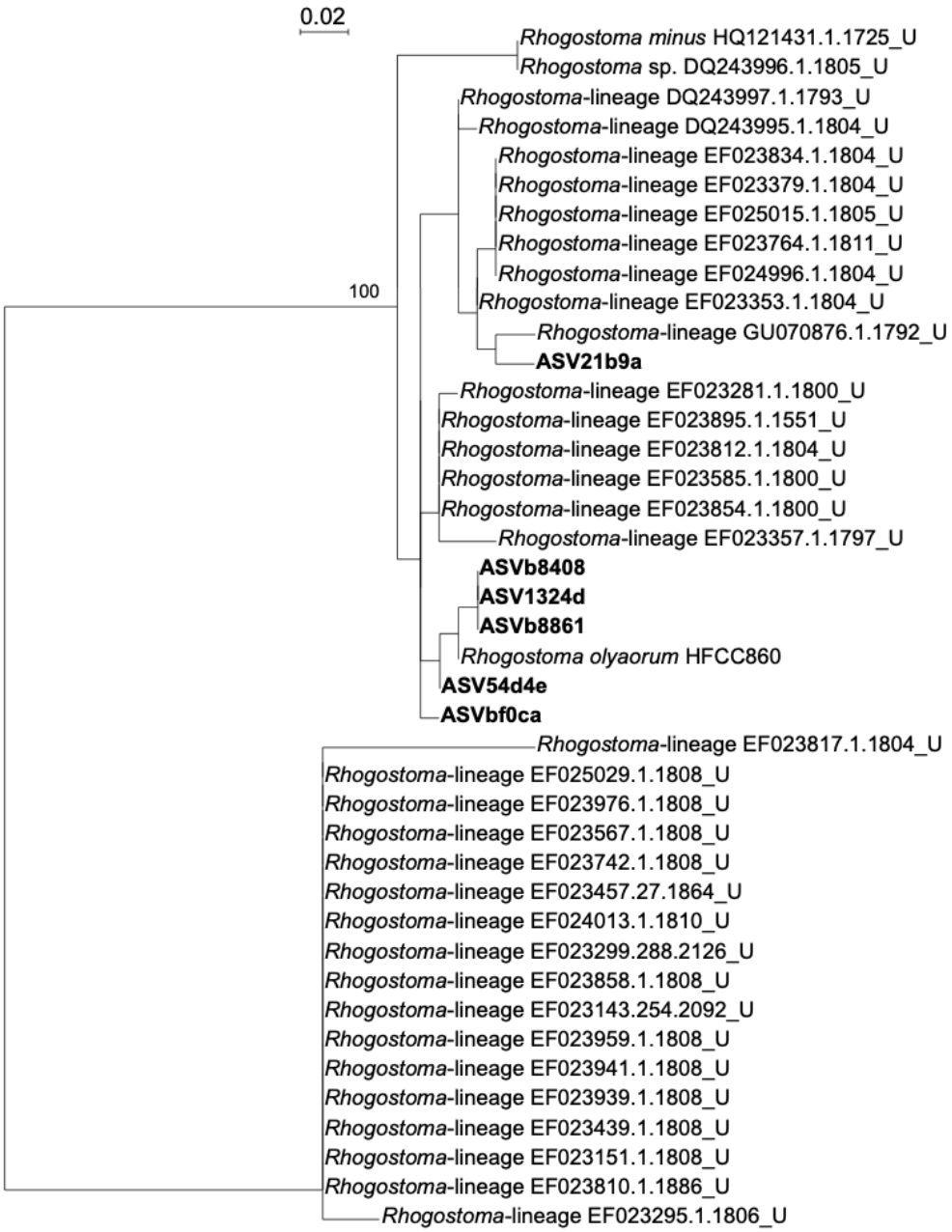
| Property | Terricolous lichen DNA | Terricolous lichen cDNA | Soil coastal cliff DNA | Microbial mat DNA |
|---|---|---|---|---|
| Vertex | 14 | 19 | 16 | 33 |
| Edges | 47 | 65 | 109 | 73 |
| Average degree | 5.28 | 6.84 | 13.62 | 4.42 |
| Average path length | 1.55 | 1.58 | 1.07 | 1.87 |
| Network diameter | 2 | 2 | 2 | 4 |
| Clustering coefficient | 0.54 | 0.74 | 0.92 | 0.1 |
| Density | 0.40 | 0.38 | 0.90 | 0.13 |
| Heterogeneity | 0.61 | 0.54 | 0.13 | 1.46 |
| Centralization | 0.59 | 0.61 | 0.09 | 0.79 |



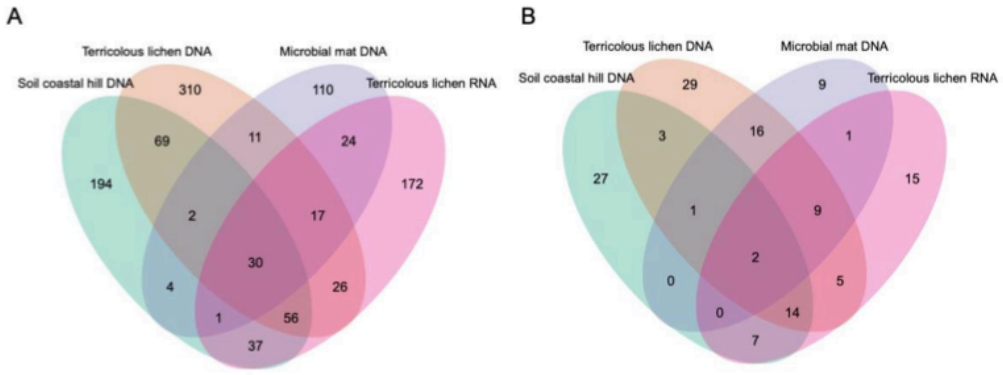

Supplementary Figure 1. Unrooted maximum likelihood phylogeny of the V9 region of the strain HFCC986 and the closest ASVs (DNA) classified as *Neobodo* and reference sequences from the genus *Neobodo* extracted from the database PR$^2$. The sequences obtained during this study are highlighted in bold.



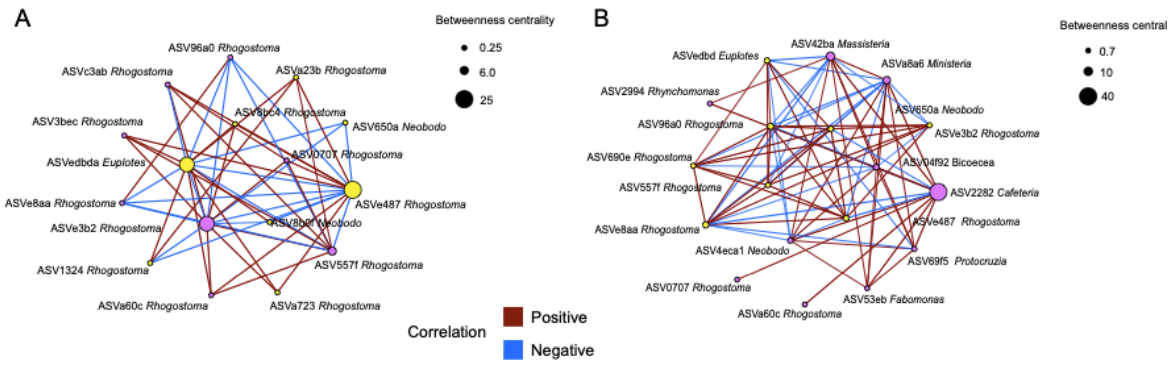

Supplementary Figure 2. Maximum likelihood unrooted phylogeny of the V9 region of the strain HFCC988 and the closest ASVs (DNA) classified as *Euplotes* and reference sequences from the genus *Euplotes* extracted from the database PR$^2$. The sequences obtained during this study are highlighted in bold.




Supplementary Figure 3. Maximum likelihood unrooted phylogeny of the ASVs (DNA) classified as *Rhogostoma* and reference sequences from the genus *Rhogostoma* extracted from the database PR$^2$ and the V9 region of the strain HFCC860 (*Rhogostoma olyaorum*). The sequences obtained during this study are highlighted in bold.




Supplementary Figure 4. The shared and unique ASVs. A: Shared and unique ASVs without filtering according to frequency. B: Shared and unique ASVs after frequency filtering.




Supplementary Figure 5. The co-occurrence of protist ASVs. A: Network based on ASVs from the station Soil coastal cliff (DNA). B: Network based on ASVs from the station Terricolous lichen (cDNA). Nodes indicate each ASV, the size of the nodes indicate the betweenness centrality and the edges indicate the type of correlation between ASVs. The colors of nodes indicate ASVs co-occurring more frequently than with other genotypes.

# Chapter 3: Protist Communities of Microbial Mats from Extreme Environments of Five Saline Andean Lagoons at High Elevation in the Atacama Desert

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# Protist communities of microbial mats from the extreme environments of five saline Andean lagoons at high altitudes in the Atacama Desert

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**Introduction:** Heterotrophic protists colonizing microbial mats have received little attention over the last few years, despite their importance in microbial food webs. A significant challenge originates from the fact that many protists remain uncultivable and their functions remain poorly understood.

**Methods:** Metabarcoding studies of protists in microbial mats across high-altitude lagoons of different salinities (4.3–34 practical salinity units) were carried out to provide insights into their vertical stratification at the millimeter scale. DNA and cDNA were analyzed for selected stations.

**Results:** Sequence variants classified as the amoeboid rhizarian *Rhogostoma* and the ciliate *Euplotes* were found to be common members of the heterotrophic protist communities. They were accompanied by diatoms and kinetoplastids. Correlation analyses point to the salinity of the water column as a main driver influencing the structure of the protist communities at the five studied microbial mats. The active part of the protist communities was detected to be higher at lower salinities (<20 practical salinity units).

**Discussion:** We found a restricted overlap of the protist community between the different microbial mats indicating the uniqueness of these different aquatic habitats. On the other hand, the dominating genotypes present in metabarcoding were similar and could be isolated and sequenced in comparative studies (*Rhogostoma*, *Euplotes*, *Neobodo*). Our results provide a snapshot of the unculturable protist diversity thriving the benthic zone of five athalossohaline lagoons across the Andean plateau.

KEYWORDS

**microbial mats, metabarcoding, protists, co-occurrence, *Rhogostoma*, *Euplotes*, salinity**

## Introduction

Microbial communities thriving in hostile environments, mainly composed of so-called extremophiles, represent hotspots of biodiversity and harbor invaluable genetic resources, which are the key to answering fundamental questions about the limits of life (Warren-Rhodes et al., 2006; Schulze-Makuch et al., 2018). Given the vast diversity of such communities, it is difficult to assess how they will respond to disturbances and to predict the possible functional consequences for the ecosystem. A significant challenge originates from the fact that many microbes remain uncultivable and their functions remain poorly

understood (Solden et al., 2016). However, modern sequencing techniques allow for the exploration of the genetic pool of microbial communities, the study of their structure and functions, and the examination of biogeographic patterns in response to contemporary environmental factors and historical contingencies (Venter et al., 2017; Schiwitza et al., 2018, 2019, 2021; Arndt et al., 2020; Rybarski et al., 2023).

High-altitude lagoons in the Andean Altiplano (15°-22° S, up to 6.170 m a.s.l.) belong to endorheic basins and include diverse microbial ecosystems such as lagoons, brines, wetlands, and salars composed of plankton and benthos enduring hostile conditions (Albarracín et al., 2015; Aran et al., 2021). Part of these microbial guilds is formed by microbial mats, which colonize precipitating salts and rocks within such ecosystems. They interact with physical-chemical gradients, such as the varying mineral concentrations characteristic of Andean water bodies, distributed throughout the ancient Atacama Desert (Demergasso et al., 2003; Farías et al., 2014; Farias et al., 2017; Rasuk et al., 2014, 2016; Fernandez et al., 2016; Saghaï et al., 2017; Vignale et al., 2022). Microbial mats have been identified as important sites for greenhouse gas exchange and complex variable ecosystems in the athalossohaline Andean salt flats, linking these complex laminar assemblages to the cycling of carbon in the remote Altiplano plateau (Dorador et al., 2018; Molina et al., 2021). The microbiology of these stratified microhabitats has been linked mainly to primary producers (cyanobacteria) and is considered to be among Earth's earliest ecosystems (Margulis et al., 1980; Dupraz et al., 2009). Various Andean microbial mats have been studied, focusing on their prokaryotic communities, including archaea and a wide range of viruses, exhibiting changes along the vertical gradient (e.g., Dorador et al., 2008, 2013; Hernández et al., 2016; Eissler et al., 2019). Considering protists, unicellular eukaryotic biosignatures have mainly been described from lower altitudes (Salar de Llamará, 750 m a.s.l.), in saline microbial mats and hypersaline lagoons harboring biosignatures classified as diatoms, bicosoecids, and ciliates as detected by metabarcoding (Saghaï et al., 2017; Rybarski et al., 2023). Efforts to isolate and sequence the cultivable and uncultivable protist diversity from saline to hypersaline lagoons unveiled a novel endemic diversity including species from Opisthokonta (choanoflagellates), Rhizaria (Cercomonadida), Discoba (percolomonads), Stramenopiles (bicosoecids and placidids), and Alveolata (ciliates) (Schiwitza et al., 2018, 2019, 2021; Arndt et al., 2020; Carduck et al., 2021; Rybarski et al., 2021, 2023; Schoenle et al., 2022; Acosta et al., 2023; Hohlfeld et al., 2023).

The increasing focus on protists all over the planet has unveiled a heterogeneous and variable diversity that can be found across different biotopes (terrestrial to aquatic), unveiling biogeographical patterns even within ecosystems (Dillon et al., 2009; Mahé et al., 2017; Schoenle et al., 2021; Singer et al., 2021; Rybarski et al., 2023). Protists have been found to follow the vertical stratification characteristics in microbial mats, assembling differentially relative to vertical physic-chemical gradients (Saghaï et al., 2017). Under the current climate change scenario, water availability is being threatened, highlighting the importance of studying the structure of protist communities living in delicate ecosystems such as the Andean microbial mats. In this study, we assess the structure of these communities at the vertical millimeter scale in the

TABLE 1 Information of the stations indicating geographical and physic-chemical properties at each station.

| Station | Altitude (m a.s.l.) | Geographic location (latitude/longitude) | Water temperature (°C) | Sediment temperature (°C) | Practical salinity units (PSU) | Resistivity (Ohm/cm) | Conductivity (mS/cm) | Total dissolved solids (TDS) |
|---|---|---|---|---|---|---|---|---|
| Helada Lagoon | 4.306 | 23°0605.3"S/67°0837.1"W | 25.1 | 24.36 | 4.51 | 124.94 | 8.06 | 3.9 |
| Amarilla Lagoon | 4.537 | 23°1312.4328/67°3615.39W | 15.5 | 17.08 | 15.51 | 146.01 | 23.21 | 11.37 |
| Salada Lagoon | 2.297 | 23°4105.2"S/68°0821.0"W | 27.2 | 25.75 | 34 | 19.45 | 51.42 | 25.1 |
| Aguas Calientes Salar | 4.232 | 23°0848.2"S/67°2509.1"W | 50.4 | 46.11 | 24.92 | 25.71 | 38.89 | 19.06 |
| Quepiaco Salar | 4.580 | 23°0456.6"S/67°3555.4"W | 10.1 | 11.5 | 7.95 | 69.93 | 14.3 | 7.07 |

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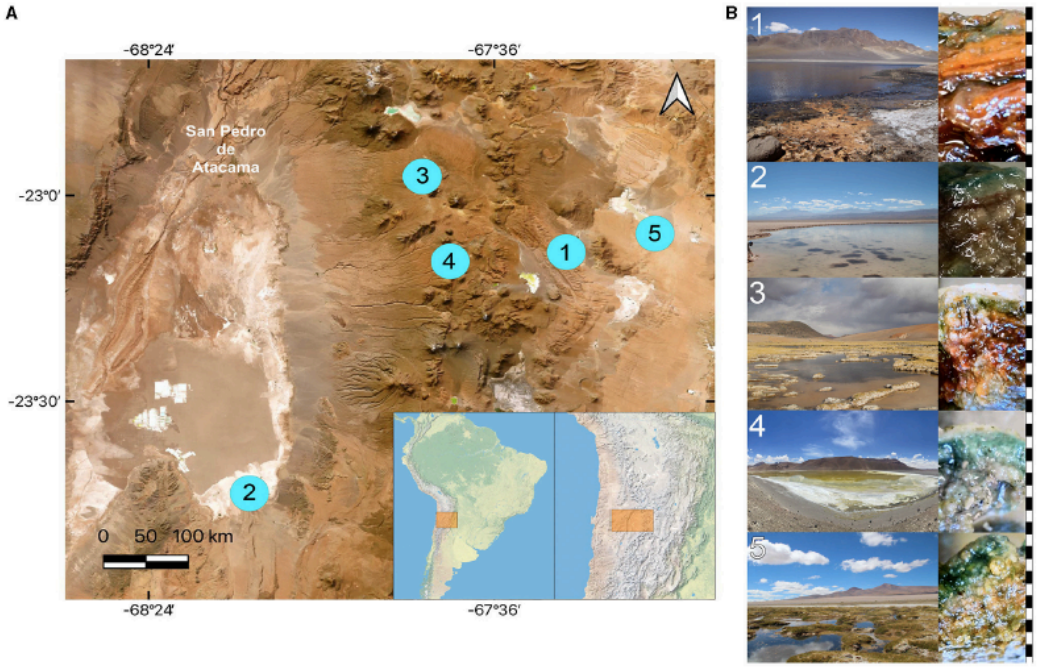


FIGURE 1
Sampling sites of microbial mat studies in the Atacama Desert. **(A)** Map showing the location of the stations. **(B)** Study sites and vertical structure of the corresponding microbial mats. 1, Aguas Calientes Salar; 2, Salada Lagoon. 3, Quepiaco Salar; 4, Amarilla Lagoon; 5, Helada Lagoon. Right side: scale in black and white indicating one millimeter intervals. Map produced and adapted from QGIS 3.28.5 - Firenze.

microbial mats of five Andean Lagoons using metabarcoding and explore their differences and their interaction with the overlying water column.

## Materials and methods

### Sample collection and molecular biology procedure

Microbial mats were sampled from the sediments below the water line in five lagoons at different altitudes across the Chilean Altiplano (Table 1). The structure of the studied microbial mats differs visibly throughout the studied sites (Figure 1B). Microbial mat layers characterized by an upper green layer were found in Helada Lagoon, Amarilla Lagoon, and Salada Lagoon, while in Quepiaco Salar, microbial mats are covered by a layer of salt and the upper green layer is thinner. In contrast, the microbial mat sampled from Aguas Calientes Salar exhibits a structure of orange and red layers. Biological samples of the microbial mats were dissected *in situ* using sterile metalware and coveralls to maintain cross-contamination to a minimum. The microbial mats were dissected to obtain three biological samples from each layer, starting from the upper layer (first millimeter), the second layer (3 mm depth), and a third layer (5 mm depth; Figure 1). The environmental parameters of the water column, such as salinity (Practical Salinity Units, PSU), resistivity (Ohm/cm), conductivity (mS/cm), and total dissolved solids (parts per trillion), were measured using an Orion Star A322 Conductivity Portable Multiparameter (Thermo Scientific) (Table 1). The sediment and water temperatures were measured using a HI935002 Dual Channel K-Type Thermocouple Thermometer (Hanna Instruments). Each replicate was homogenized in RNA later to preserve the DNA and RNA from each sample (Wang et al., 2018) in sterile 2-ml Eppendorf tubes and stored at −20°C until further analyses. The total DNA and RNA were extracted from samples using Trizol (Invitrogen), according to the manufacturer's protocol with minor modifications: Samples were homogenized in 1 ml of Trizol reagent for 10 min as a cell lysis step using a vortex adaptor. The final DNA was resuspended in 100 µl of nuclease-free water and used immediately for the amplification of the target gene. RNA was resuspended in 50 µl of nuclease-free water for downstream analyses. Total RNA was used as a template for cDNA synthesis using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher). Total concentrations of DNA, RNA, and cDNA were measured using the ND-1000 nanodrop (Peqlab Biotechnologie, Germany).

## Mock community preparation and sequencing

Aiming to assess the accuracy of the used classification method for sequencing libraries, we included an artificial sample (a mock community). This sample was composed of equimolar concentrations of the PCR-amplified V9 region from eight species from the Heteroflagellate Collection Cologne (HFCC) (Schoenle et al., 2021). The control samples included the V9 region (rDNA) amplicon aliquots of strains from the six protist supergroups Alveolata, Ancyromonadida, Rhizaria, Discoba, Stramenopiles, and Opisthokonta: *Protocruzia* sp. (MT355146), *Aristerostoma* sp. (MT081566), *Fabomonas tropica* (MT355148), *Massisteria* sp. (MT355122), Rhynchomonadidae sp. (MT355133), *Cafeteria burkhardae* (MN315604), Bicosoecida sp. (MT355117), and *Ministeria vibrans* (MT355150) (Supplementary Table 1). For this, the DNA was extracted from the strains by transferring clonal cultures (30 ml) into 50-ml centrifuge tubes (Sarstedt, Nümbrecht, Germany) and centrifuging at 4,000 × *g* at 4°C for 20 min. After discarding the supernatant, the DNA was extracted from the cellular pellet using the Quick gDNA Mini-Prep Kit (Zymo Research Corporation, United States) following the manufacturer's instructions. An aliquot of 3 µl of DNA from each strain was used as a template in a PCR reaction using 5 µM of the universal primer set 18S: Forward (5-AACCTGGTTGATCCTGCCAGT-3) and Reverse (5-TGATCCTTCGCAGGTTCACCTAC-3) (Medlin et al., 1988). The PCR conditions were the following: a denaturation step at 96°C for 120 s, then 34 cycles of 96°C for 30 s, 55°C for 45 s, 72°C for 150 s, and a final elongation step for 420 s at 72°C. The PCR products were purified using the FastGene Gel/PCR Extraction Kit (Fast Gene, Japan), and the sanger was sequenced using the 18S rDNA primer sets at GATC Biotech, Germany. The assembled sequences of the 18S rDNA gene were analyzed using the Basic Local Alignment Search Tool (RRID:SCR_004870).

## Amplification of the hypervariable region V9 SSU rDNA and next-generation sequencing

The aliquots of the DNA and cDNA from environmental samples as well as from the DNA isolated from the strains for the mock community were used as a template for the amplification of the hypervariable region V9 of the small sub-unit 18S rDNA. The amplification was carried out using the multiplex identifier-tagged (MIDs) primers 1389F and 1510R, following the PCR protocol of Amaral-Zettler et al. (2009). The PCR conditions were the following: 98°C for 30 s; 25 cycles of 10 s at 98°C, 30 s at 57°C, 30 s at 72°C, and a final step at 72°C for 600 s. The PCR products were purified using the FastGene Gel/PCR Extraction Kit (Fast Gene, Japan), and equimolar concentrations of all purified PCR products were pair-end sequenced (2 × 150 bp) in multiplexed libraries using an Illumina NovaSeq 6000 sequencer (Illumina, United States) at the Cologne Center for Genomics (CCG).

## Bioinformatic analyses

Multiplexed libraries of the reads obtained through the metabarcoding of DNA and cDNA were processed in QIIME2 (RRID:SCR_021258, Bolyen et al., 2019). The MIDs and primer sequences were removed from the libraries using the "cutadapt trim-paired" function, and sequences with an error rate higher than 0 were filtered. The raw forward and reverse reads were trimmed to exclude the primer sequences, dereplicated, and denoised into amplicon sequence variants (ASVs), using the "denoise-paired" function from the DADA2 plugin (RRID:SCR_023519, Callahan et al., 2016). This method includes a chimera-filtering step on the ASVs using the "consensus" chimera detection method on all samples, and sequences found chimeric in a sufficient fraction of samples were removed (method: isBimeraDenovo). To discern between protist taxa, the ASVs were classified using a cut-off value of 0.8 and a 97% identity to the database through the function "feature-classifier classify-consensus-blast" (Camacho et al., 2009). For this, we used the Protist Ribosomal Reference ($PR^2$) database version 4.14.0, amended with the V9 region of the 18 SSU rRNA gene sequences from the Heterotrophic Flagellate Collection Cologne (HFCC) (Schoenle et al., 2021). The ASVs classified as metazoa, archaeplastida, fungi, or unassigned were excluded from further analyses.

## Statistics and reproducibility

The alpha and beta diversity metrics were obtained through the function "qiime diversity core-metrics-phylogenetic", which relates to the lowest number of sequences. The difference in the alpha-diversity metrics (observed number of ASVs, the Shannon index) across the study sites was tested in a Kruskal-Wallis test for paired groups (study site-study site and DNA-cDNA) using the function "qiime diversity alpha-group-significance". Additionally, we tested the dissimilarity in beta diversity metrics (Unifrac metrics, Jaccard, and Bray-Curtis distances) across study sites in a permutational analysis of variance (PERMANOVA, 999 permutations) through the function "qiime diversity beta-group-significance". The output files, such as the frequency table, the sequences table, the phylogenetic tree, the assigned taxonomy, and the metadata used in QIIME2, were imported into R (version 4.3.0) and processed using the "microeco" package version 1.1.0 (Liu et al., 2021). The bar plots showing the relative abundance of the major protist lineages across study sites, as well as clustering patterns based on Euclidean distances, were plotted using the "plot_bar", setting the clustering option "TRUE". The beta diversity patterns observed through Jaccard distance were analyzed in a principal coordinate analysis (PCoA) for the DNA and DNA-cDNA-based protists community (97% of identity to the database), using the functions "cal_ordination" and "plot_ordination". Venn diagrams were generated to depict the number of shared and unique ASVs across microbial mat layers. Furthermore, a canonical redundancy analysis (RDA) with feature selection was used to evaluate the association between the environmental parameters measured in the water bodies (independent variables) and the protist taxa (dependent variables), detected through DNA and

cDNA, respectively. Due to the presence of null values, the data was normalized, scaling the abundance of the ASVs to unit variance and adding "scale = TRUE" in the "cal_ordination" function.

## Results

Overall, all the studied water bodies are mesohaline, ranking from 4.51 PSU (Helada Lagoon) up to 34 PSU (Salada Lagoon, Atacama Salar). Other parameters measured in the water column are shown in Table 1.

### Next-generation sequencing analysis

It was planned to have DNA and cDNA data sets from all sampling sites. However, cDNA could not be amplified from all stations. We successfully amplified the target phylogenetic marker mainly from the upper layers of a total of 23 microbial mat samples, distributed as follows: four samples from Helada Lagoon, three from the first layer and one from the second layer; a total of seven samples from Quepiaco Salar, three from the first layer, three from the second layer, and one from the third layer; a total of five samples from Amarilla Lagoon, three samples from the first layer and two samples from the second layer; from Aguas Calientes Salar, a total three samples from the first layer; and from Salada Lagoon, three samples from the first layer. We amplified the target gene from three out of five study sites in a total of 15 samples for cDNA. From Helada Lagoon, there were a total of four samples: one from the first layer, two from the second layer, and one from the third layer. From Quepiaco Salar, there were a total of seven samples: three from the first layer, three from the second layer, and one from the third layer. From Amarilla Lagoon, there were a total of four samples: two from the first layer and two from the second layer.

A total of over 54.6 million raw forward and reverse reads were obtained from the DNA metabarcoding of the five studied microbial mats, of which over 53.1 million (97.2%) were barcode-clipped. After quality control and denoising, a total of over 41.8 million sequences associated with 32,089 ASVs were obtained. For cDNA, a total of 24.7 million raw forward and reverse reads were obtained from three out of five of the studied microbial mats (Helada Lagoon, Amarilla Lagoon, and Quepiaco Salar). From these, ~17.5 million (70.82%) were barcode-clipped, and after quality control and denoising, we obtained a total of ~13.9 million sequences associated with 19,134 ASVs. As we included an artificial control sample of known composition (the so-called mock community), we could assess the accuracy of the taxonomic classification method used in this work. The sequences for each of the eight taxa of the mock community that could be assigned to the respective taxon contributed 3.1–24.4% to the total abundance of reads, using a diversity metric commonly used in environmental sequencing assessments (97% of identity to the database). Additionally, we detected low-frequency ASVs classified as taxa that were not included in the mock community. Such taxa were found at frequencies ranging from 0.032 to 0.047% in the different sequencing libraries. These percentages were used as a threshold for each library preparation, aiming to exclude potential spurious sequences of low abundance.

TABLE 2 The amounts of sequences and ASVs of DNA and cDNA origin obtained at critical steps of the bioinformatic analysis used in this study.

| Template | Station | Samples | Total reads | Total ASVs | Reads after filter | ASVs after filter | Protist reads | Protist ASVs | Protist reads (after filter) | Protist ASVs (after filter) |
|---|---|---|---|---|---|---|---|---|---|---|
| DNA | Helada Lagoon | 4 | 4,623,819 | 7,565 | 4,115,878 | 972 | 1,080,626 | 380 | 1,050,871 | 69 |
| | Amarilla Lagoon | 4 | 7,686,612 | 4,833 | 6,942,574 | 796 | 1,966,195 | 287 | 1,938,534 | 107 |
| | Salada Lagoon | 4 | 8,998,521 | 3,335 | 7,343,385 | 353 | 4,673,791 | 301 | 4,664,371 | 61 |
| | Aguas Calientes Salar | 4 | 6,344,391 | 3,926 | 6,050,773 | 379 | 3,639,749 | 222 | 3,621,420 | 42 |
| | Quepiaco Salar | 7 | 14,164,719 | 12,430 | 13,320,665 | 2,368 | 93,460 | 447 | 65,742 | 29 |
| cDNA | Helada Lagoon | 4 | 6,564,350 | 7,753 | 6,210,626 | 1,178 | 601,112 | 392 | 181,887 | 181 |
| | Amarilla Lagoon | 4 | 2,563,837 | 3,111 | 2,486,032 | 1,223 | 123,313 | 245 | 118,339 | 115 |
| | Quepiaco Salar | 6 | 4,848,733 | 8,270 | 4,566,137 | 2,434 | 48,239 | 320 | 40,628 | 90 |

The protist reads and ASVs correspond to the pre-filter assessment.

After applying the threshold from the mock community, our environmental samples include a total of over 37.7 million sequences (90.3% of the total sequences) associated with 4,868 ASVs (15.17% of the total ASVs), remaining from the DNA metabarcoding (Table 2), and a total of over 13.2 million sequences (94.8% of the total sequences) associated with 4,835 ASVs (25.2% of the total ASVs) remained from the cDNA metabarcoding. Using a 97% identity to the database, we identified 308 ASVs associated with 42 lineages (6.3% of the remaining ASVs after filtering) from the DNA metabarcoding, and these were associated with over 14.2 million sequences (17.6% of the remaining sequences after filtering). From the cDNA metabarcoding, we identified a total of 386 ASVs associated with 41 lineages (7.9% of the remaining ASVs after filtering), which were associated with 747,027 sequences (5.6% of the remaining sequences after filtering).

## Protist community structure in Andean microbial mats

The protist communities determined by the DNA metabarcoding were mainly dominated by Rhizaria (15.1–95.6% of the reads), Stramenopiles (0.5–69.4%), Alveolata (5–22.3%), Amoebozoa (0.9–11.3%), and Haptista, which occurred only at Quepiaco Salar (61.5% of the reads) (Figure 2). The dominant taxa detected through cDNA metabarcoding (three study sites) were Stramenopiles, ranging between 19.8% of reads for Quepiaco Lagoon, 34.4% for Helada Lagoon, and up to 59.5% for Amarilla Lagoon. Other important taxa detected by cDNA metabarcoding were Rhizaria (22–51.6% of the reads), Alveolata (6.3–11.9%), and Discoba (0.4–5.5%). Further information on the relative abundance of protist taxa that could be detected across the stations studied by DNA and cDNA is shown in Figure 3.

The analysis of ASVs of the DNA data sets shared between the different stations revealed a relatively low number either when comparing the different sites or when comparing different layers (Figure 4). The relative number of shared sequences is relatively similar when comparing unfiltered filter data sets or when a rigid (mock) filter is applied.

## Alpha diversity across the five Andean microbial mats

The overall mean number of ASVs was 10.8 with a standard deviation (SD) of 7.1, with the highest mean ASVs obtained from Amarilla Lagoon (mean = 20.2, SD = 1.1) and the least from Quepiaco Salar (mean = 2.5, SD = 1.1) (Figure 5A). The overall mean Shannon diversity obtained was 1.2 with a standard deviation of 1.4, with the highest value for Amarilla Lagoon (mean = 3.3, SD = 0.2) and the lowest for Aguas Calientes Salar (mean = 0.5, SD = 0.06). The overall phylogenetic diversity mean value was 15.7 with a standard deviation of 5.3, with the highest mean value found for Amarilla Lagoon (mean = 18.6, SD = 6.05) and the lowest for Quepiaco Salar (mean = 10.7, SD = 0.6). We tested the dissimilarity between the groups of protists detected across the study sites, considering the observed ASV numbers and Shannon diversity calculated from the DNA as well as from cDNA data sets. The amount of observed ASVs was significantly different between Amarilla Lagoon and all other study sites, and that of Quepiaco Salar was also significantly different from all the other study sites (Figure 5A). When we compared the study sites regarding the Shannon index, significant differences were found between Quepiaco Salar and Helada Lagoon on the one side and the other three study sites on the other side (Figure 5A). Regarding the results of the cDNA metabarcoding data for the three study sites investigated, there were no significant differences regarding the number of observed ASVs, while the data from Quepiaco Salar differed from the other two study sites regarding the Shannon index. The cDNA values for one station clustered very similar to the respective DNA values when compared using the principal coordinate analysis (Figure 2D).

## The structure of protist communities detected through DNA and cDNA

The dissimilarity measured between the studied layers across stations is presented in a cladogram based on the Jaccard distance, which indicates four main groups (Figure 5). Two groups were composed of samples from Quepiaco Salar (DNA and cDNA), Aguas Calientes Salar, and Salada Lagoon (DNA), and two groups were composed of Helada Lagoon, Amarilla Lagoon, and Quepiaco Salar. This last group includes samples obtained from both DNA and cDNA from Helada Lagoon and Amarilla Lagoon and samples obtained from cDNA at Quepiaco Salar (Figure 5). The principal coordinate analysis (PCoA; Figure 2C) explains 53.2% (PCo1 = 30.2%, PCo2 = 23%) of the dissimilarity between the five study sites (DNA). On the other hand, the protist communities analyzed by the DNA and cDNA for three study sites (Figure 2D) showed a pattern of dissimilarity explained by 48% (PCo1 = 31.5%, PCo2 = 16.5%). However, the differences between the communities detected through DNA and cDNA within samples from one station were not significant in all three investigated cases.

## Association of the abiotic factors and the protist communities

The results of the redundancy analysis (RDA), including the protist lineages of the DNA metabarcoding data sets in the first layer across the five stations, revealed significant correlations (Figure 6). On the other hand, the analysis of cDNA data detected no significant association between the protist taxa and environmental factors. The interpretation rate of the RDA for DNA metabarcoding was equal to 79.6% (RDA1 = 48.1%, RDA2 = 31.5%). The resistivity ($R^2 = 0.8818$, Pr = 0.001), the conductivity ($R^2 = 0.7259$, Pr = 0.003), and the salinity ($R^2 = 0.7231$, Pr = 0.003) of the water bodies were selected as significant factors ($p < 0.01$) associated with the protist composition of the studied microbial mats. Furthermore, the protist taxa associated with these environmental parameters were chlorophytes, Lobosa (Amoebozoa), Cercozoa (Rhizaria), Ciliophora, Apicomplexa and

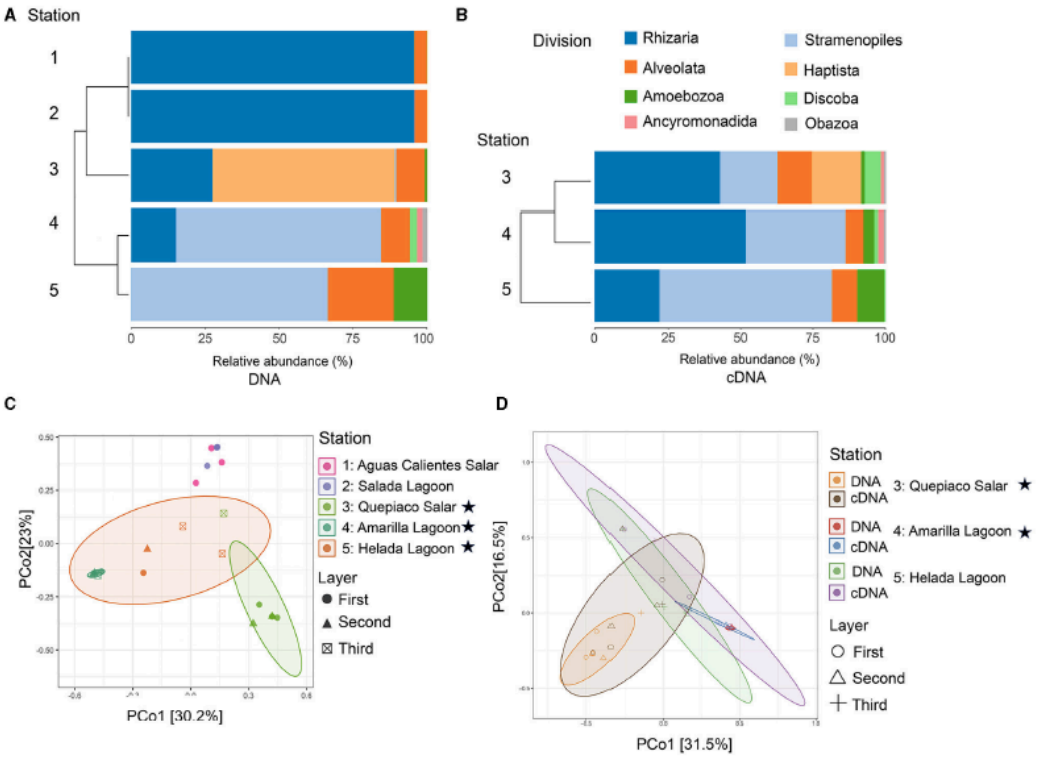


FIGURE 2
Diversity of protist taxa in microbial mats based on NGS data. **(A)** Cluster analysis and relative abundance of the major protist lineages detected through DNA (identity to the database > 97%) across the five stations. **(B)** Cluster analysis and relative abundance of the major protist lineages detected through cDNA (identity to the database > 97%) across the study sites 3, 4, and 5. Stations, 1, Aguas Calientes Salar; 2, Salada Lagoon; 3, Quepiaco Salar; 4, Amarilla Lagoon; 5, Helada Lagoon. **(C)** Principal coordinates analysis of the Jaccard distances based on DNA. **(D)** Principal coordinates analysis of the Jaccard distance between protists lineages detected through DNA and cDNA for three stations. Stars indicate stations with protist communities being significantly different from each other and from the non-labeled stations (for details see text).

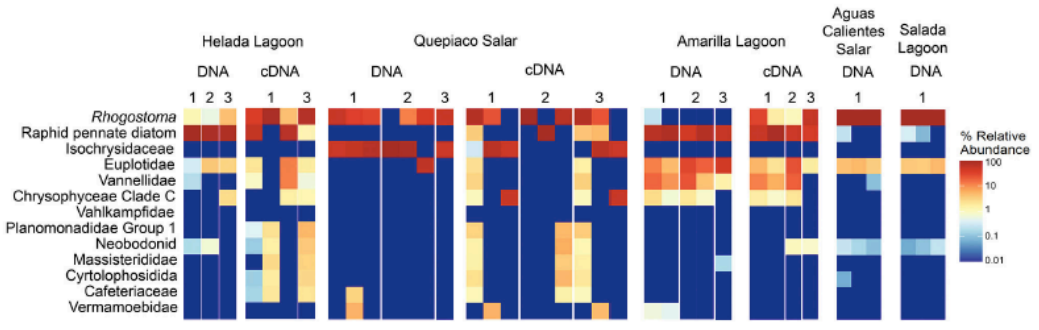


FIGURE 3
Heatmap of the relative abundance of the top 13 taxa detected in the studied layers from microbial mats based on DNA or cDNA.

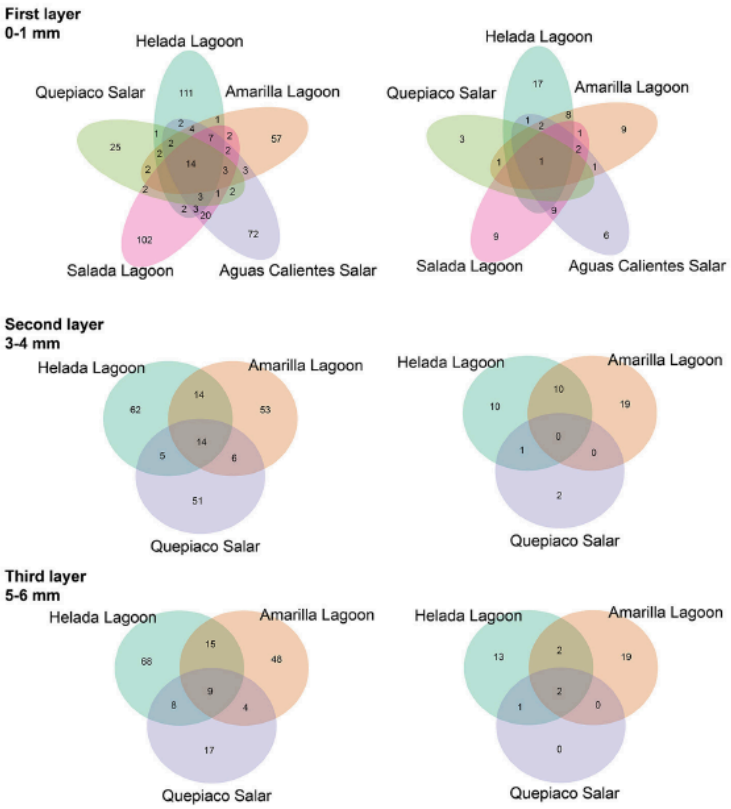


FIGURE 4
The shared amplicon sequence variants (ASVs) at each layer between stations are detected by DNA metabarcoding. **(Left)** Data without rigid filtering. **(Right)** Data filtered using the mock community-deduced threshold.

Dinoflagellata (Alveolata), Ochrophyta, Haptophyta, Discicristata, and one unclassified division of Stramenopiles.

## Discussion

In this study, we delve into the ecology of protists in the microbial mats of five Andean Lagoons with different salinities, characterizing the structure of their communities through the amplification of the V9 region (18S SSU). The primary goal was to assess, at the millimeter scale (Figure 1), the potentially resting stages as well as the active protist communities detected through the metabarcoding of DNA and cDNA in five stations. Partly, this could not be resolved, as the amplification of the target gene was successful mainly from the upper layers and from the DNA and cDNA templates only in three stations (Figure 3). Future studies aiming to study the stratification and activity of protist groups in Andean microbial mats should consider the use of both hypervariable regions (V4 and V9), as recommended for comprehensive diversity studies using environmental sequence variants (Choi and Park, 2020). Nevertheless, with our study, we could determine the ASVs shared across all stations at each studied layer (Figure 4). The restricted overlap between the different microbial mats indicates the specificity of the different aquatic habitats. The reasons for this specificity could not be fully explained in the course of the present study; however, salinity might be an important driver (see below). Overall, phototrophs and amoebae are frequent in the uppermost layers, and ciliates are common throughout all depths (DNA metabarcoding). The active protist groups (cDNA) were mainly diatoms shared between stations at the first (0–1 mm) and second (3–4 mm) layers, while *Euplotes* was the only genotype detected as active and shared by the deepest layer (5–6 mm). The functional diversity of microbes can vary across the layers of microbial mats due to changes in water and light availability, particularly for oxygen producers such as the cyanobacteria and diatoms studied in the saline lake of the Atacama Desert, the Salar de Llamará (Demergasso et al., 2003). Such variations could be responsible for the observed differences in the vertical structure of protist communities. As initially hypothesized, the studied ecosystems appeared to be

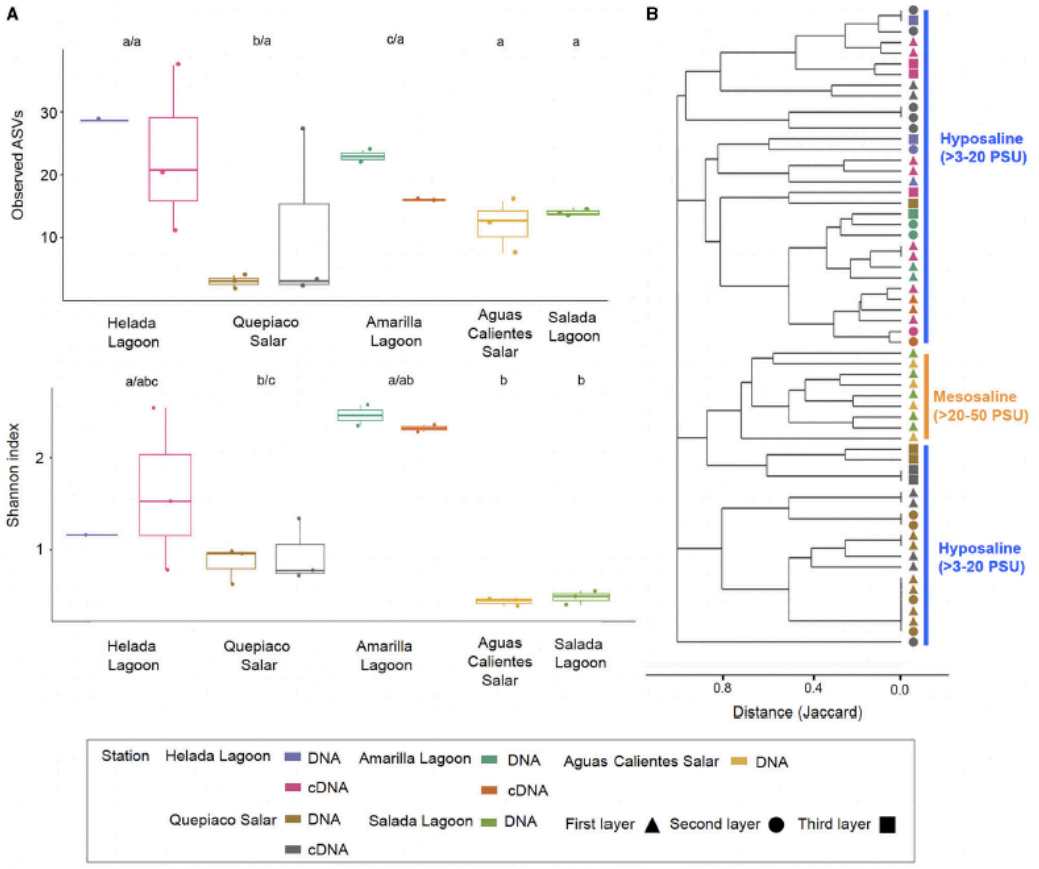


FIGURE 5
Alpha and beta diversity metrics were measured across the five stations. **(A)** Box and whiskers plots of the observed ASVs and the Shannon index measured in the first layer of microbial mats across stations. Different letters above the boxes mark significant differences, before the slash for DNA, and behind for cDNA data (Kruskal-Wallis, $p < 0.05$). **(B)** Cluster analysis based on the Jaccard distances between protist communities from each layer of microbial mats among stations ($p < 0.05$ for values >0.8). Salinity ranges are indicated on the right side.

highly underexplored regarding protists lineages. ASVs classified as eukaryotes accounted for <1% of the total sequences (at 97% identity in databases). Moreover, using a lower identity threshold (80% of identity to the databases), only up to 7.7% of the total sequences could be assigned to protist lineages. This supports the hypothesis of a high novelty in such remote ecosystems. This is in agreement with Burki et al. (2021), who suggest that such remote and underexplored environments could constitute an untapped reservoir of potential new biodiversity. Our RDA analysis revealed that factors such as salinity, conductivity, and resistivity were correlated with the detected benthic communities, which included cercozoans, ciliates, lobose amoebae, diatoms, and kinetoplastids (Figure 6). Among these, salinity is a factor often related to desiccation, shaping the community structures of microbial communities under water fluctuations (Rothrock and Garcia-Pichel, 2005; Lozupone and Knight, 2007). Other abiotic factors, such as conductivity, solar radiation, and temperature, have been associated with the community composition of microbes in hypersaline lagoons, mainly linking salinity to microbial abundance (Bryanskaya et al., 2016). Based on our results, we underscore the potential of high-throughput sequencing approaches to explore the structure of protists communities thriving in delicate and complex habitats and the limitations of the study of protists living at elevated salinity.

One essential component of biodiversity surveys using metabarcoding has been the use of an artificial sample (a mock community), aiming to identify spurious sequences (Schloss et al., 2011). This has already been implemented in the study of aquatic ecosystems throughout the Atacama and the search for patterns at the community level without blurring the diversity patterns

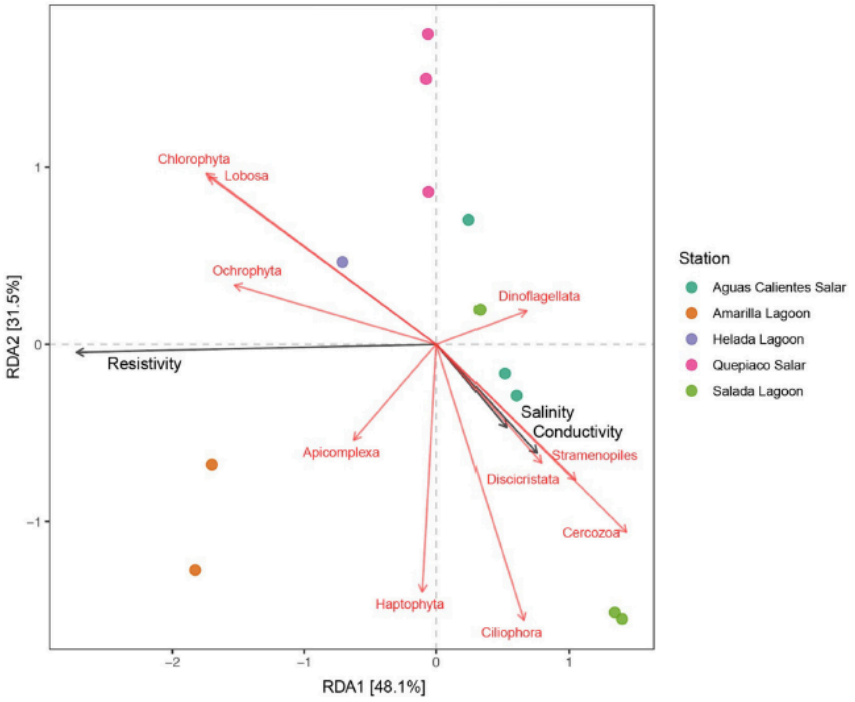


FIGURE 6
Canonical redundancy analysis (RDA) of the protist genotypes detected in the upper layer of the studied microbial mats (identity to the database > 97%). Black arrows represent the environmental variables that have a significant effect on the studied genotypes and the red arrows represent the genotypes associated with the environmental variables.

(Rybarski et al., 2023). In our study, we could assess the taxonomy of the artificial samples in three sequencing libraries for the studied microbial mats and could define a frequency threshold deduced from the frequency of reads with low abundances. We used the frequency of these reads to filter ASVs of small abundance in our environmental samples (frequency < 0.032–0.047%), affecting mainly the ASV numbers (Table 2). Approximately 9.6–18.9% of the total ASVs and between 82 and 95.2% of sequences passed this filter and were included in the diversity assessment in the downstream analyses. The correct identification of the eight strains in the mock communities additionally supported the classification method for the study of our environmental samples. With these results, we confirm the use of mock community-deduced thresholds as the backbone of studies on protist communities in different environments through metabarcoding (e.g., Fiore-Donno et al., 2019; Dünn and Arndt, 2023; Sachs et al., 2023).

## Alpha diversity and taxonomy of protists across five microbial mats

Studies on the protist diversity using metabarcoding in the Atacama Desert and the Andean region are scarce. Our results are comparable to one study on the protists diversity (hypervariable region V4) in microbial mats from the hypersaline lagoons in Llamará Salar (Saghaï et al., 2017). We can also compare our work to one study of heterotrophic flagellate communities (hypervariable region V9), detected in the waters of diverse salars across the Atacama (Rybarski et al., 2023). Those authors found a diversity of protists, reaching a minimal amount of six and up to 214 OTUs per study site. In our study, we registered lower numbers ranging between 2 and 20 ASVs per replicate. The amount of shared and unique biosignatures per station was similar (our study: 1–11 ASVs; Rybarski et al., 2023: 1–44 OTUs). Our samples reached a mean number of ASVs that could be confirmed by cDNA studies at the referred stations, reaching similar values at each station (e.g., Figure 5). Furthermore, our analysis of the shared and unique communities, including raw sequences, showed similar numbers of observed shared and unique genotypes as obtained by Rybarski et al. (2023). The registered diversity in Llamará Salar microbial mats (Saghaï et al., 2017) is low at stations of higher salinity (Simpson diversity index 0.93). In accordance with this, our results showed that the protist communities detected through DNA and cDNA metabarcoding include a low diversity at stations with elevated salinity (Figure 5).

At the millimeter scale, we detected abundant raphid diatoms, mainly in the upper layers of the microbial mats in the lagoons of Helada and Amarilla. In the active community

assessment (cDNA), they appear abundant in the upper layers (Supplementary Figure 2). Phototrophs are the main builders of microbial mats, contributing to their formation through the excretion of exopolymeric substances (EPSs) and by facilitating carbonate accumulation in organo-mineralization processes. In our dataset, we identified *Amphora*, *Navicula*, and *Nitszchia* as primary producers, which agrees with the findings from Saghaï et al. (2017). Their study documented a high occurrence of diatoms in the upper layers of microbial mats in another nearby Atacama saline lake, studied at the centimeter scale, as well as in other Andean microbial mats (Farías et al., 2014; Albarracín et al., 2015; Vignale et al., 2022). Among stramenopiles, we also detected ASVs closely related to genotypes of marine diatoms (e.g., *Skeletonema*, *Thalassiosira*, *Phaeodactylum*), freshwater diatoms (e.g., *Cymbella*, *Pseudogomphonema*), chrysomonads (e.g., *Paraphysomonas*, *Ochromonas*) and diverse unclassified raphid pennate diatoms. Saghaï et al. (2017) also found stramenopiles accompanied by choanoflagellates, bicosoecids, and ciliates, with small numbers of OTUs dominating the abundance of the protist communities. In deep layers, they detected amoebae, with Conosa reaching almost 10% of the relative abundance of protists. Interestingly, in our study in the deepest layer of Quepiaco Salar, amoebae were also detected as being active and abundant (Supplementary Figure 2, Quepiaco Salar cDNA). We assessed the shared communities of protists throughout the first, second, and third layers across the stations (Figure 4). In the first layer, a common amoeba in the DNA and cDNA sets is the thecate amoeba *Rhogostoma*. Other taxa detected through cDNA in the first layer (Helada Lagoon, Amarilla Lagoon, and Quepiaco Salar) include *Amphora*, *Euplotes*, *Vannella*, and raphid pennate diatoms. No shared protists were found in the second layer; however, *Euplotes*, *Vannella*, and *Navicula* genotypes occurred in the unfiltered data set among the shared taxa (Helada and Amarilla Lagoons, Quepiaco Salar). At the deepest layer, a genotype of the ciliate genus *Euplotes* appeared to be shared in both DNA and cDNA data sets (Supplementary Figure 2), being abundant and represented by highly similar ASVs (one nucleotide difference, Supplementary Table 3). One ASV was 100% identical to a strain isolated from the Amarilla Lagoon (HFCC988, *Euplotes* sp.). This speciose genotype, or at least a very similar one, was also reported from Llamará Salar by Saghaï et al. (2017).

A typical protistan genotype we found at the different stations belonged to the genus *Rhogostoma*, a common protist genotype that we identified through DNA and cDNA analysis and occurred mainly in the uppermost layers of the microbial mats. Species of this genus are common inhabitants of aquatic and terrestrial ecosystems (Öztoprak et al., 2020). Although we cannot indicate the presence of this genotype as a species, we can trace our result to one cercozoan genus registered earlier as an arboretum inhabitant (Howe et al., 2011) and recently to a species member of the phyllosphere of endemic cacti in the Atacama (Acosta et al., 2023). This thecofilose amoeba, *Rhogostoma*, likely predates phototrophs as the diatoms (e.g., *Amphora*) are detected in parallel. Other remarkable protist lineages are those taxa detected in Quepiaco Salar, represented by *Cafeteria*, *Isochrysis*, and *Hartmannella*, of which the first two include marine strains (Bendif et al., 2013; Schoenle et al., 2020). The occurrence of the genus *Cafeteria* has already been reported from the Atacama Desert (Schoenle et al., 2022).

## Factors associated with the benthic protists

The clustering patterns of the detected protist communities are significant based on the distance metrics shown in our PCoA analyses (Figure 2). The active DNA pool (cDNA) differed between the stations but corresponded to the total DNA pool of the respective station. However, we found, at least in part, indications for layer specificity in the activity of protists (Figure 5). Phototrophs were mainly detected in the upper layers, which supports the existence of a vertical gradient reflected in the studied distribution patterns.

Furthermore, we found that salinity plays an important role in the community structure of protists registered by metabarcoding for the different microbial mats. Differences in the salinity of the water bodies originated from different catchment areas (Ritter et al., 2018), explaining in part the uniqueness of environmental conditions in each salar/lagoon and the differences in community structure (Figure 6). The salinity is specifically tolerated by protist species from various phylogenetic lineages isolated from hypersaline lagoons and salars of the Atacama (Schiwitza et al., 2018, 2019, 2021; Carduck et al., 2021; Rybarski et al., 2021; Schoenle et al., 2022).

Another important factor that might have governed the differences in the protist community structure observed in our studies could be the differences in the community structure of prokaryotes, which are known to have a significant effect on protist communities (e.g., Glücksman et al., 2010). It has been shown that the community structure of prokaryotes can differ between the different aquatic habitats across the Atacama and the Andes, as shown by Demergasso et al. (2003), Dorador et al. (2008), and in later studies by Rasuk et al. (2014) and Farias (2016). As salinity levels may vary due to changes in water availability, the composition of active and resting taxa within these ecosystems can change significantly, altering the biodiversity of these natural ecosystems. Future studies, including the metabarcoding of 16S and 18S rRNA phylogenetic markers, could help to elucidate overall changes in the community structures of protists detected co-occurring with the prokaryotes typically found in microbial mats at different salinities characterizing the Andean range.

# Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: NCBI—PRJNA1062042.

# Author contributions

EA: Data curation, Formal analysis, Investigation, Visualization, Writing – original draft. FN: Data curation, Conceptualization, Methodology, Supervision, Writing – review & editing. CD: Writing – review & editing, Resources. HA: Writing – review & editing, Conceptualization, Data curation, Methodology, Project administration, Supervision.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2024.1356977/full#supplementary-material

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*Supplementary Material*

# 1 Supplementary Figures and Tables

## 1.1 Supplementary Tables

**Supplementary Table 1.** List of the strains from the Heteroflagellate Collection Cologne (HFCC) included in the mock communities sequenced during this work.

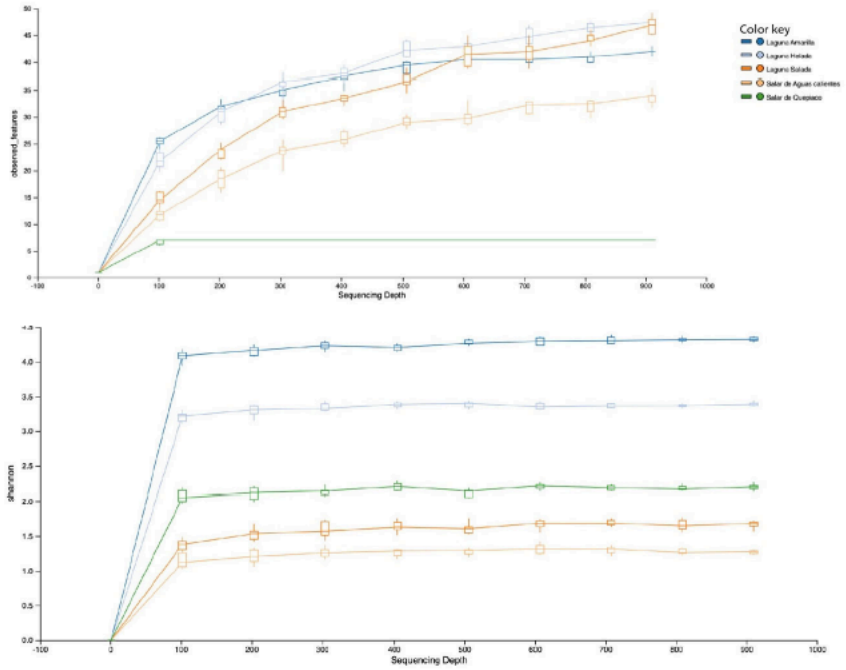
| Species | HFCC | Expedition | Accession No. |
|---|---|---|---|
| **ALVEOLATA** | | | |
| *Protocruzia* sp. | 766 | M139 | MT355146 |
| *Aristerostoma* sp. | 744 | M139 | MT081566 |
| **ANCYROMONADIDA** | | | |
| *Fabomonas tropica* | 175 | SO237 | MT355148 |
| **RHIZARIA** | | | |
| *Massisteria* sp. | 176 | SO237 | MT355122 |
| **DISCOBA** | | | |
| *Rhynchomonadidae* sp. | 171 | SO237 | MT355133 |
| **STRAMENOPILA** | | | |
| *Cafeteria burkhardae* | 203 | SO237 | MN315604 |
| Bicosoecida | 768 | M139 | MT355117 |
| **OPISTHOKONTA** | | | |
| *Ministeria vibrans* | 1787 | SO237 | MT355150 |

**Supplementary Table 2**. Identification of abundant ASVs detected by metabarcoding and their identification according to the Protist Ribosomal Reference PR$^2$ and the best hit in BLAST (NCBI).

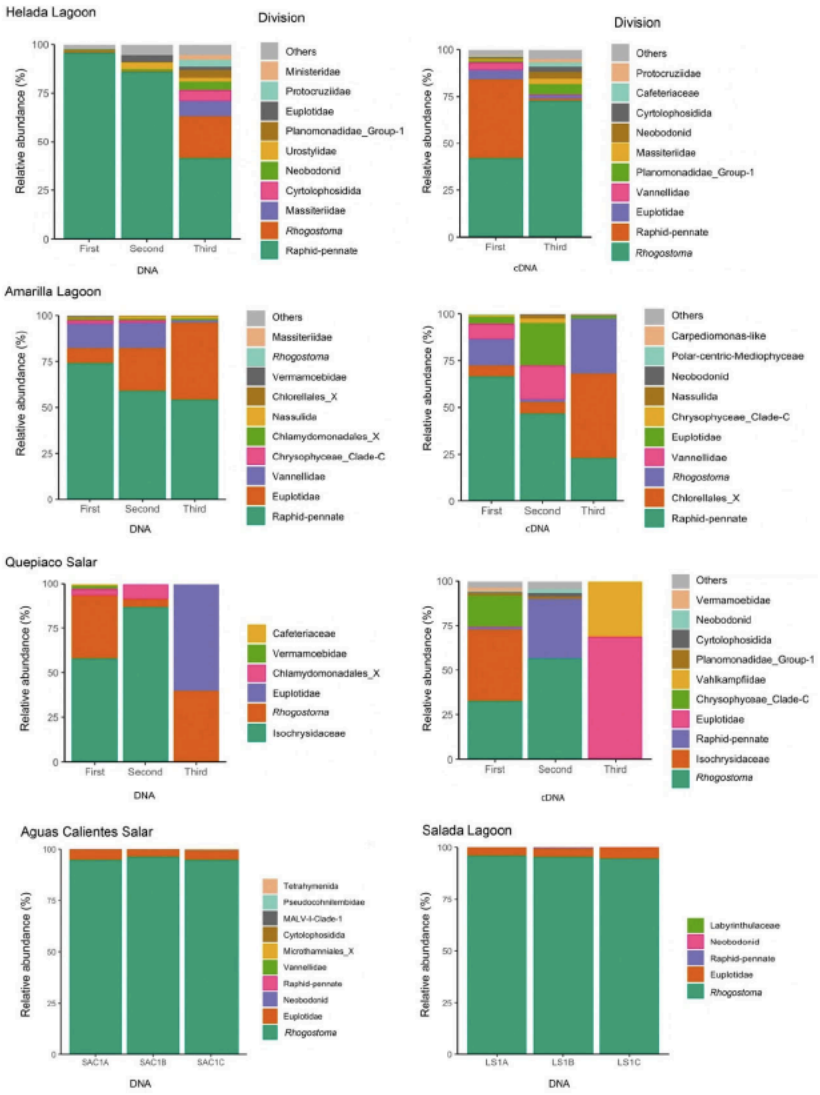
| ASV | Sequence | PR$^2$ | Best hit BLAST (NCBI) | Accesion Number |
|---|---|---|---|---|
| 0aeb77319dc51b7c5679cee98aac6cbb | CACACCGCCCGTCGCTCCTACCAATTTCGAGTGGCTCGGTGAACCTCTTTGGACTGTCGAGCAATCGCGAAATTAGAGTGAACCTGGTCACTTAGAGGAAGGAGAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCG | *Euplotes* | *Euplotes* sp. IsolateB528 | KX516712 |
| 86ed9995164224c82523 4657c61e1115 | CACACCGCCCGTCGCTCCTACCAATTTCGAGTGGCTCGGTGAACCTCTTTGGACTGTCGAGCAATCGCGAAATTAGAGTGAACCTGGTCACTTAGAGGAAGGAGAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCA | *Euplotes* | Uncultured ciliate clone ZOTU20 | MT095067 |
| 26853f2f58bfbf5dd9a981 11bac970ba | ACCGCCCGTCGTTGTTTCCGATGATGGTGCAATACAGGTGATCGGACTGACGAGCCCCTGGCTTGTCGGAAAGTTCACCGATATTTCTTCAATAGAGGAAGTAAAAGTCGTAACAAGGTAGCTGTAGGTGAACCT | *Neobodo designis* | Uncultured eukaryote clone L10.2 | AY753968 |
| 2c4aa5564987eeeb63d9 a675464358ee | ACCGCCCGTCGTTGTTTCCGATGATGGTGCAATACAGGTGATCGGATAGATGGGTGTTTCACTTGTCTAGAAGTTCACCGATATTTCTTCAATAGAGGAAGCAAAAGTCGTAACAAGGTAGCTGTAGGTGAACCT | *Neobodon id* | Cryptaulaxoides-like sp. TCG-2003 | AY425021 |
| 7cd0432cb279182befc5 6bdc71b6bab6 | ACCGCCCGTCGTTGTTTCCGATGATGGTGCAATACAGGTGATCGGACTGACGAGCCTCTGGCTTGTCGGAAAGTTCACCGATATTTCCTTCAATAGAGGAAGTAAAAGTCGTAACAAGGTAGCTGTAGGTGAACCT | *Neobodo designis* | Uncultured eukaryote clone L10.2 | AY753968 |
| af31100e08189a6c6c0b b559c9610286 | ACCGCCCGTCGTTGTTTCCGATGATGGTGCAATACAGGTGATCGGACAGACGAACCTCTGGTTTGTCTGAAAGTTCACCGATATTTCTTCAATAGAGGAAGTAAAAGTCGTAACAAGGTAGCTGTAGGTGAACCT | *Neobodo designis* | Neobodo designis isolate Dune | AY753622 |
| f62c19f04b9561776eb24 fe24e3a94b5 | CGCCCGTCGTTGTTTCCGATGATGGTGCAATGCAGGTGATCGGACAAGCAGAGGCTCTGTCTTTGCTTGAAAGTTCACCGACATTTCTTCAATAGAGGAAGCAAAAGTCGTAACAAGGTAGCTGTAGGTGAAC | *Rhynchomonas nasuta* | Rhynchomonadidae sp. HFCC173 | MT355135 |
| f1b89a1002956d332262 728c89fa7e85 | GTCGCTACTACCGATTGAATGGCTTAGTGAGCTCTCTGGGCTGTTGCGTGTTGGGGGCAACTCCGTCACGCAAACGGGAAGGAGATCAAACTTGATCATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCC | *Rhogostoma*-lineage sp | *Rhogostoma* sp. Isolate HFCC860 | OR775727 |
| e4876cbac4554cc47a78 d0987bb83174 | GTCGCTACTACCGATTGAATGGCTTAGTGAGCTCTCTGGACTGTTGCGTGTTGGGGGCAACTCCGTCACGCAAACGGGAAGGAGATCAAACTTGATCATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCC | *Rhogostoma*-lineage sp | *Rhogostoma* sp. Isolate HFCC860 | OR775727 |
| db192667fc0011815785 aa6757854e82 | GTCGCTACTACCGATTGAATGGCTTAGTGAGCTCTCTGGACTGTTGCGTGTTGGGGGCAACTCCGTCACGCAAACGGGAAGGAGATCAAACTTGATCATTTAGAGGAAGGTAAAAGTCGTAACAAGGTTTCC | *Rhogostoma*-lineage sp | *Rhogostoma* sp. Isolate HFCC860 | OR775727 |
| 26ea63322615c90fd06d c5395c434ef9 | GTCGCTACTACCGATTGAATGGCTTAGTGAGCTCTCTGGACTGTTGCGTGTTGGGGGCAACTCCGTCACGCAAACGGGGAGGAGATCAAACTTGATCATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCC | *Rhogostoma*-lineage sp | *Rhogostoma* sp. Isolate HFCC860 | OR775727 |
| 5fbe65945d8bc635112c 40fe0889ada8 | GTCGCTACTACCGATTGAATGGCTTAGTGAGCTCTCTGGACTGTTGCGTGTTGGGGGCAACTCCGTCACGCAAACGGGAAGGAGATCAAGCTTGATCATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCC | *Rhogostoma*-lineage sp | *Rhogostoma* sp. Isolate HFCC860 | OR775727 |
| 4ae9c1b363f7004798d5 1a63ff8842a5 | GTCGCTCCTACCGATTGAATGATCCGGTGAGGCCCCCGGACTGCGGCTCCGTCGCTGGTTCTTCCAGCTTTGGGGTTGCGGGAAGCTGTCCGAACCTTATCATTTAGAGGAAGGAGAAGTCGTAACAAGGTTTCC | *Isochrysis galbana* | *Isochrysis galbana* strain CCAP 927/1 | KU900225 |
| e26dbcae054f24c3de16 4299928a5b82 | GTCGCTGCTACCGATTGGATGGTCCGGTGAAATCCTCGGATAGTGAAGATCACTTTAACCTTCGGGTTGAAGCTCATTGCTAGAAGTTGATTAAACCTTATCATCTAGAGGAAGCAAAAGTCGTAACAAGGTTTCC | *Vannella cantorta* | *Platyamoeba cantorta* isolate WS1C#5 | DQ229954 |

| | | | | |
|---|---|---|---|---|
| 4537164385a9f4f04bdc1d4e68247f44 | GTCGCACCTACCGATTGAATGGTTCGGTGAAACTTTCGGACCGTGGACAGGTCGCCTTCGGGCGACTTGACCGTGGGAAGTTATTTAAACCTCATCATTTAGAGGAAGGTGAAGTCGTAACAAGGTTTCC | Chrysophyceae Clade C | *Pedospumella* sp. JBM19 | KX442747 |
| 36746617900e43b4ec1db7601939cb8a | GCCCGTCGCTCGTACCGACTGAATAGAGGTATGAATTTGATGGACCAGCGTTGCATCAAGCAATGCGAGAAAATCAACTGAATTTCTCTATTTGTAGGAACGAGAAGTCGTAACAAGGTCTTCGTAGGTGAA | Vahlkampfidae | *Tetramitus dokdoensis* 18S | KY463322 |
| 53eb12cdee4e38ed83b2a6e8bc14f439 | GTCGCTCCTACCGATTGGATGGTCCGGTGAAATCTTCGGACTGCAGCGGCGCAGCTGGTTTTCCGGCAGTGCCGCAGCGGGAAGTTGCTTAAACCTTATCATCTAGAGGAAGGAGAAGTCGTAACAAGGTTTCC | Planomonadidae | *Fabomonas tropica* | MW872733 |

## 1.2 Supplementary Figures




**Supplementary Figure 1**. Rarefaction curves obtained from the sequencing in all stations included in this study showing the number of ASVs as function of the number of sequenced reads.


Helada Lagoon
Division
Relative abundance (%)
DNA
cDNA
First
Second
Third
Others
Ministeridae
Protocruzildae
Euplotidae
Planomonadidae_Group-1
Urostylidae
Neobodonid
Cyrtolophosidida
Massiteriidae
Rhogostoma
Raphid-pennate
Amarilla Lagoon
Massiteriidae
Rhogostoma
Vermamoebidae
Chlorellales_X
Nassulida
Chlamydomonadales_X
Chrysophyceae_Clade-C
Vannellidae
Euplotidae
Carpediomonas-like
Polar-centric-Mediophyceae
Neobodonid
Chrysophyceae_Clade-C
Quepiaco Salar
Cafeteriaceae
Verrmamoebidae
Chlamydomonadales_X
Isochrysidaceae
Vahlkampfiidae
Aguas Calientes Salar
Tetrahymenida
Pseudocohnilembidae
MALV-I-Clade-1
Cytolophosidida
Microthamniales_X
Vannellidae
Raphid-pennate
Neobodonid
Salada Lagoon
Labyrinthulaceae
SAC1A
SAC1B
SAC1C
LS1A
LS1B
LS1C

**Supplementary Figure 2**. Relative abundance of the protist groups detected across layers of the studied microbial mats through DNA and cDNA. Aguas Calientes and Salada Lagoon include the three replicates detected in the first layer of the microbial mats through DNA.

## Conclusive summary and perspectives

Around the planet, the overall state of ecosystems is changing, potentially affecting human activities relying on ecosystem services which are sustained by diversity and multifunctionality (Soliveres et al., 2016). With this dissertation, new information regarding the protists in the most arid desert on Earth could be obtained. We revealed new insights into the ecology of protists and opened new venues for the future work on the tolerability of life to the extreme environments in the Atacama. Additionally, we intended to offer a starting point in the investigations on the biodiversity of habitats that could be dominated (and at least in part sustained) by the environmental functions of protists.

The work conducted during this thesis incorporate an overview on the cultivable protozoans adapted to live in remote ecosystems (Chapter 1) and give the first ideas of the patterns showed by uncultivable microbes in different habitats and latitudes across this region (Chapter 2 and 3). We make a special emphasis in the Station Terricolous Lichen (Chapter 2), as our experiments on DNA and cDNA were successful, documenting a diversity of genotypes related to protists occurring in biocrusts understudied regarding protists. Between these results we could infer a co-occurrence pattern, documenting for the first time the study of potential interaction of genotypes based on their correlation. Furthermore, we highlight the fact that although the coverage of protist lineages in soils was high (over 75 %), around a 25% was unclassified, suggesting a cryptic diversity remaining to be studied. These results are accentuated when we investigated the microbial mats datasets (i.e., over 90 % unclassified sequences), confirming the potential influence of their isolation on the diversity of protists. For those stations showing a high degree of novelty we pinpoint a potential dominance of the so-called dark matter of life, diversity which has not yet been isolated in culture (Solden et al., 2016).

We underline the studied ecosystems as newly registered habitats for protists. In the case of microbial mats, they can be seen as hotspots of biodiversity, but that depends on the ecosystem. For example, Amarilla Lagoon (Chapter 2 and Chapter 3), appear to be one of these hotspots, as it shows a pattern of high diversity through DNA which was confirmed through cDNA. On the other side, we encountered the lack of amplicons in the

stations of higher salinity, result which also could have a methodological difficulty as well as the absence of active protists. The parallel use of DNA and cDNA has been started to be implemented in biomonitoring initiatives (Pawlowski et al., 2014; Adamo et al., 2020). Nevertheless, this approach is relatively new and deserves further experimentation to test its fidelity to the true diversity across natural ecosystems. Moreover, during our enrichment and isolation of protists during the first chapter of this thesis, a much wider range of morphotypes could be observed by microscopy, however only a small fraction of these could be obtained in axenic culture. Nevertheless, the isolation of strains in pure culture is a time-consuming task and should consider the expertise on the varied lineages that can be recovered according to the data we could not show (i.e., varied protists in enrichments).

The questions we addressed at the beginning of this work could be answered, however our hypotheses, regarding the uniqueness of each extreme environment are partly supported. On one hand, soil protist detected through metabarcoding are not significantly different between each other, suggesting a common structure in the soil diversity, however further use of complementary phylogenetic markers could help to resolve this raising question. Furthermore, the diversity of protists genotypes we detected from soils is overall low, which contradict the knowledge on the diversity of the complex soil matrix (Burki et al., 2021). However, this is an arid environment, explaining partially this result characterizing our dataset. On the other hand, the communities of benthic protists can significantly vary across different water bodies, supporting our hypothesis in the studied aquatic ecosystems. As discussed in Chapter 2 and 3, initial investigations on the microbial ecology in microbial mats from these latitudes, reconstructed rich and diverse patterns, at least for prokaryotes. We used those works as background information during the selection of these stations to test our hypotheses on protists diversity, confirming in part these initial overviews. By one side the diversity was high between our dataset, nevertheless the richness of ASVs (observed ASVs, Chapter 3), was not significantly higher than those detected in soils.

We highlight the difficulty to amplify the selected phylogenetic marker for the studied stations. By one side, the sample matrix of microbial mats is complex and may include blockers in PCR reactions, however the selected method (i.e., Trizol solution), worked for the isolation of DNA and RNA from most of our environmental samples. Further efforts could test such hypothesis including specialized kits, improved for these kinds of sediments. Noteworthy is the contrast in the effectivity of the amplification of the used phylogenetic marker between the soil habitats. For example, this was not possible from the cDNA obtained from the station known as Soil Coastal Hill (Chapter 2). Regarding these samples, one fact that caught our attention was the coloration of the nucleic acids from that station, which evidently darker than all others, could be enriched in humic acids (Francioli et al., 2021). These could originate from the diverse flora living at this latitude, underlying a major difficulty to explore the active fraction of the protists in such habitats. Thus, further refinement in the extraction methods, could help to further disentangle the results we got in the first attempt to study the protistology of Andean microbial mats and arid soils. By one side regarding the active fraction of such communities at elevated salinity, as well as the assessment of the protists in a vertical gradient, virtually present in the studied structures. Further investigations could include microelectrode measurements, complementing microniches exploration and tests of association of detected genotypes to the properties changing with depth.

Although we detected a stratification measured by beta diversity metrics, these were not significant and require further validation including higher number of samples and wider phylogenetic markers. Furthermore, we aimed to associate the studied protists communities in microbial mats to the properties found in the water bodies. This task showed that at least, the protists communities detected in the uppermost layer is corelated to the salinity characterizing each habitat. These results confirm our hypothesis and confirm proposed patterns for microbes subjected to changes in salinity. On one hand, in the study of the microbial ecology in continental waters and on the other hand, those results exploring the tolerance of endemic strains of the Atacama (mentioned in discussion, Chapter 3). This result remarks the necessity to preserve such delicate ecosystems, not only threatened by

climate change, but also by the anthropogenic pressure, increasingly demanding for minerals and water characterizing these systems since former ages.

By including parallel work of enrichment during the collection of samples for metabarcoding, we could obtain endemic strains in culture and to keep them preserved in a reference collection (HFCC). According to preliminary analyses they appear to be highly novel, with HFCC986 sharing only 90.46 % of identity to the strain *Neobodo designis* (AY53623) and HFCC988 sharing only 94.4 % of identity to the closest *Euplotes* species (*E. rariseta* (FJ423449), increasing the known biodiversity of these isolated ecosystems. Given these low identities, such strains must be studied and formally described for a useful GenBank. Therefore, is very important to keep investigating the ecology of protists in this area and even more among the protistome of endangered species as the endemic cacti of the Atacama. Given the results obtained during Chapter 1, these cacti appear as a prospect for future metabarcoding or metagenomic assessments of their phyllosphere. Moreover, by measuring the phylogenetic distance between metabarcodes and information obtained in parallel from isolates, we could increase the confidence in our results. We supported thus the use of this approach which is gaining an increased attention in biomonitoring and conservation initiatives (Ruppert et al., 2019). We recommend to the future researchers, to include a wider number of biological replicates per station, aiming to elucidate new questions on the ecology of protists after this dissertation.

The combined approaches as part of this thesis gave the first overview of a vast and far from being understood extreme environment. By including genotypes detected through DNA and cDNA (from RNA), an overview of protist diversity, potentially active in the Atacama could be obtained. A more detailed study of the isolated strains from this desert, including transcriptomics and the prokaryotes that could be part of a protist microbiome would help to elucidate more in detail the patterns showed during the present work. As protists can show differences in the preferences for bacterial preys, the strains isolated during this work, are interesting starting point to shed light on inter-domain patterns, as those shown by Bock et al. (2020).

Finally, by using artificial controls in our sequencing libraries, we detected potential spurious sequences in low abundance. This helped us to elucidate a threshold for the frequency of reads, which although stringent, gave us confidence on our dataset, replicating a practice increasingly adapted in the metabarcoding approach (Dünn and Arndt, 2023; Lennartz et al., 2023; Sachs et al., 2023). Nevertheless, the occurrence of the so-called rare biosphere should be considered in future works as presented by Ramond et al. (2023). Further studies including metabarcoding approaches, should investigate the extent at which such artificial controls are beneficial for the curation of environmental molecular information when focusing on the diverse but low abundant rare microbes.

The present thesis underlines two main ideas. By one side the homogeneity of protist genotypes detected across soils and the heterogeneity of these, detected in aquatic habitats. It offers a first snapshot on the protist lineages adapted to remote and unique habitats and contribute to register reference information that can support future metagenomic investigations in the arid Atacama. Therefore, we cannot assure if endemicity theories are true or not for the studied communities. By one side, our isolates and their novelty does support this idea, but our metabarcoding of soils contradict it. However, the soil protistome we inspected could be highly adapted to this remote area.

## General References

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## Subpublications and records of achievement

Chapter 1: Acosta, E., Fincke, V., Nitsche, F., Arndt, H. Novel cercozoan and heterolobosean protists from the rhizosphere and phyllosphere of two endemic cacti from the Atacama Desert. *European Journal of Protistology, DOI: 10.1016/j.ejop.2023.126034*
The study was designed by Prof. Dr. Hartmut Arndt. The results were achieved by Victoria Fincke and the author. The author performed the analyses, microscopy and wrote the manuscript.

Chapter 2: Acosta, E., Nitsche, F., Arndt, H. (manuscript) Evidence of protists colonization in terricolous lichens, coastal hills and microbial mats highlight abundant molecular signatures related to the genera *Rhogostoma*, *Euplotes* and *Neobodo*. *Intended for European Journal of Protistology*
The study was designed by the author and Prof. Dr. Hartmut Arndt. The author was involved in sampling, performed the laboratory work, bioinformatic analyses and wrote the manuscript.

Chapter 3: Acosta, E., Nitsche, F., Dorador, C., Arndt, H. (manuscript) Protist communities of microbial mats from extreme environments of five saline Andean lagoons at high elevation in the Atacama Desert. *Submitted to Frontiers in Microbiology, Special Issue Exploring Microbial Mats Communities in Extreme Environments*
The study was planned by the author, together with Prof. Dr. Hartmut Arndt. The laboratory work and bioinformatic analyses were performed by the author under the guidance of Dr. Frank Nitsche. The author wrote the manuscript.

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## Erklärung zur Dissertation

gemäß der Promotionsordnung vom 12. März 2020

***Diese Erklärung muss in der Dissertation enthalten sein.***
**(*This version must be included in the doctoral thesis*)**

„Hiermit versichere ich an Eides statt, dass ich die vorliegende Dissertation selbstständig und ohne die Benutzung anderer als der angegebenen Hilfsmittel und Literatur angefertigt habe. Alle Stellen, die wörtlich oder sinngemäß aus veröffentlichten und nicht veröffentlichten Werken dem Wortlaut oder dem Sinn nach entnommen wurden, sind als solche kenntlich gemacht. Ich versichere an Eides statt, dass diese Dissertation noch keiner anderen Fakultät oder Universität zur Prüfung vorgelegen hat; dass sie - abgesehen von unten angegebenen Teilpublikationen und eingebundenen Artikeln und Manuskripten - noch nicht veröffentlicht worden ist sowie, dass ich eine Veröffentlichung der Dissertation vor Abschluss der Promotion nicht ohne Genehmigung des Promotionsausschusses vornehmen werde. Die Bestimmungen dieser Ordnung sind mir bekannt. Darüber hinaus erkläre ich hiermit, dass ich die Ordnung zur Sicherung guter wissenschaftlicher Praxis und zum Umgang mit wissenschaftlichem Fehlverhalten der Universität zu Köln gelesen und sie bei der Durchführung der Dissertation zugrundeliegenden Arbeiten und der schriftlich verfassten Dissertation beachtet habe und verpflichte mich hiermit, die dort genannten Vorgaben bei allen wissenschaftlichen Tätigkeiten zu beachten und umzusetzen. Ich versichere, dass die eingereichte elektronische Fassung der eingereichten Druckfassung vollständig entspricht."

Teilpublikationen:
1.Novel cercozoan and heterolobosean protists from the rhizosphere and phyllosphere of two endemic cacti from the Atacama Desert

2: Protist communities of microbial mats from extreme environments of five saline Andean lagoons at high elevation in the Atacama Desert

Datum: 27.11.2023, Name: Eduardo Acosta

## Curriculum Vitae

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**PERSONAL PROFILE**

I hold a bachelor's degree of Biotechnology (5 years study, comparable to Master degree) and have academic experience in the field of microbial ecology and bioinformatics. Nowadays, I am a PhD student at the University of Cologne researching on the microbiology of the arids soils of the Atacama Desert in a climate change scenario. My main professional interests are the data analysis and Next Generation Sequencing of microbial communities, especially from extreme environments and currently I use my knowledge on these topics to complete my PhD studies ending on early 2024

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**EDUCATION**

**University of Cologne, Cologne, Germany**

2019-2023
Ph D Thesis Title: Exploring the taxonomy, metabolic potential, and mineral influence on microeukaryotes and prokaryotes from modern and ancient ecosystems from the Atacama Desert
Thesis advisor: Prof. Dr. Hartmut Arndt
First mentor: Prof. Dr. Michael Bonkowski
Scholarship: Forschungsstipendiendien – Promotionen in Deutschland, 2019/20
Deutscher Akademischer Austauschdienst (DAAD)

**University of Antofagasta, Chile, Bachelor of Biotechnology**

2014
Thesis Title: Microbiology of sulfur oxidation in Salar de Huasco, initial studies.
Thesis advisor: Prof. Dr. Cristina Dorador Ortiz.

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**RESEARCH EXPERIENCE**

**University of Antofagasta-University of Chile, Chile**

June 2015-March 2019

Research Assistant in the Microbial Complexity and Functional Ecology Laboratory of Prof. Dr. Cristina Dorador.

Responsibilities:

- In charge of the Microbial Culture Collection, maintain strains isolated from extreme environments including bacteria, microalgae fungi and archaea.
- Preparation of scientific publications and studies on the microbial ecology of extreme environments of the Chilean Altiplano and from Southern Patagonia
- Field work including sampling of microbial mats, water samples and Sediments and soils and physic-chemical parameters.

**University of Antofagasta, Chile**

June 2018-March 2019

Research Assistant of the Project "Microbiologic control of primary stages of developing of biofouling in marine substrates" (ID14I20072). Collaboration between Universidad de Antofagasta and Aguas de Antofagasta S.A. (Prof. Dr. Fernando Silva).

Responsibilities:

- Identify microalgal strains isolated from reverse osmosis membrane samples through molecular techniques
- Identify the whole community of prokaryotes and eukaryotes thriving the reverse osmosis membranes through Environmental Sequencing approaches
- Guide undergraduate students in experiments related with genomics.

**RESEARCH EXPERIENCE**

**University of Antofagasta, Chile**

June 2014-December 2015

Formulation and award of the Project VIU130012 “Degradation platform for plastic residues by the use of microorganisms”. FONDEF, Chile.

Responsibilities:

- Identify bacterial strains isolated from oil spill and use them *in vitro* plastic degradation experiments. This includes molecular techniques and microscopy as SEM and epifluorescence.
- Assess the microbial degradation of plastics in compost experiments.
- Open a company dedicated to the commercialization of a product based by the microbial isolated proposed for plastic degradation.
- Planification and talks in schools to promote science communication of the project.

**University of Antofagasta, Chile**

August 2015-October 2015

Formulation and report of a Base Line for the National Assets Department (Chile) of the Cejar-La Piedra Lagoons. (Altiplano-Andes area)

Responsibilities:

- Monthly field work sampling and measuring of environmental parameters.
- Genomics using Next generation sequencing of the prokaryotic communities in the water samples from the lagoons
- Writing the final report

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**PUBLICATIONS**

Aguilar P., Acosta E., Dorador C., Sommaruga R. (2016). Large differences in bacterial community composition among three nearby extreme waterbodies of the high Andean plateau. Front. Microbiol 7: 976. https://doi.org/10.3389/fmicb.2016.00976

Acosta E., Fincke V., Nitsche F., Arndt H. (2023). Novel cercozoan and heterolobosean protists from the rhizosphere and phyllosphere of two endemic cacti from the Atacama Desert. *European journal of protistology* https://doi.org/10.1016/j.ejop.2023.126034

Acosta E., Nitsche F., Dorador C., Arndt H. (Submitted). Protist communities of microbial mats from extreme environments of five saline Andean lagoons at high elevation in the Atacama Desert. *Frontiers in microbiology Spec issue Microbial mats*

Acosta E., Nitsche F., Arndt H. (in preparation). Evidence of protist colonization in terricolous lichens, coastal hills and microbial mats highlight abundant molecular signatures related to the genera *Rhogostoma*, *Euplotes* and *Neobodo* across the Atacama Desert. *European journal of protistology*

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**EXTRA CURRICULAR ACTIVITIES**

**Informatic languages**

User level of python and R

**Deutsch-Uni Online**

Intensive B2 to C1 course

**Carl Duisberg Training Center Cologne**

German course B2.2 completed (six months)

**Official IELTS Test** (2018)

Level reached in the test B2

**Scientific Support** (2016-2018)

Participation in the initiative 1000 scientists 1000 classrooms, giving talks on science in schools on topics as Ecology and Informatics

**Scientific Support** (2017)

Participation in Science Festival “Fiesta de la Ciencia” as part of the group of Prof. Dr. Cristina Dorador.

**Scientific Support** (2017)

Participation in the Workshop “Conflicts and Approaches for the Research in Chile” organized by Santo Tomás University, Antofagasta Chile.

**Manager of Communications** (2017 and 2018)

In charge of organizing and execution of the “March for Science” in Antofagasta City

**Scientific Support** (2016)

Participation in Science Festival “Fiesta de la Ciencia” as part of the group of Prof. Dr. Cristina Dorador.

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**RECEIVED RESEARCH GRANTS**

**Project VIU130012 (2014)**
Plastic degradation platform from urban residues using bacterial strains"

**Research Grants 2019/2020-Until 2024**
Forschungsstipendiendien – Promotionen in Deutschland, 2019/20 Deutscher Akademischer Austauschdienst (DAAD). Full stipend for research in Germany for the completion of a PhD under the tuition of Prof. Dr. Hartmut Arndt

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**SOCIETY MEMBERSHIP**

**German Society for Protozoology (DGP):** Since January, 2020

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