Metabarcoding advances for ecology and biogeography of Neotropical protists: what do we know, where do we go?

Camila Duarte Ritter[1*], Arielli Fabrício Machado[2], Karine Felix Ribeiro[2], Micah Dunthorn[1,3]

[1] Eukaryotic Microbiology, Faculty of Biology, University of Duisburg-Essen, Universitätsstrasse 5, S05 R04 H83, D-45141 Essen, Germany.

[2] Postgraduate Program in Ecology, Biosciences Institute, Universidade Federal do Rio Grande do Sul, Avenue Bento Gonçalves, 9500 –43.113, Porto Alegre, RS, 91501-970, Brazil.

[3] Centre for Water and Environmental Research (ZWU), University of Duisburg-Essen, D-45141 Essen, Germany

*Corresponding author: Camila D. Ritter, kmicaduarte@gmail.com. Phone: +55 48991434597. Postal address: Eukaryotic Microbiology, Faculty of Biology, University of Duisburg-Essen, Universitätsstrasse 5, S05 R04 H83, D-45141 Essen, Germany.

Abstract

The Neotropical region is one of the most diverse regions of the globe in terms of macro-organismic species. Regarding the microbial world, however, little is known about the diversity and biogeography patterns of micro-organisms in the Neotropics. In this context, the study of several microbial taxonomic groups is still missing and/or incomplete, such as the protists. Our goal here was to summarize the available information of Neotropical protists, focusing on molecular data from environmental continental samples, to explore what these data evidence on their ecology and biogeography. For this, we reviewed the findings from all articles that focused on or included the terrestrial protists using metabarcoding approach and identified the gaps and future perspectives in this research field. We found that Neotropical protists diversity patterns seem to be, at least in part, congruent with that of macro-organisms and, different than plants and bacteria, just weakly explained by environmental variables. We argue that studies with standardized protocols including different biomes are necessary to fully characterize the ecology and biogeography on Neotropical protists. Furthermore, dismember evolutionary lineages and functional guilds of protists are important to better understand the relationship between diversity, dispersal abilities and functionality of particular taxa of protists in their habitats.

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Background: The tropical regions are known to harbor a higher number of species than other regions. Even so, which factors generate the latitudinal diversity gradient pattern (Pianka 1996) remains one of the biggest questions in ecology and biogeography. Moreover, even between the tropical regions the diversity is not homogeneous distributed. The Neotropics, the region that comprise the area from central Mexico to Argentina, including the Caribbean (Morrone 2014), is the most diverse area of the globe, harboring three times more flowering plant species than tropical Africa and potentially more species than tropical Africa and Asia combined (Antonelli & Sanmartín 2011). The diversity of this region is outstanding concerning the species richness across biomes (Zizka 2019) and taxonomic groups (Ceballos & Ehrlich 2006, Wiens 2007, Somveille *et al.* 2013, Zizka 2019).

The Neotropics are highly diverse in both biomes and habitats, it also includes a high number of different ecoregions, such as the Andes mountains, tropical rainforests, seasonally flooded areas, savannas, and large dry areas (Fig. 1, Hughes *et al.* 2012, Olson *et al.* 2001). Neotropics also presents strong biogeography patterns already recognized for vertebrates and plants (e.g. Lynch Alfaro *et al.* 2015, Esquivel-Muelbert *et al.* 2017, Carneiro *et al.* 2018). Between some patterns there are the west-to-east diversity gradient in the Amazonia which was suggested been explained by marine incursions (Bates 2001, Lovejoy *et al.* 2006, Antonelli *et al.* 2009), bedrock geology (Tuomisto *et al.* 2017), mountain base formation (Hoorn *et al.* 2010), soil fertility (Hoorn *et al.* 2010, ter Steege *et al.* 2006) and diversification process driven by moisture (Silva *et al.* 2019). Another pattern is the endemism areas in Amazonia (Cracraft 1985, Ribas *et al.* 2012), Atlantic Forest (Costa *et al.* 2000, Silva & Vaz-de-Mello 2020) and Cerrado savanna (Azevedo *et al.* 2016). Other patterns include the high species turnover, the increase of community dissimilarity with geographical distance, also known as the distance-decay relation in the Neotropical forests (Bohlman *et al.* 2008), body-size habitat specialization (e.g. Hillebrand & Azovsky 2001, Lafferty & Kuris 2002, Woodward *et al.* 2005, Abades *et al.* 2010) and tree species density-dependent host-specific predation and parasitism (Janzen 1970, Connell *et al.* 1971).

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Although biogeography patterns are well known for Neotropical vertebrates and plants, little is known if the biogeography of micro-organisms follows the same rules as macro-organisms. In the 90's years the idea of microbial biogeography was guided for the famous sentence "*everything is everywhere – but the environment selects*" (e.g. Finlay & Fenchel 2005, for protist overview). While the first proposition implies that micro-organisms have dispersal abilities so high that the effects of past processes are suppressed, the second assumes that current environmental characteristics select different microbial taxa according to their habitat preferences. However, the recent advance of genetics allowed a deep sampling of micro-organisms and this sentence started to be refuted (Foissner 2006, Bass *et al.* 2007, Bates *et al.* 2013, Lentendu *et al.* 2018a). Most of the idea of over-dispersal and cosmopolite occurrence of micro-organisms was due the morphology-based classification that groups several species into a "morphospecies", misidentifying and not identifying some many other species. For instance, using molecular analysis the dispersal limitation was identified in both terrestrial (Singer *et al.* 2019) and marine environments as the main factor structuring micro-eukaryotes communities (Logares *et al.* 2020).

However, although some biogeographic patterns of certain microbial groups in different regions are already known (Foissner 2006, Martiny *et al.* 2006, Azovsky & Mazei 2013, Kushwah & Thorpe 2020, Li *et al.* 2020), microbial ecology is still in its infancy and generalizations cannot be made. This knowledge gap is worrisome since micro-organisms are the richest and more abundant component in any environment (Mora *et al.* 2011), which play a pivotal role in the maintenance of ecosystems (e.g. Sherr & Sherr 2002, Petersen & Luxton 2006, Cuvelier *et al.* 2010, Steele *et al.* 2011). To understand the biogeography of micro-organisms is, therefore, crucial to understand the ecology and biogeography in general. As highlighted by O'Malley & Dupré (2007), the excessive focus on macro-organisms patterns of diversity, ecology and distribution may have distorted several aspects of our understanding about these patterns.

For instance, the classical latitudinal diversity gradient, which is the most well-known global pattern in ecology, was extensively tested in eukaryotic macro-organisms (Pianka 1996, Willig *et al.* 2003, Kreft & Jetz 2007, Jablonski *et al.* 2016), but still poorly evaluated on micro-organisms. Even so, bacteria and fungi showed similar latitudinal diversity gradient patterns to that reported for macro-organisms (Hawksworth 2001, Pommier *et al.* 2007, Fuhrman *et al.* 2008, Tedersoo *et al.* 2014, Ribeiro *et al*. 2019), and others biogeographic patterns such as

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92 endemic distributions (e.g. Whitaker *et al.* 2003, Kilroy *et al.* 2007, Ryšánek *et al.* 2015),
93 distance-decay relation (e.g. Astorga *et al.* 2012, Bahram *et al.* 2013, Zinger *et al.* 2014, Oono
94 *et al.* 2017) and allopatric speciation (e.g. Whitaker 2006, Hénault *et al.* 2017). Other studies
95 have compared the diversity patterns of micro-organisms with what is knew for macro-
96 organisms in both global (e.g. Tedersoo *et al.* 2014, Cameron *et al.* 2018, Delgado-Baquerizo
97 *et al.* 2018) and Neotropical scales (e.g. Castillo 2000, Lauber *et al.* 2009, Goffredi *et al.* 2011,
98 Navarrete *et al.* 2013, Goffredi *et al.* 2015, Dunthorn *et al.* 2017, Louca *et al.* 2017, Schimann
99 *et al.* 2017, Ritter, Faurby, *et al.* 2019), however, the study of several taxonomic groups is still
100 missing and/or incomplete, such as the protists. Even considering the soil protists in general
101 the Neotropical region is neglected. In a global review on terrestrial protists, for instance, just
102 two from the ten articles on Neotropical protists available were cited (Oliverio *et al.* 2020).

103 Our goal here was to summarize the findings concerning protistan distribution through
104 metabarcoding data from continental environmental samples in Neotropics to understand the
105 ecological processes underlying the biogeography of the protists in this region. We also wish
106 to identify the main gaps in the Neotropical protists ecology and biogeography and to shed
107 light on the already described patterns and potential prospects in this so promising study group.

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The protists: Who are they? Where do they live? How do they survive?

The protists are a paraphyletic group comprising most lineages in the eukaryotic tree of life (Keeling *et al.* 2005, Burki 2014). Several groups are closer related with macro-organisms, such the phyla Opisthosporidia, Nucleariida and Fonticula that are inside of the Holozoa, group that comprise Fungi and Metazoa (Burki 2014, Adl *et al.* 2019). Protists are the mostly non-fungi single cell eukaryotes that are over spread in the tree of life and have the potential to shed light on eukaryotic evolution (Adl *et al.* 2019).

Protists inhabit all habitats, from soils, lakes, sea, and in the bodies of other organisms (Fig. 2). They are mostly knowing to be vector of diseases such as Malaria (caused by *Plasmodium sp.*), Chagas (caused by *Trypanossoma cruzie*), Giardisis (caused by *Giardia sp.*) and Toxoplasmosis (caused by *Toxoplasma gondii*). However, the protists have a diverse lifestyle from free-living forms to parasites of other animals, plants and even other protists (Adl *et al.* 2019). They play a key role in the ecosystems, such as the primary production carried out by photosynthetic protists, which is the base of food chains in freshwater and marine environments (Worden *et al.* 2015), while the heterotrophic protists are crucial in the nutrient recycling through decomposition in water, sediments, and soils (Geisen *et al.* 2018). Furthermore, they are usually the most abundant organisms in any given location. For instance, current estimates suggested between 50,000 to 100,000 protist species in the sunlit surface layer of the global ocean, five to ten times more than for bacteria and archaea combined (deVargas *et al.* 2015). In terrestrial environments, the protists species number is more controversial, with no clear estimation of species number but an estimation of tens of thousands of individuals per gram of bulk soil (Finlay 2002; Stefan *et al.* 2014). Future perspectives include the deep phylogeny of protists and better characterization of phylogenetic and functional diversity of this amazing group(s).

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Molecular approaches to assess microbial diversity.

The difficulty to sampling, identify and test ecological and biogeographical questions in micro-organisms are mainly due to the hard-taxonomic and time-consuming identification. The taxonomic classification based on morphological characters of almost "invisible" organisms is extremely limited and needs very experienced taxonomists. Several studies on micro-organisms using microscopy, incubation and biochemistry based methods for morphological or functional identification have been done (e.g. Adl & Gupta 2006). However, due the difficulty of identification, these studies are focused in a limited group such as testate amoebae (e.g. Lansac-Tôha *et al.* 2014) or planktonic ciliates (e.g. Negreiros *et al.* 2017).

In this context, environmental high-throughput DNA sequencing (HTS) methods have revolutionized the taxonomic identification of microbes in above- and below-ground communities (Bik *et al.* 2012, Deiner *et al.* 2017), including protists (Santoferrara *et al.* 2020). One powerful method for diversity assessment in a given locality is metabarcoding. The idea of DNA barcode is based on that some DNA regions are conserved enough to have little intraspecific variation but variable enough to distinguish species, so the use of a specified DNA sequence provides taxonomic identification for a specimen (Blaxter 2004). The metabarcoding idea is similar, but instead to sequence the DNA from one specimen, we can use environmental samples, such as soil, water and air to extract all DNA present in these samples, to amplify by Polymerase Chain Reaction (PCR) and to sequence a specific DNA region to identify a phylogenetic range of organisms from a set of specimens or even entire communities (Taberlet *et al.* 2018) . These advances follow developments in the identification of barcoding sequences of species and the existence of public and relatively highly populated reference sequence databases.

From these molecular data it is possible to describe the patterns of diversity and distributions of micro-organisms on a massive scale. However, there are many factors to consider when using molecular tools in biodiversity assessments, including DNA extraction protocols that could not extract some organisms, choice of genetic marker (e.g. Pawlowski *et al.* 2012, Clarke *et al.* 2014, Elbrecht *et al.* 2016), sequencing method (e.g. Liu *et al.* 2013, Mahé *et al.* 2015, Schirmer *et al.* 2015, Ritter *et al.* 2020), and data analysis procedures (e.g. Beng *et al.* 2016, Prodan *et al.* 2020). A serious caveat for using these molecular methods for biodiversity assessments is the primer biases that oversampling same groups and under-sampling others. Another problem is the lack of taxonomic reference databases, especially for

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the tropical regions (Zinger *et al.* 2020). Without such reference databases, the recovered sequences cannot be matched to resolved taxonomic levels. Furthermore, the molecular assessment is hampered by mis-annotated reference sequences (Hofstetter *et al.* 2019), technically compromised sequences (e.g., chimeras, artificial sequences created for the groups of different DNA sequences), and reference sequences annotated at high taxonomic levels (e.g. phylum; Kang *et al.* 2010, Nilsson *et al.* 2012, Nilsson *et al.* 2016).

Although these biases, high-throughput DNA sequencing studies to biodiversity assessment form a powerful tool to explore entire communities and to understand their biotic/abiotic interactions. Even in highly diverse and poorly sampled environments, such as the Neotropics, for which reference databases are very thinly populated, the use of molecular operational taxonomic units (OTUs; Blaxter *et al.* 2005) allows for an assessment of genetic diversity and enables comparison among multiple sites (Stahlhut *et al.* 2013, Zinger *et al.* 2020, Santoferrara *et al.* 2020). The OTUs are group of sequences with high similarity (usually >97%) that represent a taxonomic unit that can correspond to one species or not (Blaxter *et al.* 2005), although other methods are available (e.g., Mahé, Rognes, *et al.* 2015, (Callahan *et al.* 2016)). DNA-based studies have the potential to overcome at least some taxonomic limitations, and have been identified as “transformative technology” for the entire field (Baird & Hajibabaei 2012). For some organisms no prior taxonomic information is available, or a complete taxonomic identification is impossible in the absence of sequence data (as often is the case with some protists). Indeed, molecular data are often the only straightforward source of taxonomic and ecological information in many groups of micro-organisms (Blaxter *et al.* 2005). More effort is needed to correct errors in reference sequences databases and the intrinsic differences between taxonomic and molecular biodiversity assessments, although some studies using metabarcoding techniques allowed to identify some biogeographical patterns on Neotropical protists.

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Biogeography of Neotropical Protists: What we know.

There are several studies using molecular tools to investigate ecological (e.g. Grossmann *et al.* 2016, Dassen *et al.* 2017, Heger *et al.* 2018) and biogeographical (e.g. Gibbons 2017, Boenigk *et al.* 2018, Singer *et al.* 2000) patterns in protists in temperate regions, however, these patterns are underexplored in the Neotropical region. Creer *et al.* (2010) were the first to sample Neotropical soil protists in one of the first environmental metabarcoding studies that evaluated the effectiveness of using Roche/454 sequencing technology to uncover the meiofauna in specific and complex eukaryotic communities in general. They sampled four sites in a secondary plot at La Selva Biological Station in Costa Rica (Fig. 1), as well as marine littoral benthic off the south coast of England. The 18S-rRNA primers that were used were designed to primarily amplify the Nematoda (Porazinska *et al.* 2009), which was the targeted meiofaunal taxon in this study. Although not designed to amplify all eukaryotes broadly, the primers also amplified numerous protist taxa. In their taxonomic assignment of the OTUs, the lumped all of the OTUs assigned to the protists as "protozoa", but did not break that group down into smaller taxa.

Later, Bates *et al.* (2013) published the first study targeting protists communities using metabarcoding approach including the Neotropics with the goal to investigate the diversity and biogeographic patterns of soil protists. They sampled several regions in the Americas, including the Neotropics regions of the Caribbean, the Southwest of the Amazon and the Northeast of the Argentina (Fig. 1). They found a biogeographical pattern for soil protists with just one of the 1,014 OTUs found having a cosmopolitan distribution. Furthermore, they found the environmental factors, such as edaphic (e.g. pH) and climatic (e.g. temperature) variables, knowing to strongly affect the diversity of plants, animals and soil bacteria had just a moderate effect on soil protistan diversity, while soil moisture was the most important, yet moderate, edaphic variable to explain protistan diversity.

Even with Bates *et al.* (2013) showing the potential of molecular studies revealing the ecological and biogeographical patterns of protists, the next studies targeting protists were published four years later (Simão *et al.* 2017, Mahé *et al.* 2017). Simão *et al.* (2017) sampled four bromeliads phytotelmata (plant-container habitats) in the Atlantic forest of Southern Brazil (Fig. 1). They used primers to amplify the V9 region of the 18S-rRNA locus (Nolte *et al.* 2010) in the Illumina DNA sequencing platform to survey the eukaryotic communities, especially ciliates, inhabiting these bromeliads phytotelmata. They found remarkably diverse eukaryotic

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communities, with Arthropoda and Ciliophora showing the highest abundance. Moreover, a high abundance of both free-living protists (ciliate genera *Tetrahymena* and *Glaucoma*) and animal parasites (the apicomplexan gregarines and the genus *Trypanosoma*) was found. They argue that the high abundance of animal parasitic protists in bromeliad tanks indicates that these organisms and their vectors use phytotelmata as a common habitat. Their results showed a hidden diversity of eukaryotes in bromeliad phytotelmata, even with limited sampling (just four phytotelmatas), shedding light on the studies of plant-protist-animal interactions.

Mahé *et al.* (2017) sampled multiple lowland tropical rainforests using Illumina sequencing technology with the aim of uncovering protistan diversity in Neotropical soils. They sampled over two years in older growth plots in La Selva Biological Station, Barro Colorado Island in Panama, and Tiputini Biodiversity Station in Ecuador (Fig. 1). The primers that were used were designed to amplify the hypervariable V4 region of the 18S-rRNA locus in all eukaryotes. The protistan soil communities in all three countries were found to be dominated by OTUs taxonomically assigned to the parasitic Apicomplexa, which are all parasites of animals (Rueckert *et al.* 2019). Although some of these apicomplexans are from the Haemospororida, which includes *Plasmodium* and close relatives that infect arthropods and vertebrates, most of the apicomplexans are from the Gregarinasina the predominantly infect arthropods and other invertebrates.

Mahé *et al.* (2017) suggested that this massive diversity of apicomplexans could potentially contribute to more animal species co-existing together in the tropical forests because of density-dependent parasitism. This “Mahé-Dunthorn” hypothesis for animals mirrors the Janzen-Connell hypothesis (Janzen 1970, Connell *et al.* 1971) for density-dependent in host-specific predation and parasitism contributing to more tropical tree species being able to co-exist. It should be noted that there are other hypotheses for high animal species co-existence in tropical forests, but these have focused on how the increased number of plant species affects herbivorous insects, and to a lesser extent other arthropods (Novotny *et al.* 2006, Basset *et al.* 2012, Becerra 2015), but not all of the high animal diversity in tropical forests can be explained or predicted by plants alone. In contrast to the apicomplexans, there were few parasitic oomycetes OTUs in the protists soil communities in three countries. Mahé *et al.* (2017) argued that there were too few oomycetes to be an important group for the density-dependent host-specific parasitism under the Janzen-Connell hypothesis. Although oomycetes were long thought to be major host-specific parasites (Freckleton & Lewis 2006), they were

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also not found to have an effect on plant communities in a fungicide- and insecticide-study in Belize (Bagchi *et al.* 2014).

Using the same data as Mahé *et al*. (2017), Lentendu *et al*. (2018) evaluated the taxa-area relationships and the distance-decay relationships on soil Neotropical protists (Fig. 1). While the the taxa-area relationships measure the increasing number of species or richness with the increase of sampled area (Arrhenius 1921, Drakare *et al.* 2006), the distance-decay relationships has the focus in the community and measures the increase of communities' dissimilarity with the increase of distance (Morlon *et al.* 2008, Soininen *et al.* 2007). These models were tested in macro-organisms in tropical forests showing a high alpha (local - Condit *et al.* 1996, Basset *et al.* 2012) and low beta (regional - Plotkin *et al.* 2000, Condit *et al.* 2002) diversity. For the parasitic and free-living protists a similar high alpha and low beta diversity pattern was found among Neotropical forests (Lentendu *et al*. 2018). These results showed the congruence with Neotropical biogeographic patterns between macro and micro-organisms and indicate that these organisms are spatially structured, at least in part, for the same general process. Yet, which process mold this Neottropica diversity patterns should be further investigated.

de Araujo *et al.* (2018) sampled soil Neotropical protists from transitions zones between the Tropical Dry Forests and the Brazilian Cerrado (Fig. 1), the most diverse savanna in the world in terms of animals and plants (Furley 1999, Myers *et al*. 2000), sampling four vegetation zones in the Sete Cidades National Park, Brazil. They also used Illumina sequencing technology with the aim of uncovering protistan diversity and co-occurrence in Neotropical savanna. The primers that were used were designed to amplify the hypervariable V9 region of the 18S-rRNA locus in all eukaryotes. Considering the vegetation coverage the Brazilian Cerrado can be classified in four vegetation succession zones: from grass, grass and shrub, shrub and tree, and tree-dominated climax vegetation zone (Coutinho 1978, Furley 1999). These zones show a plant diversity gradient (de Araujo *et al.* 2018) and are also related with animal diversity (Mares & Ernest 1995, da Silva & Bates 2002, but see Nogueira *et al.* 2009). Using this vegetation zone classification, de Araujo *et al.* (2018) compared soil protists richness and microbiome complexity, combining protists with prokaryotic and fungal sequences, through co-occurrence network analysis. Both protistian richness and microbiome complexity were higher in tree-dominated zones (de Araujo *et al.* 2018). Also, the soil protists composition was different between zones with the plant-parasites and omnivorous being more abundant in

grass zones and animal parasites in grass-shrub zones (de Araujo *et al.* 2018). They suggested that protists are key soil microbiome components and, in agreement with Mahé-Dunthorn hypothesis (Mahé *et al.* 2017), that vegetation succession towards climax vegetation, and consequently higher animal and plant diversity, is stimulated by higher loads of animal and plant pathogens. Furthermore, the authors suggested higher system stability with an increase in microbiome complexity.

Ritter *et al*. (2019a, b) also compared patterns between animals and plants with soil Neotropical micro-organisms. They used primers designed to amplify the hypervariable V7 region of the 18S-rRNA, targeting eukaryotes in general (Guardiola *et al.* 2015), with Illumina sequencing technology. They sampled litter and soils in 39 plots at four localities across a large longitudinal range in Brazilian Amazonia (Fig. 1). Localities were selected to maximize west-to-east diversity gradient in Amazonia (ter Steege *et al.* 2003, Bass *et al.* 2010, Hoorn *et al.* 2010, Zizka *et al.* 2018) and the number of vegetation (habitat) types. The habitat types included in their analysis have characteristic biota and environmental conditions (ter Steege & Hammond 2001, Haugaasen & Peres 2006, Assis *et al.* 2015, Adeney *et al.* 2016, Myster 2016). These habitats include, in a decreasing macro-organisms' diversity gradient: non-flooded rainforests (terra-firme), forests seasonally flooded by fertile white waters (várzeas) or by unfertile black waters (igapós), and naturally open areas associated with white sand soils (campinas). They found that micro-organisms richness (including protists) and community composition differ significantly among localities and habitats, and that habitat type strongly structured microbial composition than locality. Ritter, Zizka, *et al.* (2019) detected a different habitat gradient from what they expected initially, but as expected they found a west-to-east longitudinal gradient for microbial richness and community composition. The authors, in another study, explicitly test the birds and tree diversity against protists (and others micro-organisms) diversity (Ritter, Faurby, *et al.* 2019). Their results showed that the currently accepted diversity patterns in Amazonia just partially match for macro- and micro-organisms. Furthermore, these data were used to test soil chemical-physical variables to explain the richness and diversity of Amazonian micro-organisms (Ritter *et al.* 2018). They found a positive correlation for pH and a negative correlation for soil organic carbon content with respect to microbial diversity, suggesting that physicochemical soil properties can predict, to some extent, microbial soil and litter diversity in Amazonia. However, the author did not test physicochemical soil properties directly with just protistan diversity.

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Zinger *et al.* (2019) explored the role of environmental selection (i.e., soil properties, biotic interactions) and stochastic distance-dependent neutral processes (i.e., demography, dispersal) in shaping soil communities, including protists, considering the effect of body sizes. Body size is known to be important to determine ecological and biogeographical patterns in the organisms (e.g. Hillebrand & Azovsky 2001, Lafferty & Kuris 2002, Woodward *et al.* 2005, Abades *et al.* 2010). In this study, they sampled 1,132 soils from a 12 ha Neotropical forest plot in Nouragues Ecological Research Station, French Guiana (Fig. 1). They amplified the hypervariable V7 region of the 18S-rRNA, targeting eukaryotes in general (Guardiola *et al.* 2015), and also included other primers set to amplify other organisms, such as Archea, Bacteria and Viriplantae (Zinger *et al.* 2019), using Illumina sequencing technology. They found that the distribution of protists is primarily stochastic, suggesting that, at least on a regional scale (12 ha), neutral processes are important factors to shape the protistan soil community. Other weak but significant drivers of the soil protistan richness and composition include aluminum, topography, and plant species. Together, these studies showed a mix of deterministic and stochastic factors shaping Neotropical protistan biogeography and ecology. It also highlights the need of more extensive studies to understand the patterns and drivers of the distribution of Neotropical protists.

It is also worth mentioning that even more scarce is the knowledge about the biogeography of aquatic protists in the Neotropics. As far as we know, only three articles published so far have explored distribution patterns of neotropical protists in continental waters through metabarcoding (Filker *et al.* 2016, Filker *et al.* 2017, Lentendu, Buosi, *et al.* 2018). Filker *et al*. (2016) studied planktonic protists of high-mountain lakes in the Chilean Altiplano using the V4 region of the SSU rDNA locus, and Lentendu *et al*. (2018) studied planktonic protists in Brazilian lakes using the V3 region of the 18S-rRNA locus. In these two articles, the authors have found that the freshwater protists were not globally distributed, but that different communities exhibited particular taxonomic compositions both within the Neotropical (Lentendu *et al*. 2018) and when compared with other regions of the globe (Filker *et al*. 2016). The third article explored the distribution of halophilic aquatic protists, using the V4 region of the SSU rDNA locus, in shallow salt ponds with different degrees of salinity from South America and Europe (Filker *et al.* 2017). In this study, salinity was more important than geography in structuring protistan communities. Moreover, a high rate of endemism was observed.

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Gaps and prospects:

The Neotropical biogeographic patterns in protists seem to be, at least in part, congruent with that of macro-organisms (Lentendu *et al*. 2018a) despite the enormous difference between these groups. These similarities can be explained by similar filters or by biotic-interactions, for instance, parasites protists must have similar distribution that their hosts. These finds are extremely important and should be further investigated to understand whether they represent the same processes at work or if different processes lead to the same patterns between these groups. Common themes in Neotropical biogeography of macro-organisms, such as the impact of uplifting the Andes on the distribution and evolution of biotas (Antonelli et al 2009) and the Great American Biotic Interchange (Stehli & Webb 2013) remain unexplored for Neotropical protists. In addition, there is a huge difference within the group itself, which deserves to be better explored.

Protists are a highly diverse group not just in terms of the number of species but also in terms of functional ecology that may affect their distribution (e.g. Weisse 2017, Adl *et al*. 2019). For example, what differences can we expect in the ecological and biogeographical patterns between different groups of protists? This type of approach would be very interesting for the field. It is possible to explore the differences between biogeographic patterns in protists with different types of mobility (e.g. ciliate versus flagellate), reproduction (several types of asexual and sexual reproduction cycles), cell shape (several types of cell wall/theca), cell organization (unicellular, filamentous and colonial) metabolism (heterotrophic, autotrophic and mixotrophic) and preferences habitat (wetlands, aquatic, terrestrial and in association with other organisms). For instance, the extent to which the biogeography of aquatic protists is driven by the same factors as terrestrial protists is an important issue to be investigated. Cconsidering the several types of aquatic ecosystems (freshwater and salt reservoirs, ponds, lakes and rivers), it is important to compare the protistan diversity and composition in these environments in order to fully characterize this group in the Neotropics. Furthermore, protists are great models to generate new insights of Neotropical ecology and biogeography due to some species having high dispersal rates (Geisen *et al.* 2014) that allow quantifying the relative importance of niche, stochastic, and historical processes in structuring biological communities. On the other hand, endemic protists (Ryšánek *et al.* 2015) can be great models for testing the role of speciation, local adaptation and dispersal limitation (Singer *et al.* 2019, Logares *et al.* 2020). Also, due to

their short generation time and consequently high speciation rates, they can potentially lead to the convergence of ecological and evolutionary time scales.

Due to the highly diverse life modes in protists, it is possible to test the role of biotic-abiotic factors in their distribution. For instance, multiple abiotic factors drive the protists communities being the soil moisture recognized as the main factor for soil protists (Bates *et al.* 2013, Geisen *et al.* 2015, de Araujo *et al.* 2018). Although abiotic factors are important, the biotic factor as species interactions are fundamental to determine the protists communities (e.g. Bezemer *et al.* 2006, Simão *et al.* 2017, Zinger *et al.* 2019). In this context we suggest some potential ecological and biogeographical tests, such as the use of ecological co-occurrence analysis (Mikhailov *et al.* 2019), that could help to unravel the patterns and drivers of Neotropical protists diversity and distributions. For instance, de Araujo et al. (2018) showed the relationship between plants and protists, adding the influence of different environmental zones and the co-occurrence patterns between micro-organisms. The study by Simão *et al.* (2017) reveals an important point for advances in studies of Neotropical protists, showing a hidden diversity of eukaryotes in phytotelmata of bromeliads, which can be explored in future studies covering other biotic interactions as well as comparing differences on environmental factors that effects these interactions along Neotropical ecoregions. These studies reveal the importance of studies embracing different interactions between protists and other organisms, as well as between environments as key components of the ecosystem. Therefore, there is still a gap to be filled in relation to these issues, representing good perspectives for future studies.

Beyond the community dissimilarity and diversity patterns, it is also important to include abundance metrics, since density-dependent factors are crucial to understand the biogeographic and ecological patterns (Martiny *et al.* 2006). Density-dependent factors include competition, predation and parasitism (Ricklefs 2008). Even so, most evidence for density dependence diversity control is plant-based (e.g. Hector *et al.* 1999, Hooper *et al.* 2005). However, a study showed that while niche complementarity and density-dependent effects can produce a diversity-productivity saturation curve in plants, soil-transmitted micro-organisms were the major determinants of the relationship (Schnitzer *et al.* 2011). On the other hand, diversity in biological communities is also a historical product of immigration, diversification and extinction (Fukami & Morin 2003, Fukami *et al.* 2007). However, these processes are still poorly studied in protists, even less so in the Neotropical region. Therefore, we highlight the

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need for more extensive studies to understand the patterns and drivers of the distribution of Neotropical protists, covering these regions with gaps, as well as the points highlighted above.

Another important gap to be filled in biogeographic studies of Neotropical protists is the sample coverage throughout this widely diverse region. Much of the tropical rainforest region of eastern South America (covering the North of the Atlantic Forest) still needs to be studied, mainly including metabarcoding (Fig. 1). It will also be important to fill the study gaps in Neotropical savannas, which have their only sampling in the Cerrado biome (Central Savannah of Brazil), as well as the Dry Forests. Also the altitude gradient, that strongly structure macro-organisms (Mateo *et al.* 2012, Li *et al.* 2019, Veintimilla *et al.* 2019, (Villamarín *et al.* 2020) and some micro-organisms (Meng *et al.* 2013, Siles & Margesin 2016, Peay *et al.* 2017, Shen *et al.* 2020) may be tested for protists. In addition, the temperate regions of the Neotropics also lack biogeographic studies using molecular protist approaches (Fig. 1). The development of molecular tools has the potential to overcome these gaps and give a broader vision of biogeography of Neotropical protists.

Even with molecular advances we are far way to get a general picture of Neotropical protists biogeography and ecology. Although more than 350 articles using metabarcoding approaches that include protists are published until now (Santoferrara *et al.* 2020), for our knowledge, there are just ten articles including terrestrial protists in the Neotropics (Fig. 1). Among them, we have several sampling designs, different primers set and sequencing methods that make comparisons impossible. To grasp the protistan biogeographic and ecological patterns, studies with standardized methods across the different eco-regions comparing the geographical and environmental distance (Martiny *et al.* 2006) are essential. In this sense, it is important to note that although many primers were not designed to amplify widely all eukaryotes, the primers also amplified numerous protist taxa, making it an advantage to biogeographical studies of protists.

Beyond standardizing primer to study in a broader scale Neotropical protist, new techniques, yet underexplored in the Neotropics, can help to unveil protistan biogeography and ecology and may overcome some biases of PCR. For instance, metatranscriptomics, the RNA sequencing of environmental samples, uses a primer-free approach that can pick up different parts of the same gene and also uncover taxa not amplified by common primers (Geisen *et al.* 2015, Cristescu 2019). Others techniques including PCR-free targeted-sequencing (Shokralla *et al.* 2016, Giebner *et al.* 2020), non-targeted, reduced-representation of whole genome (Hand

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444 *et al.* 2015), and whole-genome skimming (Coissac *et al.* 2016). In addition, single-molecule
445 DNA sequencing technologies such as Oxford Nanopore and Pacific Biosciences (PacBio) can
446 sequence bigger fragments allowing a better taxonomic resolution (Thompson & Milos 2011,
447 Ritter *et al.* 2020). The advances in molecular based studies have the potential to allow further
448 investigation of the distribution of Neotropical protists and their drivers.

449

450 **Conclusions:** Here we review the available information of protists and put it together to
451 understand the ecological and biogeographical patterns of the Neotropical protists. Together
452 our review shows: 1) much more information is needed to explore the Neotropical protists
453 diversity, ecology and biogeography; 2) Neotropical protists diversity patterns seem to be, at
454 least in part, congruent with that of macro-organisms (Lentendu et al. 2018, Ritter, Faurby, et
455 al. 2019); 3) environmental variables weakly explain protists distribution in both regional
456 (Zinger et al. 2019) to more broad scale (Bates et al. 2013, Lentendu et al. 2018b, Ritter et al.
457 2018, Ritter, Zizka, et al. 2019), however, standardized studies including different biomes are
458 necessary to better address these patterns; and 4) studies with focus on protist that split at least
459 the main groups that could identify families or genera are important to better understanding the
460 ecosystem function of each group in their habitats.

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References

ABADES, R., G. LAUFER, M. LOUREIRO, and P. A. MARQUET. 2010. Food web structure and body size : trophic position and resource acquisition. 147–153.

ADENEY, J. M., N. L. CHRISTENSEN, A. VICENTINI, and M. COHN-HAFT. 2016. White-sand ecosystems in Amazonia. Biotropica 48: 7–23.

ADL, M. S., and V. S. GUPTA. 2006. Protists in soil ecology and forest nutrient cycling. Can. J. For. Res. 36: 1805–1817.

ADL, S. M. ET AL. 2019. Revisions to the Classification, Nomenclature, and Diversity of Eukaryotes. J. Eukaryot. Microbiol. 66: 4–119.

ALFARO, J. W. L., L. CORTÉS-ORTIZ, A. DI FIORE, and J. P. BOUBLI. 2015. Special issue: Comparative biogeography of Neotropical primates. Mol. Phylogenet. Evol. 82: 518–529. Available at: http://dx.doi.org/10.1016/j.ympev.2014.09.027.

ANTONELLI, A., J. A. A. NYLANDER, C. PERSSON, and I. SANMARTÍN. 2009. Tracing the impact of the Andean uplift on Neotropical plant evolution. Proc. Natl. Acad. Sci. 106: 9749–9754.

ANTONELLI, A., and I. SANMARTÍN. 2011. Why are there so many plant species in the Neotropics? Taxon 60: 403–414.

DE ARAUJO, A. S. F., L. W. MENDES, L. N. LEMOS, J. E. L. ANTUNES, J. E. A. BESERRA, M. DO C. C. P. DE LYRA, M. DO V. B. FIGUEIREDO, Â. C. DE A. LOPES, R. L. F. GOMES, W. M. BEZERRA, V. M. M. MELO, F. F. DE ARAUJO, and S. GEISEN. 2018. Protist species richness and soil microbiome complexity increase towards climax vegetation in the Brazilian Cerrado. Commun. Biol. 1: 135. Available at: http://www.nature.com/articles/s42003-018-0129-0.

ARRHENIUS, O. 1921. Species and area. J. Ecol. 9: 95–99.

ASSIS, R. L., T. HAUGAASEN, J. SCHÖNGART, J. C. MONTERO, M. T. F. PIEDADE, and F. WITTMANN. 2015. Patterns of tree diversity and composition in Amazonian floodplain paleo-várzea forest. J. Veg. Sci. 26: 312–322.

ASTORGA, A., J. OKSANEN, M. LUOTO, J. SOININEN, R. VIRTANEN, and T. MUOTKA. 2012. Distance decay of similarity in freshwater communities: do macro-and microorganisms follow the same rules? Glob. Ecol. Biogeogr. 21: 365–375.

AZEVEDO, J. A. R., P. H. VALDUJO, and C. DE C. NOGUEIRA. 2016. Biogeography of anurans and squamates in the Cerrado hotspot: coincident endemism patterns in the richest and most impacted savanna on the globe. J. Biogeogr. 43: 2454–2464.

AZOVSKY, A., and Y. MAZEI. 2013. Do microbes have macroecology? Large-scale patterns in the diversity and distribution of marine benthic ciliates. Glob. Ecol. Biogeogr. 22: 163–172.

BAGCHI, R., R. E. GALLERY, S. GRIPENBERG, S. J. GURR, L. NARAYAN, C. E. ADDIS, R. P. FRECKLETON, and O. T. LEWIS. 2014. Pathogens and insect herbivores drive rainforest plant diversity and composition. Nature 506: 85–88.

BAHRAM, M., U. KOLJALG, P. COURTY, A. G. DIEDHIOU, R. KJØLLER, S. POLME, M. RYBERG, V. VELDRE, and L. TEDERSOO. 2013. The distance decay of similarity in communities of ectomycorrhizal fungi in different ecosystems and scales. J. Ecol. 101: 1335–1344.

BAIRD, D. J., and M. HAJIBABAEI. 2012. Biomonitoring 2.0: a new paradigm in ecosystem assessment made possible by next-generation DNA sequencing. Mol. Ecol. 21: 2039–2044.

BASS, D., T. A. RICHARDS, L. MATTHAI, V. MARSH, and T. CAVALIER-SMITH. 2007. DNA evidence for global dispersal and probable endemicity of protozoa. BMC Evol. Biol. 7: 1–13.

BASS, M. S., M. FINER, C. N. JENKINS, H. KREFT, D. F. CISNEROS-HEREDIA, F. SHAWN, N. C. A. PITMAN, P. H. ENGLISH, K. SWING, G. VILLA, A. DI FIORE, C. C. VOIGT, and T. H. KUNZ. 2010. Global Conservation Significance of Ecuador ' s Yasunı National Park. 5.

516 BASSET, Y., L. CIZEK, P. CUÉNOUD, R. K. DIDHAM, F. GUILHAUMON, O. MISSA, V. NOVOTNY,
517 F. ØDEGAARD, T. ROSLIN, and J. SCHMIDL. 2012. Arthropod diversity in a tropical forest.
518 Science (80-.). 338: 1481–1484.

519 BATES, J. M. 2001. Avian diversification in Amazonia: evidence for historical complexity and
520 a vicariance model for a basic diversification pattern. Divers. biológica e Cult. da Amaz.
521 119–137.

522 BATES, S. T., J. C. CLEMENTE, G. E. FLORES, W. A. WALTERS, L. W. PARFREY, R. KNIGHT,
523 and N. FIERER. 2013. Global biogeography of highly diverse protistan communities in
524 soil. ISME J. 7: 652–659. Available at: http://dx.doi.org/10.1038/ismej.2012.147.

525 BECERRA, J. X. 2015. On the factors that promote the diversity of herbivorous insects and
526 plants in tropical forests. Proc. Natl. Acad. Sci. 112: 6098–6103.

527 BENG, K. C., K. W. TOMLINSON, X. H. SHEN, Y. SURGET-GROBA, A. C. HUGHES, R. T.
528 CORLETT, and J. W. F. SLIK. 2016. The utility of DNA metabarcoding for studying the
529 response of arthropod diversity and composition to land-use change in the tropics. Sci.
530 Rep. 6: 1–13. Available at: http://dx.doi.org/10.1038/srep24965.

531 BEZEMER, T. M., C. S. LAWSON, K. HEDLUND, A. R. EDWARDS, A. J. BROOK, J. M. IGUAL, S.
532 R. MORTIMER, and W. H. VAN DER PUTTEN. 2006. Plant species and functional group
533 effects on abiotic and microbial soil properties and plant-soil feedback responses in two
534 grasslands. J. Ecol. 94: 893–904.

535 BIK, H. M., D. L. PORAZINSKA, S. CREER, J. G. CAPORASO, R. KNIGHT, and W. K. THOMAS.
536 2012. Sequencing our way towards understanding global eukaryotic biodiversity. Trends
537 Ecol. Evol. 27: 233–243. Available at: http://dx.doi.org/10.1016/j.tree.2011.11.010.

538 BLAXTER, M. L. 2004. The promise of a DNA taxonomy. Philos. Trans. R. Soc. B Biol. Sci.
539 359: 669–679.

540 BLAXTER, M., J. MANN, T. CHAPMAN, F. THOMAS, C. WHITTON, R. FLOYD, and E. ABEBE.
541 2005. Defining operational taxonomic units using DNA barcode data. 1935–1943.

542 BOENIGK, J., S. WODNIOK, C. BOCK, D. BEISSER, C. HEMPEL, L. GROSSMANN, A. LANGE, and
543 M. JENSEN. 2018. Geographic distance and mountain ranges structure freshwater protist

communities on a European scale. Metabarcoding and Metagenomics 2: e21519.

BOHLMAN, A. S., F. W. LAURANCE, G. N. S. LAURANCE, E. M. H. ASCIMENTO, M. P. FEARNSIDE, and A. ANDRADE. 2008. Importance of soils, topography and geographic distance in structuring central Amazonian tree communities. J. Veg. Sci. 19: 863–874.

BURKI, F. 2014. The eukaryotic tree of life from a global phylogenomic perspective. Cold Spring Harb. Perspect. Biol. 6.

CALLAHAN, B. J., P. J. MCMURDIE, M. J. ROSEN, A. W. HAN, A. J. A. JOHNSON, and S. P. HOLMES. 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nat. Methods 13: 581–583.

CAMERON, E. K. ET AL. 2018. Global gaps in soil biodiversity data. Nat. Ecol. Evol. 2: 1042–1043.

CARNEIRO, L., G. A. BRAVO, N. ARISTIZÁBAL, A. M. CUERVO, and A. ALEIXO. 2018. Molecular systematics and biogeography of lowland antpittas (Aves, Grallariidae): The role of vicariance and dispersal in the diversification of a widespread Neotropical lineage. Mol. Phylogenet. Evol. 120: 375–389. Available at: https://doi.org/10.1016/j.ympev.2017.11.019.

CASTILLO, M. M. 2000. Influence of hydrological seasonality on bacterioplankton in two neotropical floodplain lakes. Hydrobiologia 437: 57–69.

CEBALLOS, G., and P. R. EHRLICH. 2006. Global mammal distributions, biodiversity hotspots, and conservation. Proc. Natl. Acad. Sci. 103: 19374–19379.

CLARKE, L. J., J. SOUBRIER, L. S. WEYRICH, and A. COOPER. 2014. Environmental metabarcodes for insects: In silico PCR reveals potential for taxonomic bias. Mol. Ecol. Resour. 14: 1160–1170.

COISSAC, E., M. PETER, S. L. HOLLINGSWORTH, and P. TABERLET. 2016. From barcodes to genomes: extending the concept of DNA barcoding. Mol. Ecol. 25: 1423–1428.

CONDIT, R., S. P. HUBBELL, J. V LAFRANKIE, R. SUKUMAR, N. MANOKARAN, R. B. FOSTER, and P. S. ASHTON. 1996. Species-area and species-individual relationships for tropical trees: a comparison of three 50-ha plots. J. Ecol. 549–562.

572 CONDIT, R., N. PITMAN, E. G. LEIGH, J. CHAVE, J. TERBORGH, R. B. FOSTER, P. NÚNEZ, S.
573 AGUILAR, R. VALENCIA, and G. VILLA. 2002. Beta-diversity in tropical forest trees.
574 Science (80-.). 295: 666–669.

575 CONNELL, J. H., P. J. DEN BOER, and G. R. GRADWELL. 1971. Dynamics of populations. Cent.
576 Agric. Publ. Doc. Wageningen, Netherlands. chapter role Nat. enemies Prev. Compet.
577 exclusion some Mar. Anim. rain For. trees 298–313.

578 COSTA, L. P., Y. L. R. LEITE, G. A. B. DA FONSECA, and M. T. DA FONSECA. 2000.
579 Biogeography of South American forest mammals: endemism and diversity in the
580 Atlantic Forest 1. Biotropica 32: 872–881.

581 COUTINHO, L. M. 1978. O conceito do cerrado. Rev. Bras. Botânica.

582 CRACRAFT, J. 1985. Historical biogeography and patterns of differentiation within the South
583 American avifauna: areas of endemism. Ornithol. Monogr. 49–84.

584 CREER, S., V. G. FONSECA, D. L. PORAZINSKA, R. M. GIBLIN-DAVIS, W. SUNG, D. M. POWER,
585 M. PACKER, G. R. CARVALHO, M. L. BLAXTER, P. J. D. LAMBSHEAD, and W. K.
586 THOMAS. 2010. Ultrasequencing of the meiofaunal biosphere: Practice, pitfalls and
587 promises. Mol. Ecol. 19: 4–20.

588 CRISTESCU, M. E. 2019. Can Environmental RNA Revolutionize Biodiversity Science?
589 Trends Ecol. Evol. xx: 1–4. Available at:
590 https://linkinghub.elsevier.com/retrieve/pii/S0169534719301557.

591 CUVELIER, M. L. ET AL. 2010. Targeted metagenomics and ecology of globally important
592 uncultured eukaryotic phytoplankton. Proc. Natl. Acad. Sci. 107: 14679–14684.

593 DASSEN, S., R. CORTOIS, H. MARTENS, M. DE HOLLANDER, G. A. KOWALCHUK, W. H. VAN
594 DER PUTTEN, and G. B. DE DEYN. 2017. Differential responses of soil bacteria , fungi ,
595 archaea and protists to plant species richness and plant functional group identity. 4085–
596 4098.

597 DEINER, K., H. M. BIK, E. MÄCHLER, M. SEYMOUR, A. LACOURSIÈRE-ROUSSEL, F.
598 ALTERMATT, S. CREER, I. BISTA, D. M. LODGE, and N. DE VERE. 2017. Environmental
599 DNA metabarcoding: Transforming how we survey animal and plant communities. Mol.

Ecol. 26: 5872–5895.

DELGADO-BAQUERIZO, M., A. M. OLIVERIO, T. E. BREWER, A. BENAVENT-GONZÁLEZ, D. J. ELDRIDGE, R. D. BARDGETT, F. T. MAESTRE, B. K. SINGH, and N. FIERER. 2018. A global atlas of the dominant bacteria found in soil. Science (80-.). 359: 320–325. Available at: http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L620229913%0Ahttp://dx.doi.org/10.1126/science.aap9516.

DEVARGAS, C. ET AL. 2015. Eukaryotic plankton diversity in the sunlit ocean. Science (80-.). 348: 1261605–1261605.

DRAKARE, S., J. J. LENNON, and H. HILLEBRAND. 2006. The imprint of the geographical, evolutionary and ecological context on species–area relationships. Ecol. Lett. 9: 215–227.

DUNTHORN, M., H. KAUSERUD, D. BASS, J. MAYOR, and F. MAHÉ. 2017. Yeasts dominate soil fungal communities in three lowland Neotropical rainforests. Environ. Microbiol. Rep. 9: 668–675.

ELBRECHT, V., P. TABERLET, T. DEJEAN, A. VALENTINI, P. USSEGLIO-POLATERA, J.-N. BEISEL, E. COISSAC, F. BOYER, and F. LEESE. 2016. Testing the potential of a ribosomal 16S marker for DNA metabarcoding of insects. PeerJ 4: e1966.

FILKER, S., D. FORSTER, L. WEINISCH, M. MORA-RUIZ, B. GONZÁLEZ, M. E. FARÍAS, R. ROSSELLÓ-MÓRA, and T. STOECK. 2017. Transition boundaries for protistan species turnover in hypersaline waters of different biogeographic regions. Environ. Microbiol. 19: 3186–3200.

FILKER, S., R. SOMMARUGA, I. VILA, and T. STOECK. 2016. Microbial eukaryote plankton communities of high-mountain lakes from three continents exhibit strong biogeographic patterns. Mol. Ecol. 25: 2286–2301.

FINLAY, B. J. 2002. Global dispersal of free-living microbial eukaryote species. Science (80-.). 296: 1061–1063.

FINLAY, B. J., and T. O. M. FENCHEL. 2005. Cosmopolitan Metapopulations of Free-Living

628 Microbial Eukaryotes. Supplementary Information. Protist 155: e1–e38.

629 FOISSNER, W. 2006. Biogeography and dispersal of micro-organisms: A review emphasizing
630 protists. Acta Protozool. 45: 111–136.

631 FRECKLETON, R. P., and O. T. LEWIS. 2006. Pathogens, density dependence and the
632 coexistence of tropical trees. Proc. R. Soc. B Biol. Sci. 273: 2909–2916.

633 FUHRMAN, J. A., J. A. STEELE, I. HEWSON, M. S. SCHWALBACH, M. V. BROWN, J. L. GREEN,
634 and J. H. BROWN. 2008. A latitudinal diversity gradient in planktonic marine bacteria.
635 Proc. Natl. Acad. Sci. 105: 7774–7778.

636 FUKAMI, T., H. J. E. BEAUMONT, X. X. ZHANG, and P. B. RAINEY. 2007. Immigration history
637 controls diversification in experimental adaptive radiation. Nature 446: 436–439.

638 FURLEY, P. A. 1999. The nature and diversity of neotropical savanna vegetation with
639 particular reference to the Brazilian cerrados. Glob. Ecol. Biogeogr. 8: 223–241.

640 GEISEN, S., A. M. FIORE-DONNO, J. WALOCHNIK, and M. BONKOWSKI. 2014. Acanthamoeba
641 everywhere: high diversity of Acanthamoeba in soils. Parasitol. Res. 113: 3151–3158.

642 GEISEN, S., E. A. D. MITCHELL, S. ADL, M. BONKOWSKI, M. DUNTHORN, F. EKELUND, L. D.
643 FERNÁNDEZ, A. JOUSSET, V. KRASHEVSKA, D. SINGER, F. W. SPIEGEL, J. WALOCHNIK,
644 and E. LARA. 2018. Soil protists: A fertile frontier in soil biology research. FEMS
645 Microbiol. Rev. 42: 293–323.

646 GEISEN, S., A. T. TVEIT, I. M. CLARK, A. RICHTER, M. M. SVENNING, M. BONKOWSKI, and T.
647 URICH. 2015. Metatranscriptomic census of active protists in soils. ISME J. 9: 2178–
648 2190. Available at: http://dx.doi.org/10.1038/ismej.2015.30.

649 GIBBONS, S. 2017. Metapopulation theory provides new insight into microbial biogeography.

650 GIEBNER, H., K. LANGEN, S. J. BOURLAT, S. KUKOWKA, C. MAYER, J. J. ASTRIN, B. MISOF,
651 and V. G. FONSECA. 2020. Comparing diversity levels in environmental samples: DNA
652 sequence capture and metabarcoding approaches using 18S and COI genes. Mol. Ecol.
653 Resour.

654 GOFFREDI, S. K., G. E. JANG, and M. F. HAROON. 2015. Transcriptomics in the tropics: Total

RNA-based profiling of Costa Rican bromeliad-associated communities. Comput. Struct. Biotechnol. J. 13: 18–23. Available at: http://dx.doi.org/10.1016/j.csbj.2014.12.001.

GOFFREDI, S. K., A. H. KANTOR, and W. T. WOODSIDE. 2011. Aquatic Microbial Habitats Within a Neotropical Rainforest: Bromeliads and pH-Associated Trends in Bacterial Diversity and Composition. Microb. Ecol. 61: 529–542.

GROSSMANN, L., M. JENSEN, D. HEIDER, S. JOST, E. GLÜCKSMAN, H. HARTIKAINEN, S. S. MAHAMDALLIE, M. GARDNER, D. HOFFMANN, D. BASS, and J. BOENIGK. 2016. Protistan community analysis: Key findings of a large-scale molecular sampling. ISME J. 10: 2269–2279.

GUARDIOLA, M., M. J. URIZ, P. TABERLET, E. COISSAC, O. S. WANGENSTEEN, and X. TURON. 2015. Deep-sea, deep-sequencing: metabarcoding extracellular DNA from sediments of marine canyons. PLoS One 10: e0139633.

HAND, B. K., W. H. LOWE, R. P. KOVACH, C. C. MUHLFELD, and G. LUIKART. 2015. Landscape community genomics: Understanding eco-evolutionary processes in complex environments. Trends Ecol. Evol. 30: 161–168. Available at: http://dx.doi.org/10.1016/j.tree.2015.01.005.

HAUGAASEN, T., and C. A. PERES. 2006. Floristic, edaphic and structural characteristics of flooded and unflooded forests in the lower Rio Purús region of central Amazonia, Brazil. Acta Amaz. 36: 25–35.

HAWKSWORTH, D. L. 2001. The magnitude of fungal diversity: the 1· 5 million species estimate revisited. Mycol. Res. 105: 1422–1432.

HEGER, T. J., I. J. W. GIESBRECHT, J. GUSTAVSEN, J. CAMPO, C. T. E. KELLOGG, K. M. HOFFMAN, K. LERTZMAN, W. W. MOHN, and P. J. KEELING. 2018. High-throughput environmental sequencing reveals high diversity of litter and moss associated protist communities along a gradient of drainage and tree productivity. 20: 1185–1203.

HÉNAULT, M., C. EBERLEIN, G. CHARRON, É. DURAND, L. NIELLY-THIBAULT, H. MARTIN, and C. R. LANDRY. 2017. Yeast Population Genomics Goes Wild: The Case of Saccharomyces paradoxus. *In* Population Genomics: Microorganisms. pp. 207–230,

684 Springer.

685 HILLEBRAND, H., and A. I. AZOVSKY. 2001. Body size determines the strength of the
686 latitudinal diversity gradient. 3: 251–256.

687 HOFSTETTER, V., B. BUYCK, G. EYSSARTIER, S. SCHNEE, and K. GINDRO. 2019. The
688 unbearable lightness of sequenced-based identification Available at:
689 http://link.springer.com/10.1007/s13225-019-00428-3.

690 HOORN, C., F. P. WESSELINGH, H. STEEGE, M. A. BERMUDEZ, A. MORA, J. SEVINK, I.
691 SANMARTÍN, C. L. ANDERSON, J. P. FIGUEIREDO, C. JARAMILLO, D. RIFF, F. R. NEGRI, H.
692 HOOGHIEMSTRA, J. LUNDBERG, T. STADLER, T. SÄRKINEN, and A. ANTONELLI. 2010.
693 Amazonia through time: Andean uplift, climate change, landscape evolution, and
694 biodiversity. Science (80-.). 330: 927–931.

695 HUGHES, C. E., R. T. PENNINGTON, and A. ANTONELLI. 2012. Neotropical plant evolution:
696 assembling the big picture. Bot. J. Linn. Soc. 171: 1–18.

697 JABLONSKI, D., S. HUANG, K. ROY, and J. W. VALENTINE. 2016. Shaping the Latitudinal
698 Diversity Gradient: New Perspectives from a Synthesis of Paleobiology and
699 Biogeography. Am. Nat. 189: 1–12.

700 JANZEN, D. H. 1970. Herbivores and the number of tree species in tropical forests. Am. Nat.
701 104: 501–528.

702 KANG, S., M. A. MANSFIELD, B. PARK, D. M. GEISER, K. L. IVORS, M. D. COFFEY, N. J.
703 GRÜNWALD, F. N. MARTIN, C. A. LÉVESQUE, and J. E. BLAIR. 2010. The Promise and
704 Pitfalls of Sequence-Based Identification of Plant-Pathogenic Fungi and Oomycetes.
705 Phytopathology 100: 732–737.

706 KEELING, P. J., G. BURGER, D. G. DURNFORD, B. F. LANG, R. W. LEE, R. E. PEARLMAN, A. J.
707 ROGER, and M. W. GRAY. 2005. The tree of eukaryotes. Trends Ecol. Evol. 20: 670–
708 676.

709 KILROY, C., B. J. F. BIGGS, and W. VYVERMAN. 2007. Rules for macroorganisms applied to
710 microorganisms: patterns of endemism in benthic freshwater diatoms. Oikos 116: 550–
711 564.

712 KREFT, H., and W. JETZ. 2007. Global patterns and determinants of vascular plant diversity.
713 Proc. Natl. Acad. Sci. 104: 5925–5930. Available at:
714 https://www.pnas.org/content/pnas/104/14/5925.full.pdf?gca=0608361104v1&sendit=G
715 et All Checked Abstract%28s%29.

716 KUSHWAH, M. S., and S. THORPE. 2020. A clearer picture of microbial biogeography. Nat.
717 Rev. Microbiol. 18: 318.

718 LAFFERTY, K. D., and A. M. KURIS. 2002. Trophic strategies , animal diversity and body size.
719 17: 507–513.

720 LANSAC-TÔHA, F. A., L. F. M. VELHO, D. M. COSTA, N. R. SIMÕES, and G. M. ALVES. 2014.
721 Structure of the testate amoebae community in different habitats in a neotropical
722 floodplain. Braz. J. Biol. 74: 181–90. Available at:
723 http://www.ncbi.nlm.nih.gov/pubmed/25055100.

724 LAUBER, C. L., M. HAMADY, R. KNIGHT, and N. FIERER. 2009. Pyrosequencing-based
725 assessment of soil pH as a predictor of soil bacterial community structure at the
726 continental scale. Appl. Environ. Microbiol. 75: 5111–5120.

727 LENTENDU, G., P. R. B. BUOSI, A. F. CABRAL, B. TREVIZAN SEGÓVIA, B. RAMOS MEIRA, F.
728 M. LANSAC-TÔHA, L. F. M. VELHO, C. D. RITTER, and M. DUNTHORN. 2018. Protist
729 Biodiversity and Biogeography in Lakes From Four Brazilian River–Floodplain
730 Systems. J. Eukaryot. Microbiol.

731 LENTENDU, G., F. MAHÉ, D. BASS, S. RUECKERT, T. STOECK, and M. DUNTHORN. 2018.
732 Consistent patterns of high alpha and low beta diversity in tropical parasitic and free-
733 living protists. Mol. Ecol. 27: 2846–2857.

734 LI, N., H. CHU, Y. QI, C. LI, X. PING, Y. SUN, and Z. JIANG. 2019. Alpha and beta diversity of
735 birds along elevational vegetation zones on the southern slope of Altai Mountains:
736 Implication for conservation. Glob. Ecol. Conserv. 19: e00643.

737 LI, S., P. WANG, Y. CHEN, M. C. WILSON, X. YANG, C. MA, J. LU, X. CHEN, J. WU, and W.
738 SHU. 2020. Island biogeography of soil bacteria and fungi: similar patterns, but different
739 mechanisms. ISME J. 1–11.

LIU, S., Y. LI, J. LU, X. SU, M. TANG, R. ZHANG, L. ZHOU, C. ZHOU, Q. YANG, Y. JI, D. W. YU, and X. ZHOU. 2013. SOAPBarcode: Revealing arthropod biodiversity through assembly of Illumina shotgun sequences of PCR amplicons. Methods Ecol. Evol. 4: 1142–1150.

LOGARES, R., I. M. DEUTSCHMANN, P. C. JUNGER, C. R. GINER, A. K. KRABBERØD, T. S. B. SCHMIDT, L. RUBINAT-RIPOLL, M. MESTRE, G. SALAZAR, and C. RUIZ-GONZÁLEZ. 2020. Disentangling the mechanisms shaping the surface ocean microbiota. Microbiome 8: 1–17.

LOUCA, S., S. M. S. JACQUES, A. P. F. PIRES, J. S. LEAL, A. L. GONZÁLEZ, M. DOEBELI, and V. F. FARJALLA. 2017. Functional structure of the bromeliad tank microbiome is strongly shaped by local geochemical conditions. Environ. Microbiol. 19: 3132–3151.

LOVEJOY, N. R., J. S. ALBERT, and W. G. R. CRAMPTON. 2006. Miocene marine incursions and marine/freshwater transitions: Evidence from Neotropical fishes. J. South Am. Earth Sci. 21: 5–13.

MAHÉ, F. ET AL. 2017. Parasites dominate hyperdiverse soil protist communities in Neotropical rainforests. Nat. Ecol. Evol. 1: 1–8. Available at: http://dx.doi.org/10.1038/s41559-017-0091.

MAHÉ, F., J. MAYOR, J. BUNGE, J. CHI, T. SIEMENSMEYER, T. STOECK, B. WAHL, T. PAPROTKA, S. FILKER, and M. DUNTHORN. 2015. Comparing High-throughput Platforms for Sequencing the V4 Region of SSU-r DNA in Environmental Microbial Eukaryotic Diversity Surveys. J. Eukaryot. Microbiol. 62: 338–345.

MAHÉ, F., T. ROGNES, C. QUINCE, C. DE VARGAS, and M. DUNTHORN. 2015. Swarm v2: highly-scalable and high-resolution amplicon clustering. PeerJ 3: e1420.

MARES, M. A., and K. A. ERNEST. 2019. American Society of Mammalogists Population and Community Ecology of Small Mammals in a Gallery Forest of Central Brazil Published by : American Society of Mammalogists Stable URL : https://www.jstor.org/stable/1382745 REFERENCES Linked references are ava. 76: 750–768.

MARTINY, J. B. H., B. J. M. BOHANNAN, J. H. BROWN, R. K. COLWELL, J. A. FUHRMAN, J. L.

769 GREEN, M. C. HORNER-DEVINE, M. KANE, J. A. KRUMINS, and C. R. KUSKE. 2006.
770 Microbial biogeography: putting microorganisms on the map. Nat. Rev. Microbiol. 4:
771 102–112.

772 MATEO, R. G., Á. M. FELICÍSIMO, J. POTTIER, A. GUISAN, and J. MUÑOZ. 2012. Do stacked
773 species distribution models reflect altitudinal diversity patterns? PLoS One 7: e32586.

774 MENG, H., K. LI, M. NIE, J.-R. WAN, Z.-X. QUAN, C.-M. FANG, J.-K. CHEN, J.-D. GU, and B.
775 LI. 2013. Responses of bacterial and fungal communities to an elevation gradient in a
776 subtropical montane forest of China. Appl. Microbiol. Biotechnol. 97: 2219–2230.

777 MIKHAILOV, I. S., Y. R. ZAKHAROVA, Y. S. BUKIN, Y. P. GALACHYANTS, D. P. PETROVA, M.
778 V SAKIRKO, and Y. V LIKHOSHWAY. 2019. Co-occurrence networks among bacteria and
779 microbial eukaryotes of Lake Baikal during a spring phytoplankton bloom. Microb.
780 Ecol. 77: 96–109.

781 MORA, C., D. P. TITTENSOR, S. ADL, A. G. B. SIMPSON, and B. WORM. 2011. How many
782 species are there on earth and in the ocean? PLoS Biol. 9: 1–8.

783 MORLON, H., G. CHUYONG, R. CONDIT, S. HUBBELL, D. KENFACK, D. THOMAS, R.
784 VALENCIA, and J. L. GREEN. 2008. A general framework for the distance–decay of
785 similarity in ecological communities. Ecol. Lett. 11: 904–917.

786 MORRONE, J. J. 2014. Cladisti biogeography of the neotropical regions.pdf. 30: 202–214.

787 MYERS, N., R. . MITTERMEIER, C. . MITTERMEIER, G. A. . DA FONSECA, and J. KENT. 2000.
788 Biodiversity hotspots for conservation priorities. Nature 403: 853: 853–858.

789 MYSTER, R. W. 2016. The physical structure of forests in the Amazon Basin: a review. Bot.
790 Rev. 82: 407–427.

791 NAVARRETE, A. A., E. E. KURAMAE, M. DE HOLLANDER, A. S. PIJL, J. A. VAN VEEN, and S.
792 M. TSAI. 2013. Acidobacterial community responses to agricultural management of
793 soybean in Amazon forest soils. FEMS Microbiol. Ecol. 83: 607–621.

794 NEGREIROS, O. P., B. T. SEGOVIA, F. M. LANSAC-TÔHA, B. R. DE MEIRA, P. R. B. BUOSI, A. F.
795 CABRAL, H. S. SILVA, F. A. LANSAC-TÔHA, and L. F. M. VELHO. 2017. Structure and
796 dynamic of planktonic ciliate community in a large Neotropical river: the relevance of

the pluviosity and tributaries in the biodiversity maintenance. Acta Limnol. Bras. 29.

NILSSON, R. H., L. TEDERSOO, K. ABARENKOV, M. RYBERG, E. KRISTIANSSON, M. HARTMANN, C. L. SCHOCH, J. A. A. NYLANDER, J. BERGSTEN, T. M. PORTER, A. JUMPPONEN, P. VAISHAMPAYAN, O. OVASKAINEN, N. HALLENBERG, J. BENGTSSON-PALME, K. M. ERIKSSON, K.-H. LARSSON, E. LARSSON, and U. KÕLJALG. 2012. Five simple guidelines for establishing basic authenticity and reliability of newly generated fungal ITS sequences. MycoKeys 4: 37–63. Available at: http://mycokeys.pensoft.net/articles.php?id=1186.

NILSSON, R. H., C. WURZBACHER, M. BAHRAM, V. R. M. COIMBRA, E. LARSSON, L. TEDERSOO, J. ERIKSSON, C. D. RITTER, S. SVANTESSON, M. SÁNCHEZ-GARCÍA, M. RYBERG, E. KRISTIANSSON, and K. ABARENKOV. 2016. Top 50 most wanted fungi. MycoKeys 12.

NOGUEIRA, C., G. R. COLLI, and M. MARTINS. 2009. Local richness and distribution of the lizard fauna in natural habitat mosaics of the Brazilian Cerrado. 83–96.

NOLTE, V., R. V. PANDEY, S. JOST, R. MEDINGER, B. OTTENWAELDER, J. BOENIGK, and C. SCHLOETTERER. 2010. Contrasting seasonal niche separation between rare and abundant taxa conceals the extent of protist diversity. Mol. Ecol. 19: 2908–2915.

NOVOTNY, V., P. DROZD, S. E. MILLER, M. KULFAN, M. JANDA, Y. BASSET, and G. D. WEIBLEN. 2006. Why are there so many species of herbivorous insects in tropical rainforests? Science (80-.). 313: 1115–1118.

O'MALLEY, M. A., and J. DUPRÉ. 2007. Size doesn't matter: Towards a more inclusive philosophy of biology.

OLIVERIO, A. M., S. GEISEN, M. DELGADO-BAQUERIZO, F. T. MAESTRE, B. L. TURNER, and N. FIERER. 2020. The global-scale distributions of soil protists and their contributions to belowground systems. 1–11.

OLSON, D. M., E. DINERSTEIN, E. D. WIKRAMANAYAKE, N. D. BURGESS, G. V. N. POWELL, E. C. UNDERWOOD, J. A. D'AMICO, I. ITOUA, H. E. STRAND, and J. C. MORRISON. 2001. Terrestrial Ecoregions of the World: A New Map of Life on EarthA new global map of terrestrial ecoregions provides an innovative tool for conserving biodiversity. Bioscience

51: 933–938.

OONO, R., A. RASMUSSEN, and E. LEFÈVRE. 2017. Distance decay relationships in foliar fungal endophytes are driven by rare taxa. Environ. Microbiol. 19: 2794–2805.

PAWLOWSKI, J., S. AUDIC, S. ADL, D. BASS, L. BELBAHRI, C. BERNEY, S. S. BOWSER, I. CEPICKA, J. DECELLE, and M. DUNTHORN. 2012. CBOL protist working group: barcoding eukaryotic richness beyond the animal, plant, and fungal kingdoms. PLoS Biol 10: e1001419.

PEAY, K. G., C. VON SPERBER, E. CARDARELLI, H. TOJU, C. A. FRANCIS, O. A. CHADWICK, and P. M. VITOUSEK. 2017. Convergence and contrast in the community structure of Bacteria, Fungi and Archaea along a tropical elevation–climate gradient. FEMS Microbiol. Ecol. 93.

PETERSEN, H., and M. LUXTON. 1986. A Comparative Analysis of Soil Fauna Populations and Their Role in Decomposition Processes. Oikos 39: 288.

PIANKA, E. R. 2002. Latitudinal Gradients in Species Diversity: A Review of Concepts. Am. Nat. 100: 33–46.

PLOTKIN, J. B., M. D. POTTS, W. Y. DOUGLAS, S. BUNYAVEJCHEWIN, R. CONDIT, R. FOSTER, S. HUBBELL, J. LAFRANKIE, N. MANOKARAN, and L. H. SENG. 2000. Predicting species diversity in tropical forests. Proc. Natl. Acad. Sci. 97: 10850–10854.

POMMIER, T., B. CANBÄCK, L. RIEMANN, K. H. BOSTRÖM, K. SIMU, P. LUNDBERG, A. TUNLID, and Å. HAGSTRÖM. 2007. Global patterns of diversity and community structure in marine bacterioplankton. Mol. Ecol. 16: 867–880.

PORAZINSKA, D. L., R. M. GIBLIN-DAVIS, L. FALLER, W. FARMERIE, N. KANZAKI, K. MORRIS, T. O. POWERS, A. E. TUCKER, W. A. Y. SUNG, and W. K. THOMAS. 2009. Evaluating high-throughput sequencing as a method for metagenomic analysis of nematode diversity. Mol. Ecol. Resour. 9: 1439–1450.

PRODAN, A., V. TREMAROLI, H. BROLIN, A. H. ZWINDERMAN, M. NIEUWDORP, and E. LEVIN. 2020. Comparing bioinformatic pipelines for microbial 16S rRNA amplicon sequencing. PLoS One 15: e0227434.

RIBAS, C. C., M. MALDONADO COELHO, B. T. SMITH, G. S. CABANNE, F. M. D'HORTA, and L. N. NAKA. 2012. Towards an integrated historical biogeography of the Neotropical lowland avifauna: combining diversification analysis and landscape evolution.

RICKLEFS, R. E. 2008. The economy of nature. Macmillan.

RITTER, C. D., M. DUNTHORN, S. ANSLAN, V. XAVIER, L. TEDERSOO, R. HENRIK, and A. ANTONELLI. 2020. Advancing biodiversity assessments with environmental DNA : Long-read technologies help reveal the drivers of Amazonian fungal diversity. 00: 1–16.

RITTER, C. D., S. FAURBY, D. J. BENNETT, L. N. NAKA, H. TER STEEGE, A. ZIZKA, Q. HAENEL, R. H. NILSSON, and A. ANTONELLI. 2019. The pitfalls of biodiversity proxies: Differences in richness patterns of birds, trees and understudied diversity across Amazonia. Sci. Rep. 9: 1–13.

RITTER, C. D., A. ZIZKA, C. BARNES, R. H. NILSSON, F. ROGER, and A. ANTONELLI. 2019. Locality or habitat? Exploring predictors of biodiversity in Amazonia. Ecography (Cop.). 42: 321–333.

RITTER, C. D., A. ZIZKA, F. ROGER, H. TUOMISTO, C. BARNES, R. H. NILSSON, and A. ANTONELLI. 2018. High-throughput metabarcoding reveals the effect of physicochemical soil properties on soil and litter biodiversity and community turnover across Amazonia. PeerJ 2018: e5661.

RUECKERT, S., S. V PIPALIYA, and J. B. DACKS. 2019. Evolution: parallel paths to parasitism in the apicomplexa. Curr. Biol. 29: R836–R839.

RYŠÁNEK, D., K. HRČKOVÁ, and P. ŠKALOUD. 2015. Global ubiquity and local endemism of free-living terrestrial protists: phylogeographic assessment of the streptophyte alga K lebsormidium. Environ. Microbiol. 17: 689–698.

SANTOFERRARA, L., F. BURKI, S. FILKER, R. LOGARES, M. DUNTHORN, and G. B. MCMANUS. 2020. Perspectives from Ten Years of Protist Studies by High-Throughput Metabarcoding. J. Eukaryot. Microbiol.

SCHIMANN, H., C. BACH, J. LENGELLE, E. LOUISANNA, S. BARANTAL, C. MURAT, and M. BUÉE. 2017. Diversity and Structure of Fungal Communities in Neotropical Rainforest

Soils: The Effect of Host Recurrence. Microb. Ecol. 73: 310–320. Available at: http://dx.doi.org/10.1007/s00248-016-0839-0.

SCHIRMER, M., U. Z. IJAZ, R. D'AMORE, N. HALL, W. T. SLOAN, and C. QUINCE. 2015. Insight into biases and sequencing errors for amplicon sequencing with the Illumina MiSeq platform. Nucleic Acids Res. 43.

SCHNITZER, S. A., J. N. KLIRONOMOS, J. HILLERISLAMBERS, L. L. KINKEL, P. B. REICH, K. XIAO, M. C. RILLIG, B. A. SIKES, R. M. CALLAWAY, S. A. MANGAN, E. H. VAN NES, and M. SCHEFFER. 2011. Soil microbes drive the classic plant diversity-productivity pattern. Ecology 92: 296–303.

SHEN, C., A. GUNINA, Y. LUO, J. WANG, J. HE, Y. KUZYAKOV, A. HEMP, A. T. CLASSEN, and Y. GE. 2020. Contrasting patterns and drivers of soil bacterial and fungal diversity across a mountain gradient. Environ. Microbiol.

SHERR, E. B., and B. F. SHERR. 2002. Significance of predation by protists in aquatic microbial food webs. Sherr e Sherr, 2002.pdf. 293–308.

SHOKRALLA, S., J. F. GIBSON, I. KING, D. J. BAIRD, D. H. JANZEN, W. HALLWACHS, and M. HAJIBABAEI. 2016. Environmental DNA analysis using PCR amplified marker genes has been a key application of high-throughput sequencing (HTS). However , PCR bias is a major drawback to gain accurate qualitative and quantitative biodiversity data . We developed a PCR-free. 1–28.

SILES, J. A., and R. MARGESIN. 2016. Abundance and diversity of bacterial, archaeal, and fungal communities along an altitudinal gradient in alpine forest soils: what are the driving factors? Microb. Ecol. 72: 207–220.

SILVA, J. L. DA, and F. Z. VAZ-DE-MELLO. 2020. Areas of endemism in the Brazilian Atlantic Forest based on the distribution of dung beetles (Coleoptera, Scarabaeidae, Scarabaeinae). Iheringia. Série Zool. 110.

DA SILVA, J. M. C., and J. M. BATES. 2002. Biogeographic Patterns and Conservation in the South American Cerrado : A Tropical Savanna Hotspot. 52.

SILVA, S. M., A. T. PETERSON, L. CARNEIRO, T. C. T. BURLAMAQUI, C. C. RIBAS, T. SOUSA-

910 NEVES, L. S. MIRANDA, A. M. FERNANDES, F. M. D'HORTA, and L. E. ARAÚJO-SILVA.
911 2019. A dynamic continental moisture gradient drove Amazonian bird diversification.
912 Sci. Adv. 5: eaat5752.

913 SIMÃO, T. L. L., A. G. BORGES, K. A. GANO, A. G. DAVIS-RICHARDSON, C. T. BROWN, J. R.
914 FAGEN, E. W. TRIPLETT, R. DIAS, C. A. MONDIN, R. M. DA SILVA, E. EIZIRIK, and L. R.
915 P. UTZ. 2017. Characterization of ciliate diversity in bromeliad tank waters from the
916 Brazilian Atlantic Forest. Eur. J. Protistol. 61: 359–365. Available at:
917 https://doi.org/10.1016/j.ejop.2017.05.005.

918 SINGER, D., E. A. D. MITCHELL, R. J. PAYNE, Q. BLANDENIER, C. DUCKERT, D. FERNÁNDEZ,
919 B. FOURNIER, C. E. HERNÁNDEZ, G. GRANATH, and H. RYDIN. 2000. Dispersal
920 limitations and historical factors determine the biogeography of specialized terrestrial
921 protists. 0–2.

922 SINGER, D., E. A. D. MITCHELL, R. J. PAYNE, Q. BLANDENIER, C. DUCKERT, L. D.
923 FERNÁNDEZ, B. FOURNIER, C. E. HERNÁNDEZ, G. GRANATH, and H. RYDIN. 2019.
924 Dispersal limitations and historical factors determine the biogeography of specialized
925 terrestrial protists. Mol. Ecol. 28: 3089–3100.

926 SOININEN, J., R. MCDONALD, and H. HILLEBRAND. 2007. The distance decay of similarity in
927 ecological communities. Ecography (Cop.). 30: 3–12.

928 SOMVEILLE, M., A. MANICA, S. H. M. BUTCHART, and A. S. L. RODRIGUES. 2013. Mapping
929 global diversity patterns for migratory birds. PLoS One 8: e70907.

930 STAHLHUT, J. K., J. FERNÁNDEZ-TRIANA, S. J. ADAMOWICZ, M. BUCK, H. GOULET, P. D.
931 HEBERT, J. T. HUBER, M. T. MERILO, C. S. SHEFFIELD, T. WOODCOCK, and M. A. SMITH.
932 2013. DNA barcoding reveals diversity of hymenoptera and the dominance of
933 parasitoids in a sub-arctic environment. BMC Ecol. 13.

934 TER STEEGE, H. T., N. PITMAN, D. SABATIER, H. CASTELLANOS, P. HOUT, D. C. DALY, M.
935 SILVEIRA, O. PHILLIPS, R. VASQUEZ, T. V. ANDEL, and J. DUIVENVOORDEN. 2003. A
936 spatial model of tree alpha-diversity and tree density for the Amazon. Biodivers.
937 Conserv. 12: 2255–2277.

938 TER STEEGE, H., and D. S. HAMMOND. 2001. Character convergence, diversity, and

disturbance in tropical rain forest in Guyana. Ecology 82: 3197–3212.

TER STEEGE, H., N. C. A. PITMAN, O. L. PHILLIPS, J. CHAVE, D. SABATIER, A. DUQUE, J.-F. MOLINO, M.-F. PRÉVOST, R. SPICHIGER, and H. CASTELLANOS. 2006. Continental-scale patterns of canopy tree composition and function across Amazonia. Nature 443: 444.

STEELE, J. A., P. D. COUNTWAY, L. XIA, P. D. VIGIL, J. M. BEMAN, D. Y. KIM, C. E. T. CHOW, R. SACHDEVA, A. C. JONES, M. S. SCHWALBACH, J. M. ROSE, I. HEWSON, A. PATEL, F. SUN, D. A. CARON, and J. A. FUHRMAN. 2011. Marine bacterial, archaeal and protistan association networks reveal ecological linkages. ISME J. 5: 1414–1425. Available at: http://dx.doi.org/10.1038/ismej.2011.24.

STEFAN, G., B. CORNELIA, R. JÖRG, and B. MICHAEL. 2014. Soil water availability strongly alters the community composition of soil protists. Pedobiologia (Jena). 57: 205–213.

TABERLET, P., A. BONIN, E. COISSAC, and L. ZINGER. 2018. Environmental DNA: For biodiversity research and monitoring. Oxford University Press.

TEDERSOO, L. ET AL. 2014. Global diversity and geography of soil fungi. Science 346: 1052–3. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25430752.

THOMPSON, J. F., and P. M. MILOS. 2011. The properties and applications of single-molecule DNA sequencing. Genome Biol. 12: 217.

TUOMISTO, H., K. RUOKOLAINEN, J. VORMISTO, A. DUQUE, M. SÁNCHEZ, V. V. PAREDES, and O. LÄHTEENOJA. 2017. Effect of sampling grain on patterns of species richness and turnover in Amazonian forests. Ecography (Cop.). 40: 840–852.

VEINTIMILLA, D., M. A. NGO BIENG, D. DELGADO, S. VILCHEZ-MENDOZA, N. ZAMORA, and B. FINEGAN. 2019. Drivers of tropical rainforest composition and alpha diversity patterns over a 2,520 m altitudinal gradient. Ecol. Evol. 9: 5720–5730.

VILLAMARÍN, C., M. RIERADEVALL, and N. PRAT. 2020. Macroinvertebrate diversity patterns in tropical highland Andean rivers. Limnetica 39: 677–691.

WEISSE, T. 2017. Functional diversity of aquatic ciliates. Eur. J. Protistol. 61: 331–358. Available at: https://doi.org/10.1016/j.ejop.2017.04.001.

966 WHITAKER, R. J. 2006. Allopatric origins of microbial species. Philos. Trans. R. Soc. B Biol.
967 Sci. 361: 1975–1984.

968 WHITAKER, R. J., D. W. GROGAN, and J. W. TAYLOR. 2003. Geographic barriers isolate
969 endemic populations of hyperthermophilic archaea. Science (80-.). 301: 976–978.

970 WIENS, J. J. 2007. Global patterns of diversification and species richness in amphibians. Am.
971 Nat. 170: S86–S106.

972 WILLIG, M. R., D. M. KAUFMAN, and R. D. STEVENS. 2003. Latitudinal Gradients of
973 Biodiversity: Pattern, Process, Scale, and Synthesis. Annu. Rev. Ecol. Evol. Syst. 34:
974 273–309. Available at:
975 http://www.annualreviews.org/doi/10.1146/annurev.ecolsys.34.012103.144032.

976 WOODWARD, G., B. EBENMAN, M. EMMERSON, J. M. MONTOYA, J. M. OLESEN, A. VALIDO,
977 and P. H. WARREN. 2005. Body size in ecological networks. 20.

978 WORDEN, A. Z., M. J. FOLLOWS, S. J. GIOVANNONI, S. WILKEN, A. E. ZIMMERMAN, and P. J.
979 KEELING. 2015. Rethinking the marine carbon cycle: factoring in the multifarious
980 lifestyles of microbes. Science (80-.). 347.

981 ZINGER, L. ET AL. 2019. Body size determines soil community assembly in a tropical forest.
982 Mol. Ecol. 28: 528–543.

983 ZINGER, L., A. BOETIUS, and A. RAMETTE. 2014. Bacterial taxa-area and distance-decay
984 relationships in marine environments. Mol. Ecol. 23: 954–964.

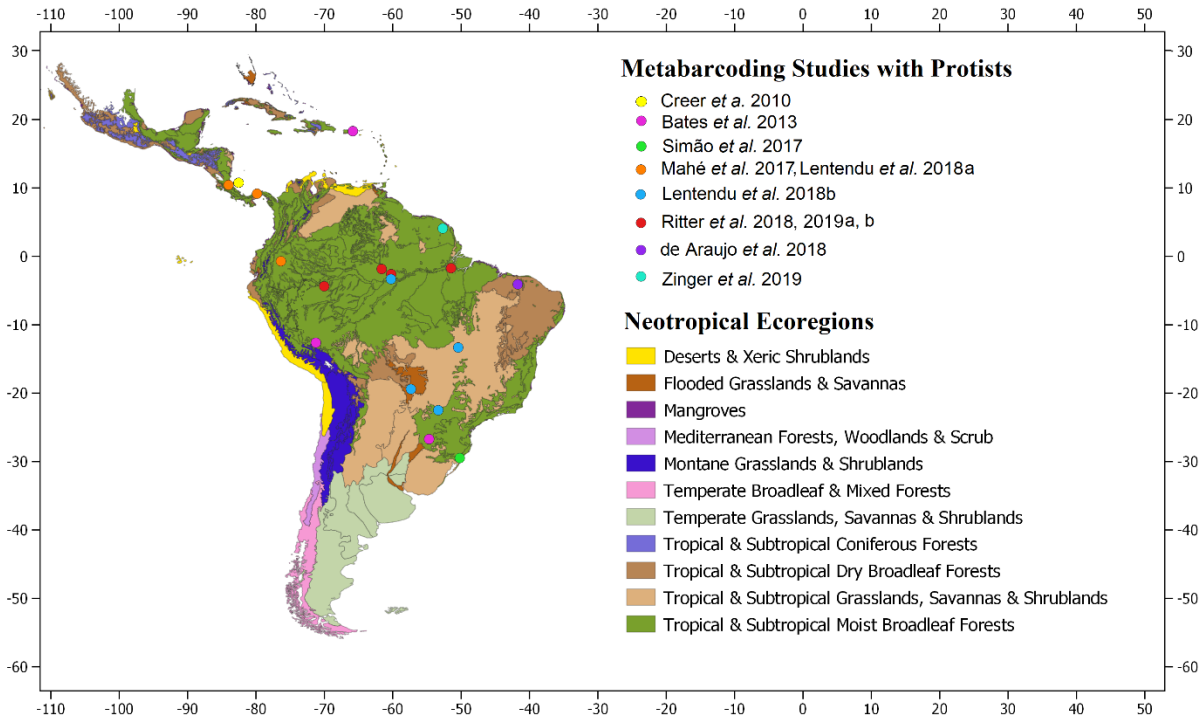
985 ZINGER, L., J. DONALD, S. BROSSE, M. A. GONZALEZ, A. IRIBAR, C. LEROY, and C. MARTINS
986 LOPES. 2020. Advances and prospects of environmental DNA in neotropical rainforests.
987 Adv. Ecol. Res. 62: 331–373.

988 ZIZKA, A. 2019. Big data suggest migration and bioregion connectivity as crucial for the
989 evolution of Neotropical biodiversity. Front. Biogeogr. 0: 0–7. Available at:
990 https://escholarship.org/uc/item/25h0w1cx.

991 ZIZKA, A., H. TER STEEGE, M. DO C. R. PESSOA, and A. ANTONELLI. 2018. Finding needles in
992 the haystack: where to look for rare species in the American tropics. Ecography (Cop.).
993 41: 321–330.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 25 July 2020 doi:10.20944/preprints202007.0608.v1

994 **Figures:**



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996 **Figure 1. Neotropical Ecoregions with the areas that samples protists.** The circles represent
997 the studies location. Studies that used the same data are cited a by the side f the other. The
998 yellow circle that represents Creer et al. (2010) is the side of Mahe et al. (2017) because they
999 are done at the same station but not with same methodology and the exactly same locations. It
1000 is possible to observe the little number of studies on Neotropic with a concentration on
1001 Neotropical forests. A big gap on sampling of other ecoregions such as Andes, dry areas,
1002 temperated Neotropical regions, deserts and xeric vegetation is notable.

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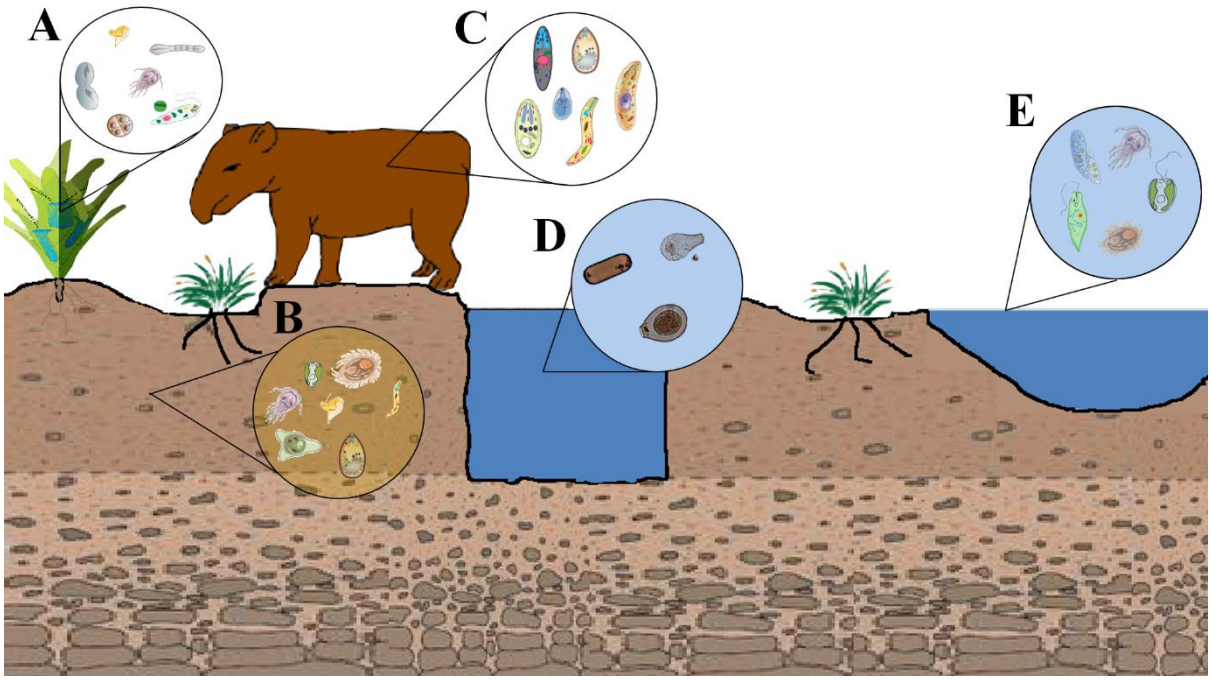

Figure 2. Schematic design of the main groups of protists found in the different environments in Neotropics. The circles represent the zoom showing the most common protists in each environment. A) the phytotelmata of bromeliads plants where was found several Ciliophora and Flagellates; B) soil protists that are mostly represented by Alveolata (mostly Apicomplexa), Dinophycea, Cercozoa and Ciliophora; C) the animal bodies that are occupied by parasitic protists such Apicomplexa; D) river environments that presents several species of Testade Amoebae; and E) lakes with the dominance of Discoba (mainly Euglenidaes), Ciliophora and Ochrophyta.