Journal of
Plankton Research

plankt.oxfordjournals.org

J. Plankton Res. (2015) 37(3): 645–655. First published online March 23, 2015 doi:10.1093/plankt/fbv016

Planktonic eukaryote molecular diversity: discrimination of minerotrophic and ombrotrophic peatland pools in Tierra del Fuego (Argentina)

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Received November 7, 2014; accepted February 19, 2015

Corresponding editor: John Dolan

We investigated the composition of the smallest size fraction (<3 μm) of eukaryotic plankton communities of five pools located in the Rancho Hambre peat bog in Argentinean Tierra del Fuego with an IlluminaHiSeq massive sequencing approach applied to the v9 region of the eukaryotic SSU rRNA gene. Communities were generally dominated by chrysophytes, with a good representation of Perkinsea and Cercozoa clade NC-10. A community composition analysis performed using GUniFraC separated minerotrophic and ombrotrophic sites, reflecting perfectly the classification of the sites based on environmental data. However, this separation disappeared when more weight was given to abundant phylotypes, suggesting that subordinate phylotypes were responsible for site discrimination. The 5% best indicators for, respectively, minerotrophic and ombrotrophic environments were searched using an IndVal analysis. Among these, autotrophic taxa were more common in minerotrophic environments, whereas mixotrophic taxa represented best ombrotrophic water bodies. However, the ecological traits of many taxa have still not been determined, and still needs to be investigated for a better understanding of freshwater systems ecology.

KEYWORDS: unknown diversity; next-generation sequencing; community; mixotroph; protist; algae; parasitoid

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INTRODUCTION

Traditional limnological studies on microbial eukaryotic plankton were first restricted to net-sized taxa, and then to those that were identifiable with light microscopy, typically algae and ciliates. Heterotrophic nanoflagellates and small autotrophs/mixotrophs are known to exhibit an immense diversity, yet they were typically pooled together into a small number of genera, as these organisms lack morphologically discriminating traits. However, it has been demonstrated that genera such as *Chlorella* (Huss *et al.*, 1999; Krienitz *et al.*, 2004), *Spumella* and *Ochromonas* (Boenigk *et al.*, 2005; Cavalier-Smith and Chao, 2006) include genetically and functionally diverging forms. Also parasitic and parasitoid taxa can only be observed when they protrude outside their hosts, like some life stages in chytrids. Therefore, diversity of planktonic communities has been largely underestimated.

The last decade saw the advent of DNA-based studies to characterize environmental eukaryotic diversity. These approaches revealed an immense diversity in freshwater systems (Richards *et al.*, 2005; Slapeta *et al.*, 2005), showing also the existence of many previously unknown deep clades. In the last 5 years, the development of massive sequencing technologies such as pyrosequencing or Illumina allowed a more in-depth picture of existing diversity by providing thousands to millions of reads representing arguably the whole extent of eukaryotic diversity present in one sample (Amaral-Zettler *et al.*, 2009; Behnke *et al.*, 2011). Indeed, the application of so-called next-generation sequencing (NGS) technologies indeed brought a better insight on diversity, bridging sequence and observation data is still not straightforward (Bachy *et al.*, 2013; Stoeck *et al.*, 2014). Beside the intrinsic interest of estimating total environmental diversity, there is a need to relate sequence data to a specific niche or role in the food web. Consequently, as large amounts of unknown sequences are revealed, it can be expected that our vision of freshwater ecosystem functioning may be challenged. For instance, the discovery of a wealth of sequences related to parasitoids in lakes suggests the importance of these organisms in regulating populations and nutrient cycling (Brate *et al.*, 2010; Mangot *et al.*, 2011). Beyond this example, trophic strategies of organisms (i.e. autotrophy/heterotrophy etc.) can be inferred in some cases by a careful examination of the taxonomic position of the organisms from which sequences derived. Also, successful adaptive strategies can be deducted from indicator OTUs for different sets of environmental conditions. These facts highlight not only the existence of unknown organisms, but also of unsuspected mechanisms ruling nutrient cycling in freshwaters (Sime Ngando and Niquil, 2011).

Peatlands are wetland ecosystems characterized by the accumulation of slowly decomposing organic matter (peat) mostly under cold, wet and anoxic conditions. The areas where peat is actively formed, termed "peat bogs" are frequently dominated by the moss *Sphagnum magellanicum* in Tierra del Fuego and host often shallow, acidic, humic pools, which in turn can display a range of abiotic features. Five pools with different morphometric characteristics from Rancho Hambre peat bog were thoroughly studied over two consecutive ice-free periods (October–April) between 2008 and 2010 regarding their physical and chemical features (Gonzalez Garraza *et al.*, 2012) as well as the variations in abundance, biomass and composition of their plankton communities (Quiroga *et al.*, 2013). Due to the impossibility of identifying morphologically the smallest fraction of organisms, their composition remained unknown until now. Here, we explored the environmental molecular diversity of these small (i.e. <3 μm) eukaryotes. We sequenced the v9region of the SSU rRNA gene using Illumina'sHiSeq technology, and related community composition with physicochemical parameters. We hypothesized that the minerotrophic (i.e. influenced by water of subterranean origin) versus ombrotrophic (i.e. exclusively fed by precipitation) character of water bodies would largely determine the composition of the smallest eukaryotes, as this factor proved crucial for structuring trophic webs in these environments (Quiroga *et al.*, 2013). Furthermore, we characterized taxonomically and determined the trophic strategies of the best indicator organisms for each water body type.

METHOD

Sampling

Five pools located within Rancho Hambre peat bog (RH; 54°44′ 52.87″S 67°49′ 29.44″W), undergoing a long-term ecological survey (Fig. 1) were sampled on 1 November 2012 during the austral late spring. They have been labeled RH1-5 and include two minerotrophic pools connected to the underlying aquifer and three ombrotrophic ones (fed only by rainwater); they have been described in detail in González Garraza (González Garraza, 2012). On account of the homogeneous physical and chemical properties of each pool, one series of samples was taken from one point at the shore in each of them. Samples for chemical and biological analysis were collected using 2-L acid-washed plastic bottles pre-rinsed with pool water and then transported to the laboratory under cold, dark conditions. Temperature, pH and conductivity were measured *in situ* with a multiparametric probe, HachSension 156 (Hach, USA).

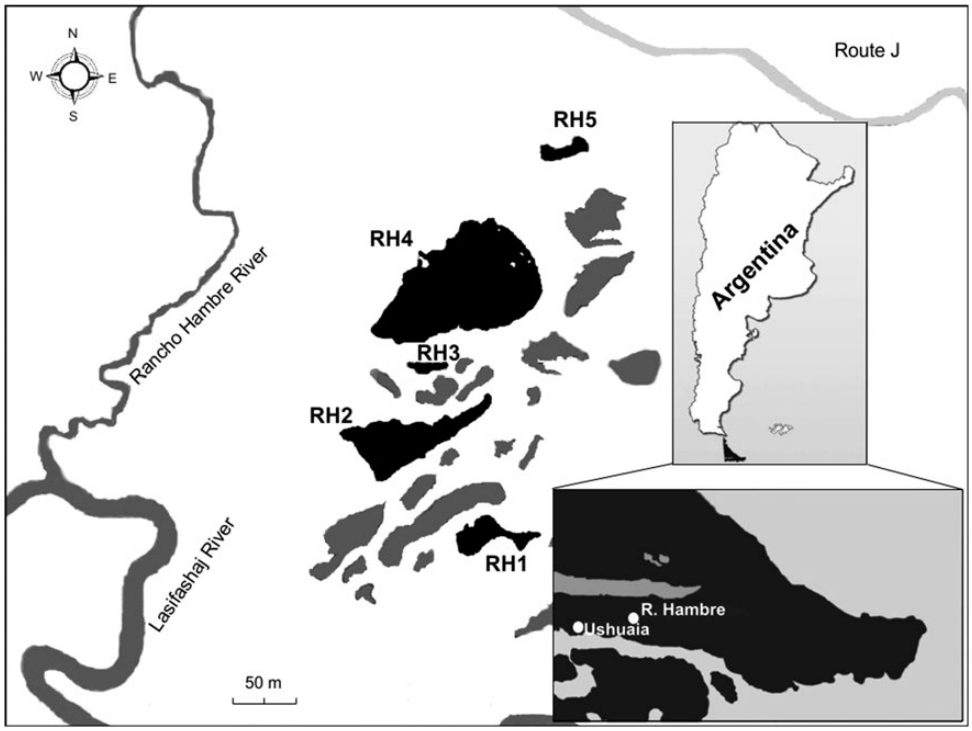

Fig. 1. Map representing the respective positions of the different water bodies and their geographic location (Mataloni *et al.*, 2015).

Lab methods

Dissolved reactive phosphorus (DRP), total phosphorus (TP) ammonia (NH_4-N), nitrate (NO_3-N), absorbance at 440 nm (A440) and total hardness (TH) were determined as described in González Garraza (González Garraza, 2012). Dissolved inorganic nitrogen was the sum of NH_4-H + NO_3-N. Dissolved organic carbon (DOC) was determined from filtered water with the high-temperature Pt catalyst oxidation method (TOC-L, Shimadzu) following Sharp *et al.* (Sharp *et al.*, 1993).

DNA extraction, PCR and sequencing

In the laboratory, 20-μm-net-filtered plankton samples were prefiltered through 3-μm pore-size polycarbonate filters to exclude as many larger organisms as possible. The filtrate was deposited onto 0.2-μm nitrocellulose filters. These were placed in cryovials and stored at −80°C until analysis. Environmental DNA was extracted by cutting nitrocellulose filters into ∼1 mm^2 pieces which were subsequently transferred into the columns of a MoBio (Carlsbad, USA) Power Soil™ extraction kit directly as recommended in Lara *et al.* (Lara *et al.*, 2009). A PCR protocol aimed at amplifying the v9 region of the SSU rRNA gene of eukaryotes present in the samples after the recommendations of Amaral-Zettler *et al.* (Amaral-Zettler *et al.*, 2009), using Promega's GoTaq polymerase (without proofreading activity). Sequencing was performed with Illumina'sHiSeq technology, using V3 chemistry (Fasteris, Geneva, Switzerland).

Sequences treatment

Sequences were sorted for quality by keeping only sequences without ambiguous nucleotides based on a phred score threshold of <28 (with a custom script). Sequences were then clustered into OTUs using the dbc454 program (Pagni *et al.*, 2013). The following setups were used: minimum number of sequence by OTU: 5; distance cutoff: 1−7 and step: 0.2. OTUs were aligned against sequences from the PR^2 database as downloaded on 24 June 2014 (Guillou *et al.*, 2013) to determine their taxonomic affiliation using SWIPE (Rognes, 2011) with the following parameters: reward: 1; penalty: −3; gap open: 2 and gap extend: 3. The OTUs assigned to Metazoa and Embryophyceae were discarded for further analysis. In order to remove rare clusters in an objective way, we determined the inflection point of the OTUs rank abundance curve from the combined data and removed all sequences that were less abundant; that threshold was found with a piecewise linear regression (Toms and Lesperance, 2003; Chiu *et al.*, 2006). We verified that the diversity reached saturation in each sample

with the function "rarecurve" as implemented in the vegan package (Oksanen *et al.*, 2012) in R.

Once assigned, the frequency of each OTU was established for every sample. The taxonomic assignations of OTUs were then verified individually against the NCBI database. A functional category (i.e. trophic mode) was assigned to OTUs whose sequences branched within a group in which all members share the aforementioned trophic mode with a custom script.

Numerical analysis

Pie charts were built to show the gross taxonomic composition encountered in each sample (Fig. 2). Non-correlated physicochemical parameters were selected for the remaining analyses. The parameters were selected based on correlation tests done for every pair of two parameters. We removed iteratively the most significant parameter until the lower *P*-value was higher than 0.05. These were pH, conductivity, DRP, TP, TH and DOC. An unsupervised random forest analysis (1 million trees) was used to classify the five water bodies according to the selected physicochemical parameters (Breiman, 2001). A dendrogram was computed on the basis of the distance between samples (Fig. 3).

In parallel, samples were also classified according to the composition of their communities using GUniFrac v 1.0 (Chen *et al.*, 2012). The parameter α was set to vary between 0 and 1. This parameter varies between a presence–absence analysis of communities ($\alpha = 0$) to giving each OTU the weight corresponding to the number of occurrences in the set of sequences, which corresponds to taking into account only the most common sequences. The phylogenetic tree required for the GUniFrac analysis was built with ExaMLv 2.0.4 (Stamatakis, 2014) based on an alignment obtained from the program Clustal Omega (Sievers *et al.*, 2011), with a gamma distribution of rate heterogeneities. A dendrogram was built for eight values of α, namely 0, 0.14, 0.29, 0.43, 0.57, 0.71, 0.86 and 1.

For each group of samples discriminated by dendrograms, based on both physicochemical parameters and community composition, we calculated indicator values for each OTU using the IndVal method (Dufrene and Legendre, 1997). This method determines the most characteristic organisms for each environment and evaluates their specificity with a score ranging from 0 to 1, 1 being an organism present only in a given environment and totally absent from the others. The 5% most characteristic organisms (39 OTUs), i.e. the OTUs that had the highest indicator score were selected and their abundance was represented in a heat map. The color represents the proportion of these OTUs in the different samples.

RESULTS

Physical and chemical characterization of water bodies

Field observations showed that among the three large water bodies, only RH1 and RH4 had inflows and/or outflows (i.e. natural surface channels), while RH2 as well as shallow RH3 and RH5, did not and received water only through rainfall and snow. In November 2012 sampling, the water temperature of the five pools showed intermediate values regarding their respective ranges, reflecting late spring-early summer conditions (Table I). Values of conductivity, TH, nutrient concentrations and organic matter were within the same range in all water bodies, and fell within the previously established variation range for these environments. On the other hand, pH and DOC clustered the pools into two groups: less acid and humic (RH1 and RH4, pH = 6.7 and 6.8, DOC = 0.42 and 0.33, respectively) and more acid and humic (RH2, RH3 and RH5, pH = 5.9, 5.1 and 5.8, DOC = 0.56, 0.63 and 0.61, respectively), showing the minerotrophic character of the first group of pools and the ombrotrophy of the second.

Taxonomic composition of communities

Environmental DNA survey gave a total of 732 109 sequence reads for all five (RH1-5) samples. From these, 205 274 sequences were kept after quality check. Sequence clustering resulted in 3291 different OTUs. From these, we removed 94 which belonged to Embryophyceae or Metazoa. After removing the rare sequences, we kept 783 OTUs for statistical analysis. Sequences related to Chrysophyceae were the most abundant in all samples with the exception of RH2 and, overall, represented the most common group. The parasitoid clade Perkinsea dominated RH2 and was present in all waterbodies, although in lower abundances. Phagotrophic taxa were represented by ciliates and bicosoecids. Cercozoa, which belonged almost exclusively to the environmental clade 10 (Bass *et al.*, 2009) were also well represented, reaching high proportions in the total number of reads in samples RH1, RH4 and RH5. Autotrophs were mostly represented by chlorophytes and bolidophyceae and many sequences of osmotrophic organisms (mostly Fungi) were also present (Fig. 2).

Numerical analysis

Random forest analysis based on environmental data grouped on one hand ombrotrophic samples RH2, 3 and 5, RH3 and 5 being the most similar and, on the other hand, minerotrophic RH1 and RH4. The GuniFrac analysis on community data and resulting dendrogram

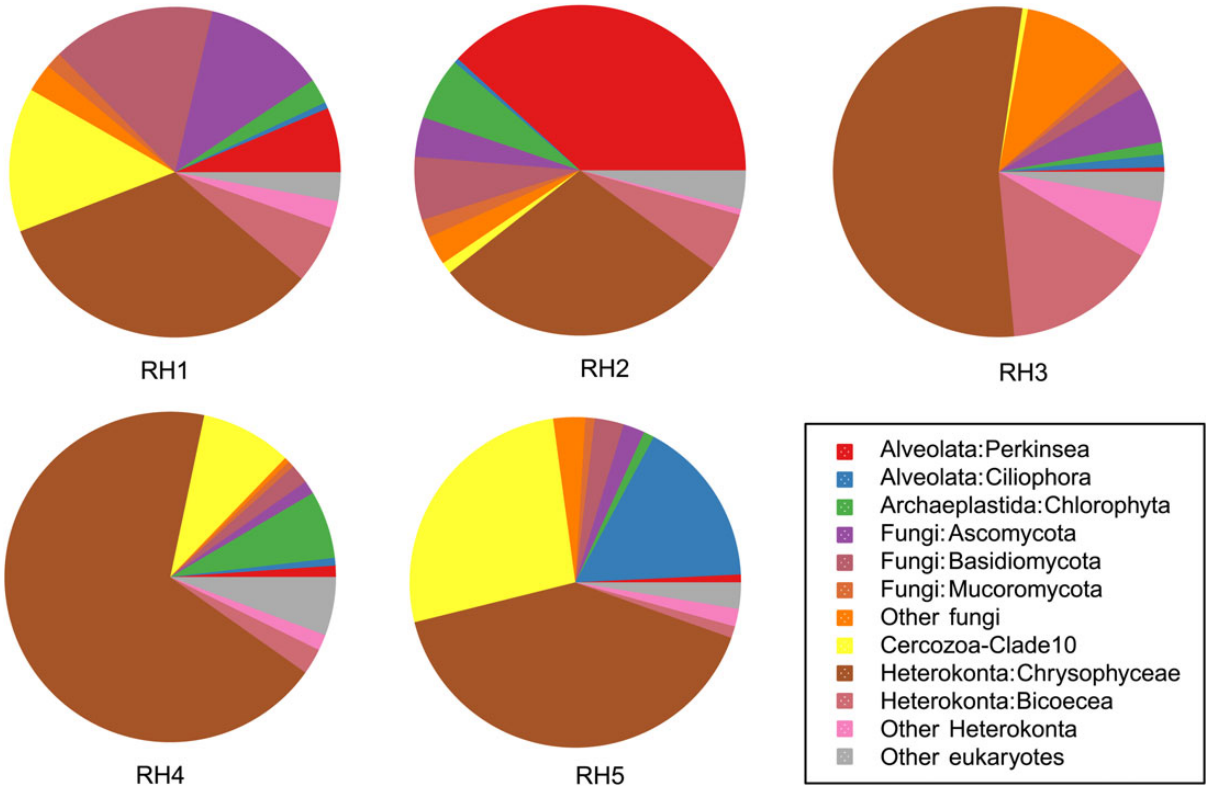

Fig. 2. Pie charts representing the high-level taxonomic composition of the communities in the five water bodies RH1-5.

showed the same pattern when parameter α varied from 0 to ~0.4, corresponding to a situation where a relatively lower weight is given to abundant OTUs. Higher values showed a completely different topology where ombrotrophic and minerotrophic communities are intermixed, $\alpha = 1$ corresponding to the situation where weight of OTUs corresponds exactly to numbers of reads (Fig. 3).

The 5% most characteristic OTUs comprised all trophic types (Figs 4 and 5). Sequences related to osmotrophic organisms where rare, occurred in the ombrotrophic pools and were represented by a basidiomycetous yeast related to *Rhodotorula* and a Mucorale (previously zygomycete) that belongs to genus *Mortierella*. Phagotrophic organisms were equally represented in both minerotrophic and ombrotrophic environments, with respectively 13 versus 11 OTUs. However, the taxonomic composition of this trophic group changed drastically between the two types of environment; small bacterivores were mainly represented by chrysophytes from genus *Paraphysomonas* in minerotrophic environments and free-swimming bicosoecids (i.e. Pseudodendromonadales) in ombrotrophic pools. Parasitoids of planktonic protists included Cryptomycota and Perkinsea in both environments, and some parasites of plants and invertebrates appeared also (respectively, a Plasmodiophorid and an Entomophthoromycota), possibly infecting organisms from the zooplankton. Pigmented organisms were divided into mixotrophic and strictly phototrophic organisms. Minerotrophic water body indicators included five autotrophic OTUs versus one in ombrotrophic systems. In contrast, mixotrophic indicator OTUs were far more represented in ombrotrophic pools than in minerotrophic, with respectively 3 and 15 OTUs. Still, the trophic mode of 13 OTUs could not be determined, which represents ~17% of all sequence reads.

DISCUSSION

Taxonomic and functional composition of planktonic communities

A high abundance and diversity of sequences related to Chrysophyceae in oligotrophic freshwater systems has been observed in many environmental DNA surveys (Richards *et al.*, 2005; Charvet *et al.*, 2012), and less so in more eutrophic systems where plankton had been filtered following a protocol similar to the one described here (Slapeta *et al.*, 2005; Lepere *et al.*, 2006). Notably, they were very abundant in a pristine oligotrophic peat bog

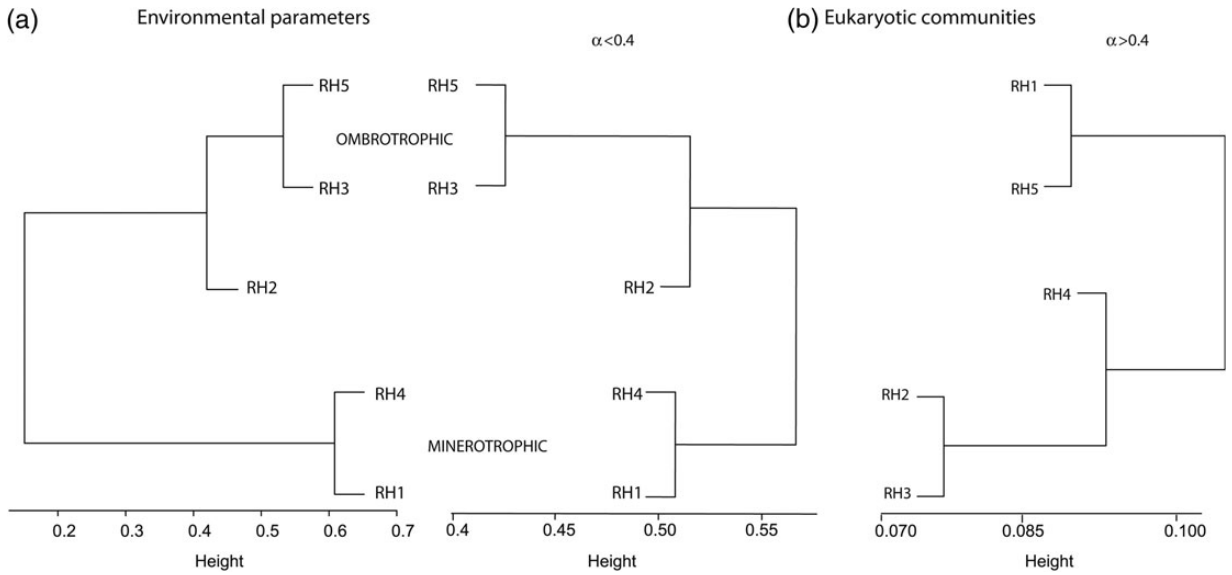

Fig. 3. Correspondence between dendrograms based on (**a**) Random forest analysis of environmental parameters (DRP, TP, TH and A440 nm) and (**b**) communities as determined by GUniFrac analysis, with respectively a low (<0.4) and a high (>0.4) α parameter. When α is low, the dendrogram based on environmental patterns corresponds perfectly to the one based on communities.

pool in Switzerland, an environment which resembles strongly the water bodies described in this study (Lara *et al.*, 2011). Their abundance in the Rancho Hambre pools corroborates the importance of chrysophyceae in oligotrophic and acidic freshwater systems. Perkinsea are also a common group in freshwaters (Brate *et al.*, 2010; Mangot *et al.*, 2011; Sime Ngando and Niquil, 2011). Their dominance in sample RH2 may be due to a local peak of abundance limited in time, as it has been shown to occur in a peat bog (Lara *et al.*, 2011). Ciliates also reach high numbers in one single lake, i.e. RH5, but occur in limited amounts in the other water bodies, possibly for the same reasons as for Perkinsea. A study that follows microeukaryotic populations through time could show if these high sequence abundances in certain lakes are persistent in time. In contrast, Clade 10 cercozoans are consistently found in high numbers in all water bodies. This clade has, to date, only been detected in the picoplanktonic fraction of oligotrophic (Richards *et al.*, 2005) and meso-eutrophic lakes (Lefranc *et al.*, 2005; Lefevre *et al.*, 2008). These organisms appear also in high numbers in these studies, which suggests an important role in planktonic communities. However, they were not detected in surveys of planktonic communities based on morphological identification, probably because of their small size. Therefore, they have never been isolated, and nothing is known about their morphology, trophic strategy or life cycle.

The difficulty of relating DNA sequences and lifestyle traits is a pervasive problem for the interpretation of environmental DNA sequences. Chrysophytes, for instance, have lost their photosynthetic ability several times in their evolutionary history, switching from a mixotrophic to a heterotrophic state. Consequently, small bacterivorous nanoflagellates that were previously grouped mostly within genera *Spumella* and *Monas* are now scattered over the chrysophycean tree (Boenigk *et al.*, 2005). Consequently, not all chrysophyte OTUs could be assigned to trophic modes in this study, either because mixotrophic and heterotrophic branches are intermingled in certain subgroups, or because no sequence has been related to a given morphotype (Richards *et al.*, 2005). Likewise, the correspondence between parasite sequences and their hosts would be desirable to understand population fluctuations in planktonic communities. Although the correspondence between Cryptomycota SSU rRNA gene sequences and the ability to parasitize oomycetes and chytrids (Held, 1981), diatoms (Jones *et al.*, 2011) and Amoebozoa (Corsaro *et al.*, 2014) has been demonstrated, the host range of the majority of these organisms still remains unknown. Likewise, Perkinsea, which have been only recently shown to be widespread in freshwaters (Brate *et al.*, 2010; Mangot *et al.*, 2011; Sime Ngando and Niquil, 2011) have still not been characterized in that sense, except for frog tadpole pathogen (Davis *et al.*, 2007). These gaps in knowledge call for detailed studies of the organisms that build the planktonic communities that include isolation, cultivation and also bar-coding. A better knowledge of the organisms will open the way for an improved understanding of the population dynamics of the plankton ecosystems.

Table I: Morphometric and physicochemical features of the five pools from Rancho Hambre peat bog (Tierra del Fuego)

Pools	RH 1	RH 2	RH 3	RH 4	RH 5
Latitude (S)	54° 44′ 52.87″	54° 44′ 48.61″	54° 44′ 46.75″	54° 44′ 41.51″	54° 44′ 39.35″
Longitude (W)	67° 49′ 29.44″	67° 49′ 31.66″	67° 49′ 32.21″	67° 49′ 31.69″	67° 49′ 26.7″
Maximum length (m)	81.9	162.9	50.7	195.7	34.5
Maximum width (m)	28.5	66.2	10.5	122.9	12.7
Maximum depth (cm)	127	165	35	150	33
Perimeter (m)	238	445	115	555	162
Area (m^2)	1824	5976	137	16 190	542
SDI	1.6	1.6	2.1	1.2	2.0
Temperature (°C)	10.4 (2.3–17.5)	9.5 (1.1–15.9)	9.5 (3.2–24.9)	9.6 (3.3–14.9)	10.4 (1.7–19.7)
pH	6.7 (5.0–7.1)	5.9 (3.8–5.9)	5.1 (3.6–5.4)	6.8 (5.2–7.0)	5.8 (4.1–5.8)
Conductivity (μS cm^{-1})	16 (14–50)	21 (9–40)	13 (10–82)	20 (16–60)	16 (5–50)
TH (mg equiv. $CaCO_3$ L^{-1})	38 (7–41)	32 (7–46)	48 (8–48)	26 (11–43)	35 (11–36)
DIN (μM)	1.6 (0.5–7.3)	1.6 (0.5–17.1)	1.6 (0.7–7.4)	1.5 (1.4–7.6)	2.4 (0.0–5.2)
TP (μM)	9.9 (3.7–9.9)	9.2 (3.0–10.7)	11.0 (2.9–11.0)	11.0 (2.9–11.0)	13.9 (2.5–13.9)
DRP (μM)	1.3 (0.8–2.7)	1.0 (0.7–2.5)	0.7 (0.7–4.2)	0.7 (0.5–1.9)	1.9 (0.6–1.9)
DOC (mM)	0.42 (0.42–0.80)	0.56 (0.42–0.75)	0.63 (0.23–1.22)	0.33 (0.33–0.50)	0.61 (0.32–1.04)

November 2012 values are given, with minimum and maximum values recorded from October 2008 to April 2010 in parentheses (Gonzalez Garraza, 2012).

TH, total hardness (Ca^{2+} + Mg^{2+}); TN, total nitrogen; DIN, dissolved inorganic nitrogen; TP, total phosphorus; DRP, dissolved reactive phosphorus; DOC, dissolved organic carbon.

Correlation between community composition and environmental parameters

Hydrological condition of the water bodies influenced environmental parameters, which in turn shaped planktonic communities. When α parameter in GUniFrac was set between 0 and 0.4 (i.e. ranging from a presence–absence analysis to an intermediate weight given to abundant OTUs in each sample), the dendrogram based on community composition corresponded perfectly to the one built on abiotic parameters. In contrast, when more weight was given to abundant OTUs by setting α above 0.4, the topology of the dendrogram changed totally. This suggests that the most common OTUs are not influenced by differing physicochemical parameters of the water bodies, but subordinate OTUs are. They are therefore the best indicators for minero-ombrotrophic conditions. The differential response of rare and common species to environmental communities has been studied in many different models, and results differ widely between cases. For instance, in plants from grassland communities, richness patterns of rare species were less predictable than those of common species (Lennon *et al.*, 2011). Conversely, in benthic stream macroinvertebrates, both rare and common species react in the same way (Siqueira *et al.*, 2012). A recent study on chironomid larvae in subtropical reservoirs showed that rare species underwent stronger niche selection and reacted more strongly to environmental fluctuation than common ones (Petsch *et al.*, 2015). Accordingly, we suggest that abundant species may have wider ecological niches and can grow in minerotrophic and ombrotrophic pools alike. Less common OTUs, in turn, are probably more specialized and thus indicate better the trophic level of the ponds. Alternatively, decreasing the influence of frequent species by decreasing α reduces the effect of local peaks of abundance, such as the ones observed for Perkinsea and Ciliates (Fig. 2).

Characteristic organisms for minerotrophic and ombrotrophic pools

The 5% most characteristic OTUs for (i) minerotrophic and (ii) ombrotrophic pools comprised organisms that used all trophic strategies: autotrophy, heterotrophy, mixotrophy, osmotrophy and parasitism. However, proportions of each varied considerably. Characteristic mixotrophs were clearly more frequent in ombrotrophic environments, where they represented more than one-third of all OTUs selected for specificity versus only three in the minerotrophic pools. In contrast, only one strictly autotrophic indicator organism was present in ombrotrophic environments (a member of the Sphaeropleales), against five in the minerotrophic water bodies (Fig. 5). A higher diversity of mixotrophs in ombrotrophic environments can be paralleled to higher abundances, as it has been already widely documented in freshwater plankton through microscopic observation, including Argentinean Patagonia and Tierra del Fuego (Saad *et al.*, 2013). Altogether, these results suggest that mixotrophy is a winning strategy in oligotrophic systems.

There was roughly the same number of heterotrophic environment-specific organisms in pools of both trophic status (respectively, 13 in minerotrophic versus 11 in

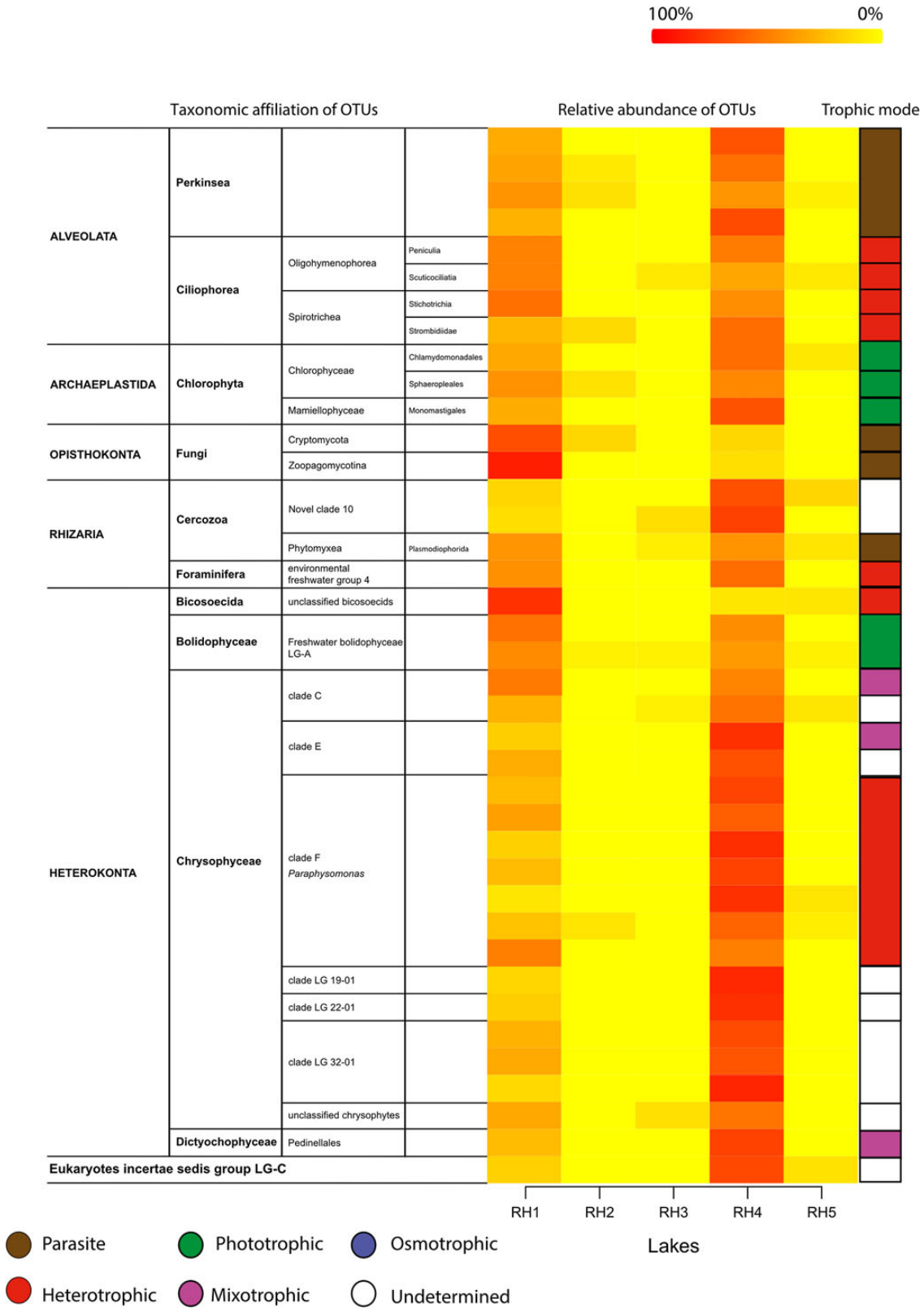

Fig. 4. Taxonomical affiliation of the OTUs which have been found to be the 5% best indicators for minerotrophy. The heat map indicates relative proportions of each read, from rare to frequent (see reference bar above). Inferred trophic types are shown on the right, next to the corresponding OTU.

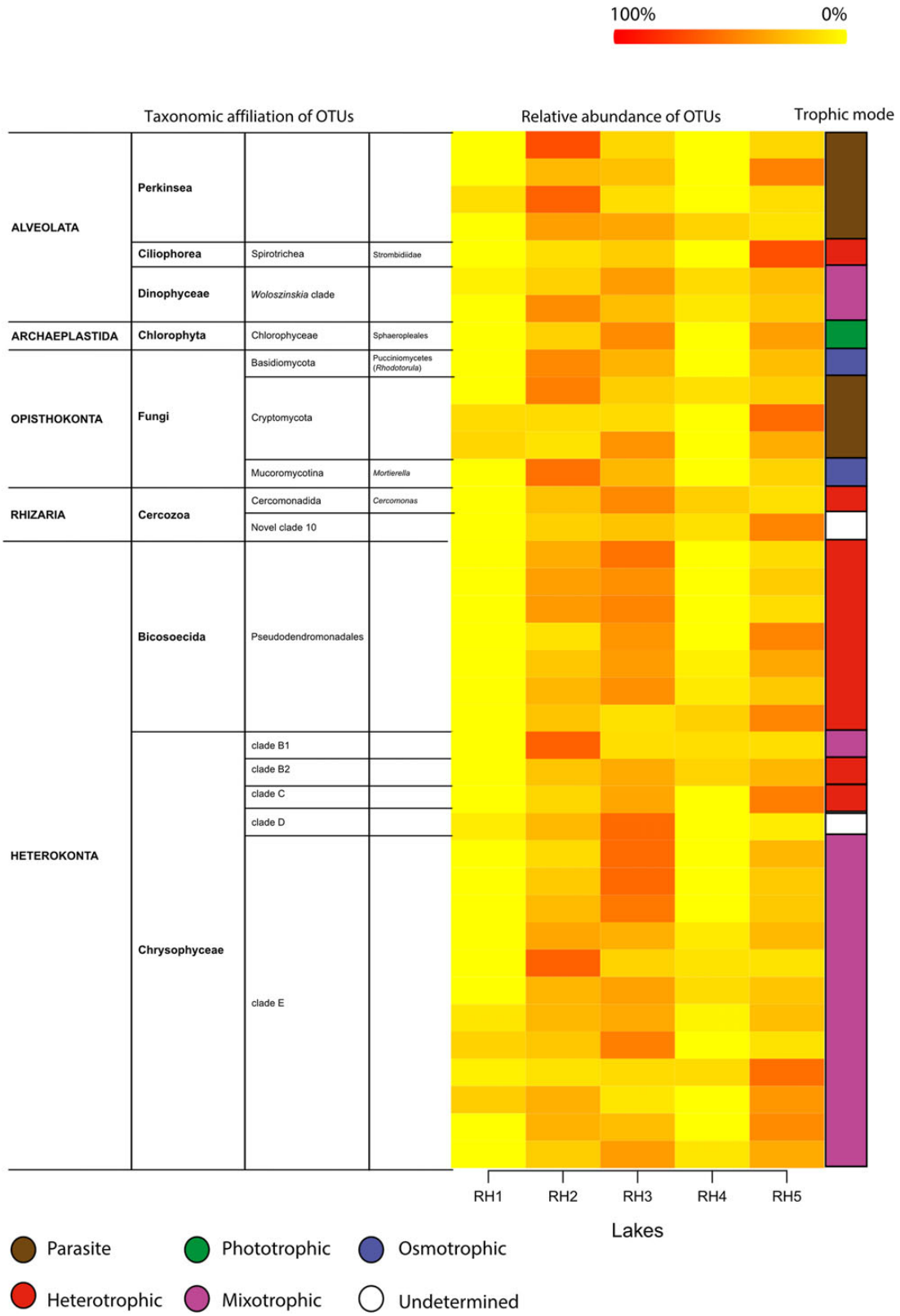

Fig. 5. Taxonomical affiliation of the OTUs which have been found to be the 5% best indicators for ombrotrophy. The heat map indicates relative proportions of each read, from rare to frequent (see reference bar above). Inferred trophic types are shown on the right, next to the corresponding OTU.

ombrotrophic habitats), but their composition clearly differed. In the case of bacterivorous nanoflagellates, Bicosoecids (i.e. Dendromonadales), which here characterize ombrotrophic environments, have been found also in another diversity study of a peat bog based on environmental DNA (Lara *et al.*, 2011). In contrast, minerotrophic water bodies were characterized by the chrysophyte genus *Paraphysomonas* (Scoble and Cavalier-Smith, 2014), a group of nanoflagellates with characteristic self-secreted silica scales which has been found abundantly in the highly mineralized Lake Alchichica in Mexico (Couradeau *et al.*, 2011). They are also abundant in marine systems (Mazei and Tikhonenkov, 2006). As *Paraphysomonas* leave remains (silica scales of characteristic shape) that are preserved in the long run, their presence in core sediments can be used to infer ancient status of water bodies and past hydrographic conditions.

Only a few osmotrophs were selected as most characteristic organisms. Only one OTU corresponding to a yeast (*Rhodotorula* sp.) and one filamentous fungus (related to *Mortierella* sp.) appeared, in spite of the fact that total reads related to Ascomycetes and Basidiomycetes are very abundant. These two fungal genera are very widespread and are common in freshwater systems, and can therefore be considered as forming part of the indigenous communities (Cray *et al.*, 2013). A possible explanation for the absence of more filamentous fungi is that organisms develop outside the pools and their spores are carried by the wind and fall randomly within one or another pool.

Characteristic parasitoid organisms were represented by Perkinsea and Cryptomycota; sequences from these two groups have been often recorded in freshwater environmental DNA surveys (Van Hannen *et al.*, 1999; Lefevre *et al.*, 2008; Lara *et al.*, 2010). Their presence as best indicator organisms probably reflects that of a host which is, in turn, specific to a certain environment. Their abundance may reveal an important role in regulating host populations, as well as a re-mobilization of nutrients (Lefevre *et al.*, 2008). Understanding their exact role in planktonic community dynamics requires a thorough study of their life cycles and host range, a task that requires isolation and cultivation of potential hosts as well as detection of the parasite. Environmental DNA surveys provide an unprecedentedly deep insight into the taxonomic diversity in planktonic microeukaryotic diversity. In that respect, they are indispensable. However, their interpretation still requires "traditional protistology skills," which have perhaps never been as useful as nowadays.

ACKNOWLEDGEMENTS

Thanks are due to the Dirección General de Recursos Hídricos de la Provincia de Tierra del Fuego, the Centro Austral de Investigaciones Científicas (CADIC) and Dr Daniel Fernández for most valuable logistic support. The help of Engineer Sergio Camargo in the fieldwork was much appreciated.

FUNDING

This work has been funded by the Swiss National Science Foundation (project FN 31003A-143960/1) given to E.L. Field work was financed by the CONICET project PIP 11220090100050.

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