Soil Biology and Biochemistry 147 (2020) 107837

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: http://www.elsevier.com/locate/soilbio

Short Communication

High-throughput sequencing of litter and moss eDNA reveals a positive correlation between the diversity of Apicomplexa and their invertebrate hosts across alpine habitats

David Singer [a,b,c,1], Clément Duckert [a,*,1], Petr Heděnec [a,d], Enrique Lara [a,e], Erika Hiltbrunner [f], Edward A.D. Mitchell [a,g]

[a] Laboratory of Soil Biodiversity, University of Neuchâtel, Rue Emile-Argand 11, CH, Neuchâtel, 2000, Switzerland
[b] Department of Zoology, Institute of Biosciences, University of São Paulo, 05508-090, São Paulo, Brazil
[c] Ottawa-Carleton Geoscience Centre and Department of Earth Sciences and Carleton University, Ottawa, Canada
[d] Institute for Environmental Studies, Faculty of Sciences, Charles University, Benátská 2, CZ12800, Prague, Czech Republic
[e] Real Jardín Botánico, CSIC, Plaza de Murillo 2, 28014, Madrid, Spain
[f] Institute of Botany, Department of Environmental Sciences, University of Basel, Schönbeinstrasse 6, CH, Basel, 4056, Switzerland
[g] Jardin Botanique de Neuchâtel, Chemin du Perthuis-du-Sault 58, CH, Neuchâtel, 2000, Switzerland

ARTICLE INFO

Keywords:
Soil biodiversity
Soil microbes
Invertebrates
Swiss Alps
Protists
Parasites

ABSTRACT

A high diversity of Apicomplexa was recently found in tropical soils presumably reflecting the diversity of their invertebrate hosts, but such patterns have not been explored in colder regions. We analysed the diversity of Apicomplexa and their potential metazoan hosts in litter and mosses collected in 11 different alpine habitats using an eDNA metabarcoding approach. The abundance and diversity of Apicomplexa phylotypes and of their potential invertebrate hosts were positively correlated. This confirms that eDNA metabarcoding is a useful tool to explore the unknown biodiversity of free-living eukaryotes, as well as potential host-parasite interactions. Future studies should aim at describing this diversity using a combination of morphological and molecular approaches.

Metabarcoding of environmental DNA (eDNA) is a powerful tool to explore the diversity of soil organisms, as shown by recent studies revealing that soils host an immense diversity of protists (Bates et al., 2013; de Araujo et al. 2018; Oliverio et al., 2020) and Metazoa (Fierer, 2017; Müller et al. 2019). This approach is especially useful to evaluate the diversity of poorly known groups such as Apicomplexa (Mahé et al., 2017; Geisen, 2015). Apicomplexa are obligate host-specific parasites of invertebrates, as well as vertebrates, including livestock and humans (del Campo et al., 2019; Simdyanov et al., 2017). It has been suggested that the richness of Apicomplexa-related sequences should be proportional to the diversity of their hosts, as illustrated by the immense diversity found in Neotropical forest soils (Mahé et al., 2017).

If this hypothesis were correct, such correlations should also be observed in other biomes. To test this hypothesis, we conducted a study at ca. 2500 m a.s.l. In the Furka pass region of the Swiss Alps. This region is characterised by a contrasted topography, bedrock and soil types resulting in high diversity of alpine habitats, plants and invertebrate communities across short distances (Hiltbrunner and Körner, 2018). We collected three to four samples of litter or mosses from 11 different habitats surrounding the Alpine Research Station Furka (ALPFOR) (Table 1, Supp. Table 1) in July 2012. We extracted eDNA using a MoBio PowerSoil extraction kit (Carlsbad, CA, USA) according to the manufacturer instructions. We assessed the phylotype richness of eukaryotes using a metabarcoding approach targeting the V9 region of the 18S rRNA gene using the eukaryotic primers 1380F/1510R (Amaral-Zettler et al., 2009). The PCR amplicons were sequenced with Illumina HiSeq 2000 (Fasteris, Geneva, Switzerland).

The eDNA reads were filtered, quality-checked, clustered into phylotypes using SWARM (Mahé et al., 2015) and taxonomically assigned with the PR^2 database ((Guillou et al., 2012) using VSEARCH (Rognes et al., 2016). We extracted all phylotypes assigned to Metazoa and Apicomplexa. As 18S rDNA sequences of parasites such as Apicomplexa are highly divergent, classical assignation based on pairwise similarity can often be unreliable. Therefore, we constructed a reference tree that comprises all complete Apicomplexa 18S rRNA gene sequences available

* Corresponding author.
E-mail address: clement.duckert@unine.ch (C. Duckert).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.soilbio.2020.107837
Received 14 February 2020; Received in revised form 15 April 2020; Accepted 23 April 2020
Available online 29 April 2020

Table 1
Habitat type and phylotype richness of Apicomplexa parasites and their hosts in the central Swiss Alps close to the Furka pass.

Habitat No.	Habitat description/Dominant plant species	Elevation [m.a.s.l]	Latitude N#	Longitude E#	Metazoa±SD	Apicomplexa± SD
1	Acidic grassland (*Nardus stricta*)	2455	46°34′41″	8°25′14″	154 ± 35	48 ± 13
2	Acidic species-rich grassland *(Nardus stricta/Calluna vulgaris)*	2493	46°34′43″	8°25′13″	209 ± 49	74 ± 21
3	Acidic ridge (*Loiseleuria procumbens*)	2468	46°34′42″	8°25′11″	220 ± 42	63 ± 18
4	Species-rich grassland on calcareous soil (*Festuca violacea*)	2392	46°34′33″	8°25′19″	191 ± 71	60 ± 20
5	Acidic grassland steep N-facing solifluction (*Carex curvula*)	2427	46°34′18″	8°25′12″	173 ± 22	59 ± 13
6	Acidic grassland (*Carex curvula*)	2491	46°34′01″	8°24′48″	177 ± 36	55 ± 11
7	Nutrient-rich grassland (*Agrostis schraderiana*)	2494	46°33′45″	8°24′48″	210 ± 75	57 ± 16
8	Snow-bed on acidic soil (*Salix herbacea*)	2432	46°34′41″	8°25′19″	218 ± 43	63 ± 16
9	Glacier forefield	2508	46°33′27	8°24′49″	173 ± 24	58 ± 13
10	Calcareous ridge (*Elyna myosuroides*)	2468	46°34′22″	8°24′49″	204 ± 59	70 ± 20
11	Fen	2433	46°34′32″	8°25′07″	258 ± 35	65 ± 17

#Coordinates are given for the centre of the selected habitat areas.

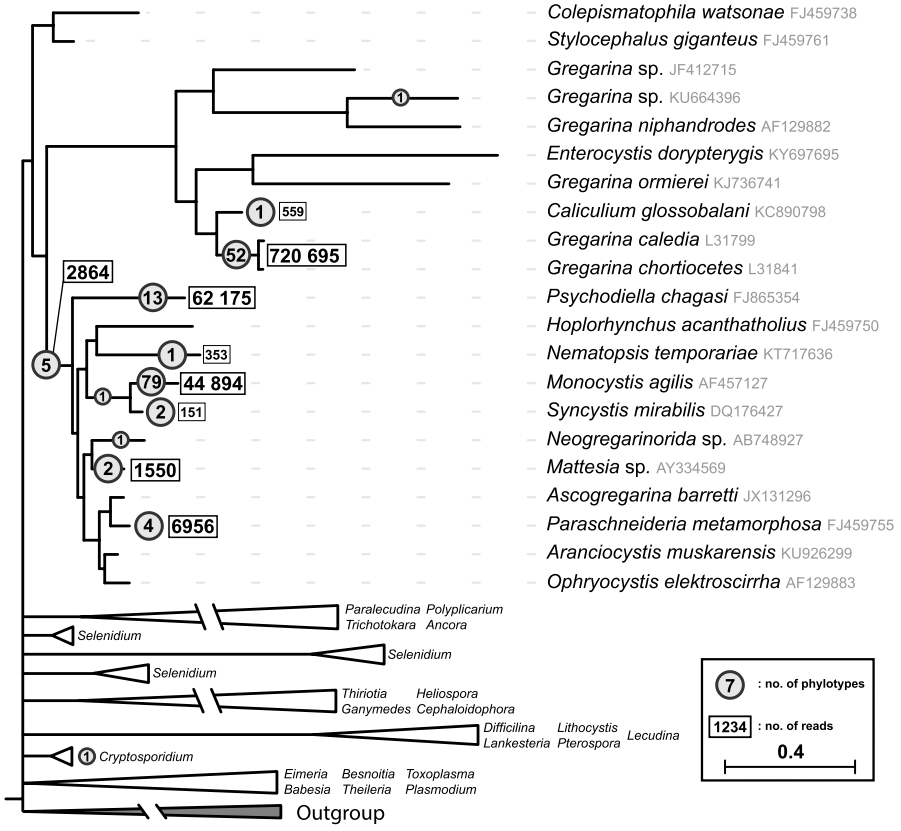

Fig. 1. Phylogenetic tree of DNA reference sequences of Apicomplexa and phylotypes from an eDNA Illumina sequencing of the V9 region of the 18S rRNA gene from 41 soil litter and moss samples collected in 11 different alpine habitats in the Furka Pass region (Switzerland). Only phylotypes with over 100 reads are shown. Numbers in circles represent numbers of phylotypes affiliated to an identified organism and numbers in squares represent the total number of reads.

on GenBank as well as some *bona fide* Alveolata and other phyla as outgroups. Then, we used the Evolutionary Placement Algorithm as implemented in RAxML v.8.2.10 to determine the phylogenetic position of the potential apicomplexan phylotypes (Stamatakis, 2014). The data are available in the NCBI Sequence Read Archive under the BioProject number PRJNA623507 and Supplementary Table 1.

We used linear regression models to test whether the abundance of phylotypes of Apicomplexa and of their putative metazoan hosts were correlated. In order to circumvent potential methodological biases due to the variation of read numbers per sample, we calculated a corrected value of the phylotype richness as the normalized residuals of the number of phylotypes minus the predicted number of phylotypes based on the total number of reads in that same sample, following a similar logic as Tedersoo and co-authors (2014) (see Supplementary methods 1). All statistical analyses were performed in R version 3.5.1.

We obtained a total of 181 phylotypes of Apicomplexa (879,886 reads) and 856 phylotypes of Metazoa (1,180,871 reads). Phylogenetic reconstruction clustered Apicomplexa phylotypes into two main lineages (Fig. 1), the Actinocephaloidea and the Gregarinoidea (superfamilies proposed by Simdyanov et al., 2017), which include species that are mainly reported as parasites of terrestrial invertebrates, especially insects. While most phylotypes were associated to insect (e.g. *Gregarina*) or Annelida (*Monocystis*) parasites, two were associated to vertebrate parasites (*Nematopsis* and *Cryptosporidium*) and one phylotype was associated to a clade that recently transitioned from terrestrial to marine invertebrate hosts (*Caliculium*) (Wakeman et al., 2014). The majority of the apicomplexan reads (78.8%) were clustered into a single phylotype associated to genus *Gregarina* (GenBank L31799 and L31841), while 4.5% of the reads (39,407 sequences), could not be assigned with confidence to any known Apicomplexa lineage.

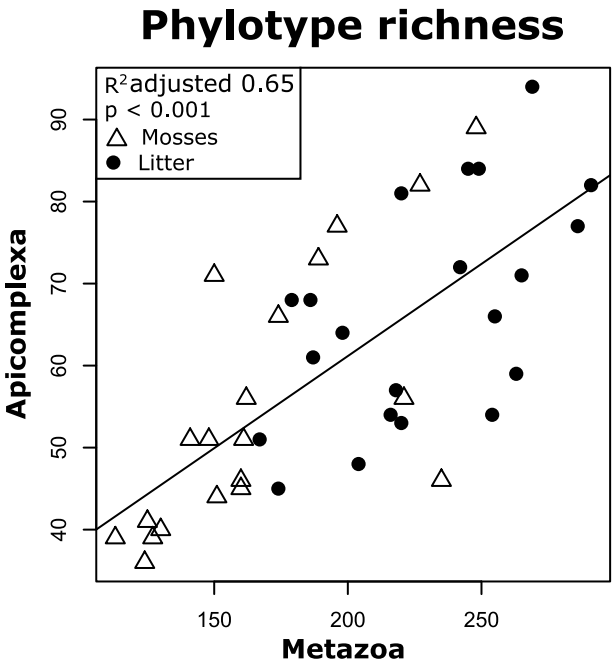

Fig. 2. Relationship between the phylotype (V9 region of the 18S rRNA) richness of Apicomplexa and Metazoa from 41 soil litter and mosses samples collected in 11 alpine habitats in the Furka Pass region (Switzerland). The line shows the slope of the linear regression.

As a whole, the number of Apicomplexa reads was significantly correlated to the number of Metazoa reads, as shown in a simple linear regression model (adjusted $R^2 = 0.64$, $p < 0.001$; Fig. 2). The correlation between the corrected diversity of Apicomplexa and Metazoa remained significant, which strengthens the validity of the analysis by removing potential methodological biases (Supp. Fig. 1). The correlations were also significant for litter samples and for both data sets combined but not for moss samples ($p = 0.11$; Supp. Fig. 1). Although we observed a positive correlation between the number of, respectively, Apicomplexa and Metazoa phylotypes *versus* all eukaryotes phylotypes, which can be interpreted as sequencing biases (Supp. Methods 1), this correlation disappeared when the correction factor was applied to the alpha diversity (Supp. fig 2; Supp. Methods 1), demonstrating the robustness of our results.

The highest number of Apicomplexa phylotypes was found in the acidic grassland with *Calluna* spots (habitat no.2) and in the calcareous ridge (habitat no. 10): two contrasting habitats that do not share similar vascular plant or moss species (Table 1). The highest number of Metazoa phylotypes was detected in the fen (habitat no. 11) which had the highest number of individual flies and midges, and the highest number of families of Diptera (Hiltbrunner and Körner, 2018).

Our results support the hypothesis that the diversity of soil Apicomplexa may reflect the diversity of Metazoa (and especially invertebrates) in an ecosystem (Mahé et al., 2017). Although Apicomplexa diversity cannot be directly compared between the two studies, notably because of the genetic markers used (V9 in our study *versus* V4 in the study of Mahé et al. (2017), see (Dunthorn et al., 2012)) the genetic diversity obtained in our samples (Fig. 1) was also high and included many different clades of Apicomplexa. Even though the number of samples and sequencing depth in the study of Mahé et al. (2017) are larger than in this study, we sampled 11 contrasted alpine habitats differing in their characteristics and two contrasted types of samples litter versus mosses. Still, while most Neotropical Apicomplexa reads and phylotypes were branching at internal nodes in the reference tree, the majority of alpine Apicomplexa were placed on or very close to the tips (Fig. 1). This suggests the existence of a higher genetic novelty in Neotropical Apicomplexa, probably reflecting also a lack of knowledge of their hosts.

This study shows that inferred host-parasite relationships based on eDNA metabarcoding is a powerful approach to explore the diversity of poorly known taxa and for inferring potential host-parasite or other biotic interactions. These findings call for further research on host-parasite interactions by combining traditional methods (species morphology and barcoding) of soil fauna analysis with meta-barcoding of eDNA to characterise the level of interactions between the hosts and their parasites.

Declaration of competing interest

The material in this manuscript is original research, has not been previously published and has not been submitted for publication elsewhere while under consideration for Soil Biology & Biochemistry. The authors declare no competing financial interests.

Acknowledgement

This work was funded by a Swiss Government Excellence Scholarship 2017–2018 (AW:2017.0308:FCS/CFBE/ESKAS:FCS scholarship) to PH, the Swiss National Science Foundation project P2NEP3_178543 to DS and by a grant by the Consejería de Educación, Juventud y Deporte, Comunidad de Madrid "Atracción de Talento Investigador" (2017-T1/AMB-5210) to EL.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2020.107837.

References

Amaral-Zettler, L.A., McCliment, E.A., Ducklow, H.W., Huse, S.M., 2009. A method for studying protistan diversity using massively parallel sequencing of V9 hypervariable regions of small-subunit ribosomal RNA genes. PloS One 4, e6372. https://doi.org/10.1371/journal.pone.0006372.

Bates, et al., 2013. Global biogeography of highly diverse protistan communities in soil. The ISME Journal.

de Araujo, et al., 2018. Protist species richness and soil microbiome complexity increase towards climax vegetation in the Brazilian Cerrado. Nature.

del Campo, J., Heger, T.J., Rodríguez-Martínez, R., Worden, A.Z., Richards, T.A., Massana, R., Keeling, P.J., 2019. Assessing the diversity and distribution of apicomplexans in host and free-living environments using high-throughput amplicon data and a phylogenetically informed reference framework. Frontiers in Microbiology 10, 2373. https://doi.org/10.3389/fmicb.2019.02373.

Dunthorn, M., Klier, J., Bunge, J., Stoeck, T., 2012. Comparing the hyper-variable V4 and V9 regions of the small subunit rDNA for assessment of ciliate environmental diversity. The Journal of Eukaryotic Microbiology 59, 185–187. https://doi.org/10.1111/j.1550-7408.2011.00602.x.

Fierer, N., 2017. Embracing the unknown: disentangling the complexities of the soil microbiome. Nature Reviews Microbiology 15, 579. https://doi.org/10.1038/nrmicro.2017.87.

Geisen, et al., 2015. Not all are free-living: high-throughput DNA metabarcoding reveals a diverse community of protists parasitizing soil metazoa. Molecular Ecology.

Guillou, et al., 2012. The Protist Ribosomal Reference database (PR^2): a catalog of unicellular eukaryote Small Sub-Unit rRNA sequences with curated taxonomy. Nucleic acids research.

Hiltbrunner, E., Körner, C., 2018. HOTSPOT FURKA - Biologische Vielfalt im Gebirge, Alpine Forschungs-und Ausbildungsstation Furka und Universität Basel, ISBN 978-3-033-06701-1. CH-4056 Basel.

Mahé, F., de Vargas, C., Bass, D., Czech, L., Stamatakis, A., Lara, E., Singer, D., Mayor, J., Bunge, J., Sernaker, S., Siemensmeyer, T., Trautmann, I., Romac, S., Berney, C., Kozlov, A., Mitchell, E.A.D., Seppey, C.V.W., Egge, E., Lentendu, G., Wirth, R., Trueba, G., Dunthorn, M., 2017. Parasites dominate hyperdiverse soil protist communities in Neotropical rainforests. Nat. Ecol. Evol. 1 https://doi.org/10.1038/s41559-017-0091, 0091.

Mahé, F., Rognes, T., Quince, C., de Vargas, C., Dunthorn, M., 2015. Swarm v2: highly-scalable and high-resolution amplicon clustering. PeerJ 3, e1420. https://doi.org/10.7717/peerj.1420.

Müller, et al., 2019. Meiofaunal diversity in the Atlantic Forest soil: A quest for nematodes in a native reserve using eukaryotic metabarcoding analysis. Forest Ecology and Management.

Oliverio, et al., 2020. The global-scale distributions of soil protists and their contributions to belowground systems. Science Advances.

Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahé, F., 2016. VSEARCH: a versatile open source tool for metagenomics. PeerJ 4, e2584. https://doi.org/10.7717/peerj.2584.

Simdyanov, T.G., Guillou, L., Diakin, A.Y., Mikhailov, K.V., Schrével, J., Aleoshin, V.V., 2017. A new view on the morphology and phylogeny of eugregarines suggested by the evidence from the gregarine *Ancora sagittata* (Leuckart, 1860) Labbé, 1899 (Apicomplexa: eugregarinida). PeerJ 5, e3354. https://doi.org/10.7717/peerj.3354.

Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30, 1312–1313. https://doi.org/10.1093/bioinformatics/btu033.

Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yorou, N.S., Wijesundera, R., Ruiz, L.V., Vasco-Palacios, A.M., Thu, P.Q., Suija, A., Smith, M.E., Sharp, C., Saluveer, E., Saitta, A., Rosas, M., Riit, T., Ratkowsky, D., Pritsch, K., Põldmaa, K., Piepenbring, M., Phosri, C., Peterson, M., Parts, K., Pärtel, K., Otsing, E., Nouhra, E., Njouonkou, A.L., Nilsson, R.H., Morgado, L.N., Mayor, J., May, T.W., Majuakim, L., Lodge, D.J., Lee, S.S., Larsson, K.-H., Kohout, P., Hosaka, K., Hiiesalu, I., Henkel, T. W., Harend, H., Guo, L., Greslebin, A., Grelet, G., Geml, J., Gates, G., Dunstan, W., Dunk, C., Drenkhan, R., Dearnaley, J., De Kesel, A., Dang, T., Chen, X., Buegger, F., Brearley, F.Q., Bonito, G., Anslan, S., Abell, S., Abarenkov, K., 2014. Global diversity and geography of soil fungi. Science 346, 1256688. https://doi.org/10.1126/science.1256688.

Wakeman, K.C., Reimer, J.D., Jenke-Kodama, H., Leander, B.S., 2014. Molecular Phylogeny and Ultrastructure of Caliculium glossobalani n. gen. et sp. (Apicomplexa) from a Pacific Glossobalanus minutus (Hemichordata) Confounds the Relationships Between Marine and Terrestrial Gregarines. The Journal of Eukaryotic Microbiology 61, 343–353. https://doi.org/10.1111/jeu.12114.