Published in final edited form as:

Environ Microbiol Rep. 2017 April ; 9(2): 113–119. doi:10.1111/1758-2229.12507.

Phylogenetic and ecological diversity of apusomonads, a lineage of deep-branching eukaryotes

Guifré Torruella, David Moreira, and Purificación López-García

Ecologie Systématique Evolution, CNRS, Université Paris-Sud, AgroParisTech, Université Paris-Saclay, 91400 Orsay, France

Summary

Apusomonads are a mysterious group of heterotrophic gliding biflagellates branching deeply in the eukaryotic tree of life as sister group to opisthokonts (including animals, fungi and a variety of unicellular protists). Despite their evolutionary interest, their diversity and ecology remain largely unknown, with very few described species and environmental sequences in databases. Most environmental 18S rRNA gene-based studies generally fail to identify apusomonad sequences, which might be due to primer bias, low abundance and/or to the fact that their biotopes remain poorly explored. We have carried out an extensive search of 18S rRNA genes using an apusomonad-specific primer in a wide variety of ecosystems. Our study significantly broadens the diversity of apusomonads showing that, despite being mostly rare protists, they often dwell in freshwater and marine benthic environments, generally associated with low-oxygen concentrations. Apusomonads have been identified in environments across a wide salinity range. Some operational taxonomic units (OTUs), occurring in both marine and freshwater ecosystems, seem truly euryhaline, indicating that members of this deep-branching lineage easily cross such ecological barriers.

Keywords

protist; Apusozoa; deep-sea; sediment; freshwater; cold seep; early evolution

Introduction

Apusomonads are a group of heterotrophic protists that branch deeply in the eukaryotic tree. Less than a dozen cultured species are available; they encompass flagellates that glide on surfaces likely powered by their posterior flagellum and graze on bacteria using protruding ventral pseudopodia (Heiss et al 2015). Phylogenetic analyses using multiple markers retrieved from the few apusomonad genomic and transcriptomic data available place them as sister to the opisthokonts (Brown et al 2013, Cavalier-Smith et al 2014, Paps et al 2013, Torruella et al 2012), one of the few recognized eukaryotic supergroups (together with Amoebozoa, Excavata, Archaeplastida and SAR – Stramenopiles-Alveolata-Rhizaria)

For correspondence: Purificación López-García, puri.lopez@u-psud.fr; Guifré Torruella, guifre.torruella@u-psud.fr; Tel. +33(0)169157608, Fax: +33(0)169154697.

Conflict of Interest

The authors declare no conflict of interest.

including, among others, animals and fungi. Apusomonads also possess genes known to be involved in animal multicellularity (e.g. (Sebe-Pedros et al 2010). Their study might therefore reveal important clues to understand early evolutionary transitions in eukaryotes.

Despite their evolutionary interest, their diversity, biology and ecology remain very poorly known and their classification obscured by certain confusion. First grouped as Apusomonadida based on morphology and ultrastructure (Karpov and Mylnikov 1989), early 18S rRNA phylogenetic analyses already suggested an evolutionary relationship with opisthokonts (Cavalier-Smith and Chao 1995). Subsequent, though weakly supported, phylogenetic analyses pointed to a sisterhood of apusomonads with another group of biflagellates, the Ancyromonadida (Atkins et al 2000). Hence, they were all included in a newborn taxon Apusozoa (Cavalier-Smith and Chao 2003) together with other *incertae sedis* flagellates (Mantamonadida or Rigifilida) (Yabuki et al 2013). However, while some later studies still supported the Apusomonadida-Ancyromonadida sisterhood (Paps et al 2013), more recent phylogenomic analyses suggest that apusomonads and ancyromonads do not branch together and question the validity of Apusozoa as a monophyletic clade (Cavalier-Smith et al 2014, Torruella et al 2015). Unfortunately, public databases such as GenBank (<http://www.ncbi.nlm.nih.gov/>), SILVA (Yilmaz et al 2014) or PR2 (Guillou et al 2013) still include Apusozoa as a taxon. This can mislead ecological inferences derived from large-scale metabarcoding analyses, since seemingly distinct lineages are grouped under the same umbrella. Solving this problem requires extending the known diversity for former Apusozoa clades (better taxonomic sampling correlates with more resolved phylogenies) and acquiring specific information about the ecological preferences of each of these clades.

Apusomonad 18S rRNA gene sequences have been only sporadically detected in classical environmental studies, mostly from marine settings, especially deep-sea sediments (López-García et al 2003, Not et al 2008, Takishita et al 2007) or oxic/anoxic interfaces in the water column (Orsi et al 2012), but also soil (Lesaulnier et al 2008). Deep-sea sediment sequences were particularly distantly related to sequences from cultured species, suggesting that the diversity of apusomonads might be undersampled. In recent comprehensive analyses including sequences from cultured species and environmental samples, a total of 16 OTUs (defined at 98% nucleotide identity) were unambiguously identified as apusomonads (Cavalier-Smith and Chao 2010, Heiss et al 2015). Nonetheless, some recent metabarcoding analyses report marginal abundances for 'Apusozoa', some of which might correspond to apusomonads (de Vargas et al 2015, Simon et al 2015b, Steichen et al 2014), while recent analyses of coastal sediments reveal higher relative abundances of apusomonads (Massana et al 2015). This, again, might indicate that this group of heterotrophic flagellates suffers from a biased representation in databases. This bias could result from different, non-mutually exclusive, causes, including primer bias (universal eukaryotic primers might amplify apusomonad sequences less efficiently), low abundance (not necessarily correlating with low diversity) or biased exploration of natural environments (protists from marine sediments or freshwater systems have been far less studied by molecular surveys than oceanic plankton).

Results and Discussion

To contribute clarifying the phylogenetic diversity and ecological distribution of apusomonads, we carried out an extensive exploration of this group in a wide collection of environmental samples. To get a comprehensive reference sequence set for apusomonads, we first retrieved previously identified nearly complete 18S rRNA gene sequences (Cavalier-Smith and Chao 2010, Heiss et al 2015) and searched for additional apusomonad sequences in the GenBank's non-redundant database, including missannotated sequences. We identified several new environmental sequences in this way, representing 29 OTUs at a 98% nucleotide identity threshold (Figure 1). Although some sequences were obtained from soil or groundwater-derived biofilms, most of them were retrieved from oxic/anoxic transition zones in the water column, deep-sea sediments, cold seeps or associated mats (Orsi et al 2012, Sauvadet et al 2010, Takishita et al 2010). We additionally identified 7 new OTUs in high-throughput environmental data (pyrosequences, PRJNA259710) from shallow, small freshwater ecosystems (Simon et al 2015b).

Taking into account this environmental information, we selected a collection of samples that was enriched in benthic and suboxic settings, and represented different ecosystems across wide-ranging salinity gradients, from freshwater to brackish, marine and hypersaline systems (Table 1 and Supplementary Table S1). Based on available sequences, we designed a specific primer for apusomonad 18S rRNA genes (APU-1R: 5'-CTTCCTTTGGTTAAACAC-3') that we combined with one general eukaryotic primer (EK-42F: 5'-CTCAARGAYTAAGCCATGCA) to amplify a large portion of this marker gene from environmental samples. Amplicons were then cloned and fully sequenced (yielding ~1350 bp, and up to ~2200 bp for some 18S rRNA genes containing large insertions) in order to make more accurate phylogenetic analyses (Supplementary Methods; GenBank accession numbers KY077689-KY077739).

Apusomonads were only detected in 18 out of more than 70 samples tested. As suspected, apusomonad sequences were mainly amplified from marine or freshwater sediments or associated biofilms, indicating a preference for low-oxygen environments or redox clines (Table 1). Nonetheless, we were unable to amplify apusomonad sequences from several other, similar marine sediment samples (Supplementary Table S1). This suggests that apusomonads tend to occur in very low abundance, which would explain the stochastic amplification success in sediment samples. This is consistent with low reported abundances in several environmental studies using general eukaryotic primers (Orsi et al 2012, Sauvadet et al 2010, Takishita et al 2010), although moderate abundances have been observed in some coastal sediments (Massana et al 2015). In addition, we failed to amplify apusomonad sequences from the Black Sea anoxic water column as well as from lithifying and non-lithifying microbial mats from hot, acidic, alkaline or hypersaline settings (Supplementary Table S1), suggesting that these kind of environments are excluded from their ecological preferences.

Despite their seemingly low prevalence, we detected a rather wide apusomonad diversity. We identified a total of 61 OTUs grouped in 30 clades, of different phylogenetic depth, that were well supported in different phylogenetic analyses (Figure 1; Supplementary Figures

S1-S3 and Table S2). From the 61 apusomonad OTUs identified, 36 were defined by environmental sequences deposited in GenBank and/or our new sequences (59% of apusomonad OTUs), and 27 contained sequences produced in this work (44%). This considerably extends the known diversity for the group. This diversity will be likely increased with as sequences from 18S rRNA (V4/V9)-based metabarcoding datasets from various environments become increasingly available, as is already observed with the inclusion of short apusomonad metabarcoding reads from coastal sediments (Massana et al 2015) in our 18S rDNA reference tree (Figure S4). In this study, we decided to consider the clade APU-01 as the earliest divergent apusomonad lineage because, in our 18S rDNA phylogenetic analyses, it systematically branched as sister to the rest of apusomonad clades, albeit with moderate support, and far from other deep-branching protist lineages included in the outgroup (Figures 1 and S1). However, APU-01 might well correspond to a new, deep-branching protist lineage deserving exploration on its own. Among the rest of apusomonad clades, some are moderately fast-evolving, such as APU-02 and APU-17, from hydrothermal sediments, APU-10 (marine water), APU-20 (intertidal pond) or APU-21 (freshwater samples). However, APU-29 and, especially, APU-30 (*Thecamonas-Chelonemonas* clade, likely corresponding to the Thecamonadinae; Heiss et al 2015) are very fast-evolving compared to the rest of apusomonads. The reasons for this acceleration of evolutionary rate are unclear, since the cultured species of this clade correspond to free-living protists that thrive in similar environments to the rest of apusomonads.

Regarding the ecological distribution of apusomonads, while some OTUs and clades appear consistently marine (e.g. APU-11, APU-24, APU-28, APU-30), and others typical of freshwater or freshwater/soil habitats (APU-04, APU-08, APU-21, APU-27), several clades and even OTUs encompass sequences of both, marine and freshwater, origin (e.g. APU-03, APU-13-15). The most extreme case corresponds to APU-09, which we tentatively and informally name 'Ubiquimonas', a single OTU that is present in soil, freshwater and marine sediments, including cold seep and hydrothermal sediments, from different oceanic regions (Figure 1).

Our study shows that the use of group-specific primers is extremely valuable to unravel the diversity and ecological distribution of low-abundant protist lineages, as has been shown in the past for other clades such as cercozoans (Bass and Cavalier-Smith 2004), marine diplomonads (Lara et al 2009) or freshwater haptophytes (Simon et al 2013). Apusomonads are indeed a relatively diverse group of low-abundant dwellers in marine and freshwater sediments. They thus belong to the so-called 'rare biosphere', a reservoir of underexplored phylogenetic diversity and poorly evaluated ecological importance (Logares et al 2015).

Apusomonads are more frequently detected in freshwater, cold seep and hydrothermal sediments (Table 1), suggesting that they are particularly well-adapted to microaerophilic conditions in areas of increased primary productivity and/or rich in organic matter. In general, apusomonads are detected in biotopes representing a wide range of salinities, from freshwater to hypersaline conditions. Moreover, some apusomonads seem to be truly euryhaline, occurring in marine and freshwater environments indistinctly. They thus add to the increasing number of protist groups that can easily cross the marine-freshwater barrier (Simon et al 2015a), questioning previous ideas that such transitions are rare (Logares et al

2009). Retrieving in culture representatives of euryhaline OTUs, such as ‘Ubiquimonas’, would allow exploring the molecular adaptations underlying that ecological flexibility.

Cultured species of apusomonads are able to grow under atmospheric oxygen concentrations (Heiss et al 2015), some species (e.g. *Thecamonas trahens*) harbor standard mitochondria based on genome data (Derelle and Lang 2012) and a marginal fraction of "apusozoan" sequences have been identified in the oxygenated seawater column (López-García et al 2003, Not et al 2008, Takishita et al 2007); de Vargas et al 2015). However, the preferred environmental distribution of apusomonads also suggests an adaptation to oxic/anoxic transition zones and oxygen-depleted environments (Table 1) (López-García et al 2003, Massana et al, 2015, Not et al 2008, Orsi et al 2012, Sauvadet et al 2010, Simon et al 2015b, Takishita et al 2007, Takishita et al 2010). Many apusomonads might thus be aerobic protists adapted to low oxygen partial pressures. Nonetheless, the identification of some apusomonads in anoxic samples, such as cold seeps and associated *Beggiatoa*-like mats (Table 1) might also suggest that some of them are (facultative) anaerobes. It would be thus interesting to elucidate whether some apusomonads have modified mitochondria, similar to other deep-branching eukaryotes, e.g. breviate (Stairs et al 2014), sharing the same kind of biotopes. Likewise, obtaining genomic/transcriptomic data from the newly identified lineages (from cultures or single cells) will be important for phylogenomic analyses tending to resolve the deepest areas of the eukaryotic tree (Burki 2014) and to elucidate whether the last common eukaryotic ancestor had already some potential for multicellularity. Interestingly, early-branching eukaryotic lineages (e.g. apusomonads, breviate, ancyromonads) seem to be prevalent in oxygen-depleted environments. Therefore, extending studies on the phylogenetic diversity, biology and ecology of these rare and poorly known ancient groups may not only help reconstructing the early history of eukaryotes but also better constraining the environmental conditions where the first eukaryotes evolved.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We are grateful to L. Jardillier, P. Bertolino and the Parc Naturel Régional de la Haute Vallée de Chevreuse for sampling help in several freshwater environments, to A. Vereschaka for Bismarck, Titanic and Black Sea samples, to J. Garcia-Ruiz and J.M. Lopez-Garcia for a joint field trip to the Atacama desert and to P. Henry and L. Gasperini for inviting PLG to participate in MARNAUT2007 and Marmara2010 research cruises. We also thank Ramon Massana for sharing with us metabarcoding sequences of apusomonads from coastal sediments. This work was funded by the European Research Council (ERC) under the European Commission 7th Framework Program ERC Grant Agreement 322669 ‘ProtistWorld’.

References

- Atkins MS, McArthur AG, Teske AP. Ancyromonadida: A new phylogenetic lineage among the protozoa closely related to the common ancestor of metazoans, fungi, and choanoflagellates (Opisthokonta). *J Mol Evol.* 2000; 51:278–285. [PubMed: 11029072]
- Bass D, Cavalier-Smith T. Phylum-specific environmental DNA analysis reveals remarkably high global biodiversity of Cercozoa (Protozoa). *Int J Syst Evol Microbiol.* 2004; 54:2393–2404. [PubMed: 15545489]

- Brown MW, Sharpe SC, Silberman JD, Heiss AA, Lang BF, Simpson AG, et al. Phylogenomics demonstrates that breviate flagellates are related to opisthokonts and apusomonads. *Proc Biol Sci.* 2013; 280:20131755. [PubMed: 23986111]
- Burki F. The eukaryotic tree of life from a global phylogenomic perspective. *Cold Spring Harb Perspect Biol.* 2014; 6:a016147. [PubMed: 24789819]
- Cavalier-Smith T, Chao EE. The opalozoan *Apusomonas* is related to the common ancestor of animals, fungi and choanoflagellates. *Proc Royal Soc London B.* 1995; 261:1–6.
- Cavalier-Smith T, Chao EE. Phylogeny of choanozoa, apusozoa, and other protozoa and early eukaryote megaevolution. *J Mol Evol.* 2003; 56:540–563. [PubMed: 12698292]
- Cavalier-Smith T, Chao EE. Phylogeny and evolution of apusomonadida (protozoa: apusozoa): new genera and species. *Protist.* 2010; 161:549–576. [PubMed: 20537943]
- Cavalier-Smith T, Chao EE, Snell EA, Berney C, Fiore-Donno AM, Lewis R. Multigene eukaryote phylogeny reveals the likely protozoan ancestors of opisthokonts (animals, fungi, choanozoans) and Amoebozoa. *Mol Phylogenet Evol.* 2014; 81:71–85. [PubMed: 25152275]
- de Vargas C, Audic S, Henry N, Decelle J, Mahe F, Logares R, et al. Ocean plankton. Eukaryotic plankton diversity in the sunlit ocean. *Science.* 2015; 348:1261605. [PubMed: 25999516]
- Derelle R, Lang BF. Rooting the eukaryotic tree with mitochondrial and bacterial proteins. *Mol Biol Evol.* 2012; 29:1277–1289. [PubMed: 22135192]
- Guillou L, Bachar D, Audic S, Bass D, Berney C, Bittner L, et al. The Protist Ribosomal Reference database (PR2): a catalog of unicellular eukaryote Small Sub-Unit rRNA sequences with curated taxonomy. *Nucleic Acids Res.* 2013; 41:D597–D604. [PubMed: 23193267]
- Heiss AA, Lee WJ, Ishida K, Simpson AG. Cultivation and characterisation of new species of apusomonads (the sister group to opisthokonts), including close relatives of *Thecamonas* (*Chelonemonas* n. gen.). *J Eukaryot Microbiol.* 2015; 62:637–649. [PubMed: 25912654]
- Karpov SA, Mylnikov AP. Biology and ultrastructure of colourless flagellates Apusomonadida ord. n. *Zool Zh.* 1989; 68:5–17. (in Russian).
- Lara E, Moreira D, Vereshchaka A, Lopez-Garcia P. Pan-oceanic distribution of new highly diverse clades of deep-sea diplomonads. *Environ Microbiol.* 2009; 11:47–55. [PubMed: 18803646]
- Lesaulnier C, Papamichail D, McCorkle S, Ollivier B, Skiena S, Taghavi S, et al. Elevated atmospheric CO₂ affects soil microbial diversity associated with trembling aspen. *Environ Microbiol.* 2008; 10:926–941. [PubMed: 18218029]
- Logares R, Brate J, Bertilsson S, Clasen JL, Shalchian-Tabrizi K, Rengefors K. Infrequent marine-freshwater transitions in the microbial world. *Trends Microbiol.* 2009; 17:414–422. [PubMed: 19726194]
- Logares R, Mangot JF, Massana R. Rarity in aquatic microbes: placing protists on the map. *Res Microbiol.* 2015; 166:831–841. [PubMed: 26598213]
- López-García P, Philippe H, Gaill F, Moreira D. Autochthonous eukaryotic diversity in hydrothermal sediment and experimental micro-colonizers at the Mid-Atlantic Ridge. *Proc Natl Acad Sci USA.* 2003; 100:697–702. [PubMed: 12522264]
- Massana R, Gobet A, Audic S, Bass D, Bittner L, Boutte C, et al. Marine protist diversity in European coastal waters and sediments as revealed by high-throughput sequencing. *Environ Microbiol.* 2015; 17:4035–4049. [PubMed: 26119494]
- Not F, Latasa M, Scharek R, Viprey M, Karleskind P, Balague V, et al. Protistan assemblages across the Indian Ocean, with a specific emphasis on the picoeukaryotes. *Deep-Sea Research Part I-Oceanographic Research Papers.* 2008; 55:1456–1473.
- Orsi W, Song YC, Hallam S, Edgcomb V. Effect of oxygen minimum zone formation on communities of marine protists. *ISME J.* 2012; 6:1586–1601. [PubMed: 22402396]
- Paps J, Medina-Chacon LA, Marshall W, Suga H, Ruiz-Trillo I. Molecular phylogeny of unikonts: new insights into the position of apusomonads and ancyromonads and the internal relationships of opisthokonts. *Protist.* 2013; 164:2–12. [PubMed: 23083534]
- Sauvadet AL, Gobet A, Guillou L. Comparative analysis between protist communities from the deep-sea pelagic ecosystem and specific deep hydrothermal habitats. *Environ Microbiol.* 2010; 12:2946–2964. [PubMed: 20561018]

- Sebe-Pedros A, Roger AJ, Lang FB, King N, Ruiz-Trillo I. Ancient origin of the integrin-mediated adhesion and signaling machinery. *Proc Natl Acad Sci U S A*. 2010; 107:10142–10147. [PubMed: 20479219]
- Simon M, Lopez-Garcia P, Moreira D, Jardillier L. New haptophyte lineages and multiple independent colonizations of freshwater ecosystems. *Environ Microbiol Rep*. 2013; 5:322–332. [PubMed: 23584973]
- Simon M, Jardillier L, Deschamps P, Moreira D, Restoux G, Bertolino P, et al. Complex communities of small protists and unexpected occurrence of typical marine lineages in shallow freshwater systems. *Environ Microbiol*. 2015a; 17:3610–3627. [PubMed: 25115943]
- Simon M, Lopez-Garcia P, Deschamps P, Moreira D, Restoux G, Bertolino P, et al. Marked seasonality and high spatial variability of protist communities in shallow freshwater systems. *ISME J*. 2015b; 9:1941–1953. [PubMed: 25853803]
- Stairs CW, Eme L, Brown MW, Mutsaers C, Susko E, Dellaire G, et al. A *SUF* Fe-S cluster biogenesis system in the mitochondrion-related organelles of the anaerobic protist *Pygssuia*. *Curr Biol*. 2014; 24:1176–1186. [PubMed: 24856215]
- Steichen JL, Schulze A, Brinkmeyer R, Quigg A. All aboard! A biological survey of ballast water onboard vessels spanning the North Atlantic Ocean. *Mar Pollut Bull*. 2014; 87:201–210. [PubMed: 25176277]
- Takishita K, Yubuki N, Kakizoe N, Inagaki Y, Maruyama T. Diversity of microbial eukaryotes in sediment at a deep-sea methane cold seep: surveys of ribosomal DNA libraries from raw sediment samples and two enrichment cultures. *Extremophiles*. 2007; 11:563–576. [PubMed: 17426921]
- Takishita K, Kakizoe N, Yoshida T, Maruyama T. Molecular evidence that phylogenetically diverged ciliates are active in microbial mats of deep-sea cold-seep sediment. *J Eukaryot Microbiol*. 2010; 57:76–86. [PubMed: 20002870]
- Torruella G, Derelle R, Paps J, Lang BF, Roger AJ, Shalchian-Tabrizi K, et al. Phylogenetic relationships within the Opisthokonta based on phylogenomic analyses of conserved single-copy protein domains. *Mol Biol Evol*. 2012; 29:531–544. [PubMed: 21771718]
- Torruella G, de Mendoza A, Grau-Bove X, Anto M, Chaplin MA, del Campo J, et al. Phylogenomics Reveals Convergent Evolution of Lifestyles in Close Relatives of Animals and Fungi. *Curr Biol*. 2015; 25:2404–2410. [PubMed: 26365255]
- Yabuki A, Ishida K, Cavalier-Smith T. *Rigifila ramosa* n. gen., n. sp., a filose apusozoan with a distinctive pellicle, is related to Micronuclearia. *Protist*. 2013; 164:75–88. [PubMed: 22682062]
- Yilmaz P, Parfrey LW, Yarza P, Gerken J, Pruesse E, Quast C, et al. The SILVA and “All-species Living Tree Project (LTP)” taxonomic frameworks. *Nucleic Acids Res*. 2014; 42:D643–648. [PubMed: 24293649]

Originality – significance statement

Extending studies on the phylogenetic diversity, biology and ecology of rare and poorly known ancient eukaryotic groups may help reconstructing their early evolutionary history and better constraining the environmental conditions where the first eukaryotes evolved. Here, we focus on apusomonads, an ancient eukaryotic group sister to the opisthokonts (including animals, fungi and a variety of unicellular protists) that branches deeply in the eukaryotic tree. Despite apusomonads belong to the "rare biosphere", being usually low-abundant, they are widely distributed in marine and freshwater sediments, having a preference for low-oxygen environments. Our work significantly extends the known apusomonad diversity and reveals that they can cope with different salinity extremes, easily transgressing the marine-freshwater barrier.

Europe PMC Funders Author Manuscripts

S3) are indicated (APU-1 to APU-30). Note that branches of clades APU-29 and APU-30 are reduced to a third of their original length. Asterisks indicate 18S rRNA sequences having one or more large (>300 bp) indels.

Table 1

Samples and ecosystems where apusomonad 18S rRNA genes were amplified from. Operational taxonomic units (OTUs) were defined at a 98% sequence similarity threshold.

Sample name	Number of OTUs detected	Nature	Location	Coordinates	Reference
<i>Freshwater systems</i>					
LCL	3	Sediment from La Claye, freshwater pond on peaty substrate	Parc Naturel Régional de la Haute Vallée de Chevreuse, France	48°36'31.72"N, 01°56'17.33"E	this work
EV	1	Sediment from Etang des Vallées, shallow lake	Parc Naturel Régional de la Haute Vallée de Chevreuse, France	48°41'23.0"N, 01°54'59.2"E	this work
SR	1	Sediment from Saint Robert, village pond	Parc Naturel Régional de la Haute Vallée de Chevreuse, France	48°39'54.82" N, 01°56'45.28"E	this work
SA	5	Sediment from Ru Saint Anne, forest brook	Parc Naturel Régional de la Haute Vallée de Chevreuse, France	48°36'45.91"N, 01°58'16.61"E	this work
RSA25	4	Ru Saint Anne, water	Parc Naturel Régional de la Haute Vallée de Chevreuse, France	48°36'45.91"N, 01°58'16.61"E	Simon et al, 2015
PR1	1	Peat bog, Praz-Rodet	Praz-Rodet, Switzerland	46°33' N, 06°10' E	Lara et al, 2011
PR4	3	Peat bog, Praz-Rodet	Praz-Rodet, Switzerland	46°33' N, 06°10' E	Lara et al, 2011
<i>Marine systems</i>					
A4	4	Sand from small cove	Mediterranean Sea, Campello, Spain	38°25'56.44"N, 0°22'57.48"W	this work
I3	3	Sediment from intertidal pond	Mediterranean Sea, Campello, Spain	38°25'54.14"N, 0°22'49.08"W	this work
LC3	3	Carbonate substrate from active hydrothermal chimney	Lost City hydrothermal field, Atlantic Ocean, 750 m depth	30°07'N, 42°07' W	López-García et al, 2007
RB7	4	Hydrothermal sediment adjacent to mussel bed	Rainbow site, Mid-Atlantic Ridge, 2 264 m depth	36°6'N, 33°11'W	López-García et al, 2003
Ma30	4	Sediment surface	Marmara Sea, Çınarçık Basin, 600 m depth	40°46.798'N, 29°6.138' E	this work
Ma50	4	Cold seep sediment, reduced, black sediment/water interface	Marmara Sea, Çınarçık Basin, 1 193 m depth	40°46.80' N, 29°6.23'E	this work
Ma56	6	<i>Beggiatoa</i> -like microbial mat on cold seep reduced sediment surface	Marmara Sea, Çınarçık Basin, 1 196 m depth	40°46.80' N, 29°6.23'E	this work
MNTsu	1	Sediment surface, cold seep	Marmara Sea, Çınarçık Basin, 1 248 m depth	40°42'99"N, 29°06'97"E	Chevalier et al, 2013
MNT12	5	Sediment 1-2 cm below surface, cold seep	Marmara Sea, Çınarçık Basin, 1 248 m depth	40°42'99"N, 29°06'97"E	Chevalier et al, 2013
Ma10_SN4S_0	2	Methane seep, sediment surface	Marmara Sea, Izmit gulf, 161 m depth	40°43.722'N, 29°23.198' E	this work
Ma10_M06_0	2	Marine sediment surface	Marmara Sea, Izmit gulf, 80 m depth	40°44.077' N, 29°25.575' E	this work