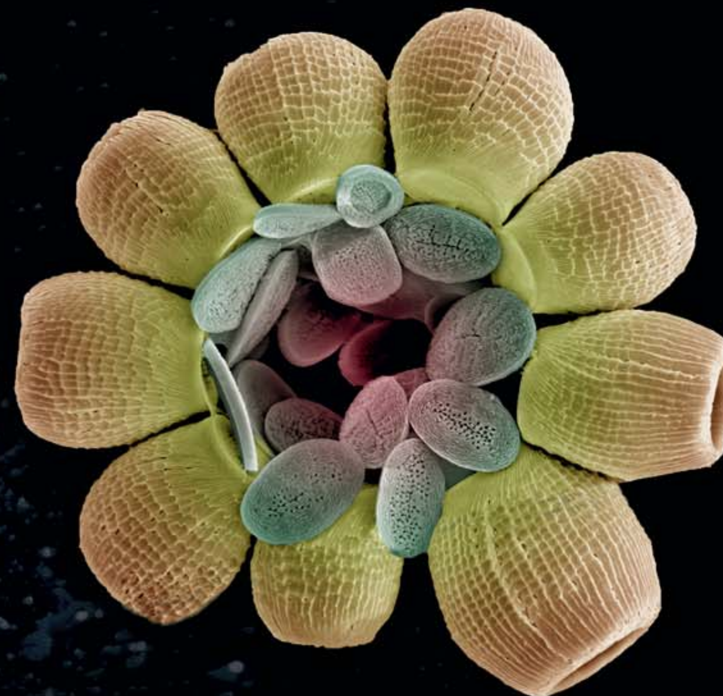
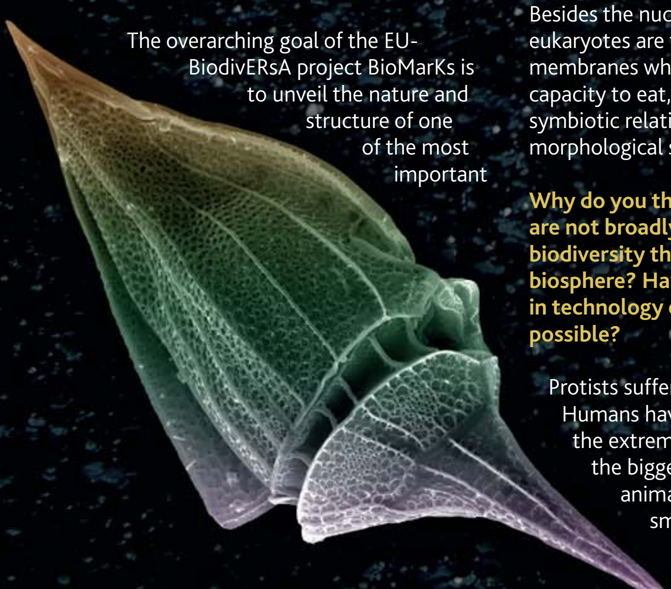
Breaking the surface waters



BioMarKs Coordinator, **Dr Colomban de Vargas**, and Program Manager, **Christophe Boutte** express their hope to draw greater attention to the relatively overlooked subject of unicellular eukaryotes – explaining how subtle changes in their biodiversity can greatly influence the entire marine ecosystem

Firstly, can you outline the objectives of the Biodiversity of Marine Eukaryotes (BioMarKs) project? Furthermore, for those who are unaware, what are marine eukaryotes?

The overarching goal of the EU-BiodivERsA project BioMarKs is to unveil the nature and structure of one of the most important biodiversity compartments in our biosphere – the marine eukaryotes. Eukaryotes are all organisms whose cell or cells enclose their DNA into a nucleus which protects the genome and allows its great increase in complexity. Besides the nucleus, the main characteristic of eukaryotes are their highly plastic and dynamic membranes which dramatically enhance cells' capacity to eat, move, establish all sorts of symbiotic relationships, and build up complex morphological structures.



Why do you think unicellular eukaryotes are not broadly studied regarding the biodiversity they represent within the biosphere? Have any recent advances in technology or knowledge made this possible?

Protists suffer from a middle-child syndrome! Humans have always been attracted by the extremes; biologists have studied the biggest forms of life (plants and animals) for three centuries, and the smallest (bacteria and viruses) for the last century. Protists, with their intermediate size-range, from 1 µm to a few mm, have been largely ignored. Given their billion year diversification preceding the evolution of animals and plants, protists possibly comprise millions of species, if not billions.

Could you describe some of the tools, technologies and techniques that you have employed in your studies?

The strength of BioMarKs is to bridge traditional research methods of sampling and detecting marine protists with the most advanced technologies in molecular ecology. In this way we establish an invaluable link between the traditional expert-based taxonomy and the novel automatic methods that will allow rapid and nearly exhaustive examination of protistan biodiversity in any ecosystem. Briefly, collections tools consist of traditional Niskin bottles, plankton nets, and sediment box corers. Protists are then concentrated onto membranes or into various liquid preservatives, before further laboratory examination of their DNA, RNA, and cellular structures.

Can you outline the nine EU coastal locations at which you conducted the study, and your reasons for selecting them?

Our nine collection sites are spread all along the European coastline from Spitsbergen to the Black Sea, with seven locations between these points in Norway, France, Spain, and Italy. We focused on coastal waters because protists play fundamental ecological roles that directly impact European societies. Photosynthetic protists support most of the primary productivity sustaining oceanic food webs and fish production. They have large genomes with thousands of genes producing organic compounds which impact marine ecosystem functioning, human health (water and sea-

Assessing protist **biodiversity**

The **BioMarKs** consortium has gathered protist samples all over Europe, from Spitzberg in the north to the Black Sea in the south, in an effort to understand the vast effects of these fascinating microorganisms

THE WELL-KNOWN adage 'big things come in small packages' rings particularly true when discussing the marine microorganisms known as protists. Many protists such as microalgae are photosynthetic and are essential primary producers in marine ecosystems, particularly in the plankton where they play a pivotal role. Members of this kingdom are amongst the main causes of human death (Plasmodium), have built up the oil reservoirs of the planet, as well as massive carbonate deposits used to construct some of our cities. A greater understanding of the immense and unexplored possibilities of protists also has the potential to be an outstanding source of innovation for green energies, pharmaceutics, cosmetics, and nanotechnology.

Despite their importance, a disproportionately low number of studies exist based on protists' far-reaching applications. To address the knowledge gap, the Biodiversity of Marine Eukaryotes (BioMarKs) project was set up in June 2009, consisting of 11 EU research institutes. The team aims to assess the taxonomic depth, environmental significance, human health and economical implications of protistan biodiversity.

One of the main aims of BioMarKs is precisely to assess the limit of marine protistan biodiversity as a whole

RULE OF THREE

Protists – also known as unicellular eukaryotes – originated in the oceans possibly around 1 billion years ago from ancestral eukaryotes that evolved into at least 10 mega-divisions that are still present and abundant in all of Earth's ecosystems. The vast majority of lineages within these divisions are made of protists; however, two independent lineages also gave rise to plants and animals.

BioMarks' approach to grasping the full implications of protists and their biodiversity is threefold. First, the team of researchers from France, the UK, Spain, Italy, Switzerland, Germany and Norway will collect three cellular size-fractions covering the whole protistan community, from the smallest to the largest cells. Next, the scientists will have the massive task of sequencing genetic markers so that the entire biodiversity is unveiled within each size-fraction. Finally, they will need to compare three complementary views of biodiversity based on DNA, RNA, and morphological analyses.

This trio of endeavours combine to maximise the chance of not only uncovering most unknown protistan diversity, but also to reveal the main actors, their role and their morphology. BioMarKs' coordinator Dr Colomban de Vargas is eager to highlight the point of the group's work: "One of the main aims of BioMarKs is precisely to assess the limit of marine protistan biodiversity as a whole," he says. "Today, the revolution in high-throughput DNA sequencing and imaging technologies make this dream of reaching one of the last frontiers in planetary biodiversity possible."

To start the process, BioMarKs had to assess protist biodiversity at three separate depths – subsurface, deep-chlorophyll maximum and surface sediment – using innovative rDNA sequencing protocols based on ILLUMINA and 454 sequencing technologies which are today capable of sequencing billions of DNA bases in a few days. Biomarkers use both rDNA and reverse transcribed rRNA general eukaryote and group-specific markers, thus allowing them to analyse both the range and complexity of marine protists at different classification levels. The team will additionally provide statistical analyses of the ecological forces shaping marine protest biodiversity through an array of physical, chemical, and biological metadata from the same samples.

JOINING THE LOCALS

The aforementioned samples are gathered from nine EU coastal water sites ranging from

food toxicity), and economy (coastal tourism, fisheries, ballast water), and which represent significant, but largely unexplored, potential for future green energies and pharmaceutics. Our sampling plan maximised the geographical and environmental scales of exploration, while being constrained by the location of EU marine laboratory providing access to boats and laboratories necessary for the collection and preparation of samples.

You have analysed protist biodiversity at three depths; subsurface, deep-chlorophyll maximum and surface sediment. Have you made any significant discoveries about the ecological diversity of protists?

We have established a unique collection – and EU baseline – of combined genetic and morphological data which will serve as a reference framework for BioMarKs and future studies of protistan diversity. We have just achieved the first round of DNA and RNA sequencing of samples representing all size fractions, depths, and locations. We had to develop original bioinformatics pipelines to link each DNA sequence to a taxonomic name, and then cluster sequences by similarity to assess the rate of novelty and measure biodiversity. Although these analyses are still in progress, we have already discovered many novel and ancient lineages of unknown protists, and significant cryptic diversity in groups that were supposedly well characterised. In addition, we observed an unexpected rate of endemism, as well as extensive planktonic protistan diversity in the sediments, which seem to play the role of a biodiversity reservoir for the fast-growing planktonic and ecologically-relevant populations.

What information have you gathered concerning the analysis of ecological forces shaping marine protist biodiversity?

This is the next step: weighing which forces, between the geographical distances, the local physico-chemical conditions, and/or the biotic interactions, are determining the composition and richness of protistan communities. It is too early to draw conclusions, but answering this question is fundamental, as it will allow understanding of how protistan biodiversity evolves and will change through local and global changes. Changes in marine protistan diversity will in turn greatly influence marine ecosystem functioning, biogeochemical cycling, and ecosystem services.

BioMarKs

BIODIVERSITY OF MARINE EUKARYOTES

OBJECTIVES

BioMarKs integrates 11 EU research institutes specialising in eukaryotic microbial taxonomy and evolution, marine biology and ecology, genomics and molecular biology, bioinformatics, as well as marine economy and policy, to assess the least explored biodiversity compartment in the biosphere: the unicellular eukaryotes, or protists.

PARTNERS

Colomban de Vargas, Jean-Michel Claverie, Richard Christen, Patrick Wincker, John Dolan, CNRS and CEA – Station Biologique de Roscoff; Institut de Microbiologie de la Méditerranée; Genoscope; Université de Nice; Observatoire Océanologique de Villefranche/Mer, France
Bente Edvardsen, University of Oslo, Norway
Ramon Massana, Institut de Ciencies del Mar, Spain
Tom Richards, David Bass, Natural History Museum, UK
Adriana Zingone, Wiebe Kooistra, Stazione Zoologica Anton Dhorn, Italy
Thorsten Stoeck, University of Kaiserlautern, Germany
Jan Pawlowski, Université de Genève, Switzerland

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CONTACT

Dr Colomban de Vargas
Project Coordinator

Station Biologique de Roscoff
Team Evolution of Plankton and PaleoOceans
Place Georges Teissier
29680 Roscoff, France

T +33 647 392 404
E vargas@sb-roscoff.fr

www.biomarks.eu

COLOMBAN DE VARGAS, Research Director at CNRS, Station Biologique de Roscoff, has a PhD in molecular ecology and evolution at the University of Geneva, Switzerland (2000). He joined the CNRS in 2006 and created the EPPO team which uses high-throughput DNA sequencing and imaging technologies toward a system biology understanding of the oceans.

Spitzbergen in Norway to the Black Sea. In 2009, the project's first year, two sampling trips were completed in Olso, Norway and Naples, Italy. By the end of 2010 more than 3,000 samples dedicated to chemical, morphological and molecular analyses had been collected. It is expected that at the end of BioMarKs' duration, more than 5 billion protist sequences will be produced.

Gathering the necessary samples proved to be one of the biggest endeavours for the researchers: "The collection of high-quality, consistent, morpho-genetic samples from three depths at all these locations was extremely challenging and took up most of our energy over the first 18 months of the project," de Vargas recalls.

Collaboration with local institutions and researchers played a vital role in collecting samples. In Bulgaria, BioMarKs worked alongside the Oceanographia Institute of Varna. This partnership was of particular importance working in the Black Sea's special circumstances. A low salinity and oxygen concentration in the sea meant the team needed the aid of local scientists to understand the specific sampling strategies required in the area.

Further afield in Spitsbergen, Norway, BioMarKs conducted its sampling alongside the European Project on Ocean Acidification (EPOCA) which is funded by the EU. Here, BioMarKs' sampling specifically aimed to study the total marine protist community in Arctic waters and how the patterns of community change under various levels of pCO_2 perturbation.

SPREADING THE WORD

In addition to working alongside local researchers, BioMarKs emphasises the importance of including stakeholders in the project's progress. The project holds regular talks to give presentations to private stakeholders to facilitate their involvement both financially and in terms of scientific collaborations. A tri-annual newsletter is also sent out to keep everyone involved abreast of announcements and to follow up on projects.

The BioMarKs consortium also holds regular meetings, the last of which took place in Barcelona at the end of March of this year. At this time, the project released its first data: "This meeting aimed to have a first common look at the data and the various analyses made by each partner," explains de Vargas. "We also wanted to set up a collective and clean dataset, to manage the work made by each lab with the set of data in the next months, and to start the redaction of a consortium high rank paper article."

Colomban de Vargas and his team are also involved in the TARA-oceans expedition through the French ANR project POSEIDON. The same work that has been carried out for BioMarKs (multifraction and depth sampling for protists, high throughput sequencing and imaging, collection of metadata) will be completed, but at the world ocean level.

A FUTURE RESOURCE

With the sampling portion of the project wrapped up, BioMarKs is now looking towards its final years. In that time, de Vargas expects a significant proportion of the team's focus will be on providing the scientific community with simple, online tools to easily assess and understand protistan diversity as revealed by the emerging technologies generating very-high throughput DNA sequencing and imaging data. This is the first essential step before spreading knowledge to policy makers and societies.

At the conclusion of the project, BioMarKs plans to host a three day workshop to concentrate on the synthesis of significant results as well as to facilitate a discussion dedicated to the ecosystemic services provided by the biodiversity of eukaryotes.

BioMarKs' research and contributions are not limited to its four-year life-span, however. Data and methods produced by the project will serve as the basis for future surveys of marine biodiversity change. Additionally, the database created during the project will serve as a reference for scientists and anyone with an interest in the field for years to come. It will become the world's largest community resource for marine unicellular eukaryotic biodiversity. The reference will be a platform for current and future projects on the subject and seeks to not only preserve an invaluable traditional EU knowledge-base but also boost the European community to the vanguard of eukaryote microbial ecology research.

THE BIOMARKS TEAM