VETENSKAPSRÅDET
THE SWEDISH RESEARCH COUNCIL

Kansliets noteringar Kod	Dnr
2008-29765-62739-34	

2008
Project Research Grant

Area of science
Other areas
Announced grants
Biodiversa's Pan European Call

VETENSKAPSRÅDET
THE SWEDISH RESEARCH COUNCIL

Kod
2008-29765-62739-34

Name of Applicant
DE VARGAS, Colomban

Date of birth
710825-

Coordinator

Name(Last name, First name)
DE VARGAS, Colomban

Date of birth
710825-

Gender
Male

Email address
vargas@sb-roscoff.fr

Position
Research scientist (CR1)

Phone
(+33)0298292528

WORKING ADDRESS

University/corresponding, Department, Section/Unit, Address, etc.
CNRS DR17
Station Biologique de Roscoff
UMR7144
Place Georges Teissier
29682 Roscoff, France

DESCRIPTIVE DATA

Project title, English (max 200 char)
BioMarKs: Biodiversity of Marine EuKaryotes

Keywords
Marine, Protist, Plankton, 454-sequencing, Ocean Acidification

Research areas
*ERA-net Biodiversa

OTHER PARTNERS

Name(Last name, First name)	University/corresponding, Department, Section/Unit, Addressetc.
MASSANA, Ramon e-mail: ramonm@cmima.csic.es	Institut de Ciències del Mar CSIC Requested funding budget for this partner (in k€): 119
RICHARDS, Tom e-mail: T.A.Richards@exeter.ac.uk	University of Exeter Requested funding budget for this partner (in k€): 265
CLAVERIE, Jean-Michel e-mail: Jean-Michel.Claverie@univmed.fr	CNRS DR12 Requested funding budget for this partner (in k€): 191
KOOISTRA, Wiebe e-mail: kooistra@szn.it	Stazione Zoologica Anton Dohrn Requested funding budget for this partner (in k€): 198
DOLAN, John e-mail: dolan@obs-vlfr.fr	CNRS DR20 Requested funding budget for this partner (in k€): 119
EDVARDSEN, Bente	University of Oslo

e-mail: bente.edvardsen@bio.uio.no

Requested funding budget for this partner (in k€): 145

Name(Last name, First name)	University/corresponding, Department, Section/Unit, Addressetc.
STOECK, Thorsten e-mail: stoeck@rhrk.uni-kl.de	University of Kaiserslautern Requested funding budget for this partner (in k€): 0

Name(Last name, First name)	University/corresponding, Department, Section/Unit, Addressetc.
, e-mail:	Requested funding budget for this partner (in k€):

Name(Last name, First name)	University/corresponding, Department, Section/Unit, Addressetc.
, e-mail:	Requested funding budget for this partner (in k€):

Name(Last name, First name)	University/corresponding, Department, Section/Unit, Addressetc.
, e-mail:	Requested funding budget for this partner (in k€):

Name(Last name, First name)	University/corresponding, Department, Section/Unit, Addressetc.
, e-mail:	Requested funding budget for this partner (in k€):

Name(Last name, First name)	University/corresponding, Department, Section/Unit, Addressetc.
, e-mail:	Requested funding budget for this partner (in k€):

Name(Last name, First name)	University/corresponding, Department, Section/Unit, Addressetc.
, e-mail:	Requested funding budget for this partner (in k€):

Name(Last name, First name)	University/corresponding, Department, Section/Unit, Addressetc.
, e-mail:	Requested funding budget for this partner (in k€):

Name(Last name, First name)	University/corresponding, Department, Section/Unit, Addressetc.
, e-mail:	Requested funding budget for this partner (in k€):

Name(Last name, First name)	University/corresponding, Department, Section/Unit, Addressetc.
, e-mail:	Requested funding budget for this partner (in k€):

Name(Last name, First name)	University/corresponding, Department, Section/Unit, Addressetc.
, e-mail:	Requested funding budget for this partner (in k€):

ENCLOSED APPENDICES

A, S

VETENSKAPSRÅDET
THE SWEDISH RESEARCH COUNCIL

Kod
2008-29765-62739-34

Name of Applicant
DE VARGAS, Colomban

Date of birth
710825-

APPLIED FUNDING: THIS APPLICATION

Total Budget (k€):	3055
Total Requested Budget (k€):	1767

VETENSKAPSRÅDET
THE SWEDISH RESEARCH COUNCIL

Kod
2008-29765-62739-34

Name of Applicant
DE VARGAS, Colomban

Date of birth
710825-

POPULAR SCIENCE DESCRIPTION

BioMarKs integrates 8 EU research institutes and 30 EU experts 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 taxonomic depth, environmental significance, human health and economical implications of arguably the least explored biodiversity compartment in the biosphere: the unicellular eukaryotes or protists. Marine protists typically live in huge populations with rapid turnover. They may build complex (in)organic skeletal structures which profoundly impact biogeochemical cycles and climate; they have complex genomes with thousands of genes producing molecules which influence marine ecosystem functioning, human health and economy, and which represent outstanding potential for future green energies, pharmaceutics and cosmetics. Based on phenotypic data, marine protists comprise <200k “species”. However, exploration of ribosomal (r) DNA clone libraries over the last decade has revealed ever-increasing biodiversity in both novel lineages and groups which were believed to be species-poor. BioMarKs will reassess coastal marine protist biodiversity using massive rDNA sequencing integrated into a network of taxonomic expertise and comprehensive contextual phenotypic and environmental metadata. 454-pyrosequencing technology permits acquisition of several hundred thousand sequence tags in a single run, providing the prospect, for the first time, of conducting nearly-exhaustive surveys of microbial diversity and population dynamics. In collaboration with GENOSCOPE (France), we propose to use 45X 454-runs (18 to 45 million sequence-tags) to assess protist biodiversity at 3 depths (subsurface, deep-chlorophyll maximum, surface sediment) in 9 EU coastal water sites from Spitzbergen to the Black Sea. An incremental sequencing strategy will maximize the depth of genetic exploration, based on both rDNA and reverse transcribed rRNA general eukaryote and group-specific markers, in order to analyze both diversity and abundance/activity of marine protists at different taxonomic levels. A suite of physical, chemical, and biological metadata from the same samples will allow statistical analyses of the ecological forces shaping marine protist biodiversity. Microscopy analyses, as well as downstream PCR-based and FISH molecular analyses of archive DNA, RNA, and cellular material (again from the same samples) will allow anchoring of the genetic data into high quality phenotypic, phylogenetic, and ecological quantitative frameworks. This general strategy will be used to (i) establish a baseline of protist biodiversity in EU coastal waters, (ii) measure biodiversity change in marine protist communities facing ocean acidification, (iii) evaluate the impact of ballast water and pollution on marine protist biodiversity. In addition to significantly enhancing our basic knowledge of eukaryote biodiversity and ecology (Who? How Many? When? Where? Why?), BioMarKs will provide baseline data and new methods for future surveys of marine biodiversity change and for evaluation of its ecological and economic cost. The BioMarKs database will become the largest world community resource on marine unicellular eukaryotic biodiversity, providing a reference platform for current and future projects dealing with this important biodiversity compartment, and elevating the European community to the forefront of marine eukaryote microbial ecology. By connecting the most modern sequencing technology to EU experts in protist taxonomy, BioMarKs will valorize an invaluable traditional EU knowledge-base. Finally BioMarKs will actively promote the diffusion of its data and new methods to a wide range of stakeholders and for scientific and public education. Several international research programs on marine biodiversity, major genetic databases and protist culture collections, governmental and private agencies involved in legislation and monitoring of coastal marine waters, foundations and companies with interest in marine biotechnologies, as well as key scientific personalities in the fields of marine science and biodiversity have already expressed their interest in BioMarKs. The BioMarKs consortium plan to produce at least 25 high impact publications, a co-authored book “Biodiversity of Marine Eukaryotes”, and a public exhibition “The Coevolution of Marine Protists and the Planet Earth” by the end of the program.

VETENSKAPSRÅDET
THE SWEDISH RESEARCH COUNCIL

Kod
2008-29765-62739-34

Name of applicant
DE VARGAS, Colomban

Date of birth
710825-

Title of research programme
BioMarKs: Biodiversity of Marine EuKaryotes

Appendix A

Research programme

Vetenskapsrådet, SE-103 78 Stockholm, tel. +46 (0)8 546 44 000, vetenskapsradet@vr.se

Research for the understanding of European Biodiversity
A Network of Research Funding Agencies in 14 European Countries
BiodivERsA is funded as an ERA-net project within the European Union's 6th Framework Programme for Research

APPENDIX A
FULL PROPOSAL APPLICATION FORM
Please fill in this form in English

Pan-European call for international research projects on biodiversity linking scientific advancement to policy and practice

Please respect the page and word limits: any additional material will not be assessed by the reviewers
NB. Full proposals must be received by 16 June, 24 CET (midnight)

I.A Administrative details

What is a partner ?
Note that according to the country, a "partner" can be :
- a researcher,
- an institution,
- a laboratory, a department of an institution.

Please consult your national contact point to make sure you comply with specific national rules

For funding, there are 3 categories of partners:

1. **Partners from countries eligible for direct funding (designated Partners 1, 2… N) :** Austria, Estonia, France, Germany, Hungary, Italy, the Netherlands, Norway, Portugal, Spain, Sweden and the United Kingdom.

2. **Partners from countries ineligible for direct funding**, **but subcontracted by a Partner 1, 2…N** (designated ***Partners 1a, 2a… Na***)(e.g. *partner 1a* is subcontracted by **partner 1**)

3. ***Fully self-financed*** partners from any country who bring their own secured budget. (designated ***partner A, B***)

Coordinator – Partner 1					
Researcher in charge					
Family name	De Vargas		**First name**	Colomban	
Title	PhD		**Gender**	Male	
Phone	+33 2 98 29 25 28		**E-mail**	*vargas@sb-roscoff.fr*	
Web site	http://www.sb-roscoff.fr/Phyto/				
Research institute / Company	Centre National de la Recherche Scientifique Délégation Bretagne Pays de la Loire (CNRS-DR17)				
Status: Private or public?	Public				
Division / Department	Station Biologique de Roscoff UMR 7144 - Adaptation et Diversité en Milieu Marin				
Street name and number	Place Georges Teissier				
PO Box	B.P. 74	**Postal code**	29682	**Cedex**	
Town	ROSCOFF		**Country**	FRANCE	
Team members involved in the project (when the partner is an institution, a laboratory, a department)					
Team member 1 : VAULOT, Daniel, male, PhD, +33 2 98 29 23 34, vaulot@sb-roscoff.fr **Team member 2 :** NOT, Fabrice, male, PhD, +33 2 98 29 25 28, not@sb-roscoff.fr **Team member 3 :** GUILLOU, Laure, female, PhD, +33 2 98 29 23 79, lguillou@sb-roscoff.fr **Team member 4:** SIMON, Nathalie, female, PhD, +33 2 98 29 25 34, simon@sb-roscoff.fr **Team member 5:** PROBERT, Ian, male, PhD, +33 2 98 29 25 28, probert@sb-roscoff.fr					

Partner 2			
Researcher in charge			
Family name	Massana	**First name**	Ramon
Title	PhD	**Gender**	male
Phone	+34 93 230 95 22	**E-mail**	ramonm@icm.csic.es
Web site	http://www.icm.csic.es/bio/projects/icmicrobis/massana		
Research institute / Company	Institut de Ciències del Mar, CSIC		

Status: Private or public?		Public			
Division / Department		Department of Marine Biology and Oceanography			
Street name and number		Passeig Marítim de la Barceloneta, 37-49			
PO Box		**Postal code**	08003	**Cedex**	
Town	BARCELONA		**Country**	SPAIN	
Team members involved in the project (when the partner is an institution, a laboratory, a department)					
Team member 1 : Pedrós-Alió, Carlos, male, PhD, +34 93 230 95 97, cpedros@icm.csic.es **Team member 2 :** Forn, Irene, female, Technician, +34 93 230 95 00, forn@icm.csic.es					

Partner 3					
Researcher in charge					
Family name	Richards		**First name**	Tom	
Title	PhD		**Gender**	male	
Phone	+44 (0)1392 263756		**E-mail**	*T.A.Richards@exeter.ac.uk*	
Web site	http://www.projects.ex.ac.uk/ceem/				
Research institute / Company		University of Exeter			
Status: Private or public?		Public			
Division / Department		Centre for Eukaryotic Evolutionary Microbiology			
Street name and number		Stocker Road			
PO Box	Not applicable	**Postal code**	EX4 4QD	**Cedex**	Not applicable
Town	EXETER		**Country**	UK	
Team members involved in the project (when the partner is an institution, a laboratory, a department)					
Team member 1 : Bass, David, male, PhD, , +44(0)1392 263756 , david.bass@zoo.ox.ac.uk **Team member 2 :** Soanes, Darren, male, PhD, +44(0)1392 263497, d.m.soanes@exeter.ac.uk					

Partner 4					
Researcher in charge					
Family name	Claverie		**First name**	Jean-Michel	
Title	PhD		**Gender**	male	
Phone	+33(0)4.91.82.54.20		**E-mail**	Jean-Michel.Claverie@univmed.fr	
Web site	http://www.igs.cnrs-mrs.fr				
Research institute / Company	Centre National de la Recherche Scientifique Délégation Provence Corse (CNRS-DR12)				
Status: Private or public?	Public				
Division / Department	Structural and Genomic Information Laboratory – UPR 2589				
Street name and number	Parc Scientifique de Luminy - 163 Avenue de Luminy - Case 934				
PO Box		**Postal code**	13288	**Cedex**	09
Town	MARSEILLE		**Country**	FRANCE	
Team members involved in the project (when the partner is an institution, a laboratory, a department)					
Team member 1 : Ogata, Hiroyuki, male, PhD, +33 04 91 82 54 20, Hiroyuki.Ogata@igs.cnrs-mrs.fr **Team member 2 :** Audic, Stephane, male, PhD, +33 04 91 82 54 20, Stephane.Audic@igs.cnrs-mrs.fr					

Partner 5			
Researcher in charge			
Family name	Kooistra	**First name**	Wiebe
Title	PhD	**Gender**	male
Phone	+ 39 081 58 33 271	**E-mail**	*kooistra@szn.it*
Web site	http://www.szn.it/		
Research institute / Company	Stazione Zoologica Anton Dohrn		
Status: Private or public?	Public		
Division / Department	Ecology and Evolution of Plankton		
Street name and number	Villa Comunale 1		

PO Box	Not applicable	**Postal code**	80121	**Cedex**	Not applicable
Town	NAPLES		**Country**	ITALY	
Team members involved in the project (when the partner is an institution, a laboratory, a department)					
Team member 1: Montresor, Marina, female, PhD, +39 081 58 33 259, mmontr@szn.it **Team member 2:** Zingone, Adriana, female, PhD, +39 081 58 33 295, zingone@szn.it **Team member 3:** Sarno, Diana, female, PhD, +39 081 58 33 282, diana@szn.it **Team member 4:** Falciatore, Angela, female, PhD, +39 081 58 33 268, afalciat@szn.it					

Partner 6					
Researcher in charge					
Family name	Dolan		**First name**	John	
Title	PhD		**Gender**	male	
Phone	+33 (0)4 93 76 38 22		**E-mail**	dolan@obs-vlfr.fr	
Web site	http://www.obs-vlfr.fr/LOV/				
Research institute / Company	Centre National de la Recherche Scientifique Délégation Côtes d'Azur (CNRS-DR20)				
Status: Private or public?	Public				
Division / Department	Laboratoire d'Oceanographie de Villefranche/Mer - CNRS Microbial Ecology – UMR 7093				
Street name and number	Port de la Darse				
PO Box	B.P. 08	**Postal code**	06234	**Cedex**	Not applicable
Town	VILLEFRANCHE/MER		**Country**	FRANCE	
Team members involved in the project (when the partner is an institution, a laboratory, a department)					
Team member 1 : Lemee, Rodolphe, male, PhD, +33 (0)4 93 76 38 39, lemee@obs-vlfr.fr **Team member 2 :** Claustre, Hervé, male, PhD, +33 (0)4 93 76 38 29, claustre@obs-vlfr.fr					

Partner 7					
Researcher in charge					
Family name	Edvardsen		**First name**	Bente	
Title	Professor, PhD		**Gender**	female	
Phone	+47 22857038		**E-mail**	bente.edvardsen@bio.uio.no	
Web site	http://www.bio.uio.no				
Research institute / Company	University of Oslo				
Status: Private or public?	Public				
Division / Department	Department of Biology / Group Microalgae				
Street name and number	**Blindernveien 31**				
PO Box	1066 Blindern	**Postal code**	0316	**Cedex**	Not applicable
Town	OSLO		**Country**	NORWAY	
Team members involved in the project (when the partner is an institution, a laboratory, a department)					
Team member 1 : Shalchian-Tabrizi, Kamran, male, PhD, 47 22854564, kamran@ulrik.uio.no **Team member 2 :** Eikrem, Wenche, female, PhD, 47 22854531, wenche.eikrem@bio.uio.no **Team member 3 :** Bjørklund, Kjell R., male, PhD, +47 22851669, k.r.bjorklund@nhm.uio.no					

Self financed Partner A			
Researcher in charge			
Family name	Stoeck	**First name**	Thorsten
Title	PhD	**Gender**	male
Phone	+49 (0)631 205-2502	**E-mail**	*stoeck@rhrk.uni-kl.de*
Web site	http://web.mac.com/stoeck_lab/		
Research institute / Company	University of Kaiserslautern		
Status: Private or public?	Public		
Division / Department	Department of Ecology		
Street name and number	Erwin-Schroedinger Street, Building 14		

PO Box	Not applicable	**Postal code**	67653	**Cedex**	Not applicable
Town	KAISERLAUTERN		**Country**	GERMANY	
Team members involved in the project (when the partner is an institution, a laboratory, a department)					

Please insert as many copies of the above table as necessary for other partners B, C…

I.B : Time to be dedicated to the project per member

In the following table, please specify the names and countries of each partner.

Partners	Teams	Time to be dedicated to the project in % of total working time
Partner organisation 1 CNRS-DR17 Station Biologique de Roscoff FRANCE	Colomban de Vargas Daniel Vaulot Fabrice Not Laure Guillou Nathalie Simon Ian Probert	50% 15% 35% 20% 15% 20%
Partner organisation 2 Institut de Ciències del Mar, CSIC, SPAIN	Ramon Massana Carlos Pedrós-Alió Irene Forn	35% 15% 30%
Partner organisation 3 University of Exeter UK	Tom Richards David Bass Darren Soanes	5% 60% 10%
Partner organisation 4 CNRS-DR12 Structural and Genomic Information Laboratory FRANCE	Jean-Michel Claverie Hiroyuki Ogata Stephane Audic	30% 30% 30%
Partner organisation 5 Stazione Zoologica Anton Dohrn ITALY	Wiebe Kooistra Marina Montresor Adriana Zingone Diana Sarno Angela Falciatore	15% 5% 5% 5% 5%
Partner organisation 6 CNRS-DR20 Laboratoire d'Oceanographie de Villefranche/Mer France	John Dolan Rodolphe Lemée Hervé Claustre	10% 10% 5%
Partner organisation 7 University of Oslo NORWAY	Bente Edvardsen Kamran Shalchian-Tabrizi Wenche Eikrem Kjell Bjørklund	10% 10% 5% 10%
Self financed partner organisation A University of Kaiserslautern GERMANY	Thorsten Stoeck	20%

I.C : Declaration of parallel submissions of this proposal (whole or parts) to other funding programmes

Provide details of any proposal related to this BiodivERsA one, which you or another project partner have submitted to other funding opportunities, including title, funding source, extent of overlap and expected decision date.
Duplication of funding is not allowed for the same (whole or part) research project.

None of *BioMarKs* proposed research was submitted to other funding agencies.

II. Summary of the project

Theme : Please indicate which themes in the call your proposal relates to (tick the box yes/no), and indicate an approximate percentage of the themes addressed in your proposal

Themes in the BiodivERsA call	YES	%	NO
1. Global change and biodiversity dynamics	X	65	
2. Ecosystem functioning	X	10	
3. Ecosystem services	X	25	

Work packages (WP) - *Title only, detailed descriptions should be included in the project description section*

No. of WP	Responsible partner	Title
1	P1	Establishing a baseline of unicellular eukaryote (protist) biodiversity in *European* coastal waters using massive parallel sequencing correlated to contextual physical, chemical, and biological metadata.
2	P3	Applying the genetic and meta-data to environmental and evolutionary questions in marine protist diversity: how many, who, where, when and why?
3	P5	Correlating change in marine protist diversity with global (ocean acidification) and local (anthropogenic pollution, ballast water) pressures.
4	P4	Developing practical methods for monitoring eukaryotic microbial diversity change and for evaluating its economic implications.

Estimated working time (in months) per work package

No. of WP	Partner 1	Partner 2	Partner 3	Partner 4	Partner 5	Partner 6	Partner 7	Self financed partner A
1	45	20	6	35	20	10	15	2
2	35	25	17	5	6	25	8	5
3	30	7	2	4	20	7	5	1
4	28	5	5	30	5	5	5	1

Deliverables

No.	Title	Delivery date [1)]
D1	An interactive *BioMarKs* web-site to introduce the project, facilitate diffusion of information within the consortium, and provide a point of access for end-users	Month 4
D2	Models of marine eukaryote biodiversity on a group-by-group basis, including a comprehensive reassessment of the molecular	Month 18

	diversity of each group and their ecological and evolutionary significance	
D3	A check-list of microbial eukaryotic genotypes identified in EU marine coastal waters, with their potential implication(s) in terms of human health, environmental hazards, and technology	Month 24
D4	A series of bioinformatics tools to rapidly retrieve and analyse taxonomic, biodiversity, and ecological information from the molecular sequences generated.	Month 24
D5	An interdisciplinary analysis of the ecological parameters shaping marine protist diversity and distribution.	Month 27
D6	A report identifying the key ecosystemic services of marine protist biodiversity, and how this relates to the molecular sequence and environmental metadata recovered. This report will also outline practical and statistical methods for evaluating the ecological and/or economical impact of protist groups	Month 28
D7	A set of large-audience educational tools to inform EU citizens of the striking beauty, diversity and fragility of eukaryotes (other than plants and animals) and their importance to human society and environmental stability	Month 30
D8	A public access database recording millions of marine eukaryotic rDNA molecular sequence tags together with their taxonomic, biogeographical, and ecological affiliation.	Month 36
D9	Predictions of change of marine eukaryote community structures in response to anthropogenic global (ocean acidification) and local (water pollution and ballast-water) pressures.	Month 40
D10	New, highly sensitive molecular methods (taxa specific PCR, rtPCR, qPCR, and FISH) and protocols (including large-scale 454 DNA sequencing) to easily detect and quantify ecologically and/or economically important protists in the aquatic environment.	Month 42
D11	A multisdisciplinary assessment of the biogeochemical importance of marine protist biodiversity and its impact on the evolution of the Biosphere, highlighting factors important for human health and environmental sustainability	Month 42

(Use as many lines as needed)
1) Indicate month number from the start of the project, e.g. month 12, month 24…

Milestones		
No.	Title	MONTH:
M1	Kickoff meeting –intercalibration of sampling and analytical methods intercalibration	2
M2	*BioMarKs* interactive website online + letter to Stakeholders	4
M3	Collection of the first set of 28 discrete coastal EU marine samples (e DNA/RNA + metadata)	7

M4	Collection of e-DNA/RNA and metadata in collaboration with EPOCA (ocean acidification mesocosm)	8
M5	Completion of 3 exploratory 454-sequencing runs (18S rDNA/RNA) (M3 + M4 samples)	9
M6	BioMarKs genetic data quality control workshop at GENOSCOPE	10
M7	Collection of e-DNA/RNA + metadata from ballast water and polluted EU port	11
M8	Completion of 17 454-sequencing runs to complete analyses of the M3 + M4 samples	12
M9	Collection of the second set of marine coastal e-DNA/RNA + metadata	12
M10	Analyses of chemical and biological contextual data for M3 + M4 samples done	13
M11	Release of the genetic data (M5-M8) to the network of taxonomic experts	13
M12	Completion of 1 exploratory 454-sequencing runs (M7 and M9 samples)	14
M13	Completion of 7.5 454-sequencing runs to complete analyses of the M7 + M9 samples	15
M14	Analyses of chemical and biological metadata for M7 + M9 samples done	16
M15	Release of the genetic data (M12-13) to the network of taxonomic experts	16
M16	*BioMarKs* workshop #1 in Roscoff: intermediate synthesis, report back from experts on all 454-data	17
M17	Report of the *BioMarKs* workshop #1 on line and sent to stakeholders	18
M18	Completion of 1 exploratory 454-sequencing run for the analysis of group specific LSU rDNA from all samples (M3,4,7,9)	18
M19	Release of the genetic data (M18) to the network of taxonomic experts	19
M20	First round of manuscript submission (estimated 6) and press release	21
M21	Public access release of the first set of *BioMarKs* data (M3-M4 454-tags and metadata) and bioinformatics tools	21
M22	Letters of invitation to the *BioMarKs* workshop #2 (M24) sent to Stakeholders	21
M23	Report back from the *BioMarKs* consortium on the D2-tag data (M14)	21
M24	Completion of 15.5 in-depth 454-sequencing runs for D2 tags group-specific analyses of all samples	21

M25	Release of the genetic data (M21) to the network of taxonomic experts	22
M26	Annotated checklist of EU marine protists and their potential ecological impact done	24
M27	Workshop #2: *BioMarKs* ecosystem services	26
M28	Final report back from the *BioMarKs* consortium on all group-specific data	26
M29	Report of the *BioMarKs* workshop #2 on line	28
M30	Second round of manuscript submission (estimated 9) and press release	30
M31	Public access release of the second set of *BioMarKs* data (M7-9 454-sequence tags and metadata) and novel bioinformatics tools	30
M32	Re-exploration of key samples (archive DNA/RNA) using group-specific PCR of longer rDNA fragment and their phylogenetic analyses done	34
M33	Quantitative analyses of the key players using group-specific FISH done	36
M34	*Grand-public* exhibition "*The Co-Evolution of Eukaryotes and the Planet Earth*" completed	38
M35	Third round of manuscript submission (estimated 10) and press release	40
M36	Public access release of the 3dn set of *BioMarKs* data (M24)	40
M37	Report on the group-specific data and methods to key stakeholders and campaign of fundraising to pursue *BioMarKs* research	41
M38	Public-access release of the entire *BioMarKs* database and its link to large-scale genetic data providers	42
M39	Final meeting: integrating *BioMarKs* data into pluridisciplinary studies of the Earth System.	42
M40	Chapters for a co-authored book "*Biodiversity of Marine Eukaryotes*" ready for Edition/publication.	42

(Use as many lines as needed)

1) Indicate month number from the start of the project, e.g. month 12, month 24…

Duration of the project:	01/01/2009 - 30/06/2012 (42 months)

Please note that projects cannot start before 01/01/2009

III. Scientific publications

Top 5 scientific publications of the applicants relevant to the application

Partner 1 CNRS-DR17 Station Biologique de Roscoff FRANCE	1. Not, F., Valentin, K., Romari, K., Lovejoy, C., Massana, R., Töbe, K., Vaulot, D. & Medlin, L. 2007. Picobiliphytes, a new marine picoplanktonic algal group with unknown affinities to other eukaryotes. **Science** 315:252-254. 2. Falkowski, P. & de Vargas C. 2004. Shotgun sequencing in the Sea: a blast from the Past? **Science**, 304: 58-60. 3. Moon-van der Staay S.Y., De Wachter R., Vaulot D. 2001. Oceanic 18S rDNA sequences from picoplankton reveal new eukaryotic lineages. **Nature** 409: 607-610. 4. Not, F., Latasa, M., Marie, D., Cariou, T., Vaulot, D. & Simon, N. 2004. A single species Micromonas pusilla (Prasinophyceae) dominates the eukaryotic picoplankton in the western English Channel. **Applied and Environmental Microbiology** 70: 4064-4072. 5. Viprey, M., Guillou, L., Férréol, M. & Vaulot, D. 2008. Wide genetic diversity of picoplanktonic green algae (Chloroplastida) uncovered in the Mediterranean Sea by a phylum-specific PCR approach. **Environmental Microbiology** in press
Partner 2 Institut de Ciències del Mar, CSIC, SPAIN	1. Massana, R. and C. Pedrós-Alió. 2008. Unveiling new microbial eukaryotes in the surface ocean. **Curr. Opin. Microbiol.** 10.1016/j.mib.2008.04.004. 2. Massana, R., B. Karniol, T. Pommier, I. Bodaker and O. Béjà. 2008. Metagenomic retrieval of a ribosomal DNA repeat array from an uncultured marine alveolate. **Environmental Microbiology** doi:10.1111/j.1462-2920.2007.01549.x. 3. Pedrós-Alió, C. 2007. Dipping into the rare biosphere. **Science** 315:192-193 4. Massana, R., R. Terrado, I. Forn, C. Lovejoy and C. Pedrós-Alió. 2006. Distribution and abundance of uncultured heterotrophic flagellates in the world oceans. **Environmental Microbiology** 8:1515-1522. 5. Massana, R., J. Castresana, V. Balagué, L. Guillou, K. Romari, A. Groisillier, K. Valentin and C. Pedrós-Alió. 2004. Phylogenetic and ecological analysis of novel marine stramenopiles. **Applied and Environmental Microbiology**. 70:3528-3534.
Partner 3 University of Exeter UK	1. Bass, D., Howe, A., Brown, N., Barton, H., Demidova, M., Michelle, H., Li, L., Sanders, H., Watkinson, S.C., Willcock, S. and Richards, T.A. (2007) Yeast forms dominate fungal diversity in the deep oceans. **Proc. Biol. Sci**. 274, 3069-3077 2. Bass, D., Richards, T.A., Matthai, L., Marsh, V. and Cavalier-Smith, T. (2007) DNA evidence for global dispersal and probable endemicity of protozoa. **BMC Evol. Biol.** 7, 162. 3. Richards, T.A., Dacks, J.B., Jenkinson, J.M., Thornton, C.R. and Talbot, N.J. (2006) Evolution of filamentous plant pathogens: gene exchange across eukaryotic kingdoms. **Curr. Biol**. 16, 1857-1864. 4. Richards, T.A. and Bass, D. (2005) Molecular screening of free-living microbial eukaryotes: diversity and distribution using a meta-analysis. **Curr. Opin. Microbiol**. 8, 240-252. 5. Richards, T.A. and Cavalier-Smith, T. (2005) Myosin domain evolution and the primary divergence of eukaryotes. **Nature** 436, 1113-1118.
Partner 4 CNRS-DR12 Structural and Genomic Information	1. Ogata H, Claverie JM (2007) Unique genes in giant viruses: regular substitution pattern and anomalously short size. **Genome Res**.17;9:1353-61,2007 Sep - PMID:17652424 2. Raoult D, Audic S, Robert C, Abergel C, Renesto P, Ogata H, La Scola B, Suzan M, Claverie JM. (2004) The 1.2-Mb Genome Sequence of Mimivirus.

Laboratory FRANCE	**Science**. 2004 Nov 19;306(5700):1344-50. 3. Ogata H, Renesto P, Audic S, Robert C, Blanc G, Fournier PE, Parinello H, Claverie JM, Raoult D (2005) The genome sequence of Rickettsia felis identifies the first putative conjugative plasmid in an obligate intracellular parasite. **PLoS Biol**.3;8:e248,2005 Aug - PMID:15984913 4. Suhre K, Audic S, Claverie JM (2005) Mimivirus gene promoters exhibit an unprecedented conservation among all eukaryotes.**Proc Nat Acad Sci U S A**.102;41:14689-93. 5. Monier A, Larsen JB, Sandaa RA, Bratbak G, Claverie JM, Ogata H (2008) Marine mimivirus relatives are probably large algal viruses.**Virol J**.5;1:12,2008 Jan 23 - PMID:18215256
Partner 5 Stazione Zoologica Anton Dohrn ITALY	1. Kooistra W.H.C.F, Gersonde R, Medlin L.K. & Mann D.G. (2007). The origin and evolution of the diatoms: their adaptation to a planktonic existence. In: Evolution of planktonic photoautotrophs. Eds: Paul G. Falkowski and Andrew H. Knoll. Academic Press, Inc. pp. 207-249. 2. Kooistra W.H.C.F., Sarno D., Balzano S., Gu, H. Andersen R.A. & Zingone A. Global diversity and biogeography of Skeletonema species (Bacillariophyta). **Protist** 159: 177-193. 3. McDonald S.M., Sarno D., Scanlan D.J., Zingone A. (2007) Genetic diversity of eukaryotic ultraphytoplankton in the Gulf of Naples during an annual cycle. **Aquatic Microbial Ecology** 50: 75-89. 4. McDonald S.M., Sarno D., Zingone A. (2007) Identifying Pseudo-nitzschia species in natural samples using genus-specific PCR primers and clone libraries. **Harmful Algae** 6: 849-860. 5. Amato, A., Kooistra, W.H.C.F., Levialdi Ghiron J.H., Mann, D.G., Pröschold, T. & Montresor, M. (2006). Reproductive isolation among sympatric cryptic species in marine diatoms. **Protist** 158: 193-207.
Partner 6 CNRS-DR20 Laboratoire d'Oceanographie de Villefranche/Mer FRANCE	1. Dolan, J.R., Ritchie, M.R., Ras, J. 2007. The neutral community structure of planktonic herbivores, tintinnid ciliates of the microzooplankton, across the SE Pacific Ocean. **Biogeosciences** 4: 297-310. 2. Gavrilova, N., Dolan, J.R. 2007. A note on species lists and ecosystem shifts: Black Sea tintinnids, ciliates of the microzooplankton. **Acta Protozoologica**, 46:279-288. 3. Dolan, J. R. Jacquet, S., Torreton, J.-P. 2006. Comparing taxonomic and morphological biodiversity of tintinnids (planktonic ciliates) of New Caledonia. **Limnology and Oceanography**, 51:950-958. 4. Croce, O., Lamarre, M., Christen, R. 2006. Querying the public databases for sequences using complex keywords contained in the feature lines. **BMC Bioinformatics** 7:45-51. 5. Dolan J.R. 2005. An introduction to the biogeography of aquatic microbes. **Aquatic Microbial Ecology** 41: 39-48.
Partner 7 University of Oslo NORWAY	1. Burki F, Shalchian-Tabrizi K, Minge M, Skjæveland Å, Nikolaev SI, et al. (2007) Phylogenomics Reshuffles the Eukaryotic Supergroups. **PLoS ONE** 2(8): e790. doi:10.1371/journal.pone.0000790 2. Dolven, J. K. L., Lindqvist, C., Albert, V. A., Bjørklund, K. R.; Yuasa, T., Takahashi, O., Mayama, S. 2007. Molecular Diversity of Alveolates Associated with Neritic North Atlantic Radiolarians. **Protist**, 158: 65-76 3. Edvardsen, B., Eikrem, W., Shalchian-Tabrizi, K., Riisberg, I., Johnsen, G., Naustvoll, L., Throndsen, J. 2007. Verrucophora farcimen gen. et sp. nov. (Dictyochophyceae, Heterokonta) - a bloom forming ichthyotoxic flagellate from the Skagerrak, Norway. **J. Phycol**. 43(5), 1054-1070 4. Edvardsen, B., Eikrem, W., Green, J.C., Andersen, R.A., Moon-van der Staay,

	S.Y. & Medlin, L.K. 2000. Phylogenetic reconstructions of the Haptophyta inferred from 18S ribosomal DNA sequences and available morphological data. **Phycologia** 39 (1): 19-35. 5. Shalchian-Tabrizi, Kamran; Eikrem, Wenche; Klaveness, Dag; Vaulot, D; Minge, M.A.; Le Gall, Florence; Romari, Khadidja; Throndsen, Jahn; Botnen, Andreas; Massana, Ramon; Thomsen, Helge, A; Jakobsen, Kjetill S. 2006. Telonemia, a new protist phylum with affinity to chromist lineages. **Proceedings of the Royal Society of London. Series B**, Biological Sciences – 22: 1833–1842.
Self financed Partner A University of Kaiserslautern GERMANY	1. Stoeck, T., Kasper, J., Bunge, J., Ilyin, V., Leslin, C., Epstein, S.S. (2007). Protistan diversity at the top of the world. **PLoS ONE** 2(8): e728 doi:10.1371/journal.pone.0000728 2. Kolodziej, K., Stoeck, T. (2007) Cellular identity of a novel uncultured MAST-12 lineage and phylogeny of the uncultured marine stramenopile sequence clade MAST-12. **Applied and Environmental Microbiology**, 73:2718-2726. 3. Boenigk, J., Jost, S., Stoeck, T., Garstecki, T. (2007) Implications of cryptic molecular and eco-physiological diversity of protist taxa. **Environmental Microbiology**, 9:593-602 4. Stoeck, T., Breiner, H-W., Zuendorf, A., Behnke, A. (2007) A molecular approach to identify active microbes in environmental eukaryote clone libraries. **Microbial Ecology**, 53:328-339 5. Zuendorf, A., Behnke, A., Bunge, J., Barger, K. J. A., Stoeck, T. (2006). Diversity estimates of microeukaryotes below the chemocline of the anoxic Mariager Fjord, Denmark. **FEMS Microbiology Ecology**, 58:476-4911.

Other relevant publications *(max 1 page, Arial font, 11 pts, single spaced)*

By the applying research group:

Bass, D., and Cavalier-Smith, T. (2004) Phylum-specific environmental DNA analysis reveals remarkably high global biodiversity of Cercozoa (Protozoa). *International* ***Journal of Systematic and Evolutionary Microbiology*** 54: 2393-2404.

de Vargas, C., Saez, A.G., Medlin, L.K., and Thierstein, H. (2003) Super-Species in the calcareous plankton. In ***Coccolithophores : from molecular processes to global impact***. Thierstein, H.R., and Young, J.R. (eds). New York: Springer Verlag.

Dolan, J.R. (2005) Marine ecology: Different measures of biodiversity. ***Nature*** 433: E9.

Pedros-Alio, C. (2006) Marine microbial diversity: can it be determined? ***Trends in Microbiology*** 14: 257-263.

Stoeck, T., Fowle, W.H., and Epstein, S.S. (2003) Methodology of protistan discovery: from rRNA detection to quality scanning electron microscope images. ***Applied and Environmental Microbiology*** 69: 6856-6863.

Vaulot, D., Romari, K., and Not, F. (2002) Are autotrophs less diverse than heterotrophs in marine picoplankton? ***Trends in Microbiology*** 10: 266-267.

Vaulot, D., Eikrem, W., Viprey, M., and Moreau, H. (2008). The diversity of small eukaryotic marine phytoplankton. ***FEMS Microbiology Reviews*** in press

Other references relevant to the application:

Adl, S.M., Leander, B.S., Simpson, A.G.B., Archibald, J.M., Anderson, O.R., Bass, D. et al. (2005) Diversity, Nomenclature, and Taxonomy of Protists. ***Systematic Biology*** 56: 684 – 689.

Corliss, J.O. (2004) Why the world needs protists! ***Journal of Eukaryotic Microbiology*** 51: 8-22.

Cotterill, F.P.D., Al-Rasheid, K., and Foissner, W. (2008) Conservation of protists: is it needed at all? ***Biodiversity and Conservation*** 17: 427-443.

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

Huber, J.A., Mark Welch, D.B., Morrison, H.G., Huse, S.M., Neal, P.R., Butterfield, D.A., and Sogin, M.L. (2007) Microbial population structures in the deep marine biosphere. ***Science*** 318: 97-100.

Huse, S.M., Huber, J.A., Morrison, H.G., Sogin, M.L., and Mark Welch, D. (2007) Accuracy and quality of massively parallel DNA pyrosequencing. ***Genome Biology*** 8.

Krause, L., Diaz, N.N., Goesmann, A., Kelley, S., Nattkemper, T.W., Rohwer, F. et al. (2008) Phylogenetic classification of short environmental DNA fragments. ***Nucleic Acids Research*** 36: 2230-2239.

Liu, Z., Lozupone, C., Hamady, M., Bushman, F.D., and Knight, R. (2007) Short pyrosequencing reads suffice for accurate microbial community analysis. ***Nucleic Acids Research*** 35.

McHardy, A.C., Martin, H.G., Tsirigos, A., Hugenholtz, P., and Rigoutsos, I. (2007) Accurate phylogenetic classification of variable-length DNA fragments. ***Nature Methods*** 4: 63-72.

Margulies, M., Egholm, M., Altman, W.E., Attiya, S., Bader, J.S., Bemben, L.A. et al. (2005) Genome sequencing in microfabricated high-density picolitre reactors. ***Nature*** 437: 376-380.

Naeem, S., and Li, S. (1997) Biodiversity enhances ecosytem reliability. ***Nature*** 390: 507-509.

Patterson, D.J. (1999) The diversity of eukaryotes. ***American Naturalist*** 154: 96-124.

Sogin, M.L., Morrison, H.G., Huber, J.A., Mark Welch, D., Huse, S.M., Neal, P.R. et al. (2006) Microbial diversity in the deep sea and the underexplored "rare biosphere". ***Proceedings of the National Academy of Sciences of the United States of America*** 103: 12115-12120.

IV. Description of the project
IV.A. Detailed description of the research area and research plan

BioMarKs – Biodiversity of Marine euKaryotes

Background The biodiversity of marine unicellular eukaryotes (protists) is largely underestimated, misunderstood, and underexploited, despite its extreme relevance for climate change and marine ecosystem functioning, for human health (water and sea-food toxicity), economy (ballast water, coastal tourism, fisheries), and biotechnology (biofuels, bioactive molecules, nano-technology). ***Marine protist biodiversity arguably represents the least explored biodiversity compartment on Earth, despite being one of the most reactive and influential in terms of global ecology and climate change*** (Fig. 1 and caption).

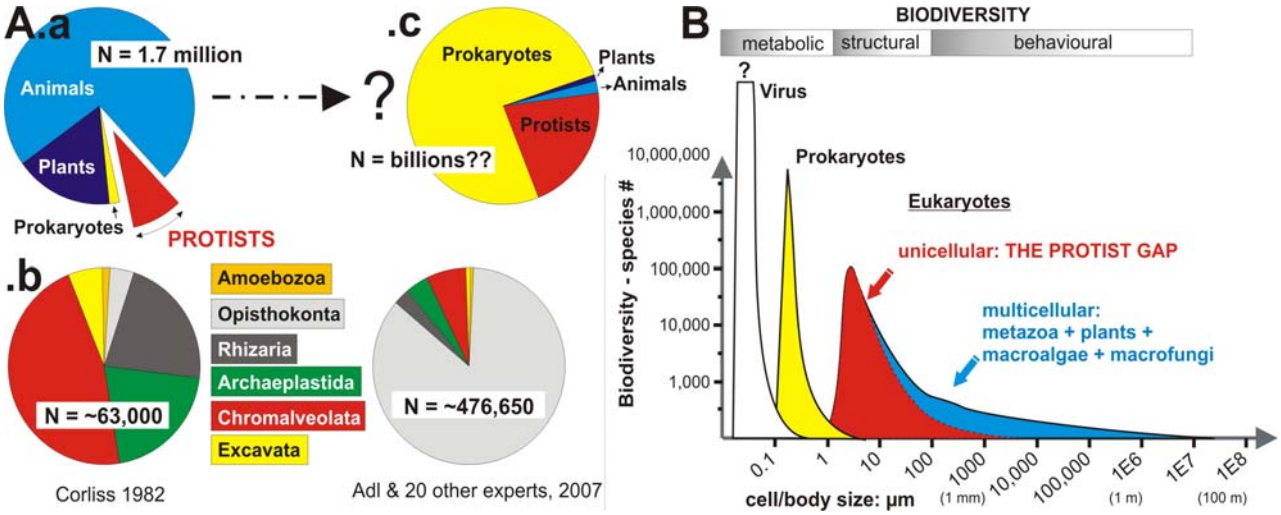


Figure 1: *The marine **protist** biodiversity **gap***. **A. a**: Total number of living phenotypically *described* species on Earth (N=1.7 million; from *International Union for Conservation of Nature*). The relatively lower numbers of unicellular (pro- and eu-karyotic) *species* principally reflects the history of biological science, dominated by *de visu* and microscopy observations since the 1600s. **b**: 1982 (single author) and 2007 (consortium) surveys of *described* species of unicellular eukaryotes and their classification among the higher level protist super-groups[1]. These censuses are highly dependant on the number (and character!) of experts working on particular groups. The 2007 picture is for instance largely skewed toward fungi and diatoms; estimation of the "real" biodiversity by the same expert consortium, based on preliminary molecular datasets and gut-feeling, reached ~18 million species. In fact, estimations based on e-rDNA PCR based clone libraries, specialties of part of the *BioMarKs* consortium for the last decade, have shown that eukaryotic microbial diversity is far greater than previously catalogued. Recent *group-specific* PCR of *shorter* e-DNA fragments (which reduces classical PCR biases) have further shown that protist biodiversity remains largely undersampled even using standard molecular approaches. Overall, recent molecular exploration of biodiversity tends to support the hypothetical distributions shown in **Ac**. and **B**. *Hypothetical* number of species (a species being defined as a distinct genome/phenotype couple at a given time) of viruses, prokaryotes, and eukaryotes in relation to cell/body size. The red area depicts the "***protist gap***", where most eukaryotic species are still hidden, despite their fundamental biogeochemical importance and their key position as a bridge between prokaryote and animal/plant biodiversities.

Recent major technological advances in parallel sequencing using micro-fabricated high-density picolitre reactors (454 sequencing) permit rapid and ever cheaper acquisition of massive amounts of DNA or rt-RNA sequence data from the environment. This new technology can analyze 400,000 ~250bp sequences with 99.5% accuracy in a single run (1 day), and 1 million ~400bp fragments per run should be available by the end of 2008. The technique also circumvents the need to use clone libraries, thus removing a strong source of artifact when analyzing e-DNA. This provides the prospect, for the first time, of conducting nearly-exhaustive surveys of microbial diversity and population dynamics on selected temporal and spatial scales. 454 data will radically alter perceptions in microbial ecology and undoubtedly provoke a step-change in understanding of the role unicellular organisms play in the Biosphere.

Hypotheses: *BioMarKs* will test 3 main interconnected hypotheses: (*1*) A major component of eukaryotic biodiversity still remains *hidden* amongst currently un-investigated marine protists, particularly in the world of small eukaryotic cells (2-10 µm) (Fig. 1B); (*2*) A large proportion of marine protist diversity evolves and resides in benthic marine habitats, where it plays the role of a reservoir and seed-bank, on ecological to geological time scales, for the climatically highly reactive and proactive planktonic biodiversity; (*3*) High biocomplexity and endemism of marine protist biodiversity are key determinants for biogeochemical cycles, ocean ecology, as well as human health, economy, and ecosystem services. The 30 scientists within the *BioMarKs* consortium propose a highly integrative and multidisciplinary approach based on 454 sequencing of protist taxonomic marker sequences from ecologically and economically relevant sites in EU coastal waters to assess the taxonomic diversity, environmental significance, and human health and economic implications of marine protist biodiversity.

Scientific objectives & work packages: Specific objectives of *BioMarKs* are:

i) to establish a comprehensive understanding of protist biodiversity in EU coastal waters and marine sediments and to detect the most ecologically relevant taxa using massive parallel rDNA/RNA sequencing integrated with environmental and phenotypic metadata.
ii) to combine the genetic and contextual data to investigate ecological and evolutionary questions in marine protist diversity : How many, Who, Where, When, and Why?
iii) to correlate change in marine protist diversity with global ocean acidification and anthropogenic pressures (pollution, nutrient input, and ballast water).
iv) to assess the ecosystem services of marine protist diversity and identify its relative role in terms of global ecology and human food, health, and biotechnology.
v) to conceive new methods and tools, based on combined massive DNA sequencing, bioinformatics, and statistics, for monitoring future eukaryotic microbial diversity change and for evaluating its economic implications.

Objectives (i) and (iii) address ***theme a*** of the *BiodivERsA* call (global change and biodiversity dynamics). In (i) we will establish a census and gold-standard baseline of marine protist biodiversity which will serve as a reference to survey future biodiversity change; in (iii) we will assess the impacts of global climate change on marine protist biodiversity and provide the framework for predicting the feedbacks of protist biodiversity change on climate. We will also evaluate coastal ecosystem responses to biological invasions (ballast water) and human-induced changes (e.g. port pollution). Objective (ii) relates to ***theme b*** (ecosystem functioning). Expert analyses of the *BioMarKs* dataset will pinpoint key ecological forces constraining marine protist biodiversity in time, space, and across habitat variation, and thus identify how protist microbial diversity relates to marine ecosystem functioning. Objectives (iv) and (v), to identify how microbial eukaryotes contribute to human ecology and assess their role in environmental sustainability, are connected to ***theme c*** (ecosystem services). The *BioMarKs* objectives are organized into 4 WPs with distinct but interrelated purposes. The ***practical*** WP1 will be led by P1, with substantial input from P2, P5, P7 for sample and metadata collection, from our external partner GENOSCOPE for massive DNA data production, and from P4 for bioinformatics support. The ***theoretical*** WP2 will be organized by P3, with statistical assistance from P4, P6, and P7. The ***ecological*** WP3 will be directed by P5, working mainly with P1. The ***economic*** WP4 will be organized by P4, through an interaction between the *BioMarKs* consortium and the *BioMarKs* stakeholders, in particular Dr. Harold Levrel (IFREMER).

***BioMarKs* general strategy** Our overarching strategy is summarized in Fig. 2. The sampling strategy was carefully designed to optimize the temporal, spatial, and ecological dimensions addressed in the main hypotheses and objectives of *BioMarKs*. Three

fundamental kinds of data will be collected: *genetic* (DNA sequences), *contextual* (ecological parameters), and *archival* (DNA, RNA, and cellular samples). The *BioMarKs* data warehouse will then be developed through an *incremental* process involving recurrent quality control and analyses of both *454* sequence tags and metadata. The most relevant genetic novelties will be verified and further characterized using the archived biological samples, and put into ecological perspective by the contextual data. This pipeline will generate a high-quality database of expert annotated taxonomic DNA tags, which will be used to address the scientific and societal questions posed in *BioMarKs*. The database and associated analyses will be shared within Europe and abroad following a strategy of high-impact outreach actions addressed to public and private end-users/stakeholders (see Communication below).

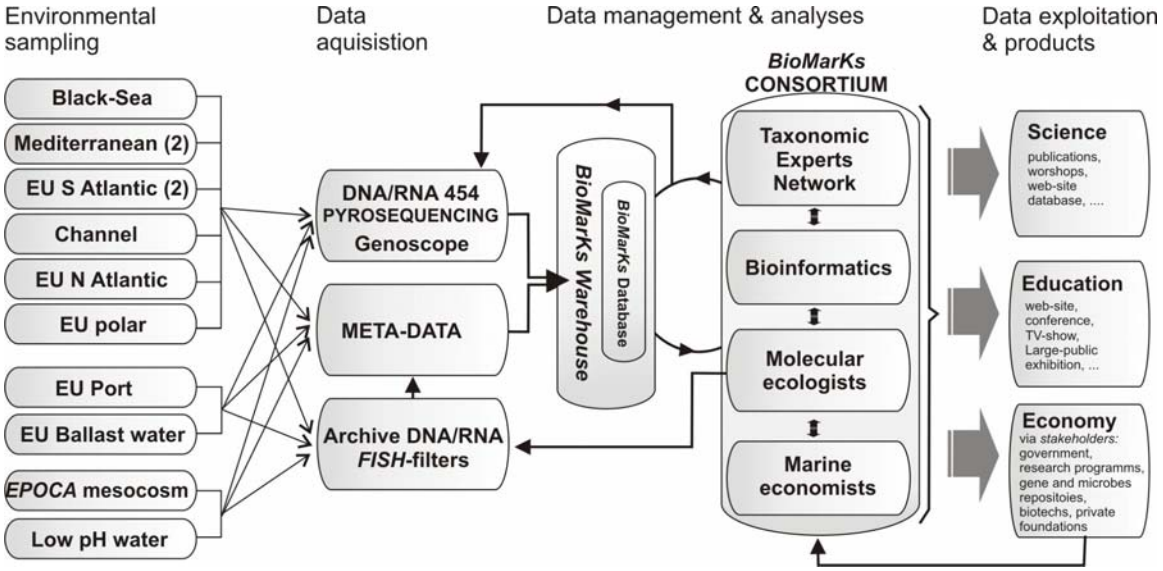


Figure 2: Flowchart summarizing the overall *BioMarKs* strategy.

Detailed work plan.

WP-1: The main goal of WP-1 is to generate DNA sequence tags representing the whole diversity of eukaryotic microbes from marine samples and to measure contextual environmental parameters for each sampling site. We propose to explore 9 coastal-water sites in the vicinity of marine institutes located along the EU coastline from Spitzbergen to the Black Sea (Fig. 3). At each site, the entire marine protist community will be sampled in Spring 2009 using Niskin bottles and plankton nets for sub-surface and deep-chlorophyll maximum (DCM) waters, and using mutli-corers for underlying surface sediments. A second round of samples will be acquired in Fall 2009. Planktonic samples will be split into 3 size-fractions, 1-3/3-20/20-1000µm, using a newly designed gravity sieving process. Total DNA and RNA will be extracted from samples from the 3 depths and 3 cellular size fractions and aliquoted for 454-sequencing and for cryopreservation as archive material. A suite of 33 physical, chemical, and biological contextual data will accompany each discrete DNA/RNA sample (Fig. 3).

Genetic exploration of 35 of these environmental samples will be undertaken using 31 *454*-sequencing runs (i.e. from ~12 and 31 million DNA sequence tags of length ~200 to 400bp, depending on technological progress by 2009) in collaboration with *GENOSCOPE* (France). *BioMarKs* consortium members have developed and are currently comparing SSU V4 and V9 general eukaryotic primers for e-DNA analysis and 454 sequencing and will identify which primer set performs best or whether a combination of primer sets is required to assay the full diversity of eukaryotic microbes.

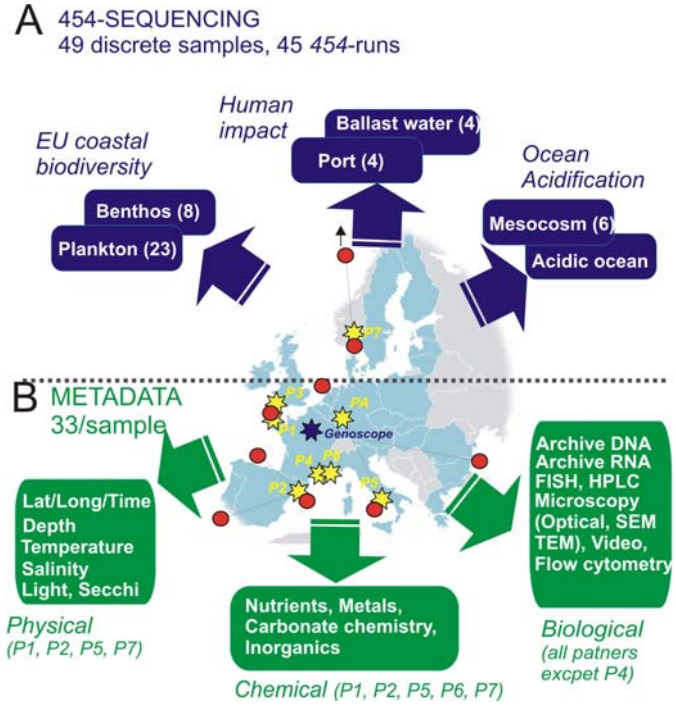


Figure 3: *BioMarKs* partners (yellow stars), sampling sites (red dots), DNA sequencing effort (A), and contextual data (B). **A**. *454* sequencing will comprise 18S rDNA, rt 18S rRNA, and 28S rDNA tags for each discrete sample. The number of *454*-runs for each sample category is given in brackets. The detailed sampling plan is available on request: vargas@sb.roscoff.fr **B**. Physical, chemical, and biological contextual metadata associated with each discrete sample. The biological metadata are particularly important in order to (i) provide the phenotypic biodiversity for each sample, (ii) provide archive DNA and FISH samples to further understand the phylogenetic position and numerical abundance of key protists revealed by 454-sequencing. A significant amount of total DNA, total RNA, and FISH-filters will be cryopreserved for testing downstream hypotheses. P1, P2, P5, and P7 will be responsible for sampling at respectively Roscoff/Varna/Svalbard, Barcelona/Faro/Giron, Naples/Rotterdam, and Oslo. They will also be responsible for acquiring the physical metadata for each sampling site. Chemical and biological downstream analyses will be performed by *BioMarKs* partners at their home institute using standardized protocols.

We will use an *incremental* and iterative approach to sequence sampling: (***i***) 18S rDNA and reverse-transcribed 18S rRNA fragments will be PCR amplified using either the V9 and/or V4 eukaryotic general primers (distinctly tagged for each discrete sample) and a multiple PCR amplification approach combined with a micro-gradient annealing temperatures and high-GC buffer in order to minimize individual PCR reaction bias. **With this dual approach, the rDNA analysis will provide data on *diversity* while the rRNA analysis will provide a picture of relative *abundance/activity* of protist taxa**. The PCR products of each discrete sample (from a given time, depth, and cell-size fraction) will be subject to >10,000 454 sequence reads enabling a first assessment of biodiversity complexity; (***ii***) analyses of sequence tag singletons and saturation curves for each sample will then allow estimation of the additional sequencing effort needed for each sample to achieve an exhaustive 454 sequence tag survey; (***iii***) the sequence tags will be processed via a bioinformatics pipeline (see below), general taxonomic affiliation identified and then the sequence data distributed to the *BioMarKs* network of taxonomists, whose expertise covers the entire biodiversity of oceanic protists. Each consortium member will then provide reports on the diversity, biological novelties and potential problems of the dataset within their group of interest. (***iv***) Protist groups with highly complex diversity profiles will then be subject to PCR and *454*-sequencing using group specific primers known to sample rDNA markers that more closely approximate ecotype diversity (e.g. LSU-D2, ITS1 or ITS2 tags). A similar incremental 454-sequencing approach (with exploratory runs followed by deeper sequencing) will be used to explore biodiversity using these faster-evolving rDNA tags. Three members of the *BioMarKs* consortium are active participants of the research program *ICoMM* (*the International Census of Marine Microbes, MBL, Woods Hole*), in which various protocols and rDNA fragments are currently being tested on calibrated samples in order to establish a standard protocol leading to a worldwide complementary and synergistic effort. *ICoMM*, which is pioneering similar approaches on marine bacteria, is a key *BioMarKs* stakeholder.

WP-2: We will recruit the unique taxonomic expertise and interdisciplinary strength of the *BioMarKs* consortium for combined analyses (direct interpretation and downstream data acquisition) of the *BioMarKs* database. Fundamental questions about the evolution and ecology of marine protists will be re-explored using a rDNA sequence sampling (and thus taxonomic depth) approaching saturation for the whole protist community and across numerous environments. All operational taxonomic units (OTUs) will be clustered into unique

types allowing for sequencing error. Each OTU will be associated to an identifier code (including Lat/Long/depth/time), taxonomic affinity (when possible), and the suite of associated metadata. Multivariate direct gradient analysis (e.g. canonical correspondence analysis) will allow investigation of relationships between environmental 'meta-data' variables and distribution of OTUs, thus identifying the environmental factors that strongly correlate with the presence and absence patterns of OTUs. Such analysis will also be conducted in order to correlate environmental characteristics with the relative abundance of genotypes using the detection rates of each genotype as derived from rRNA based protocols. Monte Carlo permutation tests will be used to identify ecological characteristics (ordination axes) and environmental variables that explain variation in OTU distribution with greater significance than chance distribution. To exclude the possibility that patterns of genotype distribution are products of geography rather than environmental selection, we will plot all sampling positions using GIS (MapInfo v8.0) and use Mantel tests to test for a correlation between compositional similarity and geographical location. The results of these statistical tests will be further investigated using secondary examination of archive biological material from the original samples. P1, P2, and PA will quantify the distribution and relative abundance of important novel marine protists (revealed key players, or rare new phyla) using group specific FISH, CARD-FISH, and COD-FISH protocols in order to further test associations between environmental variables and the relative abundance identified using the statistical analyses. To improve the estimation of phylogenetic position of any highly unique novel taxa that cannot confidently be assigned to a known phylo-group based on the *454* tag sequence alone, all partners except P4 and P6 will be involved in extending the *454* rDNA sequence read to encompass the majority of the SSU and LSU encoding sequences. This will be achieved using primers designed to be specific to the *454* sequence tag coupled with up- and/or down-stream general-eukaryote primers for PCR amplification from archived DNA in preparation for Sanger deoxy sequencing. These longer sequences will be integrated into comprehensive phylogenetic analyses. The general morphology of all highly novel OTUs will be investigated using FISH.

This strategy will enable *BioMarKs* to address several key questions in eukaryotic microbial ecology: how important is the marine *protist* biodiversity *gap*? How is marine protist biodiversity distributed throughout the tree of life, and what might be the evolutionary and/or ecological significance of this distribution? Is there a relation between cell size/volume and biodiversity in marine protists? Who are the key ecological/ biogeochemical players, and are we missing important ones? Is most marine protist biodiversity part of a "*rare biosphere*", as was recently shown for marine bacteria, and why (in other words are there really rare species, or rather is "*everything everywhere*" with less abundant species simply being dormant stages ready to start active growth under favorable conditions)? How biodiversity-rich are marine sediments as compared to overlying waters, and do they play the role of a biodiversity reservoir and seed bank for the planktonic, climatically highly reactive, protistan biodiversity, on both ecological (resting stages of planktic species) and evolutionary (benthic species with adaptive potential to become planktonic) time scales? What are the ecological parameters shaping marine protist diversity and distribution?

WP-3: Another 14 discrete planktonic samples will be analysed using 14 additional *454*-pyrosequencing runs and methods as described in WP1 and WP2, in order to **investigate how marine protist communities react to local and global anthropogenic pressures.** We will first assess the effect of *ocean acidification* on marine protist community acclimation and adaptation dynamics, in collaboration with two of our main external partners. The first set of samples will be collected in the frame of the EU-FP7 large-scale integrated project *EPOCA* (*European Project on Ocean Acidification*; http://epoca-project.eu/). The main fieldwork component of *EPOCA* consists of a pelagic CO_2 enrichment experiment using 6 mesocosms (65m^3 volume each), to be conducted near the marine station of Ny-Alesund, Spitzbergen, in

June/July 2009. A total of 8 *BioMarKs* 454-runs are budgeted to analyze both the pristine total marine protist community in these Arctic waters and underlying sediments, as well as patterns of community change under various levels of pCO_2 perturbation. The data will provide a taxonomically comprehensive overview of which marine eukaryotes are physiologically resilient to pCO_2 changes. However, oceanic protists may have high genomic adaptive capability and speciation rates, essential traits overlooked in mesocosm experiments. Therefore, we will assess protist biodiversity for its adaptive speciation to ocean acidification by sampling protist communities in what is, to our knowledge, the only modern high CO_2/low pH oceanic zone which can be used as a model system for predicting future biotic change. West and north of the Galapagos Islands, the Pacific equatorial undercurrent upwells acidic waters (pH 7.6-7.7) close to the surface. This water-mass may well contain communities of protists pre-adapted to acidic oceans, and our analyses will permit testing of the predictions based on physiological acclimation observed from the *EPOCA* CO_2 enrichment experiment. We will sample this system in collaboration with the European project *Evolution2009* on board the *Tara* (http://www.taraexpeditions.org/). This 2 year (2009-2010) *public access to science* discovery expedition around the globe, funded by *Agnes B,* celebrates the 200 year anniversary of Darwin' birth and Lamarck's work on evolution. The *BioMarKs* consortium will offer its expertise in marine protist taxonomy to *Evolution2009*, and will participate in the Galapagos leg of the cruise, this symbolically linking past (Darwin), present (*Evolution2009/BioMarKs*), and future (fate of plankton in a high-CO_2 world). Note that this Galapagos sample will not only be very complementary to the *EPOCA* samples, but will also serve as a geographical outgroup for the EU coastal-water samples.

Furthermore, we plan to reserve seven *454-runs* (up to 7 million sequence tags) to assess the effect of a major industrial port and ballast water exchange on planktonic protist biodiversity using the methods outlined in WP1-2. Ports and associated activities clearly alter the physical and chemical nature of coastal waters and *BioMarKs* will assess the effect this has on biodiversity. In particular, research on the effect of invasive organisms has been an ongoing activity in EU member states for several decades because of devastating effects on the environment. It has been estimated that a single cargo ship contains an average of >7000 genetically different species in its ballast water. This part of *BioMarKs* will be undertaken in collaboration with Dr. Marcel Veldhuis at the Royal Netherlands Institutes for Sea Research, and other international experts in the field of biological hazards related to ballast water. Our target will be the port of Rotterdam in the Netherlands (http://www.portofrotterdam.com/), which covers 10,000 hectares and is the largest logistical and industrial hub in Europe, with more than 500 scheduled services linking it with over 1000 ports worldwide. 4 total planktonic samples (integrating the water column and cell size range 1 to 1000 µm) will be analyzed along a gradient from inside the port to "natural" waters outside the port area. In addition, 3.5 *454*-runs will be used to explore the biodiversity "aging" of ballast water from a cargo-ship travelling from the Mediterranean Sea to Rotterdam. Samples will be collected at 4 time points (initial ballasting, pre- and post- open water ballast exchange, and final de-ballasting), and V4 and/or V9 rDNA and rt rRNA tags will allow analysis of changes in total protist community and in its active part.

WP-4: This package aims to develop practical and theoretical approaches to surveying marine protist biodiversity, and to evaluating its ecosystem value and societal/economical implications. As *BioMarKs* has an extremely high discovery component, this more practical part of the project, which relies on pre-analyses of data, will mostly be accomplished over the last 18 months of the project. During the first workshop (month 17), preliminary identification of how the *BioMarKs* methods and dataset could be applied to ecological, health-related, or economical evaluations will be conducted. At completion of the total 454-sequencing effort (month 22), a complete listing of all species and new genetic types discovered in the *BioMarKs* database will be compiled and used as raw material in the second *BioMarKs* workshop (month 26). This workshop will be co-organized with the help of an external

collaborator Dr. Harold Levrel from the *Marine Economics Department* at IFREMER (Brest, France). After a 1-day synthesis of significant *BioMarKs* results, 2 days of the workshop will be dedicated to discussion on the ecosystemic services provided by eukaryotes biodiversity and populations. This process should permit to develop a first *list of ecosystem services*, regarding the results of the research program at this stage. For this, we will use the *Millenium Ecosystem Assessment* framework, and in particular its classification of ecosystem services (supporting, provisioning, regulating and cultural). The *BioMarKs* data and methods should also help conceiving new practical and statistical methods to survey marine protist biodiversity in an operational way for having long term information system on ocean ecosystem services. The challenge will be to find common statistics between bioinformatics, ecology, and economy. Key stakeholders including environmental agencies will be invited to this meeting, and a workshop report will be distributed to all stakeholders and other interested parties at month 30. The new methods, tools, and statistics for monitoring future eukaryotic microbial diversity change and for evaluating its economic implications will be made available though the *BioMarKs* website.

Data management and bioinformatics Given the unprecedented scale of the *BioMarKs* eukaryotic rDNA tag sequencing campaign, of the associated metadata, and the potentially huge number of new species to be classified, "*Data Management and Bioinformatics*" (DMB) will be a critical component across the 4 work packages. DMB activities and deliverables are of three types: (***type 1***) the production of computer-based tools and databases directly linked to the internal support of experimental activities (WP1, WP3), (***type 2***) the production and use of new analytical tools required for the analysis and visualization of massive new *BioMarKs* data output (tag clustering, taxon assignment, correlation studies, etc) (WP2, WP3), (***type 3***) the design and implementation of an internet portal for the public release of *BioMarKs* activities, raw data, analyses, and offering suitable tools for further analyses by the scientific community (WP1, WP4). As various levels of bioinformatic expertise are available among P4, P6, P7, and P3, and as the analysis of the biodiversity data on different taxa by different research groups will require specific tools and approaches, DMB work will be organized in a *distributed* manner, taking advantage of a *central data warehouse* (i.e. a data repository associated to tools to extract, transform, and load data, and tools to manage and retrieve metadata), a collaborative development site (e.g. Gforge), periodical internal workshops, and coordination by a dedicated post-doctoral scientist hired for this purpose. In approximate chronological order, DMB activities will cover the following tasks:

Computational optimization of experimental design (type 1)

- Primer refinement (universal and taxon specific) (P6)
- Evaluation of sampling completeness and optimization of the tag sequencing strategy (e.g. singleton testing) (P6, P4)
- Tag sequence quality control (e.g. contamination detection, homopolymer artefacts) (P4)

Design and operation of the *BioMarKs* data warehouse (internal use) (type 1)

- Definition of a simple sequence data & metadata structure (with experimentalists)
- Definition of a controlled vocabulary (with experimentalists)
- Design/implementation of a simple interface for controlled data input (P4, P6)
- Design/implementation of a simple query interface (e.g. MySQL) (P4, P6)
- Cleaning/implementation of a reference eukaryotic rDNA sequence database derived from the available public repositories (e.g. Genbank, SILVA) (P6, P1)
- Development/testing/implementation of suitable algorithms for fast taxonomic assignment by sequence comparison of experimental short tags with the reference rDNA database (P4, P6, P7)

Design and operation of a linux-based collaborative software development server (type 1)

- Use of Gforge (gforge.org) for data and source-code internal exchange between experimentalists and bioinformaticians (P4, P6)

Multi-centric development of software tools for specific data analysis (type 2)

- Using the Gforge server for
- Quantitative/qualitative analysis and visualization of global biodiversity, data mining (e.g. PCA) (P4, P6, P7, P3, P8)
- Quantitative/qualitative analysis and visualization of taxon-specific diversity, data mining (P4, P6, P7, P3, P8)
- Quantitative correlation of biodiversity with metadata (P3, P7, P8)
- Quantitative correlation between taxa (P4)
- Mathematical modeling of biodiversity (P4)
- Production of publication-grade figures (P4, P3, P7, P8)

Design and operation of a public internet portal (type 3)

- Initial presentation of the project – rationale, goal, intended applications (P1, P4)
- Presentation of the main taxa, rDNA reference sequence database (P6, P1)
- Public taxon assignments server (against the rDNA reference sequence database) (P4, P6)
- Similarity search public server (against the *BioMarKs* tag database) (P4, P7)
- Throughout the project (contributed by all partners and under the responsibility of the program manager/outreach officer at P1): community-targeted documentation (video of sampling campaigns, microscopy images, scientist interviews), biodiversity maps, incrementing biodiversity "counter" (e.g. number of newly discovered species), popularization of the most spectacular *BioMarks* research highlights.

Close collaboration with the bioinformatics team of the *ICoMM* program will be established from the outset of *BioMarKs* to promote synergetic software development between Europe and the US.

Justification of resources requested The *BioMarKs* budget is based on precise calculation of the numbers of samples to be analyzed by 454-sequencing (GENOSCOPE), SEM and TEM (P1, P2 P5, P7, P8), flow cytometry (P1, P2), FISH (P1, P2, P7, PA), PCR and qPCR (P1, P2, P3, P5, P7, PA), HPLC (P6), and other standard chemical methods. The 45 454-runs (or up to 45 million sequence tags) requested should allow covering the taxonomic and ecological scales addressed in *BioMarKs*. The budget reflects also the relative and specific effort each partner will invest in the project, mainly in terms of man-power. P1, P2, P5, and P7 will be responsible for sampling and processing environmental DNA and associated metadata over the first year of the project. P1 requests a part-time lab technician to coordinate centralization of all samples for 454-sequencing and perform all primary tagged PCR reactions before shipping the products to GENOSCOPE. All partners except P4 and P6 will contribute to further molecular and chemical analysis of *BioMarKs* samples and validation/interpretation of the genetic dataset processed by P4. P4 will be responsible for bioinformatics, in close collaboration with P6, P3, and P7. All partners will be involved in statistical analyses and interpretation of the data. P1 and P7 are associated to national-level bioinformatics community-servers (e.g. P4, P7) through which >300 cpus of computational power is available. This available hardware, software, and expertise (in general sequence analysis, similarity searching, multiple alignment, phylogeny, large-scale tag clustering, database design and maintenance, rDNA sequence analysis, primer design, etc) will serve to jump-start the analysis of the *BioMarks* project data at no new computer equipment cost. Except for 454-sequencing, most of the *BioMarKs* budget is thus dedicated to post-doctoral salaries (total of 186 months) distributed among the network, and to lab consumables for downstream analyses of contextual and archive data.

EU added values, relevance to policy application, and importance for biodiversity issues. The *BioMarKs* project represents much more than a collection of eukaryotic rDNA genes from the oceans. The *BioMarKs* database of annotated genetic and contextual data will become the largest world community resource on marine unicellular eukaryotic biodiversity, providing a reference platform for current and future projects worldwide dealing with this

important biodiversity compartment. To our knowledge, no similar *integrated* effort exists, in particular in the US where *individual* groups are clearly leading the DNA-based exploration of marine *prokaryote biodiversity* (IcoMM, http://icomm.mbl.edu/) and metagenomes (CAMERA, http://camera.calit2.net/). Eukaryotes, unlike prokaryotes, have been studied by generations of taxonomists over the last 3 centuries and most of the few remaining experts are based in Europe or Canada. An important challenge of *BioMarKs* is to valorize this traditional and invaluable EU knowledge-base by building a link between the most advanced DNA sequencing technology and the taxonomic experts. Beyond those within the *BioMarKs* consortium, we have established a list of most EU and worldwide protist experts who will be kept informed of project results and regularly consulted on data analysis and interpretation. In addition, a significant effort of method standardization (sampling protocols, PCR primers, *454*-sequencing) will occur between the EU research institutes participating in the project (official partners and collaborating sites, Fig. 3). The unparalleled taxonomic depth in *BioMarKs* (up to 45 million tags) will also provide a supporting database and essential connection between several EU and international projects involving marine protist biodiversity (see below).

In terms of policy, The *BioMarKs* database and associated tools will be of direct relevance in several EU legislative frameworks, including:
Marine transport regulation: The discharge of ballast water is a major pathway for the transfer of alien aquatic species and potentially harmful organisms and pathogens around the world. It has been estimated that 1 - 3 billion tons of ballast water are discharged into EU ports each year, and that the economic cost due to marine bioinvaders amounts to 10s of billions €/year. The most important international marine environmental conventions relevant to this issue are Marpol 73/78, which was designed to minimize pollution of the seas, including dumping, oil and exhaust pollution, and more recently the IMO International Convention for the Control of Ship's Ballast and Sediments (2004) which requires the introduction of mandatory ballast water management from 2009, and not later than 2016, and also contains regulations concerning sea areas where ships can exchange ballast water during the transition period until treatment facilities are available: the area must be at least 200 nautical miles from the nearest land and have a water depth of at least 200 metres. Where this is not possible, ships should stay at least 50 nautical miles from the nearest land, at a water depth of 200 metres. Special areas for ballast water exchange may be designated jointly by neighbouring states. *BioMarKs* will not only directly address this question, but also provide new molecular methods and database tools which will be particularly useful in the context of the future development of ballast water treatment technologies. ***Coastal management and tourism***: Coastal zones are typically areas of intense human activity and are important in diverse economic and societal contexts. Most of this activity impacts the coastal environment, notably in terms of water quality. The most recent EU legislation in this domain is the Water Framework Directive (2000/60/EC) which requires that all inland and coastal waters must reach at least "*good*" status by 2015 and defines how this should be achieved through the establishment of environmental objectives and ecological targets. A suite of complimentary legislation related to Coastal Zone Management exists, notably the new Bathing Water Directive (2006/7/EC) and the Habitats Directive (1992/43/EC). *BioMarKs* will produce data and tools which will be directly relevant for current implementation and future refinement of these policies. ***Sea food safety***: Various toxins produced by marine protists can accumulate in larger organisms such as fish and molluscs and can ultimately affect both marine metazoan and human consumers. Monitoring for 'harmful algal blooms' (HABs) is a requirement of EU Directive 1991/492/EC and most EU states have monitoring programs to ban shellfish harvesting during HAB events or when shellfish reach a specified level of toxicity that is potentially harmful to humans. This seriously impacts fisheries and aquaculture with economic losses in the EU conservatively estimated at >100 million €/year. *BioMarKs* data will reveal the taxonomic and biogeographical complexity of potentially toxic marine protist taxa and will

provide important information on biodiversity fluxes between the sediment and water column (planktonic HABs are often seeded from resistant stages residing in sediments). Group-specific LSU rDNA D2 454-sequencing may well be *the* method of choice in the near future for rapid and low cost monitoring for the presence of hazardous microbial species from large amounts of samples. Here again, *BioMarKs* will produce essential data and tools to assist management of this important health issue.

Finally, *BioMarKs* will provide by far the largest census of marine unicellular eukaryotes from EU coastal waters ever undertaken. It will thus serve as a critical *reference baseline* for future surveys of marine protist community change using similar methods. This will be essential for global management of EU marine waters, and to monitor how marine communities react to global change. Here *BioMarKs* is at the center of several fundamental issues in global biodiversity. Protists do not only represent the largest biodiversity gap in eukaryotes, but they have been shown to rapidly react to global climate change, and in turn impose significant feedbacks on the Earth system. Indeed, many marine protists living in planetary populations with extreme turnover rates build complex nano- and micro- inorganic skeletal structures which arguably generate the largest fluxes of biological material on Earth and fundamentally impact global geochemical cycles and climate. Very little is known on how protists will react to high CO_2 oceans and how changes in protist communities will force new biogeochemical constraints on the Earth system (in terms, for instance, of primary productivity and carbon flux to the lithosphere). For the first time, these basic questions will be addressed at the level of the entire protist community, establishing solid foundations for future research in this unexplored but critical field for predicting the co-evolution of climate and biota. Furthermore, the hundreds of thousands and possibly millions of marine protists to be unveiled in *BioMarKs* likely hold a phenomenal repertoire of unknown genes. Protists have large genomes (often much larger than the human genome) with typically thousands of genes. This genetic biodiversity predates and exceeds the relatively smaller gene repertoire within plants and metazoans. Recent sequencing of oceanic metagenomes has revealed a huge unsuspected diversity of microbial species and genes in prokaryotes and viruses, but eukaryotes have not yet been included in these analyses despite their closer relationship to us. *BioMarks* will allow estimation of the global ocean eukaryotic genomic diversity, and will lay the foundation for taxonomically controlled eukaryotic metagenomics. The immense and unexplored repertoire of protist genes is potentially an outstanding source of innovation for green energies, pharmaceutics, cosmetics, and nanotechnology.

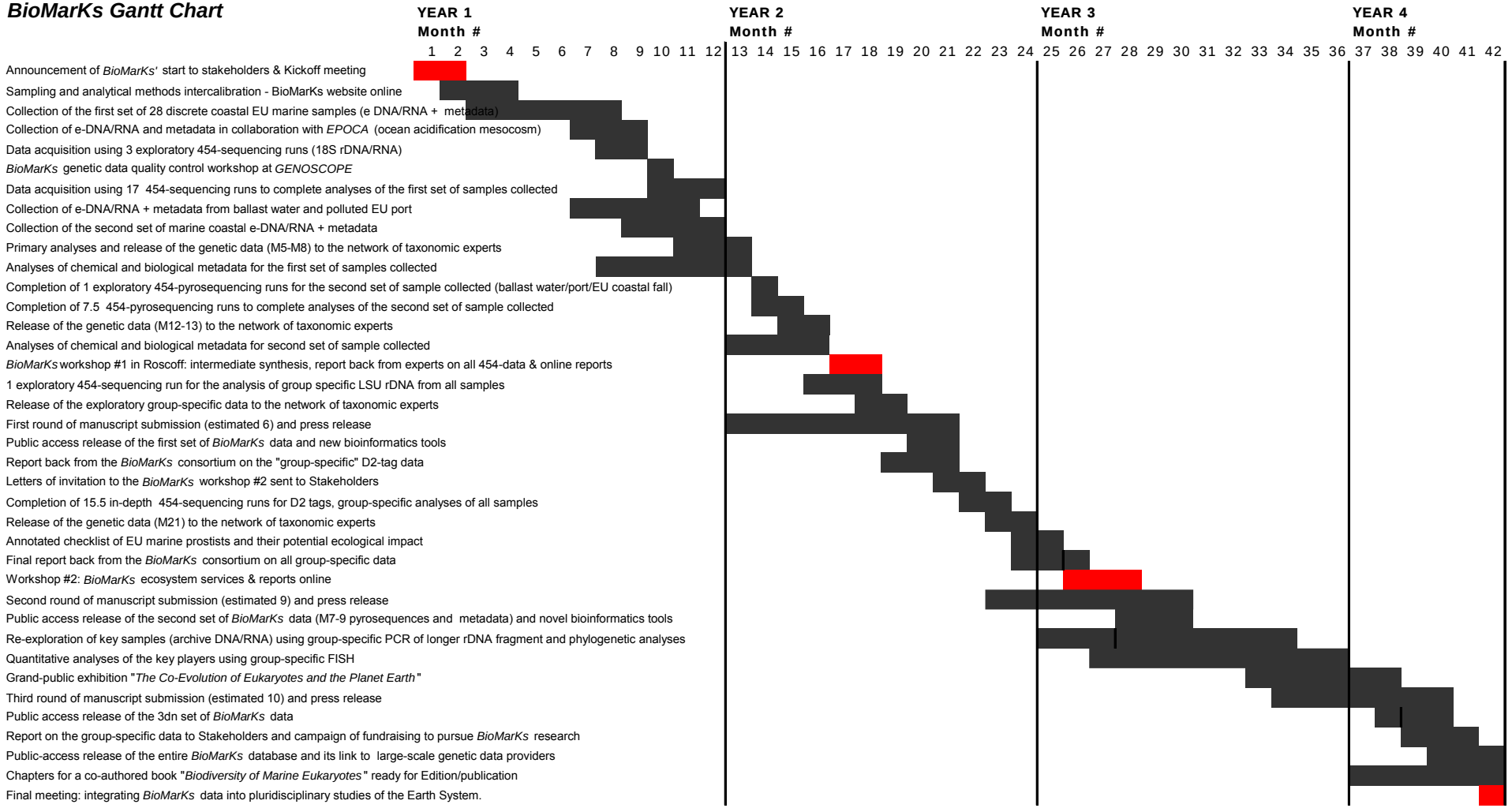
Proposed exploitation of project results *BioMarKs* data will first and foremost be the raw material for *at least* 25 high-impact scientific publications consortium members. However, the deliverables and pluridisciplinary products of the project, such as the *BioMarKs* database, bioinformatics tools, molecular methods and protocols, the checklist of EU marine protists, the *BioMarKs* ecosystemic services, and educational tools, will directly impact a broad range of end-users and stakeholders. P1 will hire an Outreach Officer who will be responsible for maintaining a constant flow of information to a broad list of potential end-users following a well-structured communication plan (see below). End-users may become stakeholders, or even collaborators if their interests do not dilute the optimal development of *BioMarKs*. Finally the *BioMarKs* database of genetic and contextual information on marine protists will be a fully open-access environment at the end of the project. We will work with major genetic data repositories and distribution centers (*SILVA*, F-O Gloeckner, Max Planck Institute, *EMBL*, Cambridge, *CAMERA*, JCraig Venter Institute, USA) to maximize the compatibility of our data with these stable and invaluable community resources for research on biodiversity.

IV.B. Communication plan

A comprehensive and professional communication strategy is clearly essential for the short-term success and longer-term impact of a modern research program. We have established direct contact with a diverse suite of key EU and international potential end-users, who were invited to become *BioMarKs* stakeholders by formally expressing their interest in specific aspects and products of the program. Among the stakeholders who responded positively are: (*i*) **key international research programs on marine biodiversity** (such as ICoMM; CAMERA; *CoML* – "*Census of Marine Life*" http://www.coml.org/; CBOL – "*Consortium for the Barcoding of Life*", http://www.barcodinglife.org/; SESAME – "*Southern EU Seas: assessing and modeling ecosystems change*", http://www.sesame-ip.eu/; or the NoE MarBEF – "*EU Network of Excellence: Marine Biodiversity and Ecosystem Functioning*", http://www.marbef.org/); (*ii*) **major international genetic databases and protist culture collections** (for example *SILVA* - the most comprehensive taxonomic rDNA database, http://www.arb-silva.de/, and repositories for marine protist cultures from USA (*CCMP*), UK (*CCAP; MBA Plymouth CC*), France (*RCC*), Australia (*CSIRO CCLM*), and Japan (*NIES MCC*); (*iii*) **international, EU, and national governmental and private agencies involved in legislation and monitoring of coastal marine waters** (e.g. IFREMER, France; Lyonnaise des Eaux; Catalan Water Agency); (*iv*) **a suite of foundations and companies with interests in marine biotechnologies**, *green* energy, pharmaceutics, and cosmetics (i.e. Pôle MER BRETAGNE, http://www.pole-mer-bretagne.com/; Pôle MER PACA, http://www.polemerpaca.com/); (*v*) **key scientific personalities in the field of marine science and biodiversity** (e.g. Paul Falkowski, Ginger Armbrust, Marcel Veldhuis, Carlos Duarte, Harold Levrel). The full list of *BioMarks* stakeholders with expressions of interest is available on request. Following these very encouraging responses, a comprehensive list of further end-users will be drawn up by the *Project Implementation Committee* (PIC) and contacted within the initial 3 months of the project. All *BioMarKs* stakeholders will receive regular information on project progress via tri-annual e-newsletters, which will also be widely distributed on relevant e-mail lists and web sites. Provision of e-newsletters will provide the opportunity to seek regular feed-back from all stakeholders; comments, suggestions and collaborative opportunities will be integrated into project functioning at the level of the PIC on a regular basis. In order to better characterize the taxonomic, ecological, and/or economical value of their own data and analyses, stakeholders will be provided with open access to the *BioMarKs* datasets within 3 months of their completion (as individual units, i.e. per sampling site, per season etc.) via the *BioMarks* web portal. Full public open access to datasets will be provided via the same source and via *GenBank* within 12 months of completion. The dramatically increasing sequencing capacity offered by advances in parallel sequencing technology will revolutionize the outlook on biodiversity issues within the next few years. All *BioMarks* partners have exemplary track records of high impact publications. We plan at least 25 scientific publications in international peer-reviewed journals, 9 of which we aim to publish in very high impact-factor (>6) journals. This will significantly contribute to placing the European research community at the forefront of this highly topical field. We also plan to be very pro-active in the essential domain of public education. The *BioMarks* web site will include a section dedicated to vulgarisation of the scientific issues surrounding the project. *BioMarks* partners will take full advantage of the Public Relations offices of their respective institutes to disseminate comprehensible information and press releases on significant advances linked to this research. Notably, we will exploit the striking beauty of marine protist micro-skeletons to assemble a large-public itinerant exhibition "*The Co-Evolution of Marine Protists and the Planet Earth*", to be distributed in major EU Museums of Natural History. The edition of a co-authored book "*Biodiversity of Marine Eukaryotes*" is planned as at the end of the project (2012). The *BioMarKs* expert consortium will also be responsible for the unicellular eukaryote data collected during the expedition *Evolution 2009* (see Work Plan). This expedition will involve important media coverage (including 5 BBC documentaries) and will be an ideal platform for wide dissemination of part of the *BioMarKs* outputs. Finally, we will make a concerted effort to foster longer-term consolidation of the *BioMarKs* approach by making a series of presentations to private stakeholders in order to involve them both financially and in terms of scientific collaborations.

V. Time schedule and working programme (use a Gantt chart or equivalent)

BioMarKs Gantt Chart



VI. Links to national and international research projects and programmes

(max. 1/2 page, Arial font, 11pts, single spaced)

Given the scope and sequencing depth of the project, *BioMarKs* will be the largest world community resource on marine unicellular eukaryotic biodiversity, providing a reference platform for all projects related to this important biodiversity compartment. All partners are successful applicants in more focused national research programs (Table 1) which will greatly benefit from and synergize through the *BioMarKs* expert database and tools. Members of the consortium also participate in several EU projects, which will either collaborate directly with (*EPOCA*, de Vargas; *MITDAL*, Edvardsen and Kooistra), or synergize with *BioMarKs*. In particular, the FP7 infrastructures project *ASSEMBLE,* which aims at enhancing trans-national access to a set of state-of-the-art European marine biological laboratories, will significantly interact with *BioMarKs*. On an international scale, *BioMarks* will be in close interaction with the *ICoMM* program (Mitch Sogin, MBL, Woods Hole, USA), which pioneered the exploration of microbial diversity using 454-sequencing, and holds the reference database for prokaryotes. P1, P6, and P8 are part of the *ICoMM* steering committee. Finally, several EU and international permanent genetic databases have expressed their strong interest in our project. Among these, *SILVA* (F-O Gloeckner, Max Plank Institute, Germany) will particularly benefit from and enhance the international value of the *BioMarKs* data.

Table 1: Example of current research programs involving *BioMarks* partners.

Research Program	Funding Agency	Partner Involved
BOOM - Biodiversity of Open Ocean Microcalcifiers	ANR (IFB), France	P1 (de Vargas)
Assessment of species-level diversity and evolution in marine phytoplankton using molecular, morphological, and fossil data.	CNRS ATIP, France	P1 (de Vargas)
PicoFUNPAC - Picoplanktonic functional diversity in the Pacific	ANR (IFB), France	P1 (Vaulot)
Aquaparadox - Aquatic protist diversity: the paradox	ANR (IFB), France	P6, P1 (Dolan, Guillou, Simon)
PicoVIR - Picophytoplankton-virus interactions in a marine ecosystem	ANR Blanc, France	P1 (Simon)
GEMMA – Genomics and ecology of marine microorganisms	MEC, Spain	P2 (Pedrós-Alió)
PROBA - Prey selection by bacterivorous protists in the microbial food web	CSIC, Spain	P2 (Massana)
CANARAD - Picoplanktonic radiolarians in the Canarian Islands	MEC, Spain	P2 (Massana)
Diversity, identity and ecological role of a novel fungal super clade	NERC, UK	P3 (Richards)
Molecular diversity of microbial eukaryotes using a large-scale parallel tag sequencing strategy	CoSyst (BBSRC/NERC), UK	P3 (Richards)
Comparative genomics and eukaryote cell evolution	Leverhulme Trust Early Career Fellowship, UK	P3 (Richards)
Bioportal - web-based service platform for phylogenomic analysis	UiO, Norway	P7 (Shalchian-Tabrizi)
EPOCA - European project on ocean acidification	EU FP-7	P1 (de Vargas, Probert)
ASSEMBLE - Association of European Marine Laboratories	EU FP-7	P5, P1 (Kooistra, Vaulot, Probert)
MITDAL - Microarrays for the detection of toxic algae	EU FP-7	P7, P5
*Plankton*Net*		
MarPlan MarBEF - Marine Biodiversity and Ecosystem Functioning	EU FP-6	P5, P1, P2, P7
ICoMM - International Census of Marine Microbes (Census of Marine Life)	Sloan Fundations, US private funds	P1, P6, PA
CMarZ - Census of Marine Zooplankton (Census of Marine Life)	Sloan Fundations, US private funds	P1
Decoding Virus Leviathans	NSF, US	P4 (Claverie)
Chlorella genomics	DOE, US	P4 (Claverie)
Emiliania huxleyi genomics	DOE, US	P4 (Claverie), P1 (de Vargas)
Large DNA virus genomics	NEDO of Japan	P4 (Claverie)

VII.A. Budget instructions

Please note that each partner will be funded by his own national funding organization.

To fill in the budget tables, please first follow the BiodivERsA rules (e.g. in Euro, include VAT, be aware of participants ineligible for funding…). For additional budget questions, please make sure to comply with the national rules (e.g. subcontracts, overheads…). ***We advise you to consult your national contact point.***

Teams from the following countries are eligible for direct funding: Austria, Estonia, France, Germany, Hungary, Italy, the Netherlands, Norway, Portugal, Spain, Sweden and the United Kingdom. **(= Partners 1, 2, … N)**

Partners from countries ineligible for direct funding :

- can be associated in the projects, **as NON-FUNDED PARTNERS, if they can bring a secured budget from a different source of funding *(specify below in the first budget table); (= Self financed partners A, B…)***

- **may be subcontracted by other partners in some cases.** Please, refer to the national rules. Some countries have specific restrictions about subcontracts (details on *BiodivERsA website (www.eurobiodiversa.org)* and your proposal will be ineligible if you do not follow national rules. ***(= partners 1a, 1b, 2a…)***

- **CANNOT REQUEST FUNDING.** In Table 1, please do not request a funding budget for countries ineligible for direct funding (***partners 1a, 1b, 2a and Self financed partners A, B)*** : indicate 0€ in column B and indicate 0% in column "Funding rate" (B/A). **The whole proposal will be ineligible if a partner from a country not participating in the call requests some funding.**

VII.B. Budget tables

Table 1 : please specify the names and countries of each partner.

Costs per partner and requested funding budget (in EURO, incl. VAT)				
Partner	**A - Total costs/expenses**[1)] **including subcontracts**	**B - Requested funding budget including subcontracts**	**Funding rate (B/A) %**	**Other funding (co-funding and its source, self funding…)**
Partner 1 CNRS-DR17 Station Biologique de Roscoff FRANCE	1 304 974.78 €	730 600 €	56 %	permanent personnel : 574 374.78 €
Partner 2 Institut de Ciències del Mar, CSIC, SPAIN	119 320 €	119 320 €	100 %	
Partner 3 University of Exeter UK	330 956.92 €	264 765.53 €	80% (including consumables and travel)	permanent personnel hours not charged to grant: 18 285.58€
Partner 4 CNRS-DR12 Structural and Genomic Information Laboratory FRANCE	417 555 €	191 248 €	46 %	permanent personnel: 174 307 € Marseille-Nice Génopole (PC-Cluster upgrade): 52 000 €
Partner 5 Stazione Zoologica Anton Dohrn ITALY	255 203 €	198 001 €	77.6 %	Permanent personnel 57 202 €
Partner 6 CNRS-DR20 Laboratoire d'Oceanographie de Villefranche/Mer FRANCE	207 165 €	118 511 €	58 %	Permanent personnel: 88 654 €
Partner 7 University of Oslo NORWAY	326 600 €	145 000 €	44 %	Permanent personnel 125 000 € Experimental work 56 750 €
Self financed partner A University of Kaiserslautern GERMANY	96 750 €	0 €	0%	96 750 €
Total	**3 058 524.7 €**	**1 767 445.5 €**	**57.8 %**	**1 243 323.4 €**

1) Total costs/expenses comprise costs or expenses for personnel (including permanent salaries), travelling, consumables, overheads (if applicable), subcontracts *etc*.; the cost calculation has to be based for each partner on its national funding rules; for questions, please contact your national contact point. **** Total of Partners 1, 2, N + A, B (total of white and yellow boxes): DO NOT include the partners 1a, 2a, 2b…** Total of all the partners (1, 2…N, 1a, 2a…, A, B)***

Table 2a

Breakdown of total costs per partner per calendar year (in Euro, incl. VAT)								
			2009	**2010**	**2011**	**2012**	**Total cost**	**Funding Request**
Partner 1 CNRS-DR17 Station Biologique de Roscoff France	Salaries	permanent	164 107 €	164 107 €	164 107 €	82 053.78 €	574 374.78 €	277 500 €
		temporary	88 800 €	88 800 €	88 800 €	11 100 €	277 500 €	
		total	252 907 €	252 907 €	252 907 €	93 153.78 €	851 874.78 €	
	Travel		10 000 €	8 000 €	8 000 €	4 000 €	30 000 €	30 000 €
	Overheads		11 652 €	11 492 €	4 192 €	764 €	28 100 €	28 100 €
	Consumables		12 500 €	10 500 €	8 000 €	4 000 €	35 000 €	35 000 €
	Equipment							
	Other	Sub-contract[2)]	180 000 €	180 000 €			360 000 €	360 000 €
		Other						
Partner 2 ICM-CSIC Spain	Salaries	permanent	0	0	0	0	0	89 320 €
		temporary	44 000 €	45 320 €	0	0	89 320 €	
		total	44 000 €	45 320 €	0	0	89 320 €	
	Travel		3 000 €	3 000 €	3 000 €	1 000 €	10 000 €	10 000 €
	Overheads		0	0	0	0	0	0
	Consumables		5 000 €	4 000 €	4 000 €	2 000 €	15 000 €	15 000 €
	Equipment		0	0	0	0	0	0
	Other	Sub-contract[2)]						
		Other	2 000 €	2 000 €	1 000 €	0	5 000 €	5 000 €
Partner 3 Exeter UK	Salaries	permanent	8 098.77 €	8 098.77 €	8 098.77 €	4 049.38 €	28 345.69 €	104 431.95 €
		temporary	29 198.36 €	29 198.36 €	29 198.36 €	14 599.17 €	102 194.25 €	
		total	37 297.13 €	37 297.13 €	37 297.13 €	18 648.55 €	130 539.94 €	
	Travel		3 632.27 €	3 632.27 €	3 632.27 €	1 816.13 €	12 712.94 €	10 170.35 €
	Overheads		48 189.31 €	48 189.31 €	48 189.31 €	24 094.67 €	168 662.60 €	134 930.08 €
	Consumables		5 440.41 €	5 440.41€	5 440.41 €	2 720.21 €	19 041.44 €	15 233.15 €
	Equipment							
	Other	Sub-contract[2)]						
		Other						
Partner 4 CNRS-DR12 Structural and Genomic Information Laboratory France	Salaries	permanent	49 802 €	49 802 €	49 802 €	24 901 €	174 307 €	139 392 €
		temporary	46 464 €	46 464 €	46 464 €		139 392 €	
		total	96 266 €	96 266 €	96 266 €	24 901 €	313 699 €	
	Travel		6 000 €	6 000 €	6 000 €	2 000 €	20 000 €	20 000 €
	Overheads		2 102 €	2 102 €	2 102 €	1 050 €	7 356 €	7 356 €
	Consumables		5 000 €	5 000 €	5 000 €	2 500 €	17 500 €	17 500 €
	Equipment		59 000 €				59 000 €	7 000 €
	Other	Sub-contract[2)]						
		Other						

Partner 5 Stazione Zoologica Anton Dohrn Naples Italy	Salaries	permanent	16 343 €	16 343 €	16 343 €	8 172 €	57 202 €	
		temporary	10 800 €	21 600 €	21 600 €	10 800 €	64 800 €	64 800 €
		total	27 143 €	37 943 €	37 943 €	18 972 €	122 001 €	
	Travel		5 000 €	5 000 €	5 000 €	5 000 €	20 000 €	20 000 €
	Overheads		16 286 €	22 766 €	22 766 €	11 383 €	73 201 €	73 201 €
	Consumables		10 000 €	10 000 €	10 000 €		30 000 €	30 000 €
	Equipment		10 000 €				10 000 €	10 000 €
	Other	Sub-contract[2)]						
		Other						
Partner 6 CNRS-DR20 LOV France	Salaries	permanent	25 329.72 €	25 329.71 €	25 329.71 €	12 664.86 €	88 654 €	
		temporary		49 200 €	49 200 €		98 400 €	98 400 €
		total	25 329.72 €	74 529.71 €	74 529.71 €	12 664.86 €	187 054 €	
	Travel		1 571 €	1 571 €	1 571 €	787 €	5 555 €	5 555 €
	Overheads		1 301.72 €	1 301.71 €	1 301.71 €	650.86 €	4 556 €	4 556 €
	Consumables		3 334 €	3 333 €	3 333 €		10 000 €	10 000 €
	Equipment							
	Other	Sub-contract[2)]						
		Other						
Partner 7 University of Oslo Norway	Salaries	permanent	34 375 €	35 375 €	36 500 €	18 750 €	125 000 €	
		temporary	35 250 €	43 600 €			78 850 €	78 850 €
		total	69 625 €	78 975 €	36 500 €	18 750 €	203 850 €	
	Travel		4 360 €	4 360 €	3 750 €	3 750 €	16 220 €	16 220 €
	Overheads		10 610 €	13 110 €			23 720 €	23 720 €
	Consumables		9 330 €	9 360 €	6 250 €	1 250 €	26 190 €	26 210 €
	Equipment							
	Other	Sub-contract[2)]						
		Other	25 375 €	31 375 €			56 750 €	
Self financed Partner A Kaiserslautern Germany	Salaries	permanent	5 500 €	5 500 €	5 500 €	2 250 €	18 750 €	
		temporary	21 000 €	21 000 €	21 000 €		63 000 €	0 €
		total	26 500 €	26 500 €	26 500 €	2 250 €	81 750 €	
	Travel							0 €
	Overheads							0 €
	Consumables		2 000 €	2 500 €	3 500 €	2 000 €	10 000 €	0 €
	Equipment		5 000 €				5 000 €	0 €
	Other	Sub-contract[2)]						0 €
		Other						0 €
Total Partners 1, 2, N + A, B *(white/yellow boxes)*			**1 036 751.56 €**	**1 043 770.54 €**	**717 970.54 €**	**231 475.05 €**	**3 058 524.7 €**	**1 767 445.5 €**

Table 2b : TOTAL AMOUNT FOR THE PROJECT
(do not consider the amounts of *partners 1a*, *2a*… already included in "subcontract budget" of partners 1, 2…N)

	Total salaries		Travel	Overheads	Consumables	Equipment	Other	
	permanent	temporary					Sub-contract	Other
Total amount of project	1 066 633.5 €	913 456.25 €	114 487.94 €	325 595.6 €	162 731.44 €	74 000 €	360 000 €	61 750 €
Total Funding request	0 €	852 693.95 €	111 945.35 €	291 863.08 €	148 943.15 €	17 000 €	360 000 €	5 000 €

Sub-contract to Partner 1:
[2)] Please, provide further information concerning "sub-contract": name of contract holder, any contract convention established between contract holder and the funding partner.

Given the scope of the genetic part of *BioMarKs*, we contacted Dr. Jean Weissenbach, director of the GENOSCOPE, to request a formal partnership between our project and this major sequencing center in Europe (http://www.genoscope.cns.fr/spip/). This was accepted and an official GENOSCOPE quote for 45x *454*-sequencing runs was emailed to: biodiversa.call@gis-ifb.org.
A convention will be established as soon as the project in funded.

Explanation and/or remarks concerning the proposed budget (table 1 and 2) :

The requested budget (~1.77 million €) is significantly reduced as compared to the pre-proposal. The main reason is that a formal collaboration has been established with the major European sequencing center, GENOSCOPE (http://www.genoscope.cns.fr/). As an external BioMarKs partner and stakeholder, GENOSCOPE offers an extremely competitive price for 454-sequencing (8000 Euros/run) and has agreed to dedicate a significant part of its machine and engineer time at no cost to the project. The 454-sequencing cost has thus been reduced to 360K€ (originally 600K€). Except for 454-sequencing, most of the BioMarKs budget is thus dedicated to post-doctoral salaries (total of 186 months) distributed among the network, and to lab consumables for downstream analyses of contextual and archive data. The category "other" contains costs for shipping cryo-samples, web-site development and publication costs. Travel costs are requested to organize yearly meetings, to partially finance the WP-4 workshops in Y-3 and Y-4, and to ensure efficient information flow and promote regular knowledge and technology transfer by post-doctoral students during visits to partner labs. Overheads were calculated according to national rules. Note that the BioMarKs german partner will self finance participation in this project since Germany does not contribute to the BiodivERsA scheme. All major equipment necessary for achievement of BioMarKs (454-sequencer, PCR machines, flow cytometer with cell sorter, electron and optical microscopes, HPLC, hardware maintenance contracts etc.) is available within the consortium at no direct cost to the project. Likewise, considerable ship-time and technician time are available at no cost.

VIII. Description of project management

Drawing on previous experience, a management structure has been designed that is simple in conception and implementation, but comprehensive in covering all key aspects of project operation and coordination. These range from informed and transparent decision making, through efficient flow of information between all project components and from the project to the EC, to ensuring the widest possible awareness of, and access to, the *BioMarks* network.

Project Management Team (PMT) *Coordinators* of this project will be Dr. Colomban de Vargas (P1) and Dr. Jean-Michel Claverie (P4). The coordinators will propose strategic and political orientations to the partners and follow-up resulting decisions and recommendations of the *Project Implementation Committee* (PIC). Both Dr. de Vargas and Dr. Claverie have experience of coordinating large national level projects and have participated as PIs in European projects. Dr. de Vargas will be the principal coordinator and will take overall responsibility for organizing and supervising the project. He will ensure the communication flow between the partners, organize and chair meetings of the PIC, and prepare the progress and financial reports with the support of the Management Office. Dr. de Vargas will be responsible for communication with the European Commission through the relevant scientific officer. The vice-coordinator, Dr. Claverie, whose scientific expertise in bioinformatics is highly complementary to that of Dr. de Vargas in protist evolution and ecology, will support the coordinator in all aspects of the scientific implementation of the project.

The Management Office will be in charge of the day-to-day management of *BioMarks* according to decisions made by the coordinators and the PIC. The office will employ a Program & Outreach Manager (*POM*). The *POM* will coordinate and collate all information necessary for periodic scientific and financial reports. The principal coordinator will assume overall responsibility for the budget, but the *POM* will oversee day-to-day financial matters, including budgeting for meetings and workshop activities. The financial reports as well as audit certificates will be gathered and prepared by the *POM* before transmission to the EC. The *POM* will have overall responsibility for implementing the dissemination activities of the project. (S)he will coordinate setting up, initial population, and subsequent updating of the *BioMarks* web portal in close collaboration with all PIs and notably with the main bioinformatics team (P4). In addition to management of the web portal, the *POM* will act as the hub for information flow between *BioMarks* partners and stakeholders (including the wider general public). This will involve coordination of identification and contact with further potential stakeholders in the first phase of the project. Subsequently, the *POM* will oversee production and distribution of the tri-annual e-newsletters (see Communication). Complimentary responsibilities will include collecting and collating feed-back from stakeholders, and publicity of research and networking activities through other channels (e-mail lists, web sites, pamphlets, direct contacts). The *POM* will also oversee internal networking activities, notably through management of the restricted-access intranet section of the web portal. Support for the *POM* is available at the CNRS including support for legal, patent and technology transfer matters. Synergistic links will also be created with the management office of the FP7 infrastructures project ASSEMBLE which will run from 2009-2013.

The Project Implementation Committee (PIC) The PIC will be the main decision-making and organisational body of the *BioMarks* management structure. It will be chaired by Dr. de Vargas, and will consist of all *BioMarks* PIs, as well as two external advisors appointed in concertation with the *BioDiversa* project officer. Decisions will be taken on project implementation from an organizational point of view, with a particular focus on administrative/legal/financial aspects. The PIC will also preside over scientific and technical

aspects of the project in order to ensure the achievement of the work plan in due time. PIC meetings will be held twice a year, of which one will be by videoconference. PIC members will be in frequent contact by e-mail, telephone, and videoconferences. Additional meetings can be held on the request of any member, and if urgent decisions need to be taken, e-mail vote will be implemented. The majority of the decisions are expected to be reached by consensus as a good working relationship already exists between partners, but if a formal vote is required then each PI will have a single vote and the Coordinator will have a casting vote in the case of a tie. PIC decisions will be taken in order to: (1) Supervise overall project and WP level implementation according to the work plan; (2) Allocate resources following the financial plan; (3) Supervise the evolution of the consortium with the exit of possible defaulting parties and entrance of new ones; (4) Resolve conflicts or risks which may emerge during the project: if a partner does not respect the deadlines for the deliverables and reports, the PIC can take the necessary measures following the Consortium Agreement; if a partner fails to deliver its outcome it will be considered as a defaulting party and the PIC can take measures following the Consortium Agreement; if resources have to be reallocated between the partners, this will be decided by the PIC; if a defaulting party leaves the project, the reallocation of resources to achieve the work plan will be decided by the PIC. The Consortium Agreement that will be negotiated and signed by all partners before the signature of the Grant Agreement will specify all details relating to the voting rules, the quorum, organization of extraordinary meetings and the minutes.

Communication Management of communication is vital for the integration of this project. Providing easy access to knowledge and communication systems is therefore an essential part of the management structure. In addition to e-mail, telephone and videoconference communication, a *BioMarks* web portal will be created comprising both open-access and restricted-access sections. The structuring impact of *BioMarKs* depends on the quality of partners and interactions between them, but also to a large extent on visibility to the research community as well as to other stakeholders (primary and secondary education, biotechnology companies, policy makers, and the general public and society at large). Visibility will be achieved by the *external* (open-access) part of the *BioMarks* web site. The main purposes of this are to: (1) provide general information about the project as well as detailed information of each partner; (2) communicate *BioMarks* activities; (3) present results of *BioMarks* joint research activities. It is also important to establish a culture of co-operation and exchange within the network. For this an intranet for *internal* communications and information will be set-up as part of the *BioMarks* web site. This restricted-access zone will contain sections dedicated to joint research tasks (ongoing debate and general circulation of information, reports and common protocols, ongoing exchanges within shared research issues, debate about the research agenda), to announcements and information concerning networking events, to minutes from project implementation committee meetings, and to annual activity reports.

Gender Issues The under-representation of women in the natural sciences is a waste of human resources and a serious obstacle for the development of sciences and for European society as a whole. It is thus of great importance to improve gender balance at all levels and to take measures to ensure equal access to all opportunities that a scientific career can offer. *BioMarks* will support the principle that criteria of excellence are independent of gender and will adhere to the gender mainstreaming strategies that have been adopted by the Commission. Approximately half of the scientists directly involved in the *BioMarKs* consortium are female.

Ethical Issues There are no specific ethical issues involved in the *BioMarKs* project.

IX. Signatures

1. Each partner MUST contact his national contact point regarding any original official paperwork required by his national funding agency.
This must be submitted in accordance with national rules and in any case as soon as possible. **You will NOT be funded without this signature**.
Further information is available on BiodivERsA website (http://www.eurobiodiversa.org)

2. **"Self financed" partners must provide evidence that their organisations will support their activities**. They should send a signed official letter of support from their Head of Department or Financial administrator (as appropriate) to the BiodivERsA secretariat. This letter must be received electronically (.pdf) by the project deadline and in hard copy 7 days later.
Further information is available on BiodivERsA website (http://www.eurobiodiversa.org)

Guidelines for applicants

Please refer to the 'call for proposals' document for eligibility and rules for joint collaborative projects; and for information on the evaluation procedure. The following aspects should be included in the application form:

I. General information on the

- Coordinator
- Partners involved

Note that eligibility criteria follow national rules.

Time to be dedicated to the project per member

Declaration of parallel submissions of this proposal (whole or parts) to other funding programmes

II. Project Summary

III. Relevant scientific publications of the applying research group

IV. Detailed description of the research area and research plan and communication plan

This should include:

1. *A short description of the hypothesis*
2. *Scientific objectives with detailed account of their relationship to the themes of the call and to ongoing relevant projects. Organize the objectives into a list so that each objective is accurately defined and quantified.*
3. *Work plan and division of work packages between the partners*
4. *Justification of resources*
5. *Relevance for the identified policy application, importance of the research for solving pressing concerns and/or issues related to biodiversity*
6. *Proposed exploitation of future project results*
7. *Dissemination of results to practitioners, policy- and decision-makers*

Please take into account the selection criteria:

- Scientific Aspects

a) Scientific quality of the proposed research
b) Novelty / Originality and innovation
c) Clarity of the hypothesis
d) Quality and suitability of the consortium
e) Level of inter/multi/trans-disciplinarity
f) Fit to thematic priorities

- Policy relevance

a) Relevance for the identified policy application, importance of the research for solving pressing concerns/issues related to biodiversity
b) Identification of end users
c) Approach to stakeholder engagement
d) Arrangements for knowledge transfer

- Project management and added value:

a) European added value
b) Feasibility and risk
c) Level of integration and collaboration
d) Relation to other projects

e) Suitability of budget requirements
f) Quality of the project governance

V. Detailed time schedule and working programme
VI. Sharing of work and cooperation with external organisations contributing to the project (if applicable)
VII. Budget
Please take national rules into account.
VIII. Description of project management
IX. Signatures

VETENSKAPSRÅDET
THE SWEDISH RESEARCH COUNCIL

Kod
2008-29765-62739-34

Dnr
2008 -

Name of applicant
DE VARGAS, Colomban

Date of birth
710825-

Reg date
2008-06-16 23:55:31

Project title
BioMarKs: Biodiversity of Marine EuKaryotes

OVR

Biodiversa's Pan European Call
Project Research Grant
*ERA-net Biodiversa

Appendix S

Signatures

2009 2010 2011 2012 2013

2009-01-01 -- 2012-06-30

Applicant | **Date**

Head of department at host University | **Clarifi cation of signature** | **Telephone**

A signature on the application is required not only from the applicant but also from the authorised representative of the host university/institution or equivalent (normally the head of the department or establishment where the research is to be conducted). The signature confirms that the department can accommodate the proposed research, position or equipment; that the costing in the application is approved for the department's part, that any proposed experimentation on human or animal subjects has been reported, and that the appplicant has reported any secondary occupations and commercial ties (s)he may have, and nothing inconsistent with good research practice has thereby emerged. The applicant must have discussed these conditions with the representative of the host university/institution or equivalent before the latter approves and signs the application.

Educational Science (not Travel and Conference grants)
Note that the signature of an authorised representative of the host university/institution or equivalent means that the latter undertakes to invest its own resources corresponding to at least one-third of the funds obtained from the Committee for Educational Science, if the application is approved. This co-funding may include existing research if this can be linked with projects in Educational Science. Host universities/institutions or equivalent that are awarded funds must annually submit a special report on how the institution has measured up to the co-financing requirement. For research conducted in collaboration with researchers from other educational institutions, the host university/institution or equivalent is responsible for all agreements with the researchers concerned.

International Postdoctoral Fellowships are administered by the Swedish Research Council. The only signature required on the application is that of the applicant.

Travel, Conference and Publication grants: The only signature required on the application is that of the applicant.

Vetenskapsrådets noteringar
Kod

2008-29765-62739-34

Vetenskapsrådet, SE-103 78 Stockholm, tel. +46 (0)8 546 44 000, vetenskapsradet@vr.se