

Biodiversité des Protistes Aquatiques: le Paradoxe

Projet ANR-07-BDIV-004

AQUAPARADOX

Programme Biodiversité 2007



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A IDENTIFICATION

Acronyme du projet	AQUAPAROX
Titre du projet	Biodiversité des Protistes Aquatiques: Le Paradoxe
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B.1 Résumé consolidé public en français

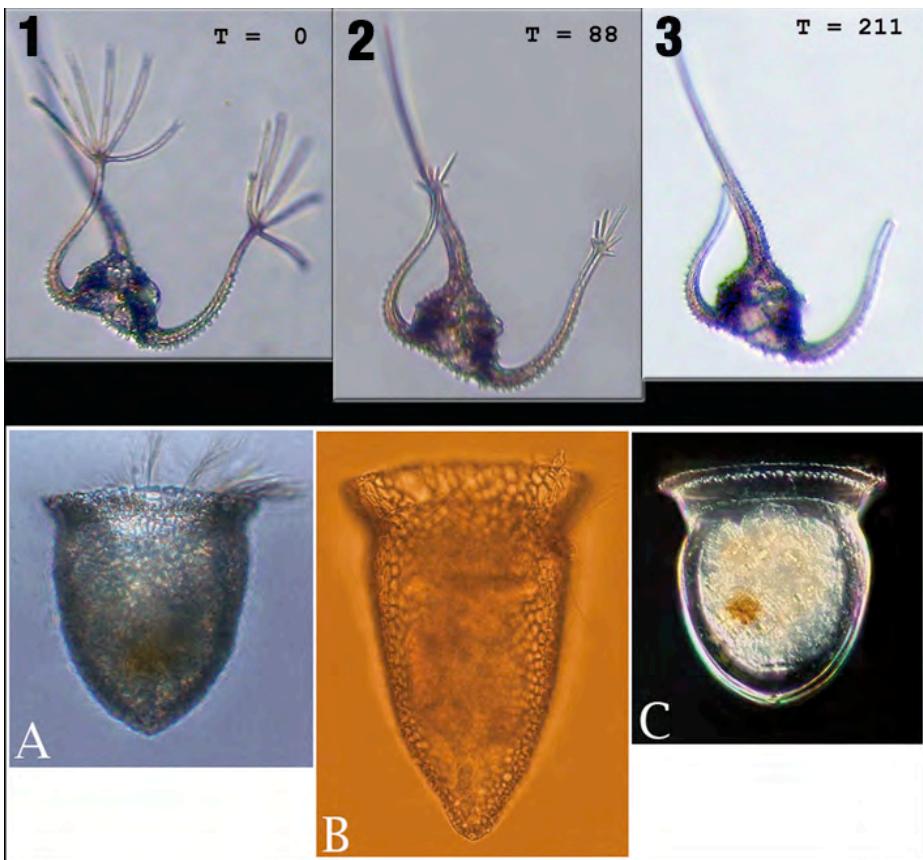
Aquaparadox: Pourquoi Autant d'Espèces dans le Plancton?

Une Etude de la Biodiversité Océanique. Les plantes et animaux microscopiques du plancton forment la base de la chaîne élémentaire dans les océans. Un litre d'eau peut contenir des milliers d'espèces ce qui par extrapolation conduit à une richesse d'espèces inexplicable dans ce monde microscopique et relativement homogène - c'est ce que l'on appelle 'le paradoxe du plancton'. Les protistes, des organismes unicellulaires, ont des caractéristiques communes : le nombre d'espèces est élevé et beaucoup semblent avoir une distribution géographique très large, voir globale. Connaître l'étendue et les raisons de cette particularité du plancton marin est essentiel pour comprendre le fonctionnement des écosystèmes océaniques et les effets du changement climatique. Nous avons examiné plusieurs questions fondamentales concernant ces organismes planctoniques en nous focalisant sur des groupes importants présentant des types fonctionnels différents : des micro-algues (des plantes), des brouteurs (des animaux) et des parasites. Y a t-il autant d'espèces que de formes morphologiques distinctes? Quel est l'influence du milieu sur la variabilité morphologique des espèces ? Certaines espèces sont-elles réellement à large distribution, ou la diversité est-elle masquée par des convergences morphologiques ? Aquaparadox était un projet de recherche fondamentale focalisé sur la biodiversité des protistes planctoniques.

Sondre le plancton à travers des études des populations naturelles *in situ* et au laboratoire.

Est-ce que les protistes et les organismes multicellulaires du plancton sont similaires en termes de distribution et de structures des populations? Nous avons examiné des populations naturelles de protistes et les avons comparées avec celles des organismes planctoniques multicellulaires les plus abondants, les copépodes. En laboratoire, nous avons étudié la variabilité des clones, issus de la même cellule. Des cultures de parasites et d'hôtes ont été établies et ont permis de comprendre comment un parasite pouvait persister en infectant des hôtes typiquement saisonniers. Nous avons appliqué les outils moléculaires pour déterminer l'identité génétique des formes identifiées sur la base de leur morphologie, et évaluer le spectre d'hôtes de certains parasites.

Résultats majeurs du projet. Nous avons démontré que les assemblages de protistes planctoniques ont une structure très similaire à celle des copépodes (multicellulaires) sur des échelles multiples du temps et de l'espace. Par exemple, ils ont le même gradient global de diversité: le nombre d'espèces est le plus élevé en zones tropicales, intermédiaire et zones tempérées, et minimal dans les zones polaires. Nous avons découvertes des exemples remarquables de morphologie variable parmi les protistes planctoniques (voir figure). Une espèce de *Ceratium*, une micro-algue, caractérisée par ses 'doigts', les résorbe à chaque couver du soleil et les reforme à l'aube. Au moins une espèce de tintinnides est connue sous des noms différents du fait de sa capacité à fabriquer des coquilles de formes différentes. Nous avons trouvé que les parasites peuvent persister dans les kystes de leur hôte pour infecter une nouvelle génération l'année suivante.



Un protiste peut avoir une morphologie remarquablement variable. La série en haut (1,2,3) montre la réabsorption des 'doigts' de *Ceratium ranipes* au coucher du soleil, un processus accompli en 3 heures. La série du bas (A,B,C) montre les types de coquilles formées par une seule espèce de cilié tintinnide. Ces organismes font 200 µm de longueur.

Production scientifique. Les découvertes sont documentées dans des dizaines d'articles (39) au sein de revues scientifiques très sélectives et de 5 thèses. Outre des publications, le projet a produit des logiciels pour l'analyse des séquences d'ADN et la construction d'arbres phylogénétiques. Une galerie d'images de protistes planctoniques, une bibliothèque virtuelle des monographies taxonomiques et des guides pour l'identification des groupes spécifiques a été créée. Tous ces produits d'AQUAPARADOX ont été mis en accès libre via <http://www.obs-vlfr.fr/LOV/aquaparadox/>

Le projet AQUAPARADOX était un projet de recherche fondamentale coordonné par John R. Dolan. Il a été réalisé par un consortium de 4 équipes: 1) Microbial Ecology and Biogeochemistry, Laboratoire d'Océanographie de Villefranche, Université Paris6 CNRS UMR 7093 (J. Dolan, R. Lemeé), 2) Microbial Diversity and Evolution, Ecologie, Systématique et Evolution, Université Paris-Sud XI CNRS UME 8079 (P. Lopez-Garcia, D. Moreira), 3) Diversity of Ocean Plankton, Adaptation et Diversité en Milieu Marin, Université Paris 6 CNRS UMR 7144 (L. Guillou, N. Simon), et 4) Virtual Biology Laboratory, Institute of Developmental Biology and Cancer, Université Nice- Sophia Antipolis CNRS UMR 6543 (R. Christen). Commencé en janvier 2008 et terminé en décembre 2011, le projet bénéficia d'une aide ANR Biodiversité de 497.000 € et d'une aide du Pôle Mer PACA de 21.000 €, pour un coût global estimé à 1,9M €.

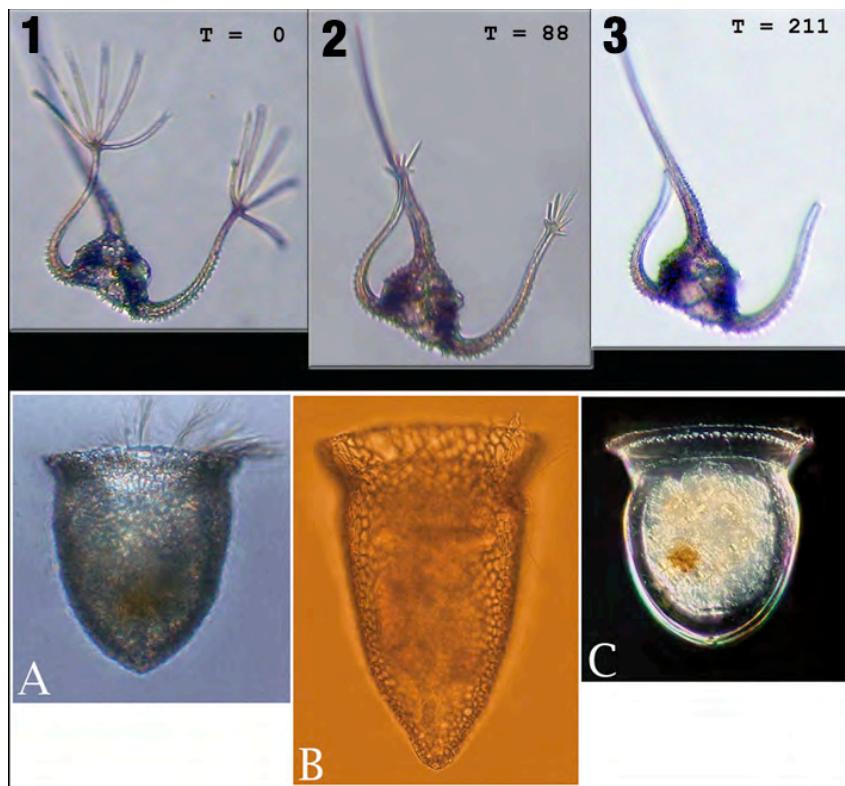
B.2 English Non-Technical Project Summary

AQUAPARADOX: Why So Many Species in the Plankton?

Examining Biodiversity in the Ocean. The microscopic plants and animals of the plankton are the base of the marine food chain. Among planktonic microbes, there seems to be an unreasonably large number of species, thousands in a single liter: 'the paradox of the plankton'. Protists of the plankton, single-celled organisms, are typical as species richness is high and each species appears to be very widely distributed. Arguably, we need to know what exists in the plankton to understand how marine food chains work and the effects of global change. We examined basic questions concerning protists of the plankton by studying particular groups typical of the different types: primary producers (plants), grazers (animals) and parasites. Are there really as many different species as there seems to be? Perhaps some species look different under different conditions. Is an organism found around the world really the same organism everywhere? Aquaparadox was a basic research project focused on protist biodiversity in the marine plankton.

Probing the plankton using field sampling, laboratory cultures, and molecular analyses. We compared the structure of natural assemblages of plankton protists to those of the most abundant multi-cellular plankton, the copepods. We sought to determine if protists differed fundamentally from multicellular plankton. We compared the characteristics of natural communities of protists assemblages and copepod assemblages on different scales of time and space. In the laboratory, we examined the morphological variability of individuals grown from a single cell. We compared strains of a single species from different areas. Parasites and hosts were grown together to see how parasites can persist in seasonal species. We used molecular analyses to establish the genetic identities of different morphological types and to verify if types appearing similar were also genetically similar. Molecular analyses were used to determine if parasites infected only a specific host species or infect different species.

Our Results. We found that protists of the plankton have a community structure very similar to that of multicellular organisms. For example, they have the same global pattern of biodiversity of a high species-richness in tropical regions, intermediate in temperate zones and low diversity near the poles. We found that some protists have a remarkably variable morphology. We discovered that a species of *Ceratium* with prominent 'fingers' absorbs its fingers at sunset only to re-form them at sunrise. We found that species of tintinnids thought to be different species, because of their distinct shells, are genetically a single species (see figures below). We established that parasitic protists can persist in the cysts of their host to re-infect the next generation.



Protist species can have remarkably variable morphologies. The top series (1,2,3) shows a *Ceratium ranipes* absorbing its 'fingers' at sunset, a process which takes about 200 minutes. The bottom series (A,B,C) shows the different shell types formed by a single species of tintinnid ciliate. These organisms are about 200 µm in size.

Project Products. Discoveries, like those mentioned above, were reported in dozens of scientific articles (39) in selective scientific journals and 5 doctoral theses. In addition, products include software developed for analysis of DNA sequences and construction of phylogenetic trees. An image gallery of over 500 images of planktonic protists was put online as well as a taxonomic resource site with PDF versions of key taxonomic references. Aquaparadox contributed to web-based guides for identification of specific groups of planktonic protists. All products can be accessed via <http://www.obs-vlfr.fr/LOV/aquaparadox/>

Descriptive information. The Aquaparadox project was a basic research project coordinated by John R. Dolan. The research consortium consisted of 4 research groups: 1) the Microbial Ecology and Biogeochemistry Group, Laboratoire d'Océanographie de Villefranche, Université Paris6 CNRS UMR 7093 (J. Dolan, R. Lemeé), 2) the Microbial Diversity and Evolution Group, Ecologie, Systématique et Evolution, Université Paris-Sud XI CNRS UME 8079 (P. Lopez-Garcia, D. Moreira), 3) the Diversity of Ocean Plankton Group, Adaptation et Diversité en Milieu Marin, Université Paris6 CNRS UMR 7144 (L. Guillou, N. Simon), and 4) the Virtual Biology Laboratory, Institute of Developmental Biology and Cancer, Université Nice- Sophia Antipolis CNRS UMR 6543 (R. Christen). The project began in January 2008 and ended in December 2011. Financing was provided by the ANR Biodiversité program (€497,000) with additional funding provided by the Pole Mer PACA (€21,000); total project cost was €1,951,000.

C. SCIENTIFIC REPORT

C.1 Abstract

The plankton is the base of the marine food chain. Among planktonic microbes, there seems to be an unreasonably large number of species, thousands in a single liter: 'the paradox of the plankton'. Protists, single-celled eukaryotic organisms, are typical of the plankton as species richness seems high and each species appears to be very widely distributed. Knowledge of what actually exists in the plankton is needed to understand how marine food chains work and the effects of global change. We examined basic questions concerning protists of the plankton by studying particular groups typical of the different types: primary producers, grazers, and parasites. Are there really as many different species as there seems to be? Perhaps some species look different under different conditions. Is an organism found around the world really the same organism everywhere? Is there a structure to assemblages of protists?

We compared the structure of natural assemblages of plankton protists to those of the most abundant multi-cellular plankton, the copepods to determine if protists differed fundamentally from multicellular plankton. In the laboratory, we examined the morphological variability of protists grown from a single cell and compared strains of a single species from different areas. Parasites and hosts were grown together to see how parasites can persist in seasonal species. We used molecular analyses to establish the genetic identities of different morphological types and to verify if types appearing similar were also genetically similar. Molecular analyses were used to determine if parasites infected only a specific host species or infect different species.

Among our results were finding that protists have a community structure very similar to that of multicellular organisms. For example, they share the same global pattern of biodiversity of a high species-richness in tropical regions, intermediate in temperate zones and low diversity near the poles. We found that some protists have a remarkably variable morphology. For example, a species of *Ceratium* with prominent 'fingers' absorbs its fingers at sunset only to re-form them at sunrise. We found that species of tintinnid ciliates thought to be different species, because of their very distinct shells, are genetically a single species. We established that parasitic protists can persist in the cysts of their host to re-infect the next generation.

Discoveries like those mentioned above were reported in dozens of scientific articles (39) in selective scientific journals and 5 Ph.D. theses. In addition to scientific publications and communications, products included software developed for analysis of DNA sequences and construction of phylogenetic trees. An image gallery of over 500 images of planktonic protists was put online as well as a taxonomic resource site with PDF versions of key taxonomic references. Aquaparadox contributed to web-based guides for identification of specific groups of planktonic protists.

C.2. Introduction

Among planktonic microbes, there seems to be an unreasonably large number of species, thousands in a single liter: 'the paradox of the plankton'. In Aquaparadox we examined aquatic protist diversity using multiple lines of evidence (morphological, genetic, and ecological) in 4 distinct taxa of protists. The assemblages or groups of protists studied were: (1) all cosmopolitan, (2) of different sizes and typical densities- spanning orders of magnitude (1 to $10^3 \mu\text{m}$ and 1 to 10^7 cells per L), and (3) members of distinct functional groups: primary producers (*Micromonas* and *Ceratium*), herbivores (tintinnid ciliates) and parasites (Syndiniales). Here are summarized some of our major research question and results with regard to each of 4 groups investigated and our efforts to synthesize macroecological patterns.

C.4 Primary Producers

a. - *Micromonas*

Micromonas pusilla is one of the most ubiquitous and cosmopolitan species of all picoeukaryotes described to date. It can dominate the picoeukaryotic community all year round in coastal systems such as the English Channel (Not et al., 2004). Studies based on the phylogenetic analysis of several genes from isolates of this species collected worldwide revealed the existence of 3 to 6 phylogenetically discrete units or clades and suggested that this taxon was a complex of cryptic species (Slapeta et al., 2006). This may explain the remarkable range of habitats colonized by *Micromonas pusilla*. However, there were no obvious biogeographical patterns among the phylogenetic clades known nor indications of any morphological, physiological or ecological differentiations between strains or populations of this species save that one small lineage was described as purely Arctic (Lovejoy et al., 2007). *Micromonas* represented an ideal model to study the potential ecological significance of 'microdiversity' in a protistan taxon.

Our work was centered around the questions: Is there an ecological significance of the genetic structure and of the genetic microdiversity in *Micromonas pusilla*? Are the phylogenetic clades described based on genetic studies true ecological species? Do they differ in morphology? Do they each occupy a specific niche in the natural environment? To answer these questions we conducted a wide-ranging investigation of organisms described as *Micromonas pusilla*. We obtained, from a large number of cultured strains, 36 new 18S and ITS rDNA sequences. Phylogenetic analysis showed the existence of 3 major clades confirming previous studies. We established the karyotypic and phenotypic traits of isolates of the three main clades A, B and C. Chromosome number ranged from 17 to 21 and differed between clades. Content in major pigments was similar between clades, but clade A alone possess an additional chlorophyll c_3 -like pigment. We found that the clades actually differed in morphology, in particular, the clades showed distinct flagellar structures (Fig. 1). Both genomic and phenotypic characters appeared homogeneous within each of the clades A, B and C. Based on these results, we have proposed a revision of the genus *Micromonas* and of the species *Micromonas pusilla* (Foulon et al., submitted).

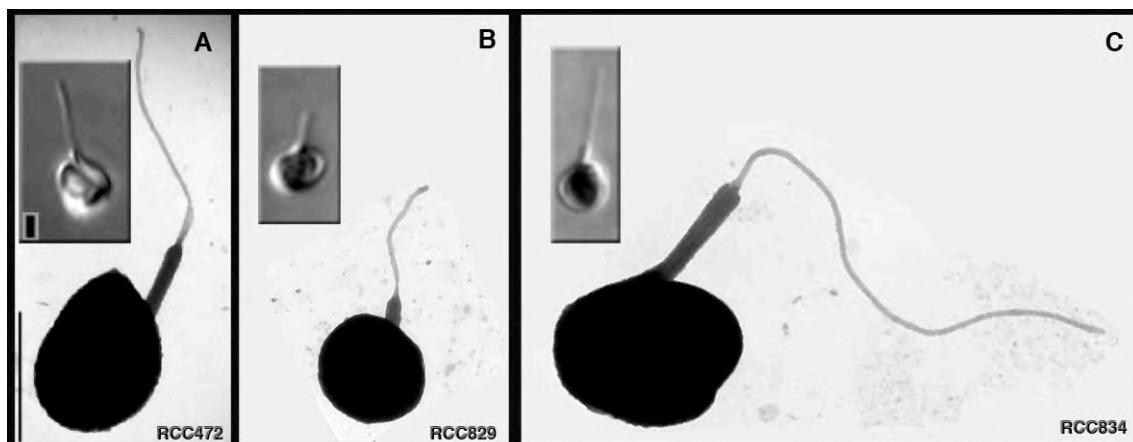


Fig. 1. The three clades of *Micromonas* (A,B,C) correspond with three distinct morphologies. TEM shadow images with inset images of light micrographs. Scale bar is 1 μ m. From Foulon et al. submitted.

The "cryptic species" of *Micromonas pusilla* were revealed to be not so 'cryptic' after all, but rather composed of forms distinguishable via morphology, pigments, and karyotype. The distinct clades of *Micromonas* have distinct seasonal cycles of abundance (Foulon et al. 2008). However, the precise differences in their ecological niches remain cryptic.

b. - Ceratium

The genus *Ceratium* Schrank is ubiquitous and the best known of the armoured marine dinoflagellates (Gomez et al. 2010). It occurs in all seas, from the poles to the tropics, both in open and coastal marine waters where it is a major component of the microphytoplankton. Based on morphology, the genus has been subdivided into more than 120 infra-genus taxa including species, subspecies, forms or varieties. Because both the spatial and temporal distribution of many species of *Ceratium* appear correlated with temperature, different forms of *Ceratium* have been considered as biological indicators of water masses and climate change. (e.g., Dodge and Marshall 1994). Based on analysis of historical and recent data, we found that *Ceratium* species are potential indicators of climate change in the Mediterranean (Tunin et al. 2009). However, while temperature is one of the main ecological factor controlling *Ceratium* species distribution, this parameter or others ranging from light to trace metal concentrations maybe associated with morphological variations (e.g. Smayda 2010).

In Aquaparadox work on *Ceratium* was centered around the questions: What are the relationships between morphology, genetics and ecology? Is a species of *Ceratium*, defined via morphological characteristics, actually a group of genetically distinct populations? Does the apparent dichotomy between 'morphs' of the same species reflect genetic difference or simply an adaptation to different environmental conditions? We established clonal cultures of *Ceratium* species to investigate morphological variability among genetically identical individuals and variability related to culture conditions. Natural populations were sampled and genetic analysis performed on single cells to link sequences with documented morphology.

We found examples of both remarkable morphological variability as well as cryptic species in *Ceratium*. One species, *C. ranipes* is characterized by its very prominent 'fingers' although 'fingerless' strains have been reported. We discovered that the species has distinct day and night morphologies. It absorbs its fingers at sunset, a process which takes about 3 hours (Fig. 2), only to re-form the fingers every dawn. We discovered that the varieties of *C. ranipes*, classically distinguished based on 'finger characteristics' (e.g. Jorgensen 1920, Taylor 1976), are but individuals caught different times of the day or night.



Fig. 2. Finger absorption in a single individual *Ceratium ranipes*. Finger morphology at the start of the dark period ($T = 0$), after 88 minutes of darkness, and 211 minutes of darkness. *C. ranipes* is about 200 μm long.

Our discovery of day and night morphologies in *Ceratium ranipes* supports a view that morphological diversity may be greater than genetic diversity, at least in *Ceratium*. However, our work sequencing individual cells from natural populations (Cras et al., in prep.) showed that in at least one 'species', *C. symmetricum*, there are cryptic species, genetically distinct groups of morphologically similar forms. Previously, the species was thought to show a continuum of morphological variation (e.g., Taylor 1976). However, the ITS sequences of 16 cells formed 3 groups indicating the existence of cryptic species of very similar morphologies with 2 of the groups appearing morphologically indistinguishable (Fig. 3).

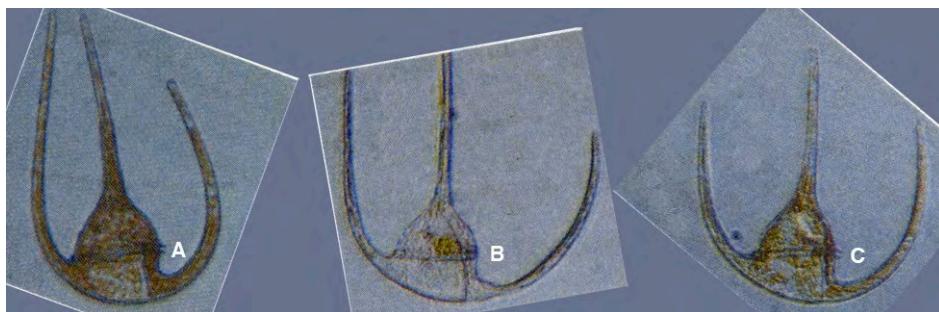


Fig. 3. Single cell sequencing (ITS) of *Ceratium symmetricum* revealed the existence of 3 clades (A, B,C). The morphological variety of *C. symmetricum* (A) previously recognized as '*coarctatum*' is genetically distinct. The two other clades (B, C) appear to be indistinguishable morphologically. *C. symmetricum* is about 150 µm long. From Cras et al., in prep.

C.5 Herbivores

Tintinnid Ciliates

Ciliates are an important component of the microzooplankton, the grazers of phytoplankton in planktonic ecosystems. Among marine planktonic ciliates are tintinnids; they are characterised by the possession of a tube or cup-shaped lorica into which the ciliate cell can withdraw. The group is composed of about 1000 described species. Characteristics of the shell or lorica (material, size, shape architecture) distinguish species. The "paradox of the plankton" applies well to tintinnids. In general, they represent but 10 % of the ciliate microzooplankton. However, species richness appears 'unreasonably' large. In small volumes of water (10 l) 16-21 species can be found (Dolan & Stoeck 2011).

In Aquaparadox we focused on the relationships between morphology, genetics and ecology in tintinnids. Is there a structure to tintinnid assemblages? Is a species of tintinnid, defined via morphological characteristics, actually a group of genetically distinct populations? Is the apparent diversity in terms of numbers of species and morphotypes related to genetic diversity?

We examined natural assemblages in open waters of the Mediterranean Sea to assess the structure and variability of a tintinnid population over a month long period. We found that the population was composed of 'core species', a set of a few species numerically dominating the population, and a large number of satellite or occasional species. Core species were of distinct oral diameters and satellite species were mostly of oral diameters similar to one of the core species (Dolan et al. 2009). The relatively abundant satellite species, may be thought of as 'understudies' of core species, ready to replace a dominant species. This result suggested that oral diameter in tintinnids is a diagnostic character of value. Subsequently, a literature review established the correlation of oral diameter in tintinnids with of a suite ecological characteristics (Dolan 2010).

The question of the relationships between morphological and genetic identity were addressed through development and application of single-cell gene marker amplification and sequencing techniques. New SSU rDNA sequences were acquired for 33 species and ITS rDNA for 17 species (Bachy et al. 2012). Analysis of this new genetic data provided the basis of a new phylogeny and showed conclusively that lorica morphology was unrelated to phylogeny. Furthermore, certain sequences were common to distinct morphologies showing that a tintinnid genotype could correspond to different phenotypes, previously thought to be distinct species of different genera (Fig. 4). Thus, in tintinnids the existing classification based on differences in lorica morphology likely over-estimate actual tintinnid diversity.

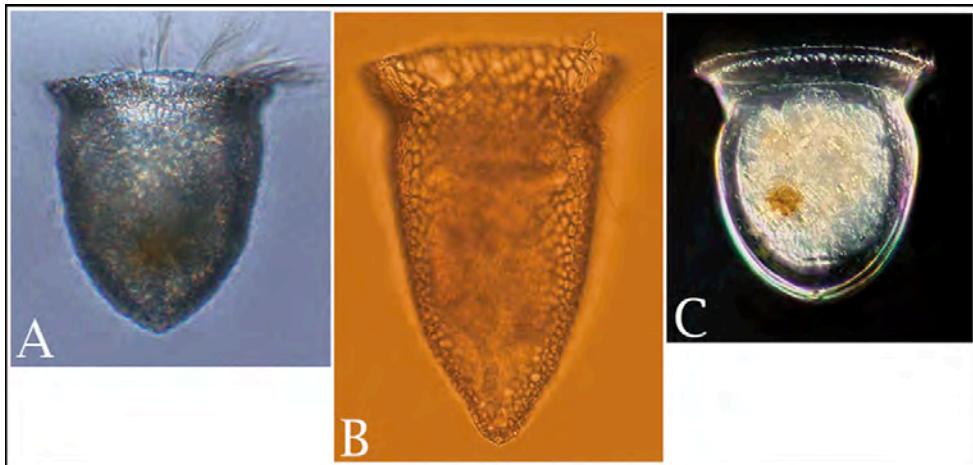


Fig. 4. The SSU rDNA sequences of tintinnids with three distinct lorica types, known as *Cyttarocylis eucercyphalus* (A), *Cyttarocylis cassis* (B) and *Petalotricha ampulla* (C) were found to be identical suggesting them to be a single species capable of forming different lorica morphologies. The loricae are 150-200 µm in length.

C.6 Parasites - Syndiniales -

Syndiniales are a group of protists which includes parasites of marine protists, those infecting dinoflagellates, ciliates, acantharians and even other parasites. In recent years many Syndiniales sequences (also known as Group II Aveolates) have been retrieved from very distinct marine environments ranging from coastal waters to the open ocean, sediments, deep anoxic waters, hydrothermal vents, etc. Thus, just as dinoflagellates and ciliates are found in nearly all marine environments, so are Syndiniales.

In Aquaparadox our efforts relied on the establishment of laboratory cultures to examine infection dynamics and the use of genetic techniques to characterise parasites found in natural host populations. We investigated a specific parasite-host system in a bloom-forming dinoflagellate, *Scrippsiella trochoidea*. We discovered how a parasite can persist in hosts which are seasonal in occurrence- infected hosts can form resting cysts (Fig. 5) allowing the parasite to develop only when the cyst germinates (Chambouvet et al. 2011). Using single cell sequencing, we examined host specificity and phylogenies to determine if host and parasites evolved together. We found no evidence of co-evolution nor any group-specific trends of host range (Bacharoff et al. 2012).

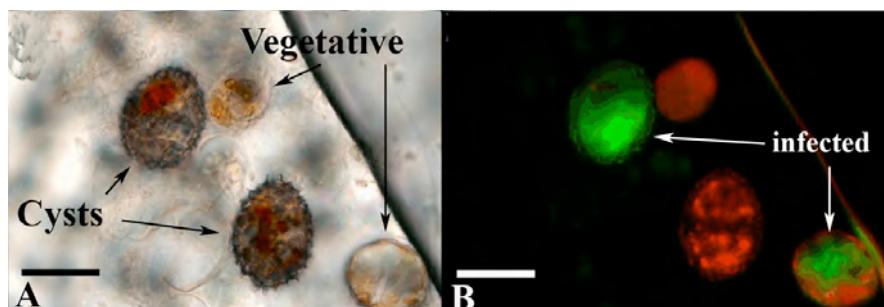


Fig. 5. Cysts and vegetative cells of *Scrippsiella trochoidea*. A Transmitted light micrograph. B. Epifluorescence micrograph showing the autofluorescence of cysts and vegetative cells. Red color is the autofluorescence of chlorophyll, green autofluorescence is the parasite, *Amoebophrya* sp. In B, the cyst in the upper left is infected by *Amoebophrya* sp., lower right is an infected vegetative cell. From Chambouvet et al 2011.

C.7 Macroecology of Planktonic Protists - *Ceratium* & *Tintinnids* -

We compared distributional patterns of protists, *Ceratium* of the phytoplankton and tintinnids of the microzooplankton, to those of copepods, the most abundant multicellular planktonic organisms. We investigated population structures and variability over a month-long period at an open-water site and found all three populations to be similar in that all were composed of 'core species', a set of a few species numerically dominating the population, and a large number of satellite or occasional species, ready to replace a dominant species (Raybaud et al. 2009). Global distributional patterns of all three groups were examined by exploiting data from a historical oceanographic campaign, the last voyage of the Carnegie (Dolan 2011). We found that *Ceratium*, tintinnids and copepods shared a similar latitudinal diversity gradient (Fig. 6).

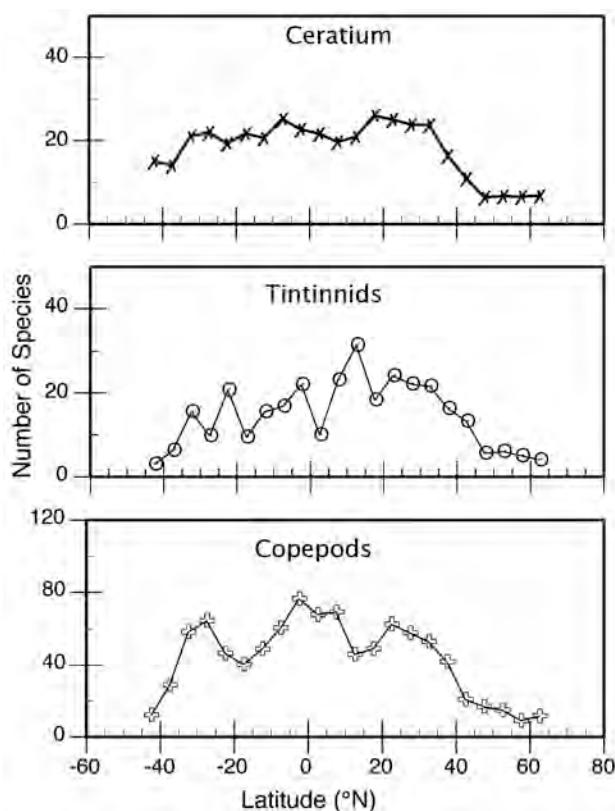


Fig. 6 Protists and multicellular organisms of the plankton show similar global patterns of species richness. The latitudinal diversity gradients of *Ceratium*, tintinnid ciliates and copepods were constructed using data from the taxonomic monographs of the Carnegie expedition. From Dolan (2011).

C.8 Conclusions

Our investigations into the diversity of planktonic protists showed firstly that protist diversity does not differ fundamentally from that of multicellular organisms in overall patterns of temporal and spatial variability. Thus, common mechanisms regulating diversity are likely at work among planktonic organisms. Secondly, we found that protist diversity is more likely to have been over-estimated than under-estimated, at least with regard to free-living (non-parasitic protists). Among planktonic protists, cryptic species may not be a common phenomenon. Careful examination of genetically distinct groups revealed correlation with morphological characteristics. Morphological variability has resulted in single taxa being described as distinct varieties, species, and genera. In contrast to the free-living forms, the diversity of parasitic forms has likely been under-estimated.

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D. LIST OF DELIVERABLES

Date de livraison	N°	Titre	Nature (rapport, logiciel, prototype, données, ...)	Partenaires (souligner le responsable)	Commentaires
Dec 2009	1	Morphological variability-	rapport	1	
Dec 2008	2	Culture	protocole	1	
ongoing	3	Geographic variability of <i>tintinnid</i> spp	rapport	1	
ongoing	4	Geographic variability of <i>Ceratium</i> spp	rapport	1	
ongoing	5	Effects of culture conditions	rapport	1	
n/a	6	Mesocosm experiments: resources vs. predators	rapport	1	Replaced by analysis of historical data
n/a	7	FISH probe data	données	1	Replaced by single-cell sequencing
Dec 2008	8	DNA amplification protocol	protocole	2	
Oct 2011	9	Sequence data molecular markers	données	2	
Dec 2008	10	Development of primers	protocole	2	
Oct 2011	11	Molecular phylogeny and geography	rapport	2	
Dec 2008	12	Adaptation of single cell DNA extraction methods	protocole	3	
Oct 2009	13	SSU sequence-based analysis of <i>Ceratium</i>	données	3	
Oct 2009	14	ITS sequences- of <i>Ceratium</i>	données	3	
	15	Syndiniales & <i>Alexandrium</i> sub-specific relationships	rapport	3	
	16	Comparison of encysted, pre-bloom & bloom populations	rapport	3	
Dec 2008	17	Creation of a Content Management System	logiciel	4	
Dec 2008	18	Determine sequence similarities	rapport	4	
Dec 2008	19	Geographic mapping tools	logiciel	4	
	20	Meta-analysis	logiciel	4	

E PROJET PRODUCTS

E.1 SUMMARY TABLES OF PRODUCTS

E.1 a. Publications & Communications (detailed in E2)

		multipartenaires	monopartenaires
International	Revues à comité de lecture	14	29
	Ouvrages ou chapitres d'ouvrage		4
	Communications (conférence)	4	13
France	Revues à comité de lecture		
	Ouvrages ou chapitres d'ouvrage		1
	Communications (conférence)		4
Actions de diffusion	Articles vulgarisation		2
	Conférences vulgarisation		
	Autres	5 thèses de Doctorat	

E.1 b. Other Products (detailed in E2)

Product type	Brief description (E.3 for details)
Molecular Phylogeny Web Tool	2008 'BLAST' server for identification of microorganisms
Molecular Phylogeny Web Tool	2008 BLAST server for design and testing of PCR primers and probes
Molecular Phylogeny Tool	2008 program EasyTree for the creation of phylogenetic trees
Molecular Phylogeny Web Tool	2008 KeyDNATools website for annotation of sequences
Identification tool	2010 Ceratium ID Website
Identification tool	2011 Dinophysid Website
Image Gallery	2009 Protist Image Gallery
Taxonomic Resource	2010 Classic Planktonic Protist Monographs website

E.2 AQUAPARADOX PUBLICATIONS ET COMMUNICATIONS

E.2.a. Articles in Peer-reviewed International Journals

2008

1) **Chambouvet, A.**, Morin, P., Marie, D., **Guillou, L.** 2008. Control of toxic marine dinoflagellate blooms by serial parasitic killers. *Science* **322**: 1254-1257.

3) **Guillou, L.**, Viprey, M., **Chambouvet, A.**, Welsh, R.M., Kirkham, A.R., Massana, R., Scanlan, D.J., Worden, A.Z. 2008. Widespread occurrence and genetic diversity of marine parasitoids belonging to Syndiniales (Alveolata). *Environmental Microbiology*, **10**: 3349-3365.

4) **Foulon, E.**, Not, F., Jalabert, F., Cariou, T., Massana, R., **Simon, N.** 2008. Ecological niche partitioning in the picoplanktonic green algae *Micromonas pusilla*: evidence from environmental surveys using phylogenetic probes. *Environmental Microbiology*, **10**: 2433-2443.

5) Montagnes, D.J.S., **Chambouvet, A.**, **Guillou, L.**, Fenton, A. 2008. Can microzooplankton and parasite pressure be responsible for the demise of toxic dinoflagellate blooms? *Aquatic Microbial Ecology*, **53**: 211-225.

2009

- 6) Andersen, V., Goutx, M., Prieur, L., **Dolan, J.R.** 2009. Short-scale temporal variability of physical, biological and biogeochemical processes in the NW Mediterranean Sea: an introduction. *Biogeosciences*, 6: 453-461.
- 7) **Dolan, J.R.**, Ritchie, M.E., Tunin-Ley, A., **Pizay, M.-D.** 2009. Dynamics of core and occasional species in the marine plankton: tintinnid ciliates of the N.W. Mediterranean Sea. *Journal of Biogeography*, **36**:887-895.
- 8) **Dolan, J.R.**, Mostajir, B., Sime-Ngando, T. 2009. Rassoulzadegan in Villefranche-sur-Mer and the 'evolution' of marine microbial ecology over three decades. *Aquatic Microbial Ecology*, **57**:221-225
- 9) **Gómez, F., P. López-García ,P.**, Nowaczyk, A., **D. Moreira, D.** 2009. The crustacean parasites *Ellobiopsis* Caullery, 1910 and *Thalassomyces* Niezabitowski, 1913 form a monophyletic divergent lineage within the Alveolata. *Systematic Parasitology* **74**:65-74.
- 10) **Gómez, F., D. Moreira, D., López-García, P.** 2009. Life cycle and molecular phylogeny of the dinoflagellates *Chytriodinium* and *Dissodinium*, ectoparasites of copepod eggs. *European Journal of Protistology* **45**:260-270.
- 11) **Gómez, F., P. López-García, P., Moreira, D.** 2009. Molecular phylogeny of the ocelloid-bearing dinoflagellates *Erythropsidinium* and *Warnowia* (Warnowiaceae, Dinophyceae). *Journal of Eukaryotic Microbiology*, **56**:440-445
- 12) **Pizay, M.-D., Lemée, R., Simon, N., Cras, A.-L.,** Laugier, J.-P., Dolan, J.R. 2009. Night and day morphologies in a planktonic dinoflagellate. *Protist*, 160: 565-575.
- 13) Raybaud, V., Tunin-Ley, A., Ritchie, M. E., **Dolan, J.R.** 2009. Similar patterns of community organization characterize distinct groups of different trophic levels in the plankton of the NW Mediterranean Sea. *Biogeosciences*, 6:431-438.
- 14) **Simon, N., Cras, A.-L., Foulon, E., Lemée, R.** 2009. Diversity and evolution of marine phytoplankton. *Comptes Rendus Biologies*, **332**:159-170.
- 15) Tunin-Ley, A., Ibanez, F., Labat, J.P., Zingone, A., **Lemée R.** 2009. Phytoplankton biodiversity and climate change in NW Mediterranean Sea: changes in dinoflagellate genus Ceratium over the last century. *Marine Ecology Progress Series*, **375**:85-90.

2010

- 16) Chevenet F., **Croce O.**, Hebrard M., **Christen R.**, Berry V. 2010. ScripTree: scripting phylogenetic graphics. *Bioinformatics*, **26**:1125-6.
- 17) **Dolan, J.R.** 2010. Morphology and ecology of tintinnid ciliates of the marine plankton: correlates of lorica dimensions. *Acta Protozoologica*, **49**: 235-244.
- 18) **Gómez, F., Moreira D. & López-García P.** 2010. Molecular Phylogeny of Noctilucid Dinoflagellates (Noctilucales, Dinophyceae). *Protist*, **161**: 466-478.
- 19) **Gómez, F., Moreira D. & López-García, P.** 2010. Molecular phylogeny of the dinoflagellates *Podolampas* and *Blepharocysta* (Peridiniales, Dinophyceae). *Phycologia*, **49**: 212-220.
- 20) **Gómez, F.** 2010. Diversity and distribution of Noctilucoid dinoflagellates (Noctilucales, Dinophyceae) in the open Mediterranean Sea. Gomez, F. 2010. *Acta Protozoologica* **49**:365-372.
- 21) **Gómez, F., P. López-García, P., Moreira, D.** 2010. *Neoceratium gen. nov.*, a new genus for all marine species currently assigned to *Ceratium* (Dinophyceae). *Protist*, **161**:35-54.
- 22) Stoeck, T., Behnke, A., **Christen, R.**, Amaral-Zettler, L., Rodriguez-Mora, M.J., Chistoserdov, A., Orsi, W., Edgcomb, V.P. 2009. Massively parallel tag sequencing reveals the complexity of anaerobic marine protistan communities. *BMC Biology*, **7**:72.

2011

- 23) Alves-de-Souza, C., Cornet, C., Nowaczyk, A., Gasparini, S., Skovgaard, A., **Guillou, L.** 2011. *Blastodinium* spp. infect copepods in the ultra-oligotrophic marine waters of the Mediterranean Sea. *Biogeosciences* 8:2125-2136.

- 24) **Chambouvet A.**, Laabir M., Sengco M., Vaquer A., **Guillou L.** Genetic diversity of the parasitic Amoebophryidae (Syndiniales) *Alexandrium catenella/tamarensse* (Dinophyceae) blooms in Thau lagoon (Mediterranean Sea, France). *Research in Microbiology*, 9:959-968.
- 25) **Chambouvet A.**, Alves-de-Souza C., Cueff V., Marie D., Karpov S., **Guillou L.** (2011). Interplay between the parasite *Amoebophrya* sp. (Alveolata) and the cyst formation of the red tide dinoflagellate *Scrippsiella trochoidea*. *Protist*, 162:637-649.
- 26) Chemidlin Prévost-Bouré, N., **Christen R.**, Dequiedt S., Mougel C., Lelièvre M., Jolivet, C., Shahbazkia, H.R., Guillou, L. Arrouays, D. 2011. Validation and Application of a PCR Primer Set to Quantify Fungal Communities in the Soil Environment by Real-Time Quantitative PCR. *PLoS ONE* 6: e24166. doi:10.1371/journal.pone.0024166
- 27) Cohu S., Thibaut T., Mangialajo L., Labat J.P., Passafiume O., Blanfuné A., **Simon N.**, Cottalorda J.M., **Lemée R.** 2011. Occurrence of the toxic dinoflagellate *Ostreopsis cf. ovata* in relation with environmental parameters in Monaco (NW Mediterranean). *Marine Pollution Bulletin*, 62:2681-2691.
- 28) **Dolan, J.R.**, Stoeck, T. Repeated sampling reveals differential variability in measures of species richness and community composition in planktonic protists. *Environmental Microbiology Reports*, 3:661-666.
- 29) **Dolan, J.R.** 2011. The legacy of the last cruise of the Carnegie: a lesson in the value of dusty old taxonomic monographs. *Journal of Plankton Research*, 33: 1317-1324.
- 30) Edgcomb, V., Orsi, W., Bunge, J., Jeon, S., **Christen, R.**, Leslin, C., Holder, M., Taylor, G.T., Suarez, P. Varela, R., Epstein, S. 2011. Protistan microbial observatory in the Cariaco Basin, Caribbean. 1. Pyrosequencing vs Sanger insights into species richness. *ISME*, 5:1344-1356.
- 31) **Gómez, F., Moreira D.,** Benzerara K. & **López-García P.** 2011. *Solenicola setigera* is the first characterized member of the abundant and cosmopolitan uncultured marine stramenopile group MAST-3, *Environmental Microbiology*, 13: 193-202.
- 32) **Gómez, F., Moreira D. & López-García P.** 2011. Molecular phylogeny of dinophysoid dinoflagellates: the systematic position of *Oxyphysis oxytoxoides* and the *Dinophysis hastata* group (Dinophysiales, Dinophyceae), *Journal of Phycology*, 47:393-406.
- 33) Lecroqa, B., Lejzerowicz, F., Bacharc, D., **Christen, R.**, Esplinge, P., Baerlocher, L., Osterasf, M., Farinelli, L. Pawlowska, J. 2011. Ultra deep sequencing of deep sea foraminiferal diversity challenges the unknowable. *Proceedings of the National Academy of Sciences (US)*, 108:13177-13182.
- 34) Pawlowski, J., **Christen, R.**, Lecroq, B., Bachar, D., Shahbazkia, H.R., Amaral-Ettler, L., Guillou, L. 2011. Eukaryotic richness in the abyss: insights from pyrotag sequencing. *PLoS ONE* 6: e18169. doi:10.1371/journal.pone.0018169.
- 35) Siano R., **Alves-de-Souza C.**, Foulon E., Bendif EIM, **Simon N.**, **Guillou L.**, Not F. Distribution and host diversity of Amoebophryidae parasites across oligotrophic waters of the Mediterranean Sea. *Biogeosciences*. *Biogeosciences*, 8:267-278.
- 2012**
- 36) Bachvaroff, T.R., Kim, S., **Guillou, L.**, Delwiche, C.F., Coats, D.W. Molecular diversity of the syndinean genus *Euduboscquella* Based on Single Cell PCR. 2012. *Applied and Environmental Microbiology*, in press.
- 37) **Bachy, C., Gómez, F., López-García, P., Dolan, J.R., Moreira, D.** 2012. Molecular phylogeny of tintinnid ciliates (Tintinnida, Ciliophora). *Protist*, in press.
- 38) **Gómez, F., Moreira D. & López-García P.** (2011) *Sinophysis* and *Pseudopalacroma* are distantly related to typical dinophysoid dinoflagellates (Dinophysales, Dinophyceae). *Journal of Eukaryotic Microbiology*, in press.
- 39) **Gomez, F., Lopez-Garcia, P., Dolan, J.R., Moreira, D.** Molecular phylogeny of the marine dinoflagellate genus *Heterodinium* (Dinophyceae). *European Journal of Phycology*, in press.
- Submitted**
- 40) Alves-de-Souza C., Varela D., Iriarte J. L., Gonzalez H., **Guillou L.** Amoebophryidae infect harmful dinoflagellates in a southern Chilean fjord dominated by diatoms. *Aquatic Microbial Ecology*.
- 41) Foulon E., Jouenne F., Desdevives Y., Houdan A., Derelle E., Le Gal F., Vaulot D., **Simon N.** 2012. Revision of the genus *Micromonas* (Manton et Parke) (Chlorophyta, Mamiellophyceae), of the species *M. pusilla* (Butcher) Manton et Parke, and description of two novel species, based on the genomic and phenotypic characterization of cultured isolates. *Protist*.

- 42) Pernice M. C, Logares R., **Guillou L.**, Massana R. Diversity and phylogenetic structure in different groups of marine protists. *Environmental Microbiology Reports*
- 43) Tunin-Ley, A., Vaugelas, J. de, Garcia, D., **Marro, S., Lemée, R.** A new collaborative web site to improve the accuracy of dinoflagellates identification: focus on the morphologically-variable genus *Ceratium* Schrank. Soumis à *ISRN Ecology*.
- In Preparation**
- 44) **Cras, A.L., Simon, N.,** Bailly, X., Tunin-Ley, A., **Dolan, J.R., Lemée, R.** Revisiting the delineation of taxa in the genus *Ceratium* Schrank (Alveolata, Dinophyceae) using ITS markers.
- 45) **Bachy, C., Dolan, J.R., López-García, P.,** Deschamps, P., **Moreira, D.** Estimating protist diversity: morphology versus 18S rRNA gene libraries and pyrosequencing: a case study using tintinnid ciliates.
- E.2.b. Book Chapters - International Titles**
- 1) **Dolan, J.R.**, Coats, D.W. 2008. Physiological diversity in widely distributed microzooplankton: Digestion in the ciliate *Euplotes vannus*. in '*Biological Oceanography Research Trends*', L.P. Mertens (ed), Nova Publishers
 - 2) **Gómez, F.** 2010. Changes in the Mediterranean phytoplankton community related to climate warming. in "*Phytoplankton responses to Mediterranean environmental changes*", N° 40, CIESM Workshop Monographs, (F. Briand, Ed.), CIEMS, Monaco, pp. 37-42.
 - 3) **Dolan, J.R.** 2012. Tintinninid Ciliates: an Introduction and Overview in *Biology and Ecology of Tintinninid Ciliates: Models for Marine Plankton*, Dolan, J.R., Agatha, S., Coats, D.W., Montagnes, D.J.S., Stocker, D.K. (eds). Wiley-Blackwell, Oxford, in press.
 - 4) **Dolan, J.R.** 2012. Diversity and Distributions of Tintinnid Ciliates. in *Biology and Ecology of Tintinninid Ciliates: Models for Marine Plankton*, Dolan, J.R., Agatha, S., Coats, D.W., Montagnes, D.J.S., Stocker, D.K. (eds). Wiley-Blackwell, Oxford, in press.
- E.2.c. Book Chapters - France**
- 1) **Chambouvet A., Guillou L.** 2009. Les *Amoebophrya*, parasitoïdes de dinoflagellés toxiques. *Toxines et Signalisation*. Rencontres en Toxinologie. Editions de la SFET. Accès libre sur le site : <http://www.sfet.asso.fr>. 73-78.
 - 2) **López-García, P. & Moreira, D.** (2010) Les grandes profondeurs. In "Aux origines de l'environnement" P.-H. Gouyon & H. Lériche (editors), Fayard, Paris, pp 146-161.
- E.2.d Articles - General Public/Popular Press**
- 1) **Guillou L.**, Alves-de-Souza C., Siano R., Gonzalez H. 2010. The ecological significance of small, eukaryotic parasites in marine systems. The ecological significance of small, eukaryotic parasites in marine systems. *Microbiology Today*. May, 2010: 93-95.
 - 2) Jacquet, S., Domaizon, I., Masquelier, S., Lepère, C., **Guillou, L., Chambouvet, A.**, Debroas, D., Sime-Ngando, T. 2011. Virus, bactéries et protistes pathogènes Le rôle insoupçonné des parasites dans le fonctionnement des écosystèmes aquatiques. *Courrier de l'Environnement de l'INRA* n°60: 33-46.
- E.2.e Communications- International Conferences**
- 2009**
- 1) **Bachy, C., Lopez-Garcia, P. Moreira, D.**, Vereshchaka, A. Diversity and vertical distribution of microbial eukaryotes in the snow, sea ice and marine waters from North Pole. *11th Symposium on Aquatic Microbial Ecology*, Piran, Solvenia, August-September, 2009.
 - 2) **Chambouvet, A., Guillou, L.** Amoebophryidae (Syndiniales), an important parasitoid group for dinoflagellates. ASLO Aquatic Sciences Meeting, Nice, France, January 2009.

3) **Cras, A.-L., Bailly, X., Simon, N., Lemée, R.** Genetic variability and molecular phylogeny of the genus *Ceratium* (planktonic dinoflagellates) inferred from single-cell analysis of its rDNA sequences. ASLO Aquatic Sciences Meeting, Nice, France, January 2009.

4) **Dolan, J.R.**, Ritchie, M.E., Raybaud, V., Tunin -Ley, A. Similar patterns of community organization characterize groups of different trophic levels in the plankton of the NW Mediterranean Sea. ASLO Aquatic Sciences Meeting, Nice, France, January 2009.

2010

5) **Dolan, J.R.** 2010. Stars, co-stars and understudies in planktonic protist communities: an analysis of niche separation among species in the tintinnid ciliate community of the Bay Villefranche (NW Mediterranean Sea). International Society of Protistologists - British Society for Protist Biology Joint Meeting, Canterbury, UK, July 2010.

6) **Bachy, C., Gomez, F., Dolan, J., Moreira, D., Lopez-Garcia, P.** 2010. Merging molecular phylogeny and classical taxonomy of tintinnid ciliates. International Society of Protistologists - British Society for Protist Biology Joint Meeting, Canterbury, UK, July, 2010.

7) **Lemée R.**, Thibaut T., Cohu S., Blanfuné A., Marro S. & Mangialajo L. (2010). Spatial and temporal variations of planktonic and epibenthic cells of *Ostreopsis cf. ovata* in NW Mediterranean (Ligurian Sea). 14th International Conference on Harmful Algae, Crete, Greece, November, 2010

8) **Dolan, J.R., Ritchie, M.** 2010. Coherent species packing of planktonic protists: Tintinnid ciliates of the oligotrophic Mediterranean Sea. AGU-ASLO-TOC Ocean Sciences Meeting, Portland OR, USA, February 2010.

9) **Guillou, L.**, Chambouvet, A. 2010. Interplay between the marine parasite Amoeboophrya (Syndiniales) survival and the sexuality of its host (*Scrippsiella trochoidea*, Dinoflagellate). AGU-ASLO-TOC Ocean Sciences Meeting, Portland OR, USA, February 2010. **Invited**

10) Guillou L. Parasitic dinoflagellates in marine systems. Society for General Microbiology meeting, Edinburgh. March 2010. **Invited**

2011

11) Cohu S., Mangialajo L., Thibaut T., Blanfuné A., **Lemée R.** (2011). *Ostreopsis cf. ovata* development in relation with depth, biotic substrate and environmental factors in the North Western Mediterranean Sea in 2008 and 2009 years – MediOs 2 program. International Congress on *Ostreopsis* Development, Villefranche-sur-Mer, France, April 2010.

12) Cohu, S., Thibaut, T., Mangialajo, L., Labat, J.P., Passafiume, O., Blanfuné, A., **Simon N.**, Cottalorda, J.M., **Lemée, R.** (2011). Impact of environmental factors on *Ostreopsis cf. ovata* dynamic during the summers 2007 and 2008 in Monaco (NW Mediterranean Sea). International Congress on *Ostreopsis* Development, Villefranche-sur-Mer, France, 6-8 April.

13) **Dolan, J.R.** 2011. Similarity in widely separated communities of planktonic protists (tintinnid ciliates). VI European Congress of Protistology, Berlin, July 2011. **Invited**

14) **Bachy, C., Lopez-Garcia, P., Dolan, J. Moreira, D.** 2011. Morphological versus molecular-based diversity surveys of planktonic microbial eukaryotes: the case of tintinnid ciliates. The 12th Symposium on Aquatic Microbial Ecology, Rostock, August, 2011.

15) **Dolan, J.R.** 2011. Using Old Data to Examine Current Questions: The Last Voyage of the Carnegie. 1st Asian Congress of Protistology (ACOP 2011), Jeju, Korea, October 2011. **Invited**

16) **Dolan, J.R.** 2011. Niche separation and the role of dominants among tintinnid ciliates, grazers of the microzooplankton. Dolan, J.R. ASLO 2011 Aquatic Sciences Meeting, San Juan, PR, Feb. 2011.

17) Mangialajo L., Ganzin N., Accoroni S., Asnaghi V., Blanfuné A., Cabrini M., Cattaneo-Vietti R., Chavanon F., Chiantore M., Cohu S., Costa E., Fornasaro D., Grossel H., Marco-Miralles F., Masó M., Reñé A., Rossi A.M., Sala M.M., Thibaut T., Totti C., Vila M., **Lemée R.** (2011). Trends in *Ostreopsis cf. ovata* proliferation along the northern Mediterranean Sea (NW Mediterranean and Adriatic Seas) coastal waters. International Congress on *Ostreopsis* Development, Villefranche-sur-Mer, France, April, 2011.

E.2.e Communications- Conférences France

2009

1) **Dolan, J. R.** Comparing ciliate community structure in the East and West (A vs.C). BOUM Post-cruise Meeting, Marseille, France, June 2009.

2) **Guillou, L.** Infection of copepods by the parasitic photosynthetic dinoflagellate *Blastodinium* during the BOUM cruise. BOUM Post-cruise Meeting, Marseille, France, June 2009.

2011

3) **Dolan, J.R.** 2011. The challenge of understanding ecological roles and diversity in heterotrophic protists of the plankton. Dolan, J.R. ANR/ARP meeting- Combining high throughput genomics, quantitative imaging, in situ oceanographic and satellite data and modeling to better understand plankton ecosystems at the planetary level. Villefranche-sur-Mer, January 2011. **Invited**

4) Tunin-Ley, A., de Vaugelas, J., Garcia, D., **Marro, S., Lemée, R.** (2011). Un nouveau site web interactif dédié à l'identification spécifique et infraspécifique des taxons du genre *Ceratium* Schrank. Colloque de la Société Phycologique de France, Villefranche-sur-Mer, France, April, 2011.

E.2.f Thèses

2008

Foulon, E., 2008. Diversité génétique, biochimique, physiologique, morphologique et écologique au sein de l'espèce picoplanctonique marine *Micromonas pusilla* (Butcher) Manton & Parke (Chlorophyta, Prasinophyceae). Thèse de l'Université Pierre et Marie Curie.

2009

Chambouvet, A. 2009. Les Amoebophryidae (Syndiniales) parasitoïdes de dinoflagellés : cycle de vie, dynamique et spécificité in situ. Thèse de l'Université Pierre et Marie Curie.

2011

Alves de Souza, C. 2011. Interactions parasitoid-host between Amoebophryidae (MALV II) and planktonic dinoflagellates in marine systems with different trophic status. Thèse de l'Université Pierre et Marie Curie en cotutelle avec l'Université Australie du Chili.

2012

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E.2.g Mémoires

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Cras, A.-L. 2009. Relations entre la diversité morphologique et la diversité génétique au sein du genre *Ceratium* (dinoflagellé phytoplanctonique). Mémoire de fin d'études Diplôme d'Etudes Supérieures. Université Pierre et Marie Curie,

2010

Bergeret J. 2010. Diversité et écologie de la picoalgue marine *Micromonas pusilla* (Chlorophyta, Mamiellophyceae) et de ses virus dans un écosystème marin côtier (Manche occidentale). IUT d'Anger, Rapport de Licence professionnelle BAEMOVA.

Cornet, C. 2010. Ecology and phylogeny of endocommensal photosynthetic dinoflagellates (*Blastodinium* spp.) infecting marine planktonic copepods. Rapport de Master 2 Université Pierre et Marie Curie.

2011

Biard, T. 2011. Contribution à la création d'un site internet de reconnaissance de Dinophysiales. Rapport de Master 1 Université Pierre et Marie Curie.

E3 Other Aquaparadox Project Products

E.3.a. Bioinformatic tools

1) BLAST server dedicated to the identification of sequences from microorganisms

<http://bioinfo.unice.fr/blast>

The server allows sequence searches by source type and origin. Thus, searches for matches can be restricted to sequences from cultured, therefore presumably correctly identified, microorganisms. The server returns annotated files of sequences directly exportable to EasyTree, our program for the construction and manipulation of phylogenetic trees (see below).

2) BLAST server for the design and testing of PCR primers and probes

<http://bioinfo.unice.fr/ohm>

The tool is used for creating, verifying and adjusting the sequences of group-specific primers and probes. The webpage includes extensive documentation and tutorials.

3) The EasyTree program for the creation of phylogenetic trees

The program uses sequences in the fasta format from the large public databases (e.g., EBI et NCBI) and re-formats them for efficient phylogenetic analysis. Annotated data files are created directly from the fasta format files or by automated requests to the NCBI using EUtils (cgi scripts). The program produces nearly automatically phylogenetic trees of publication quality which importantly can include notation of the geographic origin of sequences.

4) KeyDNATools

<http://keydnatools.com/>

A website for automatic annotation of 18sRNA sequences and the detection of chimeric sequences.

5) Web-based guides for Identification of Protists

Simple guides to common species were developed for dinoflagellates of the genus Ceratium and Dinophysid forms.

Ceratium Identification Website

http://720plan.ovh.net/~idbio/idbiov3/data/visu_test/index.php?Key_projet=a87ff679a2f3e71d9181a67b7542122c#

Dinophysiales Identification Website

http://720plan.ovh.net/~idbio/idbiov3/data/visu_test/index.php?Key_projet=4e732ced3463d06de0ca9a15b6153677

6) Image Gallery of Planktonic Protists

Image gallery of planktonic protists, free-living and parasitic. The album contains over 500 images, most are original images.

<http://www.obs-vlfr.fr/gallery/Aquaparadox>

The 'image gallery' is a linked resource on other websites:

1) International Society of Protistology, 'Portal to Protistology' , Images of Protists:

<http://protozoa.uga.edu/portal/images.html>

2) Census of Marine Zooplankton (Census of Marine Life), Galleries, Protist:

<http://www.cmarz.org/galleries.html>

3) Centre of Excellence for Dinophyte Taxonomy, Photo Collections:

http://www.dinophyta.org/index.php?option=com_content&task=blogsection&id=10&Itemid=56

Many of the images have also been contributed to other websites:

1) World Register of Marine Species: <http://www.marinespecies.org/>

2) AlgaeBase: <http://www.algaebase.org/>

3) Encyclopedia of Life: <http://eol.org/>

4) ASLO Image Library: <http://aslo.org/photopost/>

4) Soon- Website of the U. S. National Oceanographic and Atmospheric Administration (NOAA):

<http://www.photolib.noaa.gov/>

7) Classic Taxonomic Monographs WebPage

Taxonomic monographs made available for downloading as PDF files. A large number of publications (137 as of 31/12/2011) which are difficult to obtain, dating from the 1840's to the 1990's, were scanned or compiled from other sources. These works, made available on a single page, include the majority of key references, original species descriptions, and monographs concerning tintinnid ciliates, marine planktonic dinoflagellates, and other planktonic protists.

<http://www.obs-vlfr.fr/LOV/aquaparadox/html/ClassicMonographs.php>

The 'classic taxonomic monographs' page is a linked resource on other websites:

1) Centre of Excellence for Dinophyte Taxonomy, literature:

http://www.dinophyta.org/index.php?option=com_content&task=view&id=141&Itemid=97

2) The journal *Acta Protozoologica*, Bibliographic Resources:

http://www.eko.uj.edu.pl/ap/index.php?option=com_content&task=view&id=24&Itemid=54

3) Census of Marine Zooplankton (Census of Marine Life), Zooplankton Taxonomic References:

http://www.cmarz.org/resources/zooplankton_taxonomic_reference